BITES, VENOMS, AND VENOMOUS

SNAKES OF COLOMBIA



INSTITUTO NACIONAL DE SALUD

BITES, VENOMS, AND VENOMOUS SNAKES OF COLOMBIA

Editors
Teddy Angarita Sierra
Francisco J. Ruiz Gomez

Salud

Instituto Nacional de Salud

Bites, Venoms, and Venomous Snakes of Colombia / Teddy Angarita Sierra and Francisco Ruiz [eds.]; first edition. Bogotá: Instituto Nacional de Salud, 2024.

500 p. (color illustrations)

ISBN Impreso: 978-958-13-0188-1
ISBN Digital: 978-958-13-0189-8

1. Snake venoms - Colombia 2. Snakebites 3. Antivenoms 4. Viperidae 5. Elapidae 6. Biological toxins 7. Envenomation 8. Toxinology I. Angarita Sierra, Teddy II. Ruiz Gomez, Francisco.
NLM: QW630.5.V4 - AN581m

President of the Republic
Gustavo Francisco Petro Urrego

Minister of Health and Social Protection
Guillermo Alfonso Jaramillo Martinez

Director General, National Institute of Health
Dra. Diana Marcela Pava Garzon

Director of Production, National Institute of Health
Dr. Juan Felipe Bedoya Meneses

Scientific committee
Dr. Franklyn Edwin Prieto Alvarado
Director of Surveillance and Risk Analysis in Public Health, National Institute of Health.

Dr. William W. Lamar
Professor Emeritus, University of Texas at Tyler.

Dr. Thomas Defler
Professor Emeritus, Department of Biology, Universidad Nacional de Colombia, Bogota Campus.

Dr. Juan Felipe Bedoya Meneses
Director of Production, National Institute of Health.

Editors
Teddy Angarita-Sierra
Biodiversity for Society Research Group, Academic Directorate, Universidad Nacional de Colombia, at La Paz, Cesar.

Francisco J. Ruiz-Gomez
Research Group on Venomous Animals and Their Venoms, Production Department, National Institute of Health.

Authors
Adrian Marcelo Franco Vasquez, Alejandro Montañez Mendez, Amira Abultaif Kadamani, Andres C. Montes-Correa, Ariadna Rodriguez Vargas, Brayan Fahir Castillo Calderon, Camila Figueredo Salinas, Carlos Andres Bravo-Vega, Carlos Antonio Castro-Sandoval, Carolina Zona, Chris Akcali, Cristian Gonzalez Acosta, Daniel Bocanumenth, Daniel J. Sanchez-Ochoa, Daniela Garcia Cobos, Diana Marcela Walteros Acero, Dina Lucia Rivera-Robles, Esteban Alzate, Felipe Andres Toro Cardona, Fernando Vargas Salinas, Francisco J. Ruiz Gomez, Guido F. Medina-Rangel, Jackeline Rivera Gomez, Jaime Andres Pereañez Jimenez, Jose Rances Caicedo-Portilla, Juan Carlos Vega Garzon, Juan Daniel Vasquez Restrepo, Juan David Bayona Serrano, Juan David Jimenez Bolaño, Juan Jose Torres Ramirez, Juan Pablo Hurtado Gomez, Julian Alberto Rios Soto, Julian Andres Rojas Morales, Katterine Isabel Urieles Sierra, Kristian Alberto Torres-Bonilla, Laura Rubio-Rocha, Lina Maria Peña Acevedo, Luis Enrique Vera Perez, Manuel Maldonado-Contreras, Maria Carlina Castillo-Beltran, Maria F. Diago-Toro, Maria Paula Toro-Gomez, Maria Victoria Cubillos Abrahams, Mariela Osorno Muñoz, Marley Tatiana Gomez Rincon, Miguel Arevalo-Paez, Monica M. Saldarriaga Cordoba, Monica Sarmiento, Monica T. Rincon Aranguri, Nathalie Citeli, Santiago Casas-Cardona, Santiago J. Sanchez-Pacheco, Juan Sebastian Navarro Gonzalez, Sergio D. Cubides-Cubillos, Teddy Angarita Sierra, Wilmar Agudelo-Sanchez, Yelenny Lopez Aguirre.

Scientific reviewers and evaluators
Dr. Eliecer de Jesus Jimenez Charris,
Nutrition Group, Faculty of Health, Universidad del Valle.

Dra. Leidy Johana Vargas
Universidad Cooperativa de Colombia.

Dr. Alejandro Carbajal-Saucedo
Herpetology Laboratory, Faculty of Biological Sciences, Universidad Autonoma de Nuevo Leon.

Dra. Nohora Vega
Protein Research Group, Department of Chemistry, Faculty of Sciences, Universidad Nacional de Colombia.

Dr. Gerardo Corzo
Department of Molecular Medicine and Bioprocesses, Institute of Biotechnology, Universidad Nacional Autonoma de Mexico.

Dr. William W. Lamar
Universidad de Texas en Tyler.

Dra. Maria Elisa Peichoto
National Scientific and Technical Research Council (CONICET), National Institute of Tropical Medicine (INMeT) -ANLIS Dr. Carlos G Malbran, Puerto Iguazu, Misiones, Argentina.

Dr. Alexis Rodriguez Acosta
Laboratory of Immunochemistry and Ultrastructure, Anatomical Institute, Universidad Central de Venezuela.

Dra. Maria Elena Barragan
Gustavo Orces Foundation, Ecuador.

M.Sc. Juan Manuel Renjifo
Former professor of the Research Group on Management and Conservation of Neotropical Fauna, Flora and Strategic Ecosystems (MIKU), Faculty of Basic Sciences, Universidad del Magdalena.

MD. Rafael Otero-Patiño
Former professor of the Snakebite/Scorpionism Program, Faculty of Medicine and Academic Corporation for the Study of Tropical Pathologies, Universidad de Antioquia.

MD. Santiago Ayerbe
Former professor of pediatrics at the Faculty of Health Sciences and of Toxinology at the Faculty of Natural, Exact and Educational Sciences of the Universidad del Cauca.

Dr. Thomas Defler
Professor Emeritus, Department of Biology, Universidad Nacional de Colombia sede Bogota.

Dr. Osnamir Elias Bru Cordero
Dirección Académica, Universidad Nacional de Colombia sede De La Paz.

Dra. Paola Rey-Suarez
Research Group in Toxinology, Therapeutic and Food Alternatives, Faculty of Pharmaceutical and Food Sciences, Universidad de Antioquia.

Dra. Nancy Oguiura
Special Laboratory of Ecology and Evolution, Butantan Institute

Style reviewers
Franklin Ruiz — Spanish
Thomas Defler — English

Digital and printed design and layout
Maria Catalina Duran and Johanna Montes Bustos

Web design and layout
Jonathan Medina and Daiby Javier Jimenez

Art Front and back cover
Oscar A. Ramirez Ruiz

Art and illustrations
Oscar A. Ramirez Ruiz and Juliana Mendoza

Mapping
Carlos A. Bravo-Vega

Please cite as
Complete works in Spanish:
Angarita-Sierra, T., Ruiz-Gomez, F.J. Mordeduras, venenos y serpientes venenosas de Colombia. Instituto Nacional de Salud: Bogota D.C., Colombia, 2024.

Complete works in English:
Angarita-Sierra, T., Ruiz-Gomez, F.J. Bites, venoms, and venomous snakes of Colombia. Instituto Nacional de Salud: Bogota D.C., Colombia, 2024.

Chapter in Spanish:
Cubides-Cubillos, S.D; Saldarriaga-Cordoba, M.M; Pereañez, J.A; Angarita-Sierra, T. Capitulo 3. Asesinos por naturaleza: Comprendiendo los caminos evolutivos de las viboras colombianas. En: Mordeduras, venenos y serpientes venenosas de Colombia; Angarita-Sierra, T., Ruiz-Gomez, F.J, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 125-178.

Chapter in English:
Cubides-Cubillos, S.D; Saldarriaga-Cordoba, M.M; Pereañez, J.A; Angarita-Sierra, T. Chapter 3. Natural-born killers: Understanding the evolutionary paths of Colombian pitvipers. In book: Bites, venoms, and venomous snakes of Colombia; Angarita-Sierra, T., Ruiz-Gomez, F.J, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 115-164.

Copyright: © 2024 National Institute of Health
Open access publication under the terms and conditions of the Creative Commons Attribution license (CC BY-NC-ND 4.0). Avenida calle 26 No. 51-20, Zona 6, CAN Bogota, D. C., Colombia Phone: (+57) 6012207700 www.ins.gov.co

First edition 2024
ISBN HARD COPY: 978-958-13-0188-1
ISBN DIGITAL: 978-958-13-0189-8
DOI: https://doi.org/10.33610/9789581301881

Responsibility and Legal Use
The designations employed and the presentation of the content in this publication do not imply the expression of any opinion or judgment on the part of the National Institute of Health (INS). Likewise, the views expressed do not necessarily reflect the official decisions, policies, or positions of the INS. All concepts, analyses, and opinions presented herein are the sole responsibility of the respective authors.

All proprietary rights to this work belong to the National Institute of Health (INS) of Colombia. This publication is open access and is licensed under the terms of the Creative Commons Attribution-Non-Commercial-NoDerivatives 4.0 International License (CC BY-NC-ND 4.0). This means that the material may be copied and redistributed in any medium or format for non-commercial purposes only, provided that appropriate credit is given to the INS as the rights holder, no modifications or adaptations are made to the content, and all legal notices remain intact. For more information about this license, please refer to: http://creativecommons.org/licenses/by-nc-nd/4.0/.

This work complies with Colombian legal deposit regulations. It may not be used to promote or endorse specific products, services, or entities. The use of the INS institutional logo is restricted and requires prior express written authorization from the institution.

Presentation

The book "*Bites, Venoms, and Venomous Snakes of Colombia*" represents a scientific milestone that reinforces the National Institute of Health's (INS) commitment to generating and managing knowledge for public health. Based on Colombia's unique biodiversity, this work comprehensively addresses toxinology, venomics, and the epidemiology of snakebite accidents, as well as the development of antivenoms and medications derived from snake venom toxins.

This work is backed by 55 national and international scientists who generously and selflessly contributed their knowledge and experiences regarding the taxonomic, biochemical, pharmacological, ecoepidemiological, and clinical aspects of Colombian snakes of medical importance. It provides a modern foundation that promotes progress in research, education, and public health policies.

The contributions in this work synergistically align with the mission of the INS, which aims to: (1) *Produce scientific knowledge in health by characterizing and analyzing the diversity and biochemical properties of snake venoms, promoting an understanding of ophidism and its medical implications*; and (2) *Contribute to public health sovereignty by* highlighting the national development of antivenoms and innovative biomedical technologies derived from snake toxins. This positions Colombia as one of the tropical countries leading biomedical advancements to reduce the morbidity and mortality caused by ophidism, one of the most neglected tropical diseases worldwide.

Similarly, the contributions from the authors and reviewers of the ten chapters and 59 snake species profiles help consolidate the strategic objectives of the INS, which aim to: (1) *Generate and manage knowledge by providing scientific evidence that facilitates the formulation of public policies and health strategies based on Colombia's territorial needs*; (2) *Strengthen the public health surveillance system* through detailed ecoepidemiological analysis, providing critical information to identify patterns and risk factors associated with snakebite incidents; (3) *Produce goods of interest for public health* by recounting the history and analyzing advances in antivenom production, reinforcing the INS's role as a leader in the national production of these essential medicines.

Particularly, this work enhances the INS's leadership in public health by creating the Colombian Snakebite Geovisor (https://ofidismo.ins.gov.co/), a tool that uses open data to provide free access to epidemiological and environmental information that is openly available for consultation and download. This reinforces the INS's commitment to transparency, free access to public information, as well as to develop public health strategies based on scientific evidence. Furthermore, the book is available in

three different formats: a freely downloadable PDF, a web version (https://serpientes.ins.gov.co), and a limited printed edition. In this way, the INS promotes open access to the book as an educational, technical, and scientific resource for health professionals, researchers, policymakers, and the general public.

The book and the Geovisor actively implement territorial articulation principle of the National Institute of Health by providing direct access to high-quality information from the territories and for the territories. This contribution significantly impacts community education, promoting comprehensive knowledge of venomous snakes and encouraging the prevention and proper management of bites. These efforts are essential to reducing severe cases of ophidotoxicosis and strengthening the protection of the most vulnerable communities.

"*Bites, venoms, and venomous snakes of Colombia*" is a fundamental contribution to Colombia's scientific and medical advancement. Its interdisciplinary approach highlights the INS's capacity to address priority public health issues. Moreover, it strengthens the country's health sovereignty through innovation in antivenom production that responds to the territorial needs and unique challenges presented by Colombia as a biologically and culturally megadiverse country.

A step forward in safeguarding biodiversity and public health in Colombia—because public health is built with and from the territories!

Dr. Diana Marcela Pava Garzon,
Medical toxicologist
Director General
National Institute of Health

Foreword

Colombia's unique biodiversity and my friends and collaborators in the country

Colombia enjoys the richest faunal biodiversity of any country, being inhabited by 10% of the world's animal species, many of them endemic. These include more than 1,800 species of birds, 1,500 of freshwater fish, and some 460 of mammals and 340 of snakes.

Over the last 25 years, I was privileged to be introduced to some of these wonders, during visits to Bogota, Medellin, Villa de Leyva, and to Cartagena, where, in 2000, I helped to organise an International Congress of Tropical Medicine, opened by President Andres Pastrana Arango. But long before my first visit to Colombia, I had corresponded with Juan Jose Silva-Haad at Hospital Regional de Leticia. He sent me reprints of his fascinating papers, in one of which he illustrated the horrifying local tissue destruction and haemorrhage caused by *Lachesis muta* envenoming in local Amerindians. He described a distinctive syndrome of autonomic nervous system stimulation, evolving as soon as 15 minutes after the bite [1]. It comprised vasogenic shock with marked bradycardia, vomiting, diarrhoea and abdominal colic [2].

Since about 2000, my guide, philosopher, and friend in Colombia has been Juan Manuel Renjifo. His photographs and publications celebrate the animals, plants, and scenery of Colombia, especially: "*Ranas y Sapos de Colombia*" (1997); "*Anfibios y reptiles de Urrá*" (1999); and the amazing "*La Amazonia de Colombia*" (2008). Dave Theakston and I first met him at the Instituto Nacional de Salud (INS) in Bogota, to seek his help with the Liverpool School of Tropical Medicine's international initiative to develop a new Pan African Polyspecific Antivenom for the neglected and deserted snakebite victims in that vast continent [3]. Juan Manuel and his colleagues at INS, including Francisco Ruiz Gomez, and Santiago Nicholls, raised our programme's most potent candidate antivenom, successfully immunising Colombian horses against selected African snake venoms, including that of the notorious black mamba (*Dendroaspis polylepis*). Unfortunately, political problems prevented the implementation of this promising result.

The need for "Bites, Venoms, and Venomous Snakes of Colombia"

With this background of admiration for Colombian collaborators, Colombia's venomous snakes, and INS antivenoms, I was excited to hear of the plan by the Serpentarium at INS to publish "*Bites, Venoms, and Venomous Snakes of Colombia*". This ambitious project will include 10 chapters and 59 species sheets both in Spanish and English, authored by 55 experts, in three different formats: a freely downloadable pdf; a web ver-

sion; and (mercifully for those of us who still prefer hard copy) a limited print edition. A database linked to the book is the "*Geovisor Colombiano de Ofidismo*" (https://ofidismo.ins.gov.co/). It makes all the shapefiles of epidemiological information, inventories of the environmental variables, and curated distribution records of the medically important Colombian snake species, available for free download. Such a generous strategy will display the content available to a very wide, international, and grateful readership. I anticipate publication as eagerly as the world's many other snake, snakebite, and toxinology enthusiasts.

I hope that this publication may stimulate discussion towards the drawing-up of new consensus guidelines for the prevention and clinical management of snakebite envenoming in Colombia. I have observed the continuing belief in traditional shamanic treatments among rural people, and diverse approaches to medical treatment in the different Latin American countries that I have been privileged to visit (Brazil, Ecuador, Peru, Costa Rica, Martinique, Saint Lucia, and Colombia). In the absence of unifying WHO/PAHO snakebite advice, or up-to-date national guidelines in many countries, controversial therapies are still used in some centres. For example, a patient bitten by *Bothrops asper* in Colombia, who sought my advice after returning to Europe, had been treated with topical ice packs (causing frostbite incurred during heavy opiate-sedation), an anticoagulant, platelet-inhibitor, non-steroidal anti-inflammatory agent, corticosteroid, antihistamine, and prophylactic antibiotic, and had been kept immobilisation in bed. Cryotherapy has been abandoned in most countries, because it was found to cause tissue damage in rattlesnake-bite-victims in North America [4]. Anticoagulants and the other drugs have also been proscribed by most authorities, while early re-mobilisation is encouraged, to restore normal function of the bitten limb.

In advance of publication, I have been granted a limited preview of some of the chapters.

Antivenom production in Colombia

Of particular interest to me was Chapter 7: "*Venoms, Love, and Hate*" by Amira Abultaif Kadamani which gives a detailed perspective of the history of antivenom production, from Albert Calmette's original "sérum antivenimeux", first used successfully in a human victim of monocled cobra (*Naja kaouthia*) envenoming in Saigon, Vietnam in 1896; to Vital Brazil in Brazil and Clodomiro Picado in Costa Rica (early 20th century); to Bernado Samper in Colombia (from 1937), with later help by Roger Bolaños from Costa Rica, and eventual antivenom production at INS by Juan Manuel Renjifo, coordinator of the Serum Group (in the mid-1970s).

Species characteristics and the quest for clinical phenotypes of envenoming

The emphasis of this book is on the recognition and identification of Colombia's venomous species with details of their precise geographical distribution (*Geovisor Colombiano de Ofidismo*, https://ofidismo.ins.gov.co/);

and their morphology, recognition, symptoms of envenoming, treatment and epidemiology of bites, conservation etc., (provided by the individual species sheets). This accords perfectly with my career-long emphasis on trying to define the clinical phenotypes (syndromes or composite pictures of clinical effects of envenoming) by the different species. Without such detailed data, optimal treatment of severely envenomed patients, and guidance to laboratory toxinologists in discovering improved treatments that target medically-relevant toxins/epitopes, will not be possible. The hand-drawn illustrations of each species are a very attractive feature of these species sheets, and provide an opportunity to emphasise distinctive features, such as the black inverted anchor nuchal marking of *M. ancoralis*. I note that the local Spanish name for this species is "gargantilla" meaning "choker" or bejewelled regal necklace in English, also referring to this snake's neck markings. The lack of specific symptomatology and treatment advice highlights the need for prospective clinical studies of patients envenomed by expertly-identified snakes, for toxinological studies of their venomics, and, in particular, of their neutralisation by available antivenoms, and the possible role of first-aid with anticholinesterases [5].

For future use, it is encouraging that a polyspecific *Micrurus* antivenom developed in Medellin immune-recognised the venoms of M. ancoralis and 8 other Colombian *Micrurus* venoms [6], possibly expanding the range of the polyvalent coral snake antivenom developed earlier at INS [7].

Snake mythology? The dreaded surucucú/shuchúpe (Lachesis muta)

I look forward to reading the account of Colombia's, and the Western Hemisphere's, most spectacular venomous snakes, the bushmasters. Despite their reclusive and generally-inoffensive habits, it is perhaps not surprising that their formidable size (perhaps exceeding 3.6 m in total length) and the devastating effects of their venom (see above) have generated great, awe, and dread in the countries they inhabit.

A recent expedition across the Guyanese jungle, shown on UK television [8] reminded me of one of the most widespread myths surrounding these marvellous animals, their alleged "song". This was first mentioned by Evaristo Garcia in his classic "*Los Ofidios Venenosos del Cauca*" (Cali, Paris: Ch. Bouret, 1896): "Á este ofidio se refieren las leyendas más singulares, contadas por los que han viajado en los *Chocóes. Abunda en los bosques, en donde se la oye gritar sonidos semejantes al cacareo de la gallina… ronca durante el día cuando duerme envuelta en espirales*". However, in Guyana, the sound attributed to bushmasters was very different from Garcia's "cackling of a hen" or "snoring". It was a continuous high-pitched crescendo whistle, giving the travellers the impression that their camp was being surrounded by these snakes. William Lamar, who has travelled extensively in search of reptiles throughout most Latin American countries, asserts that: "From Costa Rica across South America, disparate groups of indigenous peoples, colonists, hunters, and the like all share the belief, going back nearly two centuries, that the bushmaster has a

distinctive sound"[9]. n the Western Amazon region, an explanation has been provided by the discovery of two Hylid frogs (*Tepuihyla shushupe* and *T. tuberculosa*) whose calls, in parts of Peru and Ecuador, are believed by the local people to emanate from bushmasters [10].

This is yet another example of the endless intrigue provided by snakes, and the need for the authoritative information that this welcome new publication has to offer.

Congratulations to all those involved!

Dr. David A. Warrell
University of Oxford, UK, November 2024.

References

1. Silva-Haad, J. Accidentes Humanos Por Las Serpientes de Los Géneros Bothrops y Lachesis. *Mem. Inst. Butantan* **1980**, *44*, 403–423.
2. Campbell, J.A.; Lamar, W.W. *The Venomous Reptiles of the Western Hemisphere (Vol. 2)*; Comstock Publishing: New York, 2nd edition ,2004; ISBN 978-0801441417.
3. Theakston, R.D.G.; Warrell, D.A. Crisis in Snake Antivenom Supply for Africa. *Lancet* **2000**, *356*, 2104, doi:10.1016/S0140-6736(05)74319-1.
4. McCollough, N.C.; Gennaro, J.F. Treatment of Venomous Snakebite in the United States. *Clin. Toxicol.* **1970**, *3*, 483–500, doi:10.3109/15563657008990121.
5. Vital Brazil, O.; Vieira, R.J. Neostigmine in the Treatment of Snake Accidents Caused by Micrurus Frontalis: Report of Two Cases (1). *Rev. Inst. Med. Trop. Sao Paulo* **1996**, *38*, 61–67, doi:10.1590/s0036-46651996000100012.
6. Tabares Vélez, S.; Preciado, L.M.; Vargas Muñoz, L.J.; Madrid Bracamonte, C.A.; Zuluaga, A.; Gómez Robles, J.; Renjifo-Ibañez, C.; Estrada-Gómez, S. Standard Quality Characteristics and Efficacy of a New Third-Generation Antivenom Developed in Colombia Covering Micrurus Spp. Venoms. *Toxins (Basel).* **2024**, *16*, doi:10.3390/toxins16040183.
7. Castillo-Beltrán, M.C.; Hurtado-Gómez, J.P.; Corredor-Espinel, V.; Ruiz-Gómez, F.J. A Polyvalent Coral Snake Antivenom with Broad Neutralization Capacity. *PLoS Negl. Trop. Dis.* **2018**, *13*, 1–14, doi:10.1371/journal.pntd.0007250.
8. Shepherd, L. Secret Amazon: Into the Wild Available online: https://www.lucy-shepherd.com/amazonuncharted (accessed on 21 November 2024).
9. Lamar, W.W. Lachesis Muta: Cantante y Bailarín? In Proceedings of the Primer Simpôsio. Lachesis muta: um desafio biológica e toxinólogico.; Instituto Vital Brazil: Río de Janeiro, **1998**; p. 1.
10. Ron, S.R.; Venegas, P.J.; Ortega-Andrade, H.M.; Gagliardi-Urrutia, G.; Salerno, P.E. Systematics of Ecnomiohyla Tuberculosa with the Description of a New Species and Comments on the Taxonomy of Trachycephalus Typhonius (Anura, Hylidae). *Zookeys* **2016**, *2016*, 115–154, doi:10.3897/zookeys.630.9298.

Dedication

Teddy Angarita-Sierra.— To Myriam Sierra Guerrero, my mother, and Tulio Angarita Serrano†, my father, for encouraging me to pursue my wildest dreams and to believe in the will of the impossible.

Francisco J. Ruiz Gomez.— To Maria Carlina, my wife, and Ana Maria, my daughter, the deep roots of my effort and dedication. Endless thanks for lighting up my sleepless nights and gray days, and for always believing in me

Acknowledgements

This book, in essence, is the result of the collective, intense, and passionate effort of all the authors of the chapters and species sheets who, selflessly, agreed to participate and unite their wills with the common purpose of providing a contemporary context on the knowledge surrounding snakebites, venoms, and venomous snakes in Colombia. It aims to enhance future research, strengthen the prevention, management, and treatment of snakebite incidents, and reduce the negative perceptions about snakes. For this reason, we, the editors, deeply thank the authors, as the excellence and rigor presented in this work would not have been possible without them.

To the reviewers: Dr. Eliecer de Jesus Jimenez Charris (Universidad del Valle); Dr. Leidy Johana Vargas (Universidad Cooperativa de Colombia); Dr. Alejandro Carbajal-Saucedo (Universidad Autonoma de Nuevo Leon); Dr. Osnamir Elias Bru Cordero, Dr. Thomas Defler, Dr. Nohora Vega (Universidad Nacional de Colombia); Dr. Gerardo Corzo (Universidad Nacional Autónoma de Mexico); Dr. Maria Elisa Peichoto (National Scientific and Technical Research Council); Dr. Alexis Rodriguez Acosta (Universidad Central de Venezuela); Dr. Maria Elena Barragan (Gustavo Orces Foundation, Ecuador); MSc. Juan Manuel Renjifo (Universidad del Magdalena); Dr. Paola Rey-Suarez; Dr. Rafael Otero-Patiño (Universidad de Antioquia); Dr. Santiago Ayerbe (Universidad del Cauca); Dr. Nancy Oguiura (Butantan Institute) for their critical reading, recommendations, and time invested in improving this work.

Special thanks go to our colleagues from the Venomous Animals Group of the Production Directorate of the National Institute of Health (INS) for their unconditional support, dedication, and innovative, resourceful, and meticulous contributions to this work, which honor public service and contribute to nation-building.

To the scientific committee composed of Dr. Franklyn Edwin Prieto: (Director of Surveillance and Risk Analysis in Public Health, National Institute of Health), Dr. Juan Felipe Bedoya Meneses (Director of Production, INS), Dr. William W. Lamar (Professor Emeritus, University of Texas at Tyler), and Dr. Thomas Defler (Professor Emeritus, Department of Biology, Universidad Nacional de Colombia, Bogota campus), for ensuring the excellence and quality of this work.

Likewise, we extend special thanks to the leaders of the Institute who believed in this vision and provided their institutional support, particularly Dr. Diana Marcela Pava Garzon (General Director, INS), Dr. Franklyn Edwin Prieto Alvarado (former Director of Surveillance and Risk Analysis in Public Health, INS), and Drs. Guiovanni Rubiano Garcia and Martha Lucia Ospina Martinez (former General Directors, INS).

Special gratitude to Dr. William W. Lamar (University of Texas at Tyler) for openly and generously sharing his knowledge and experiences about Colombian venomous snakes, significantly improving the species sheets and chapters, and to Dr. David Warrell (University of Oxford) for reviewing the draft manuscript and contributing a warm foreword for the book.

To the artists, graphic designers, and web developers Oscar A. Ramirez Ruiz, Maria Catalina Duran, Johanna Montes Bustos, and Jonathan Medina for imbuing this work with sensitivity, easthetic, and scientific rigor.

To Carlos A. Bravo-Vega for developing the cartography and spatial analyses, positioning this work at the forefront of knowledge.

The authors of Chapter 3 thank the Ecology and Evolution Laboratory at the Butantan Institute for their contribution to obtaining some of the DNA sequences used for phylogenetic reconstruction. Thanks also to the reviewers Thomas Defler, William Lamar, and Nancy Oguiura for their valuable input in reviewing the chapter. Furthermore, we are grateful to the institutions and funds that financed and/or supported the authors during the analysis and writing of the chapter, such as the University of São Paulo, CAPES, COLFUTURO, Universidad de Antioquia, Universidad Bernardo O'Higgins, and the National Agency for Research and Development (Fondecyt Regular Project 1220921).

The authors of Chapters 5 and 6 thank the Venomous Animals Group and the Innovation and Technological Development Group of the National Institute of Health, the Protein Research Group of the Universidad Nacional de Colombia, Universidad de Antioquia, and the Biomacromolecule Chemistry Laboratory of the National Autonomous University of Mexico for their support in the work plan, and the Universidad Nacional de Colombia, De La Paz campus, for its institutional support.

The authors of Chapter 9 thank the municipal and departmental health secretariats, as well as the Primary Data Generating Unit (UPGD), for managing the information for epidemiological surveillance in Colombia.

MD. Lina Maria Peña Acevedo, co-author of Chapter 9, thanks the Universidad de Antioquia and the San Vicente Foundation Hospital Medellin, institutions where she develops all her teaching and healthcare activities, for allowing her to learn and love everything she knows about snakebites, a passion that continues to grow. She also thanks the National Institute of Health of Colombia for entrusting her with the analysis of the information presented in this book, which significantly contributes to the understanding of snakebite cases and epidemiology in Colombia and the world.

The author of the *Bothrocophias tulitoi* fact sheet thanks the Biotechnology and Environment Group at INCCA University of Colombia for allowing me to be part of a team committed to advancing knowledge. Special thanks to Professor Teddy Angarita Sierra for his trust and support in in-

viting me to participate in this book, thus contributing to the knowledge and scientific dissemination of medically significant snakes in Colombia.

The authors of the species fact sheet for *Micrurus clarki*, *M. spurrelli*, and *Bothrops taeniatus* thank the members of the Evolution, Ecology, and Conservation (EECO) research group and the Biology Program at Universidad del Quindio for their contributions to obtaining information, text reviews, or logistical support during the development of these species' sheets.

To the Universidad Nacional de Colombia, De La Paz campus, for its institutional support during the last two years of this project.



This book and the Colombian Snakebite GeoViewer (https://ofidismo.ins.gov.co/) are part of the products derived from the research project titled *"Strengthening, Consolidation, and Generation of Knowledge of the Missionary Activities of the Serpentarium of the National Institute of Health,"* funded from 2021 to 2022 by Minciencias through Call 891 to strengthen vocations and training in Science, Technology, and Innovation (STI) for economic reactivation in the post-pandemic context of 2020.

More than just a publication, this book represents a testament to the unity of purpose of a growing group of Colombian researchers who, despite financial, technical, and social limitations that challenge scientific work daily in Colombia, persist and overcome these barriers with tenacity, passion, high academic quality, and camaraderie, contributing in a cutting-edge manner to the comprehensive understanding of Colombian snakes.

Introduction

Colombia stands as a global bastion of biodiversity, hosting approximately 10% of the world's animal species and ranking among the planet's 25 most critical biodiversity hotspots. Among its vast natural richness, the country is home to a remarkable diversity of venomous snakes that play crucial ecological and biomedical roles. However, this extraordinary natural heritage brings a series of public health challenges related to ophidism—a medical term referring to snakebite envenomation.

In response, the book, *Snakebites, Venoms, and Venomous Snakes of Colombia*, offers a scientifically comprehensive assessment of the 59 snake species of medical importance in the country. This work seeks to address the pressing need for accessible and rigorous information about these feared yet extraordinary reptiles, while also enriching our understanding of the toxinological and public health knowledge of medically important species in Colombia. This book emerges as a fundamental resource that reinforces the mission of Colombia's National Institute of Health (INS) to protect public health and contribute to global scientific knowledge.

Structured into ten chapters, the book explores taxonomic, evolutionary, biochemical, toxinological, ecoepidemiological, and historical aspects related to the development of antivenoms that neutralize envenomation caused by medically significant Colombian snake species. Based on an analysis of the latest research, it provides new evidence and relevant findings for understanding ophidism, while emphasizing the need to continue investigating unresolved questions driving scientific advancement in these fields.

Chapter 1 provides a retrospective overview of snake species relevant to public health in Colombia, including an updated taxonomy, data on their distribution, and conservation status. This chapter highlights the need to continue researching the ecology and natural history of these species, which are essential for implementing conservation measures to mitigate the risk and incidence of ophidism.

A notable section of the book is the in-depth analysis of the venom of species within the genus *Micrurus* (coralsnakes) in Chapter 2. Known for their potent neurotoxins, coralsnakes are biologically and medically fascinating. However, the significant variability in venom composition remains poorly understood. The authors call for extended studies on the ecological and evolutionary factors driving this variability and reevaluate the role of aposematic coloration (bright warning colors) in deterring predators.

Chapter 3 focuses on the evolutionary complexity and variability of venom in pitvipers (rattlesnakes, bushmasters, and lanceheads), which represent the most medically significant snakes in Colombia. Despite

their prominent role in public health, these species and their venoms have been understudied compared to venomous snakes in other tropical regions. This chapter examines their evolutionary history, the variability in venom composition and biological activity, and their potential as models for studying evolutionary biology, providing new perspectives on their adaptation to Colombia's diverse tropical landscapes.

Chapter 4 delves into colubrids (rear-fanged snakes), which are generally considered "non-venomous" snakes. Nevertheless, this chapter robustly compiles recent studies showing that many colubrids produce salivary secretions with toxins capable of causing mild but significant envenomations. Additionally, it underscores the importance of understanding the evolutionary history of their venoms, their biological significance, and their incredible pharmacological potential for the development of new drugs and biomedical applications.

The biochemical nature of snake venoms is examined in Chapter 5, focusing on key components such as metalloproteinases, phospholipases A_2, and three-finger toxins. These venom components, essential for immobilizing and digesting prey as well as deterring predators, are also responsible for the pathophysiological processes observed in snakebites. This chapter details the mechanisms of action of toxins, analyzes the composition and variability of venoms from medically significant Colombian species, and links venom biochemistry to the clinical profiles of envenomations.

Understanding this relationship has immediate implications for the clinical management of snakebite incidents, as the severity of envenomations and treatment approaches are heavily influenced by venom composition and activities.

Antivenoms remain the cornerstone for deal envenomation caused by snakebites. Chapter 6 details their development and application in Colombia, highlighting advances in the pharmaceutical production of antivenoms and the use of molecular techniques to enhance their efficacy. Given the significant venom variability within and between snake species, continued research on their protein components is essential. Furthermore, the authors explore complementary therapeutic approaches, such as immunorecognition techniques, to improve antivenom effectiveness.

The history of antivenom production in Colombia is narrated in Chapter 7, from pioneering efforts to the consolidation of structured production protocols. This chapter provides an in-depth look at the role of the INS in advancing Colombian and regional public health through the management, production, and development of antivenoms to address one of the world's most neglected tropical diseases. It captures the evolution of the INS's production capacity for antivenoms in Colombia, situating current initiatives within a historical framework that contextualizes the country's achievements and challenges in addressing snakebite envenomation.

Chapter 8 examines the unique challenges of maintaining venomous snakes in captivity for antivenom production. Drawing on decades of experience at the INS serpentarium, the authors share lessons learned about best practices to ensure the health and welfare of captive snakes. Additionally, the authors emphasize the importance of understanding the ideal comprehensive conditions for snake captivity, minimizing stress, and enabling physiological and behavioral pattern monitoring to ensure their well-being and survival. Consolidating these learnings contributes to the conservation efforts of these species and facilitates the acquisition of high-quality venom for research, development, and improvement of antivenoms.

Snakebite envenomation remains a priority public health issue in Colombia, particularly in rural areas where poverty, food insecurity, and limited access to medical care pose significant challenges. Chapter 9 provides an ecoepidemiological analysis of snakebites using data from the national surveillance system, SIVIGILA (2010–2020). The authors examine spatial and temporal patterns in snakebite occurrences, identify demographic factors associated with envenomation risk, and review clinical management practices. In line with World Health Organization guidelines, they offer recommendations for improving data collection and medical training to reduce the morbidity and mortality associated with ophidism.

Finally, Chapter 10 explores the therapeutic potential of Colombian snake venoms, focusing on their applications in cardiovascular and nervous system treatments as well as their potential in cancer and antimicrobial therapies. The authors advocate the use of omics technologies to explore the pharmacological potential of Colombian snake venoms while emphasizing the value of traditional biochemical and pharmaceutical research to discover bioactive compounds with significant medical relevance.

This ambitious project, written by 55 scientists, is presented in three different formats: a free downloadable PDF, a web version (https://serpientes.ins.gov.co), and a limited print edition. The project includes a database linked to the book called the Colombian Ophidism Geoviewer, providing free access to shapefile data with epidemiological information, environmental variables, and distribution records of medically important snake species in Colombia (https://ofidismo.ins.gov.co). Additionally, it presents 59 species profiles summarizing key biological, toxinological, and epidemiological information about these medically significant snakes in the country.

This book titled *Snakebites, Venoms, and Venomous Snakes of Colombia* thus becomes an indispensable resource for herpetologists, toxinologists, clinicians, public health professionals, and the general public. Offering a comprehensive investigation into the biological, ecological, and medical dimensions of Colombia's venomous snakes, this book aligns with the INS mission, advancing scientific understanding and reinforcing Colombia's commitment to public health and international collaboration in

the fields of toxinology and public health. The meticulous research and accessible format of this work will undoubtedly support the development of informed public health policies and promote the conservation of these misunderstood and ecologically significant creatures.

Teddy Angarita-Sierra and Francisco J. Ruiz-Gomez
Editors

Authors of the chapters



Amira Abultaif Kadamani
amiabu@hotmail.com
Academic background: Bachelor of Journalism and Communications
Professional profile: Journalist, writer, editor and documentary producer
Affliliation: Independet consultant
Chapter 7



Adrian Marcelo Franco-Vasquez
adrian.franco.vasquez@gmail.com
Academic background: Bachelor of Biology and Chemistry, Master of Science, PhD (c) in Science
Professional profile: Toxinology, Biochemistry, Proteomics and Characterization of toxins
Affliliation: Department of Chemistry of Biomacromolecules, Institute of Chemistry, National Autonomous University of Mexico
ORCID: https://orcid.org/0000-0001-6978-2176
Chapters 5, 6



Andres Camilo Montes Correa
andresc.montes@gmail.com
Academic background: Biologist, Master estudent in Biodiversity and Evolution
Professional profile: Herpetology, systematics, taxonomy, ecology, evolution, and conservation
Affliliation: Herpetology Lab, Emilio Goeldi Museum, Belém, Para, Brasil
GECOS Foundation, Santa Marta, Magdalena, Colombia. Research Group on Neotropical Ecology, Magdalena University, Santa Marta, Magdalena, Colombia
ORCID: https://orcid.org/0000-0003-3561-0426
Chapters 1



Ariadna Rodriguez Vargas
arlrodriguezva@unal.edu.co
Academic background: Physician, Master in Toxicology, PhD in Sciences - Biochemistry
Professional profile: Toxinology, Biochemistry
Affliliation: Research group on venemous animals and their venoms, National Institute of Health, Bogota, Colombia
Protein Research Group, Universidad Nacional de Colombia
ORCID: https://orcid.org/0000-0001-6636-987X
Chapters 5, 6



Camila Figueredo Salinas
cfigueredo@unal.edu.co
Academic background: Biologist and Environmental and Sanitaria Engineer, Toxicology Master student
Professional profile: Toxinology, Biochemistry
ORCID: https://orcid.org/0000-0002-3476-0943
Chapters 5, 6



Carlos Andres Bravo-Vega
ca.bravo955@uniandes.edu.co
Academic background: Chemical engineer, MSc. in Biomedical Engineering, PhD in Engineering.
Professional profile: Ecoepidemiology
Affliliation: Postdoctoral teaching assistant, research Group in Mathematical and Computational Biology (BIOMAC), Department of Biomedical Enginering, University of Los Andes
ORCID: https://orcid.org/0000-0002-5588-7396
Chapters 1, 9



Carlos Antonio Castro-Sandoval
cacastro@ins.gov.co
Academic background: Zootechnician
Affliliation: Research group on venemous animals and their venoms, National Institute of Health, Bogota, Colombia
ORCID: https://orcid.org/0000-0003-1400-2405
Chapter 8



Carolina Zona
dczonar@unal.edu.co
Academic background: Respiratory therapist, Critical care specialist, Master of Science-Pharmacology, PhD (c) in pharmaceutical sciences, Universidad Nacional de Colombia
Professional profile: Intensive care, Mechanical ventilation, Pharmacology, Toxicology
Affliliation: Research professor, Manuela Beltran University
ORCID: https://orcid.org/0000-0002-3529-5342
Chapter 10



Chris Akcali
chrisakcali@yahoo.com
Academic background: Biologist, PhD in Biological Sciences
Professional profile: Herpetologist
Affliliation: North Carolina Museum of Natural Sciences, Raleigh, North Carolina, USA
ORCID: https://orcid.org/0000-0002-8089-3501
Chapter 2



Diana Marcela Walteros Acero
dianamwalteros@hotmail.com
Academic background: Physician, Specialist in Epidemiology, Field Epidemiology, Master in Epidemiology, PhD (c) in Public Health
Professional profile: Epidemiologist and public health doctor
Affliliation: Subdirector of Surveillance INS Colombia
ORCID: https://orcid.org/0000-0002-3007-6984
Chapter 9



Francisco J. Ruiz Gomez
fruiz@ins.gov.co
Academic background: Veterinarian Doctor
Professional profile: Production and Technological Development of Antivenoms
Affliliation: Research group on venemous animals and their venoms, National Institute of Health, Bogota, Colombia
ORCID: https://orcid.org/ 0000-0002-8418-4243
Editor, Chapter 8



Jaime Andres Pereañez Jimenez
andrespj20@gmail.com
Academic background: Pharmaceutical Chemist, PhD in Basic Biomedical Sciences
Professional profile: Toxinology, Therapeutic alternatives, Biochemistry, Toxin characterization, Ophidism
Affliliation: Research group of Toxinology, therapeutic and food alternatives, Faculty of Pharmaceutical and Food Sciences, Universidad de Antioquia. Research group on Promotion and Pharmaceutical Prevention, Faculty of Pharmaceutical and Food Sciences, Universidad de Antioquia
ORCID: https://orcid.org/0000-0001-9612-2827
Chapters 3, 5, 6, 10



Juan Carlos Vega Garzon
jcvegag@unal.edu.co
Academic background: Bachelor of biology, Veterinary Doctor, Master in Microbiology, PhD in functional and Molecular biology
Professional profile: Educational technology and science communication
Affliliation: Assistant professor, Intersedes research group (Bogota and De La Paz) Genetic Control in animal health, Universidad Nacional de Colombia at De La Paz Campus, Cesar, Colombia
ORCID: https://orcid.org/0000-0002-6534-2234
Chapter 5



Juan David Bayona Serrano
juan.serrano.esib@esib.butantan.gov.br
Academic background: Biologist, PhD in Sciences - Toxinology
Professional profile: Transcriptomics, Genomics, Proteomics and Evolution of snake venom toxins
Affliliation: Laboratory of Applied Toxinology, Butantan Institute
Chapters 2, 4



Juan Jose Torres Ramirez
jtorresr@ins.gov.co
Academic background: Biologist
Professional profile: Herpetologist
Affliliation: Research group on venemous animals and their venoms, National Institute of Health, Bogota, Colombia
ORCID: https://orcid.org/0000-0002-3438-030X
Chapter 8



Juan Pablo Hurtado Gomez
juanphg@gmail.com
Academic background: Biologist, Msc Biology, PhD (c) in Biology
Professional profile: Systematics, Taxonomy, Evolution, Biogeography, Herpetology
Affliliation: Museum of Zoology, Senckenberg Natural History Collections Dresden, Dresden, Germany
ORCID: https://orcid.org/0000-0003-4351-2834
Chapter 2



Katterine Isabel Urieles Sierra
kurieles@unal.edu.co
Academic background: Physician, Specialist in Epidemiology, Master in Public Health, PhD (c) in Public Health
Professional profile: Epidemiologist and public health doctor
Affliliation: Health policy research group, Faculty of Medicine, National University of Colombia, Bogota.
ORCID: https://orcid.org/0009-0002-1345-9526
Chapter 9



Kristian Alberto Torres-Bonilla
kristiantorres19@gmail.com
Academic background: Biologist, Master in Pharmacology, PhD in Pharmacology
Professional profile: Toxinology, Biochemistry and Pharmacology of venoms and toxins
Affliliation: Laboratory of Biochemical and toxinological pharmacology, Universidade Estadual de Campinas, Brazil
ORCID: https://orcid.org/0000-0001-6476-7778
Chapter 4



Lina Maria Peña Acevedo
lina.pena@udea.edu.co
Academic background: Clinical Toxicologist, Specialist and Master in safety and occupational health. PhD (c) Clinical Medicine.
Professional profile: Clinical Toxicology
Affliliation: Titular Professor, Pharmacology and Toxicology Department Medicine School- Universidad de Antioquia
ORCID: https://orcid.org/0000-0001-5424-2927
Chapter 9



Maria Carlina Castillo-Beltran
mcastillo@ins.gov.co
Academic background: Chemical engineer
Professional profile: Antivenom development and production
Affliliation: Research group on venemous animals and their venoms, National Institute of Health, Bogota, Colombia
Chapter 6



Monica M. Saldarriaga Cordoba
monica.saldarriaga@ubo.cl
Academic background: Veterinary Doctor, Master in Biomedical Sciences, PhD in Sciences with mention in Ecology and Evolutionary Biology.
Professional profile: Molecular Biologist, Evolutionary Biology and Bioinformatics
Affliliation: Center for Research in Natural Resources and Sustainability (CIRENYS), Universidad Bernardo O'Higgins. Chile
ORCID: https://orcid.org/0000-0002-4768-4919
Chapter 3



Monica Sarmiento
msarmiento@ins.gov.co
Academic background: Veterinay Doctor, Quality management specialist, Msc in Public Health
Professional profile: Veterinary medicine of domestic species and reptiles, Production and Technological Development of Antivenoms, Public Health
Affliliation: Research group on venemous animals and their venoms, National Institute of Health, Bogota, Colombia
ORCID: https://orcid.org/0009-0005-3075-0077
Chapter 8



Sergio D. Cubides-Cubillos
sergio.cubides@usp.br
Academic background: Biologist, Master in Education, PhD in Biotechnology
Professional profile: Herpetologist, Taxonomist, Snakebites, Molecular Systematics
Affliliation: Ecology and Evolution Laboratory, Butantan Institute - São Paulo, Brazil.
ORCID: https://orcid.org/0000-0001-5214-2498
Chapter 3



Teddy Angarita Sierra
tgangaritas@unal.edu.co
Academic background: Biologist, Master in Environmental Auditing and Management, PhD in Biological Sciences
Professional profile: Herpetologist, Taxonomist, Ecologist, Conservation biology, Snakebites
Affliliation: Biodiversity for Society Research Group, Academic Direction, Universidad Nacional de Colombia at De La Paz Campus, Cesar, Colombia
ORCID: https://orcid.org/0000-0002-5978-9312
Editor, Chapters 1, 2, 3, 4, 5, 6, 8, 9, 10

Contents

Presentation V
Dr. Diana Marcela Pava Garzon

Foreword VII
David A. Warrell

Dedication XI

Acknowledgements XIII

Introduction XVII

Authors of the chapters XXI

Chapter 1 Medically important snakes in Colombia: A retrospective look at their knowledge, advances, and future perspectives 29

Chapter 2 Lineages, venom variability, and warning signals in New World Coralsnakes 83

Chapter 3 Natural-born killers: Understanding the evolutionary paths of Colombian pitvipers 115

Chapter 4 An unexplored universe: Venoms and toxins from colubrid snakes 165

Chapter 5 Intricate mixtures: Diving inside the venoms 201

Chapter 6 Snakebite therapies in Colombia: antivenoms as pivotal responses 275

Chapter 7 Venoms, love, and hate 303

Chapter 8 Venomous snakes in captivity: A history of learned lessons 349

Chapter 9 Ecoepidemiology and clinical outlook of the snakebites in the Colombian context 385

Chapter 10 The dose makes the poison: A review of the pharmacology of snake venoms 433

Abbreviations and meaning 481

Glossary 485

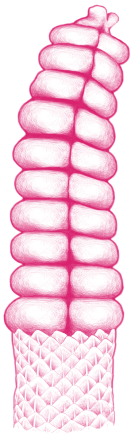
Chapter 1

Medically important snakes in Colombia: A retrospective look at their knowledge, advances, and future perspectives

Teddy Angarita-Sierra, Carlos A. Bravo-Vega, Andres C. Montes-Correa

Abstract: The knowledge about Colombian snakes has been minimally explored throughout history, focusing mostly on medically important species because of people's fear and aversion to the serious and life-threatening effects of their venomous bites. Nevertheless, many essential aspects of their natural history traits, ecology, behavior, systematics, taxonomy, distributions, ophidism, ethnobiological uses, and social perceptions remain poorly understood. Since the 2000s, increase of studies has reduced our historical gaps of knowledge, leading to significant advances in the understanding of Colombian snakes. In this chapter, we summarize the available information about the medically important species of Colombia, integrating it with information about their richness, endemicity, distributions, taxonomy, and conservation status. We provide a brief historical account of snake species richness in Colombia and a reviewed venomous species list based on latest the available information regarding the taxonomic status of medically important snake species and their biological significance. Our aim is not to resolve all current taxonomic problems of Colombian snakes, but to provide a taxonomic treatment and criteria that allocate the puzzling snake species into taxonomic entities based on the current evidence available. In addition, we review the conservation status of Colombian venomous species and propose a novel framework to address the threats and challenges for their conservation and for a reduction of snakebite as a neglected tropical disease.

Keywords: Ophidiofauna; geographic distribution; endemicity; species richness; taxonomy; conservation.



Citation: Angarita-Sierra, T.; Bravo-Vega, CA.; Montes-Correa, AC. Chapter 1. Medically important snakes in Colombia: A retrospective look at their knowledge, advances, and future perspectives. In Book: *Bites, venoms, and venomous snakes of Colombia*; Angarita-Sierra, T., Ruiz-Gomez, FJ, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 29–82. doi:10.33610/917749zmlemn

Copyright: © 2024 by the authors. Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0) license (http://creativecommons.org/licenses/by/4.0/).

Illustrations by:
Oscar A. Ramirez Ruiz

1. A brief historical account of the snake species richness in Colombia

Historically, most of Colombian snakes have been poorly studied, producing significant gaps in our knowledge about essential aspects of their natural history traits, ecology, behavior, systematics, taxonomy, distributions, ophidism, ethnobiological uses, and social perceptions. Currently, some of this topics remain poorly understood [1–3]. However, since the end of the 20th century and the first decade of the 21st century, a plethora of studies has emerged, aiming to reduce the historical deficit of understanding the snake species richness that inhabits one of the top megadiversity countries worldwide [1].

The knowledge about the snakes of Colombia is limited for multiple reasons, such as: scanty funding resources for field studies; few holotypes (i.e., the specimen on which the description of a species is based) are housed in Colombian biological collections; most of the medical important snake species have few vouchers in Colombian biological collections, in turn representing limited geographic representativeness (see below); few experts in universities and research institutions are capable of describing and identifying snakes, and teaching about their biology; vast areas of the country, as well as several type localities (i.e., the locality where the holotype of a species was collected) of most Colombian venomous snakes, have been historically inaccessible or highly risky for scientist due internal armed conflicts [4–6].

Because of the above, large regions of Colombia still lack intensive sampling, and most of the Colombian ophidiofauna has not been subject to comprehensive biological studies. As a result, significant sampling and knowledge gaps exist, rendering Colombia a “black hole” in terms of essential biological knowledge for most groups of medically significant snake species, despite its key geographical position in the historical exchange of faunas between North and South America [7], as well as being one of the countries with the highest annual rate of reported snakebites in America (see Chapter 9).

As a member of the Royal Botanical Expedition, and zoological components manager, Jorge Tadeo Lozano [8,9], was the first Colombian to study Colombian amphibians and reptiles. He particularly emphasized snake natural history, and the non-medical practices used to deal with snakebites during the end of the period of the Nuevo Reino de Granada (1538-1819) and the beginning of the Gran Colombia period (1819-1831). Thus, he became the first Colombian herpetologist [9] (see Chapter 7). Particularly, Jorge Tadeo Lozano in his publication titled “*Memorias sobre serpientes*” stated future research guidelines that would increase our understanding of snakebite and its eco-epidemiological context in Colombia (see Chapter 9), as follow [10]:

1. To study the snake anatomy for classifying venomous from non-venomous snakes, evaluating the oral structures very carefully to ensure the existence of “venom accumulator bags (=venom glands)” and hollow fangs (=solenoglyphus dentition).

2. To study the activity and effect of snake venoms, using animals of varied sizes and types, scrupulously noting all the phenomena that arise.
3. Once the effects of each venom are known, test whether they can be used in medicinal treatments for diseases, or to combat the venoms of other species.
4. Evaluate the effect of removing the venom apparatus from snakes.
5. Examine the amount and intensity of snake venoms, depending on their age, sex, size, times of year, and other circumstances.
6. Perform a chemical analysis of the "venom liquor", recognizing its nature, composition, and differences between species.
7. Study the "anti-venoms" that are commonly used, and test their effectiveness on bitten animals, or by mixing them with venoms, and observe if they decompose, disorganize, or have deprived them of their deleterious virtue.
8. Do all experiments for evaluating the guaco plant (= *Mikania guaco*), the most used "anti-venom."
9. To investigate the amount of venom required to kill an animal of each order.
10. Make a detailed study of venomous snakes to distinguish them from harmless ones, using the characteristics of number, arrangement and figures that make up their scales.
11. To find out if nature gave the snakes venom solely for self-defense and to kill their enemies, or if, as I suspect, it is a supplement to aid in digesting food instead of chewing it, which they cannot do due to the structure of their teeth that prevent it.

The Lozano's guidelines were visionary because most of his recommendations and conjectures are the currently most important and prioritized lines of research of venomous snakes, as well as dealing with snakebite envenoming [11–13] (see Chapter 3, 5, 6, 7, 9, and 10). After Lozano's visionary guidelines, national and foreign researchers attempted to describe the natural history and envenomation capacities of the snakes inhabiting the region at the beginning of the birth of the country now known as Colombia. Most of these researchers were motivated to provide an understanding of snakebite accidents and the therapies used as medical practices.

In 1870, the engineer and homeopathic doctor Silvestre B. Higgins, native of the city of Barranquilla (Atlantico department), published the book "*Culebras i reptiles venenosos: sus hábitos características i particularidades"*. This book focuses on the non-medical practices used as therapies against snakebites by Colombian healers. It also describes general aspects of the natural history traits of some venomous species and its ecoepidemiological context. He compiled the most amazing beliefs, tales, myths, and magical events regarding Colombian snakes, explaining their common names, and social beliefs that still prevail in many rural communities.

Interestingly, Higgins provided an annotated list of venomous snakes from each of the nine Colombian states (Higgins lived during a history period in which Colombia was a cluster of federal states named as Estados Unidos de Colombia 1863-1886). However, Higgins did not provide a solid

scientific foundation in his book, so venomous and non-venomous snake species were listed by common names rather than scientific ones. Thus, based on Higgins's species list, the results are difficult to apply reliably to most of the snake taxa described. However, by excluding common name synonyms, non-snake species (e.g., amphisbaenians), and non-venomous species (e.g., *Pseudoboa neuwiedii*) from Higgins's list, the number of snake species can be estimated to represent 12: *Bothrops asper*, *B. atrox*, *B. bilineatus*, *B. punctatus*, *Bothrocophias* spp., *Bothriechis schlegelii, Crotalus durissus*, *Micrurus dumerillii*, *M. mipartitus*, *Lachesis acrochorda*, *L. muta*, and *Porthidium lansbergii*.

In 1889, the physician Andres Posada Arango published the article "*Note Sur Quelques Solenoglyphes de Colombie*" in which he describes four new species from the antiquated genus *Thanatophis* (*Thanatophis patoquilla, T. sutus, T. montanus, and T. torvus*) according to his interpretation. Nevertheless, these species were poorly described and posteriorly synonymized into three genera and three different species or species complexes (*T. patoquilla* and *T. sutus* were synonymized with *Porthidium lansbergii* [14]; *T. montanus* was synonymized with *Bothrops punctatus* [15], and *T. torvus* was allocated to the genus *Bothriechis* as *B. torvus* [16]).

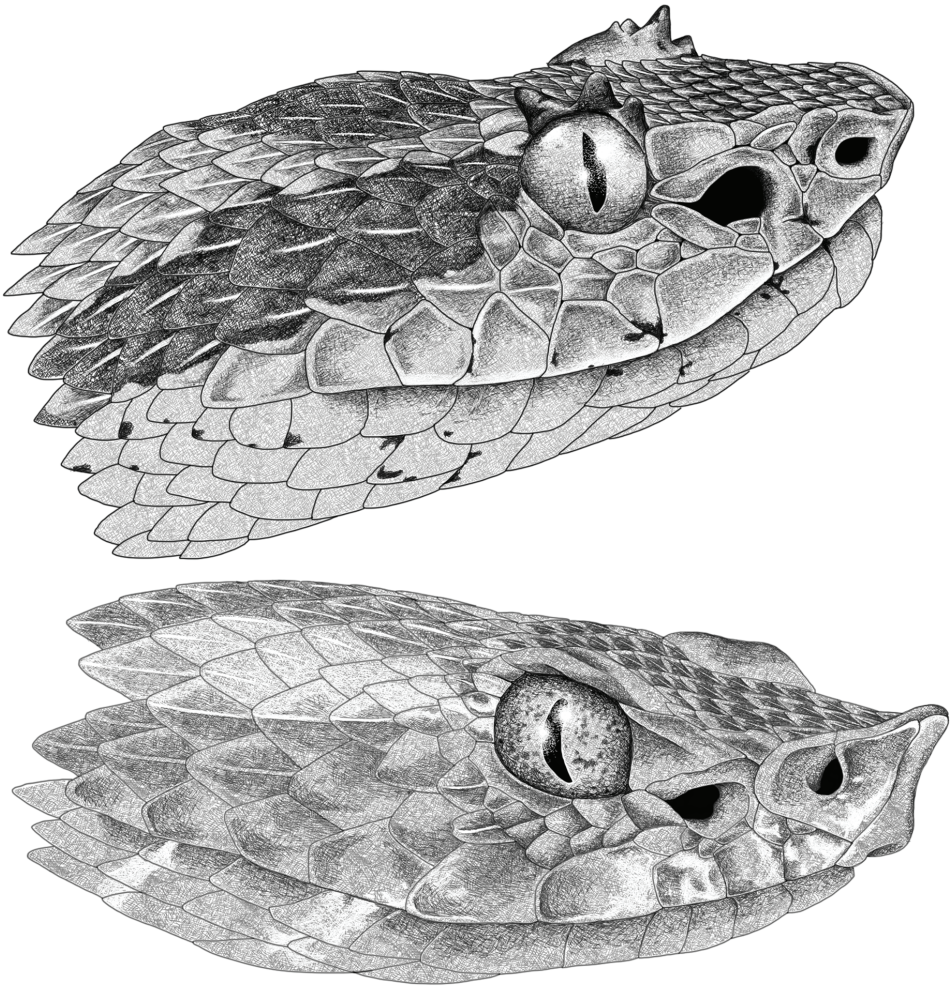


Figure 1. Illustrations of the new species proposed by Andres Posada-Arango. (Top) Head in lateral view of *Bothriechis schlegelii* species complex from Manizales, Caldas (INSV-SR-00138). (Bottom) Head in lateral view of *Porthidium lansbergii* (INSV-SR-89) from Yondó, Antioquia, Colombia. Illustrations by Oscar A. Ramirez Ruiz.

In 1896 the physician Evaristo Garcia Piedraita provided a list of medically important snake species from the Cauca department (currently Cauca, Valle del Cauca, and Putumayo departments). Dr. Garcia-Piedraita in his book "*Serpientes venenosas del Cauca*" [17], provides a detailed study of the classification of venomous snakes; and he characterizes, describes, and diagnoses the venomous apparatus, the effects of venoms, and the symptoms of envenomation caused by snakebites. Additionally, he analyses and reflects on the empirical methods used against snakebites, methods to neutralize venom, and the treatment to counteract snakebite symptoms [1]. Garcia-Piedrahita provided a list and description of 19 medically important snake species, however, discounting common name, synonyms, and non-snake species and non-venomous species from the Garcia-Piedraita's species list, a total of 11 species were reported (*Bothriechis schlegelii, Bothrops asper*, *B. rhombeatus*, *B. punctatus*, *Crotalus durissus*, *Lachesis acrochorda*, *L. muta*, *Porthidium lansbergii*, *Micrurus mipartitus*, and *M. hemprichii*).

Two decades later, Afranio do Amaral [18,19] expanded the knowledge about Colombian snakes providing a regional species list of snakes of Colombia, reporting five venomous snakes from the Santa Marta region (*Micrurus dumerillii, M. mipartitus*, *Bothrops asper*, *Crotalus durissus, Porthidium lansbergii*,) and six from the San Juan River lower Basin, Choco department (*Micrurus ancoralis*, *M. mipartitus, Bothrops asper, B. punctatus, Bothriechis schlegelii, Porthidium nasutum*). Nevertheless, until the 1940s, Niceforo Maria [20] was the first researcher who attempted to consolidate a complete species account list of Colombian snakes. He reported about 180 snake species, however, discounting subspecies from Niceforo's species, his list the number of snake species reaches 174 (pers. obs. J.D. Lynch) [1]. A total of 26 venomous snakes were reported of which 15 were elapids (with the description of *Micrurus sangilensis* as a new species), and 11 viperids.

In 1968, Federico Medem (Friedrich Johann Graf von Medem) [21] provided a comprehensive review of the historical development of herpetology in Colombia, listing all known species of amphibians and reptiles of the country, encompassing records from explorers from XVIII century up to 1968. He reported a total of 231 snake species inhabiting Colombia, of which, discounting subspecies, 10 were viperids and 13 were elapids. Medem provided detailed descriptions of their geographic distributions, habitats, natural history traits, ethnozoological data, and social beliefs.

Two years later, Peters and Orejas-Miranda [15] provided a comprehensive catalogue of the Neotropical snakes, including taxonomic keys for genera, species, and subspecies. They provided scientific names, distributions and attempted to resolve unknown or undefined taxa. Discarding subspecies, these authors reported 182 Colombian snake species of which 16 were viperids and 17 elapids. The taxonomic keys for genera, species, and subspecies remain valid for many taxa, and these continue to be used in several biology academies institutions in Latin America as an introductory approach to an understanding the taxonomic richness of Neotropical snakes.

In 1985, a new attempt to compile knowledge about Colombian venomous snakes was performed by the physician and professor of the Universidad de Antioquia, Rodrigo Angel [22] when he published the monograph "*Serpientes de Colombia: guía práctica para la clasificación y tratamiento del envenenamiento causado por sus mordeduras*". Angel reported 160 snake species for Colombia of which 13 were viperids and 31 elapids. However, discounting subspecies from Angel's species list the number of venomous snakes in Colombia reaches 36 taxa (Figure 2). This book was one of the main references for most of the medical personnel who faced snakebites in Colombia up to the end of the 20th century.

In 1988, Perez-Santos and Moreno published the first modern monograph, "Ofidios de Colombia", that extensively compiled the snake species richness of Colombia. They provided keys for their identification, descriptions of their diagnostic characters, and maps of their distributions. These authors reported 240 snake species in Colombia discounting subspecies. A total of 36 venomous species were reported (20 elapids and 16 viperids). Nevertheless, this monograph has significant shortcomings in the quality of species records, mainly because most of the distribution records and natural history information is inadequately documented, presenting questionable identifications and distributions, totally lacks cross-references of the specimens per species and museum collection numbers [23]. Despite this, the book provided comprehensive species checklists and diagnostic characters, most of it useful. It lacks a rigorous evaluation of the data used, resulting in an underestimation of species richness for the country, and adds confusion regarding their distributions within the national territory [1,23].

In 1989, Campbell and Lamar offered a comprehensive and well-supported account of venomous snake species across Latin America, including Colombia. Their work addressed several misconceptions raised by previous publications and corrected data of previous studies that often-lacked cross-references of specimens per species and museum collection numbers, or else omitted references from published data, resulting in confusing geographic distributional ranges.

Towards the end of the 20th century, Sanchez et al. [24] provided a biological review of reptile species in Colombia [24]. However, in this publication, the quality of the records used was not evaluated. This oversight led to the an underestimation of species richness for the country and confusion regarding their distributions within the national territory [1]. This resulted in an underestimated species richness for the country (Figure 2).

Since the 21st century an explosion of studies has emerged, aiming to reduce the historical deficit in understanding the snake's richness. These studies have quickly increased the number of snake species with comprehensive taxonomic reviews, as well as given the description of new species, thus, passing from ~240 to ~331 snake species (Figure 2). In 2002, Pineda and Renjifo presented and updated account of Colombian snakebite accidents, focusing on clinical reports, effects of venoms, and the

symptoms of envenomation caused by snakebites present in Colombia, providing a comprehensive overview of the clinical manifestations, epidemiological information, and how to handle snakebite accidents and their therapy and updating that previously presented by Angel in 1985. Also, these authors provided an account of 35 venomous snake species. However, they cited inaccurately the venomous snake list previously provided by Campbell and Lamar [25], missing some *Micrurus* species (Figure 2).

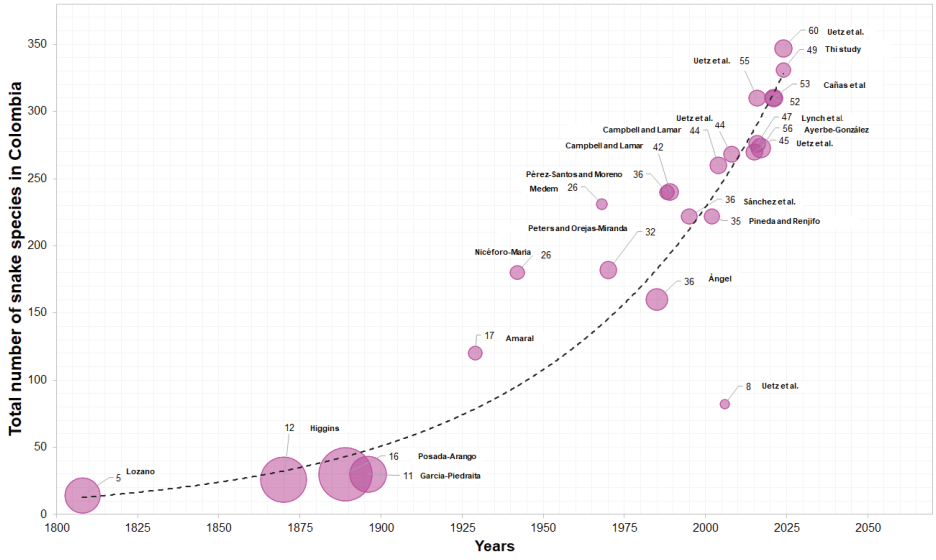


Figure 2. Overview illustrating the exponential growth of snake richness in Colombia. The size of the bubbles corresponds to the ratio of venomous snakes to total snake species, as reported by each author. The number linked with each bubble indicates the number of venomous snakes reported by that author.

In 2004, Campbell and Lamar [26], made notable advances toward understanding venomous reptiles in the Western Hemisphere. The classic book "*The Venomous Reptiles of the Western Hemisphere*" is the first modern compendium that setting the modern baseline for understanding the taxonomic conundrums of Colombian venomous snakes. This comprehensive study updated their proposal from 1989 and add significant information. Their taxonomic proposals remain valid for many species of venomous snakes distributed in Colombia.

In 2016, Cañas et al. [27], in their book "*Serpientes venenosas: lecciones aprendidas desde Colombia*", provide an interesting review of the knowledge accumulated until the 20th century and the beginning of the 21st century on the diversity, general biology, and identifying characteristics of medically significant snakes in Colombia. They describe the features that allow for distinguishing venomous snakes from non-venomous ones and offer a clinical perspective on snakebite manifestations. They are the first authors to compile toxinological advances related to venomics, biochemistry, and the biological activities of venoms from the medically important snakes in the country. Additionally, they presented epidemiological information and guidelines for managing snakebite, with emphasis on snakes distributed across the four life zones of the Valle del Cauca department. These authors indicate that 310 snake species inhabit Colombia, 53 are medically important snakes (21 vipers and 32 elapids), of which 14 species

are distributed in Valle del Cauca department. However, their list of species lacks cross-references with specimens from collections or published literature, making it impossible to trace the data's sources.

In 2021, Ayerbe [28], in his book "*Serpientes: accidentes y soluciones*" provides a detailed description of the symptoms and signs of poisoning caused by Colombian snakes, their clinical manifestations and complications in their treatment, presenting therapeutic approaches to deal with the snake accident. Within the generalities of the biology of snakes of medical importance in Colombia, Ayerbe [28] indicates that 310 species of snakes live in the country, of which 56 are of medical importance. However, similarly to Cañas et al. [27], he does not provide cross-references with specimens from collections or published literature to support his lists (e.g., *Micrurus hemprichii* versus *Micrurus ortoni*; see the explanation in the following sections of this chapter).

Since Campbell and Lamar [26], most studies of the 21st century have corrected the shortcomings of the past, providing snake accounts based on high-quality records from peer-reviewed literature, museum collection specimens, and curated digital species repositories or databases [1]. The ongoing project of Uetz, Hallermann, Hosek, and collaborators, the Reptile Database (http://www.reptile-database.org), have accelerated and consolidated the historical efforts previously undertaken by several authors, providing a modern method to annually update species lists of worldwide non-avian reptile species.

Currently, this website serves as the primary or initial option for any researcher or reptile enthusiast seeking a basic understanding of taxonomic richness, geographic distributions, specialized literature, original descriptions, pictures, and other relevant biological data. The Reptile Database representing the "modern consensus" among herpetologists worldwide about scientific name, taxonomic decisions, and distribution. No doubt, Uetz, Hallermann, Hosek et al. have made an enormous advance in compiling a colossal amount of knowledge about non-avian reptile species [29].

Nevertheless, since the Reptile Database do not provide any taxonomic assessment beyond a checklist with the feedback from some the authors who proposed it; and these species list is based on information from various sources [30], controversy about taxonomic decisions and distributions of several taxa, especially snakes, persists. As taxonomy is a dynamic scientific field, controversy always fuels its engine. Thus, the total number of snake species could change between authors or within the same authors across time according to the criteria employed by them, generating a constant expansion and contractions of the snake species list due to redundancy or missing taxa.



For example, in this book, we consider that currently in Colombia there are a total of 331 snake species, of which 49 taxa represent venomous snake species (Viperidae and Elapidae; see https://ofidismo.ins.gov.co). Our spe-

cies accounts varies from Uetz et al. [29] for 4.6% of the total snake species and 18% for venomous species account (Figure 2, Table 1). The criteria employed that explain these differences are included in the following sections of this chapter. Despite the variability in snake species lists among authors, in the 21st century, Colombia has gained an exponential understanding of its ophidiofauna, indicating that the country has begun to emerge from the "black hole" by acquiring essential snake biological knowledge. Across time, the ratio of venomous snakes to total snake species has decreased, indicating that the medical view of snakes gradually changed towards a broader biological view including all snakes in the species account list.

Furthermore, since the beginning of the 21st century this ratio has had averaged of 16.3% (9.8-20.1%) indicating that the medically important snake species in Colombia represented less than 20% of the total ophidiofauna of the country. We hope that soon, this trend continues by addressing the taxonomic uncertainties of several Colombian snake taxa. However, this historical trend also points out that our understanding of Colombian snakes is far from comprehensive, encouraging us to continue investing significant efforts due to their crypticity and the dearth of information surrounding them. We hope this challenge will be addressed by both young national and international herpetologists.

2. A megadiverse mess: The taxonomic conundrum of the medically important snake species in Colombia

One of the key stages for any person or medical practitioner facing a snakebite accident is the accurate identification of snake. According to the snake taxonomic identification, medical practitioner can provide an accurate report to the health surveillance system, to determinate the envenoming type, addressing the suitable treatment and antivenom therapy, as well as anticipating the possible clinical complications [31]. For this reason, most of the guidelines, manuals, field guides and protocols aiming to treat or manage snakebite accidents, include the snake taxonomic identification as a paramount stage into its step-by-step process (algorithm) [27,28,31–34] (see Chapter 9).

Despite this task sounding clear and easy, in tropical and megadiverse country like Colombia the task can be complicated, even more when several of the snake species have a puzzling taxonomic status due to poor scientific research and their high crypticity [1]. Additionally, the distributional of several venomous snake species can extensively overlap (sympatric species), rendering species identification through the geographical elimination method unsuitable in most cases.

Recently, several researchers have made important efforts to untangle the taxonomic problems of some Colombian snakes of medical importance [35–38]. Nevertheless, most snake diversity in the country continuous with puzzling taxonomic status, maintaining as a taxonomic baseline the classic and comprehensive taxonomic reviews made in the end of the 20th the beginning of the 21th centuries [15,23,39–44].

Most of these scientific publications conclude that Colombian populations of venomous snakes require detailed taxonomic revision [15,26,39,45–47]. Despite this, several researchers have employed synonyms and subspecific epithets as full species names without a taxonomic revision, or they lack solid evidence for the use, erection, or resurrection of a name [28,48]. Hence, this practice has caused a taxonomic instability, deepening the conundrum of Colombian snakes of medical importance, beyond the science-based changes in taxon names [49].

This phenomenon has occurred not only with Colombian snakes. Globally, taxonomic issues arise when the data used to create taxonomic decisions are shoddily presented, derive from spurious research, or lack evidence [50]. Thus, subsequent application to a broad range of endeavors, like conservation or neglected tropical diseases (e.g., ophidism), undermine the results by a lack of consistency. In addition, due to the explosion of electronic information and the social media revolution across of the first two decades of 21th century [51], a rapid dispersion and merge of scientific and non-scientific taxonomic information has occurred, making it difficult to make suitable taxonomic decisions for medical practitioners or non-herpetology professionals.

Accordingly, Kaiser et al. [50] propose that a scientific taxonomic decision must follow three main steps: (1) generate hypotheses of group membership (e.g., a species, a clade or taxon) or evolutionary relationship (e.g., sister taxa) based on available primary sources (e.g., fossil record, existing or new collections of specimens including whole animals, tissues, quantitative analyses, and DNA sequences, etc.) and the available literature; (2) test these hypotheses via appropriate, rigorous, and honest analysis of the relevant data using the scientific method; and (3) submit proposed taxonomic decisions (e.g., taxonomic rearrangements, descriptions of new species, elevation of subspecies to species rank) to peer-reviewed journals in the form of manuscripts that present the data and provide a rational justification for the proposed decisions.

Unfortunately, several high impact investigations did not follow the Kaiser et al. [50] approach, instead basing taxonomic decisions on lacking evidence, evidence shoddily presented; or worse, committing taxonomic vandalism [52,53], that means the deliberate establishment of scientific names (= hypothesis) by eschewing the scientific process described above [49,50]. In addition to these shortfalls, some taxonomic decisions have been published in predatory/fake journals or fast-track journals, which in exchange for a payment, the authors get a rapid publication and broad audience, at the expense of scientific quality, legitimacy of the taxonomic decisions, and the robustness of the peer-review process, and scientific value.

Confusion regarding species or genus names of venomous snakes could cause serious constraints in the implementation of public health schemes and snakebite treatment. For example, this can increase the underreporting of snakebite cases by classifying them under a species name that is nonscience-based, as well as masking the variability of clinical symptoms of envenoming from broadly distributed species [54–57].

Particularly in Colombia, one of the main difficulties during the diagnostic and snakebite accident treatment is the correct snake species or genus identification [1]. This arises as one of the main obstacles constraining the need for quick decision-making by a medical practitioner for the suitable antivenom therapy required, including the antivenom dosage, as well as the prevention of clinical complications during the treatment [58].

The species concept continues to be debated due to the fact that no single species concept simultaneously include all the ways, forms, and combinations in which life evolves [59]. Nevertheless, all species concepts have something in common: all consider species as a hypothesis. Hence, all taxa can be treated as "the educated assumption of some outcome based on scientific method, logic and observation" [60]. Thus, all can be the subject of testing. To treat species as hypotheses allows employing multiple lines of evidence as operational criteria (*sensu* Mayden [61]), to perform empirical testing and diagnosis of species in the nature [61–63].

According to this outlook, in this section we present a reviewed venomous species list based on the currently available information regarding the taxonomic status of the medically important snake species, encompassing colubrids (aglyphous and opisthoglyphous) viperids (solenoglyphous), and elapids (proteroglyphous) inhabiting Colombia. We conducted a detailed review based on the available literature in order to make the "best" scientific taxonomic decisions following the Kaiser et al. [50] proposal, as well as all those that clustered around the major lines of evidence to delimiting a species.

Our aim is not to resolve all current taxonomic problems of Colombian snakes, but to provide taxonomic treatment and criteria to allocate the puzzling snake species into a taxonomic scheme based on the current evidence available. This will help to medical to designate the taxonomic snake entities that cause bites in Colombia, reducing misunderstanding and misidentification, as well as the underreporting rate of snakebite [56,64] (see Chapter 9).

We hope that the next taxonomic review based on a scientifically based approach (including integrative taxonomy) and publication in a high quality peer-reviewed journal can untangle the puzzling Colombian snake species included here. All the criteria defined in this chapter will include the species taxonomic treatment throughout the chapters of this book.

2.1 Tackling the taxonomic uncertainties in the medically important snake species

During the last two decades, with the explosion of molecular techniques and analyses, the massive use of quantitative analyses, bioinformatic, high resolution computed tomographies (HRCT) aided by available free open-source software available (e.g., R, Python, Past, etc.), and the popularization of the hemipenial eversion techniques among researchers [65],

new species, genera resurrection, revalidation or elevation of species from subspecies, and species synonymies have been proposed [35,36,66–71]. These efforts, most of them including multiple lines of evidence (integrative taxonomy), have allowed us to untangle several taxonomic problems of South America venomous snakes previously pointed out by Campbell and Lamar [26], providing a notable advances toward our understanding.

Nevertheless, in comparison with these efforts, few taxonomic attempts using multiple lines of evidence have been made to resolve some puzzling Colombian snake venomous species [35,37,72]. In most Colombian species Campbell and Lamar [26] continues to be the paramount guideline for taxonomic decisions because there is no new evidence to tackle problematic species (e.g., *Micrurus dumerilli*, *M. mipartitus*, *Bothrops venezulensis*), or the new evidence provided has been shoddily presented, causing more confusion than clarity (e.g., *Bothrops ayerbei* and *Bothrops rhombeatus*; see Chapter 3).

Taxonomic problems arise from multifactorial causes. However, we summarize them in two main categories as follows:

Crypticity.— Researchers can observe insights indicating that populations of a particular species could represent different evolutionary lineages, but under the current state of knowledge, they cannot readily be distinguished or delimited due to significant uncertainties of their diagnostic characters (e.g., indistinct morphological attributes and/or ecological properties, lack of DNA data, or available quantitative analyses available) [63,73,74]. Therefore, a taxon might be considered as a complex of species that could cluster two or more species, but new lines of evidence are required for a suitable diagnosis and delimitation [74].

Data deficient.— This category groups species that: (1) since its erection and type series (the group of specimens on which the description of a species is based in addition to the holotype), no new specimens or populations are known; (2) lack or loss of type specimens; (3) species erected from a single known specimen; (4) its taxonomic name cannot be assigned with certainty to any taxonomic group because the description is insufficient for identification, and/or the original specimen is lost, or no longer exists (*nomen dubium*) [75]; (5) despite having been the object of a taxonomic revision across its known distribution (employing integrative taxonomic approach or a single line of evidence), Colombian populations of this species have not been included in these assessments; and (6) species delimitation and diagnosis has deep shortfalls and incongruences during its establishment. Therefore, conclusions made about its taxonomic status cannot be straightforwardly assumed for Colombian populations.

Using a taxonomic conservative view, the Colombian medically important snake species list is proposed here (Table 1), is based on a comprehensive revision of the available lines of evidence for the species delimitations, as detailed in the following section.

2.2 Records of Colombian medically important snake species

We retrieved and curated approximately 5,488 records of medically important snake species geolocated in Colombia from 50 sources. These references include specimens housed in biological collections, records from specialized herpetological literature (articles, books), unpublished data from field notebooks of various Colombian and foreign herpetologists, and clinical records from Colombian serpentarium dedicated to antivenom research and production (see https://ofidismo.ins.gov.co).

Colombia harbors a total of 59 medically important snake species, of which ten species are colubrid, 20 are viperids, and 29 are elapids. About 13.6% of these species bite frequently and are associated with serious and life-threatening envenoming, while the remainder of snake species rarely causes serious and life-threatening envenoming or have no reported cases (Table 1).

Table 1. Colombian medically important snake species. m: meters above sea level.

Species	Endemic	Type locality in Colombia	Department Distribution	Elevation range (m)
Snakes that bite frequently associated with serious and life-threatening envenoming				
*Bothriechis schlegelii**	No	Yes	Ant, Boy, Cal, Cau, Ch, Cor, Cu, Hui, Nar, Qui, Ris, San, Tol, Val	2—2946; $\bar{x}$ = 1832
*Bothrops asper**	No	No	Ant, Atl, Bol, Boy, Cal, Cau, Ces, Cho, Cor, Cun, LaG, Mag, NSa, Qui, Ris, San, Suc, Tol, Val	2—2200; $\bar{x}$ = 547
*Bothrops atrox**	No	No	Ama, Arau, Boy, Caq, Cas, Cun, Gua, Guav, Met, NSa, Put, Vau, Vic	57—1923; $\bar{x}$ = 308
Crotalus durissus	No	No	Ant, Ara, Atl, Bol, Boy, Ces, Cun, Hui, LaG, Mag, Met, Tol, Vic	7—1717, $\bar{x}$ = 235
*Porthidium lansbergii**	No	Yes	Ant, Atla, Bol, Boy, Cas, Ces, Cho, Cor, Hui, LaG, Mag, NSa, San, Suc, Tol	0—1243, $\bar{x}$ = 782
Porthidium nasutum	No	No	Ant, Cho, Val	12—1011, $\bar{x}$ = 307
*Micrurus dumerilii**	No	Yes	Ant, Atl, Bol, Boy, Cal, Cau, Cesar, Cho, Cor, Cun, Hui, LaG, Mag, Met, Nar, NSa, Ris, San, Suc, Tol, Val,	2—2278, $\bar{x}$ = 815
*Micrurus mipartitus**	No	Yes	Ant, Boy, Cal, Cau, Ces, Cho, Cor, Cun, Hui, Mag, Met, Nar, NSa, Ris, San, Suc, Tol, Val,	11—2606, $\bar{x}$ = 1204

Snakes that bite frequently, but rarely cause serious and life-threatening envenoming				
Dryophylax gambotensis	Yes	Yes	Atl, Bol, Ces, Cor, LaG, Mag, Suc	1—161, $\bar{x}$ = 34
Helicops angulatus	No	No	Ama, Caq, Cun, Gua, Guav, Met, Put, Vau, Vic	52—570, $\bar{x}$ = 237
*Leptodeira annulata**	No	No	Ama, Ant, Atla, Bol, Boy, Cal, Caq, Cas, Cau, Ces, Cho, Cor, Cun, Hui, LaG, Mag, Met, San, NSa, Suc, Tol, Val, Vic	3—2130, $\bar{x}$ = 646
Oxybelis fulgidus	No	No	Ama, Bol, Cat, Mag, Met, Suc, Vau	3—484, $\bar{x}$ = 220
*Erythrolamprus bizona**	No	Yes	Ant, Boy, Cal Cas, Cau, Ces Cun, Hui, Mag, Met, NSa, San, Tol, Val	14—2566, $\bar{x}$ = 900
Thamnodynastes pallidus	No	No	Ama, Cas, Met, Put, Vic	75—437, $\bar{x}$ = 162
Xenodon rabdocephalus	No	No	Ant, Cal, Cau, Cho, LaG, San, Tol, Val	3—1778, $\bar{x}$ = 483
Snakes that bite rarely, but are capable of causing serious and life-threatening envenoming				
Bothrocophias myersi	Yes	Yes	Cau, Cho, Val	12—1275, $\bar{x}$ = 224
Bothrocophias myrringae	Yes	Yes	Cun, Met	1757—2761, $\bar{x}$ = 2137
Bothrocophias tulitoi	Yes	Yes	Boy, Cas, Cun	1685—2694, $\bar{x}$ = 1969
Bothrops punctatus	No	Yes	Ant, Cal Cau, Cho, Nar, Ris,Val	4—1578, $\bar{x}$ = 569
Hydrophis platurus	No	No	Cau, Cho, Nar	-50—0, $\bar{x}$ = -5
Lachesis acrochorda	No	Yes	Ant, Boy, Cau, Cho, Nar, San, Val	2—1775, $\bar{x}$ = 655
Lachesis muta	No	No	Ama, Caq, Met, Put, Vau	24—1809, $\bar{x}$ = 352
Micrurus helleri	No	No	Ara, Caq, Cas, Cun, Met, Put, Vau	87—1338, $\bar{x}$ = 393
*Micrurus hemprichii**	No	Yes	Ama, Boy, Caq, Cas, Met, Vic	67—770, $\bar{x}$ = 311
*Micrurus lemniscatus**	No	No	Ama, Gua	79—125, $\bar{x}$ = 84
Micrurus obscurus	No	No	Ama, Caq, Gua, Guav, Met, Put, Vau, Vic	79—616, $\bar{x}$ = 243
Micrurus surinamensis	No	No	Ama, Caq, Cun, Guav, Met, Vau, Vic	52—537, $\bar{x}$ = 292

Snakes that bite rarely, and have not caused significant envenoming or have not caused documented bites

Bothrocophias campbelli	No	No	Cho, Nar, Val	32—1650, $\bar{x}$ = 428
Bothrocophias colombianus	Yes	Yes	Cho, Cau, Ris	211—2506, $\bar{x}$ = 1530
Bothrocophias hyoprora	No	No	Ama, Caq, Guav, Nar, Put, Vau	64—1202, $\bar{x}$ = 234
Bothrops bilineatus	No	No	Ama, Caq, Vau,	80—265, $\bar{x}$ = 105
Bothrops oligobalius	No	No	Ama, Caq, Guav, Vau	81—274, $\bar{x}$ = 151
Bothrops pulcher	No	No	Caq, Put	274—1725, $\bar{x}$ = 872
Bothrops taeniatus	No	No	Ama, Cun, Gua, Met, Vau, Vic	78—533, $\bar{x}$ = 155
Bothrops venezuelensis	No	No	Boy, Cas	151—1785, $\bar{x}$ = 1052
Micrurus ancoralis	No	Yes	Ant, Cal, Cho, Ris, Tol, Val,	21—707, $\bar{x}$ = 98
Micrurus camilae	Yes	Yes	Ant, Cor, San, Suc,	30—1381, $\bar{x}$ = 178
Micrurus clarki	No	No	Cau, Cho, Val	2—865, $\bar{x}$ = 257
Micrurus dissoleucus	No	No	Atl, Bol, Ces, Cor, LaG, Mag, NSa, Suc,	5—1297, $\bar{x}$ = 124
Micrurus filiformis	No	No	Ama, Caq, Cas, Gua, Guav, Met, Vau, Vic	68—438, $\bar{x}$ = 205
Micrurus isozonus	No	No	Ara, Cas, Met, Vic	4—429, $\bar{x}$ = 276
Micrurus langsdorffi	No	No	Ama, Caq, Gua, Guav, Met, Put, Vau,	81—637, $\bar{x}$ = 179
Micrurus medemi	Yes	Yes	Cun, Met	229—1599, $\bar{x}$ = 490
Micrurus multiscutatus	No	Yes	Cau, Cho, Ris, Val	67—2506, $\bar{x}$ = 425
Micrurus narduccii	No	No	Ama, Caq, Gua, Put, Vau	75—283, $\bar{x}$ = 140
Micrurus nattereri	No	No	Gua, Vau	85—229, $\bar{x}$ = 166
Micrurus nigrocinctus	No	No	Ant	6—32, $\bar{x}$ = 18
Micrurus oligoanellatus	Yes	Yes	Cau	1442
Micrurus ornatissimus	No	No	Ama, Vau	82—115, $\bar{x}$ = 93

Micrurus psyches	No	No	Ara, Met	156—452, $\bar{x}$ = 299
Micrurus remotus	No	No	Gua, Vau	83—233, $\bar{x}$ = 128
Micrurus renjifoi	Yes	Yes	Vic	115
Micrurus sangilensis	Yes	Yes	Boy, Cas, San	993—2420, $\bar{x}$ = 1660
Micrurus scutiventris	No	No	Ama	79—126, $\bar{x}$ = 114
Micrurus spurrelli	Yes	Yes	Cho	41—71, $\bar{x}$ = 51
Micrurus tikuna	No	No	Ama	78—81, $\bar{x}$ = 79
Other potentially medically important which have not caused documented bites				
Erythrolamprus aesculapii	No	No	Ama, Caq, Guav, Put, Vau	88—327, $\bar{x}$ = 169
*Leptophis ahaetulla**	No	No	Ant, Cas, Ces Cun, Hui, Met, San, Tol,	42—2171, $\bar{x}$ = 746

Colombian departments. Ama: Amazonas; Ant: Antioquia; Arc: Archipielago de San Andres, Providencia and Santa Catalina; Arau: Arauca; Bol: Bolivar; Boy: Boyaca; Cal: Caldas; Caq: Caqueta; Cas: Casanare; Cau: Cauca; Ces: Cesar; Cho: Choco; Cor: Cordoba; Cun: Cundinamarca; Gua: Guainia; Guav: Guaviare; Hui: Huila; LaG: La Guajira; Mag: Magdalena; Met: Meta; Nar: Nariño; NSa: Norte de Santander; Put: Putumayo; Qui: Quindio; Ris. Risaralda; San: Santander; Suc: Sucre; Tol: Tolima; Val: Valle del Cauca; Vau: Vaupes; Vic: Vichada.
* = species complex.

The ten Colombian departments with the greatest snake species richness are Amazonas, Vichada, Vaupes, Choco, Cauca, Caqueta, Antioquia, Cundinamarca and Valle del Cauca, ranging from 15 to 22 snake medically important species (Figure 3A). All departments encompass the most diverse ecoregions and complex topographic areas of Colombia and northern South America. However, most records are concentrated in the departments of Antioquia, Meta, and Santander because there are the political-administrative divisions of Colombia that historically have received major sampling efforts [1].

When snake richness was analyzed after splitting into elapid and viperid species, the general tendency observed was stable, and the species richness clustered over the complex topography and ecologically diverse regions. However, for viperids, the departments with the greatest snake species richness were those located in the trans-Andean region of Colombian (Choco, Valle del Cauca, Boyaca, Antioquia), while for elapids, species richness is mostly concentrated in the cis-Andean region (Meta, Amazonas Vichada, Vaupes, Caqueta; Figure 3B). This suggests intricate biographic patterns of expansion and diversification in the lineages of these venomous snake species (see Chapters 2 and 3). Antioquia, Arauca, and Meta are the departments most of the known geographic records of viperids clustered, while for elapid species, Santander, Cundinamarca, and Valle del Cauca were the departments that harbor most of the known geographic records (Figure 3C).

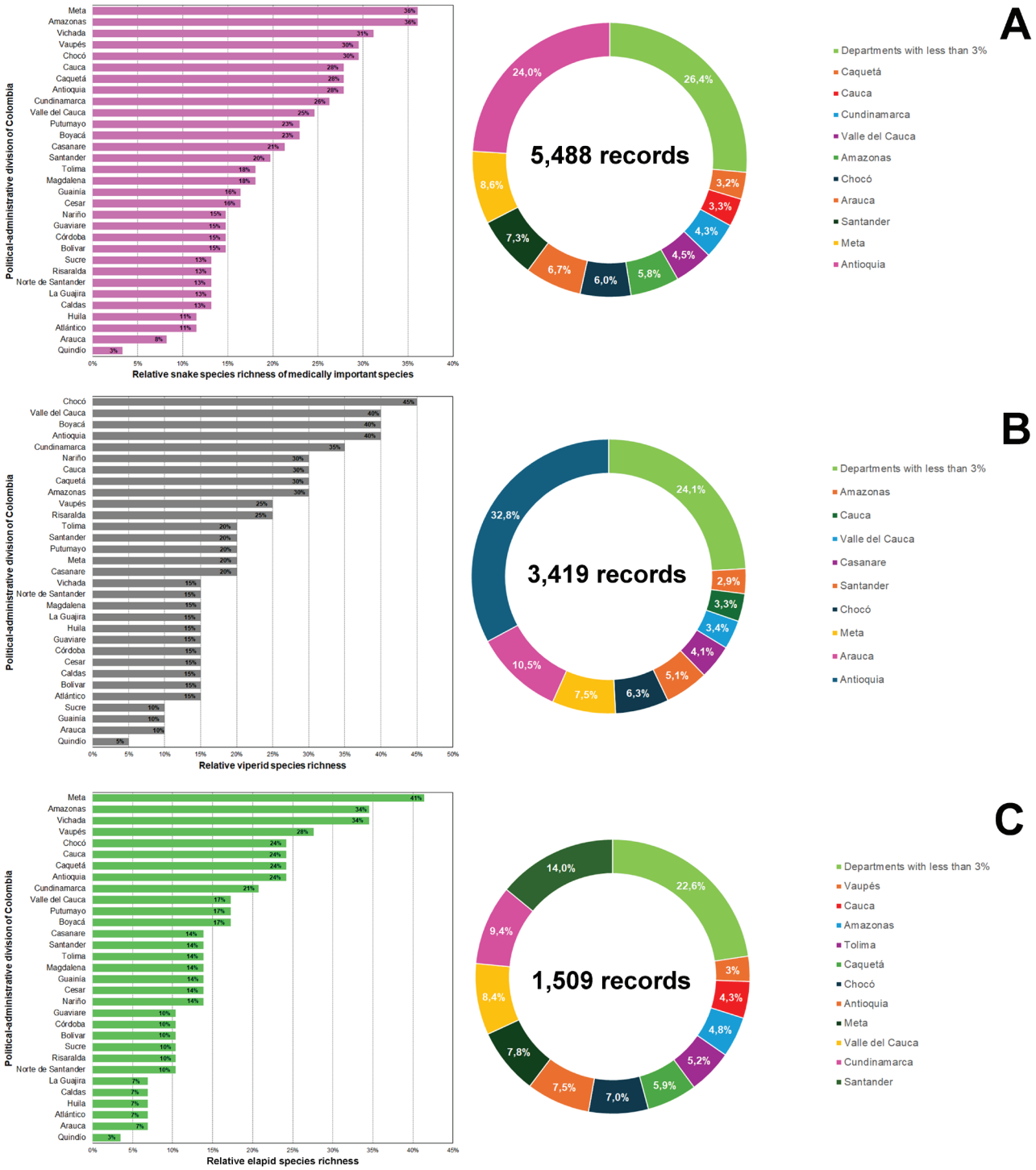


Figure 3. Species richness and records of medically important snake species of Colombia

Since political-administrative divisions are not biological constraints for species distributions, nor are they a suitable approach for estimating the geographical representativeness of medically important snake species, we divided the country into grids/pixels of 1.0 geographical degrees (110 x 110 km; WGS84 system) to analyze distribution. This approach allows for comparisons with previous studies, such as Lynch et al. [1], and future research (Figure 4).

Regardless of the taxonomic group (viperids or elapids), most of the available records come from the trans-Andean region of Colombia, highlighting a notable historical disparity in the sampling effort to understand and document the medically important snake species in the country (Figure 4A-C). Additionally, this disparity is exacerbated when comparing viperids and elapids, showing that elapids have been historically under sampled (Figure 4D).

The greatest number of historical records of venomous snakes from the Viperidae and Elapidae families is concentrated in the department of Antioquia. This department also shows the highest annual average of snakebite reports for the period 2010-2020 (see Chapter 9), indicating that it is the only department that has the most robust information on two of the most important variables for understanding snakebite as an epidemiological event [64]. In contrast, the rest of the national territory exhibits a notable under sampling of venomous snakes, with fewer than 112 records per pixel (110 km x 110 km; see Figure 4A). This represents a critical problem for understanding the total diversity of medically important snakes involved in human-snake conflict, as well as their incidence in snakebite events.

This knowledge gap exacerbates the limitations in understanding the causes of negative encounters between humans and snakes, identifying the species involved, and formulating and implementing strategies to prevent envenoming and conserve the country's snake fauna. For this reason, we recommend intensifying the sampling of medically important snakes in the short and medium term, with a focus on regions such as the southern Andes, the Pacific, the Caribbean (especially in the upper Guajira), Orinoquia, and Amazonia.

Nevertheless, ten years after the first assessment by Lynch et al. [1] that estimated the geographical representativeness of medically important snake species in the country (Figure 5A-C), researchers and institutions advocating for snake research and conservation have achieved significant advancements in the knowledge of the distributions of Colombian venomous species (Figure 5E-G). On average, they have added 91 new geographical records per department, provided the first official records for 19 of the 32 departments in Colombia, and achieved significant coverage of 87% of the national territory with at least one geographical distributional record (Figure 5D-H). For accurate and complementary information, please consult: https://ofidismo.ins.gov.co.

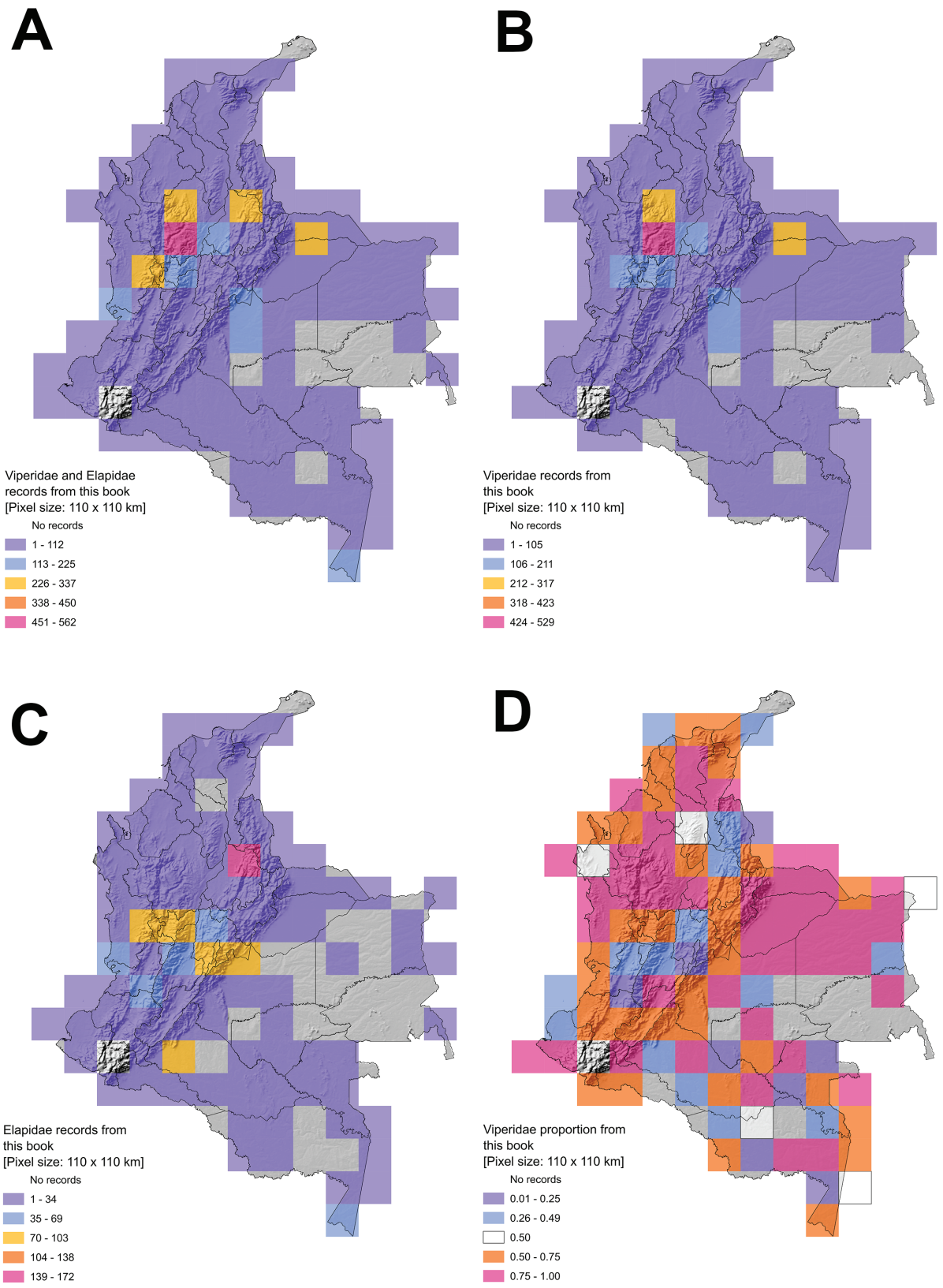


Figure 4. Sampling effort per pixel of 1.0 geographic degree (110 x 110 km; WGS84) in Colombia during 2010-2024. (A) Representativeness of viperids and elapids species. (B) Representativeness of viperids. (C) Representativeness of elapids. (D) Proportion of viperids/elapids+viperids.

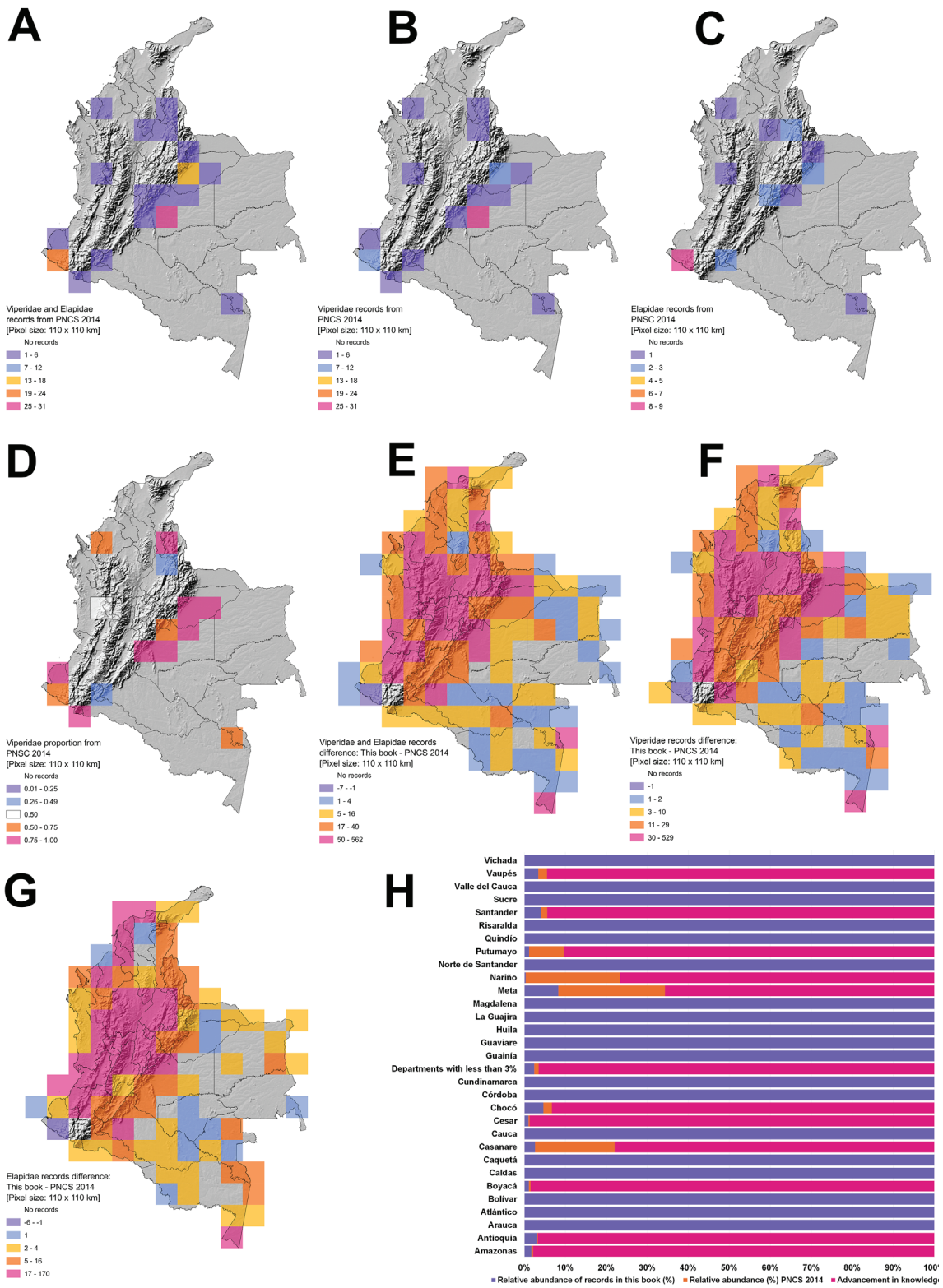


Figure 5. Comparisons between records reported in this book and Lynch et al, [1]. (A-C) Sampling effort per pixel of 1.0 geographic degree (110 x 110 km; WGS84) reported by Lynch et al. [1] for venomous snake species, viperid and elapid species. (D) Proportion of viperids/elapids+viperids per pixel according to Lynch et al. [1]. (E-G) Advancement in knowledge per pixel for venomous snake species, viperid and elapid species, respectively. (H) Comparisons per political unit between records reported in this book and those by Lynch et al. [1], showing the advancement in the knowledge of the geographic distribution of Colombian venomous snakes.

2.3 Venomous snake species out of the list

Dubious and puzzling species

Bothriechis schelegelii.— Arteaga et al. [16] recently reviewed the *B. schlegelii* species complex (i.e. *B. schlegelii* and *B. supraciliaris*), splitting it in 10 species, five of which were therein described in the publication. Despite our acknowledgement that *B. schlegelii* is a species complex, herein we refrain from using their taxonomy due to multiple inconsistencies in the species delimitation and diagnosis. One major problem is that none of the lineages they recognized as species have strong support in their phylogenetic tree (posterior probability values: Colombian node <75%-94%; Colombian intranode <50% pp). Also, the genetic distances between some of the closely related lineages proposed by them as species have genetic distances below the 4% boundary that they proposed as species delimitation criterion.

In their publication, morphological boundaries between closely related and distributed lineages are unclear, morphological characters are ambiguous and feeble when distinguishing their defined species from *B. schlegelii sensu stricto* (i.e., see Table 2 in Arteaga et al. [16], including diagnostic characters as follow: Interoculolabials, canthal scale condition, gular scale condition, loreal in contact with preocular). Although Arteaga et al. [16] (see supplementary material 1 in Arteaga et al. [16]) examined 45 characters and 400 specimens, almost 40% of their morphological matrix corresponds to missing data (meaning their actual sample size is ~160 specimens; so, a considerable portion of these characters was not examined for all specimens). Morphometric and meristic characters were poorly described, since only ranges were presented, without specifying the measures of central tendency and the presence of outliers. Likewise, most of these characters require a morphometric analysis to properly state the size and relative proportions among head scales and their diagnostic capacity. Besides, the use of diagnostic characters that depend on sample size (e.g., loreal in contact with preocular) provokes significant biases in species delimitation, as well as doubts about their geographic distributions (e.g., between *B. khwargi* and *B. klebbai*).

Color pattern is not suitable as a diagnostic character in the *B. schlegelii* species complex, as it is widely known to have several biases and constraints, such as ontogenetic shifts [26], and polychromatic intrapopulation variability [25,26,39]. Indeed, Arteaga et al. [16] are aware of this because most of their figures and descriptions of Colombian *B. schlegelii* populations clearly showed both ontogenetic and polychromatic variability. Despite this, they insisted on the use of these color patterns as diagnostic characters, even though their diagnostic capacity is low or null.

In Colombia, Arteaga et al. [16] proposed six species in their work: *B. khwargi*, *B. klebbai*, *B. rahimi*, *B. rasikusumorum*, *B. schlegelii*, and *B. torvus*. Recently, Reyes-Velasco [149], critiqued these taxonomic indicating that the genetic and morphological data fail to support the distinction of these species. Reyes-Velasco argues that the over-splitting of species due to deep intraspecific genetic variation has led to broader taxonomic inflation, as this variation may reflect clinal differences rather than species boundaries.

Reyes-Velasco [149] concludes that the new species proposed by Arteaga et al. [16] within the *B. schlegelii* group may represent geographic variants or subspecies rather than distinct species. As shown above, Reyes-Velasco's assessment supports our findings, affirming our decision to recognize only three valid species within the *B. schlegelii* complex: *B. nigroadspersus*, *B. schlegelii*, and *B. supraciliaris*. The remaining taxa proposed in recent revisions, including *B. khwargi*, *B. klebbai*, *B. rahimi*, *B. rasikusumorum*, and *B. torvus*, do not withstand more rigorous analysis and should be synonymized with *B. schlegelii*. This result has important implications for conservation efforts in Colombia and underscores the need for caution when making taxonomic changes based solely on mitochondrial DNA. Additionally, unstable taxonomic changes in a medically significant species like *B. schlegelii*, due to its association with snakebites (see Chapters 5 and 9), may complicate non-taxonomic literature, including medical and biochemical references.

We recommend that future publications dealing with the *B. schlegelii* complex refer to it as we suggest (*B. schlegelii*), and to be rigorously described the geographic origin of the reported specimens (e.g., accurate locality, coordinates). In this way, when lineages within this species complex are properly delimited, the already published data can be linked to the corresponding lineages or species.

Bothrops ayerbei and *B. rombeatus*.— Folleco-Fernández [76] tried to clarify the *Bothrops asper* species complex from the western slopes of the Cordillera Occidental by proposing *Bothrops ayerbei* and *B. rombeatus* as new members of this complex. However, the description lacked the detailed characteristics needed to clarify boundaries between these proposed species, as well as between the specimens of the type locality (Obispo, Darien, Panama). The descriptions of the specimens´ physical traits were vague, morphological analysis was misconducted, and there was no phylogenetic analysis performed. Hence, taxonomic decisions are difficult to delimit either species.

Also, there was no designation of a type specimen or a formal description of the new taxon by Garcia-Piedrahita [17]. Ramirez and Solari [77] point out that *B. rhombeatus* is a *nomen dubium* because of the lack of type material, also its troubled history that hampers a correct taxonomic assignation due the lack of crucial data on geographical distributions that hinders an understanding of possible interbreeding between *Bothrops ayerbei* and *B. rombeatus*. Additionally, according to Ramirez and Solari [77], *Bothrops ayerbei* constitutes an unavailable name following to the rules of the current International Code of Zoological Nomenclature, because the code does not accept the publication of new taxa in electronic journals before 2011.

The significance of distinguishing between these taxa as valid species was not properly discussed by Folleco-Fernández [76]. Despite this, some researchers have used molecular markers to study the venomic variation and antivenomic responses within the *B. asper* species complex, using genetic sequences of populations allocated as *B. ayerbei* and *B. rhombeatus* without a proper taxonomic delimitation or review of these specimens

[36,78,79]. Besides, these authors have not provided accessibility to the official gene databases for these sequences (e.g., GenBank, EMBL). In fact, the only research that analyzes the relationships among the three species found that *B. asper* is paraphyletic in relation to the other two [80]. Therefore, we suggest that future research includes a broad and exhaustive sampling methodology, considering a larger number of individuals from diverse habitats and employing an integrative taxonomic approach that combines more lines of evidence, allowing for a clear delimitation of these species.

Micrurus hemprichii species complex.— Historically, *Micrurus hemprichii,* similarly to most of the South American coralsnakes, has suffered several nomenclatural changes since its establishment. Bernarde et al. [81] provide a comprehensive historical summary of the nomenclatural changes for *M. hemprichii*. Since Peters and Orejas-Miranda [15] there are two recognized valid subspecies, *M. h. hemprichii* and *M. h. ortoni* Schmidt [82], both distributed in Colombia. *Micrurus h. hemprichii* ranges from the Colombo-Venezuelan savannas of the Orinoquia region to rainforests of Guiana, while *M. h. ortoni* is distributed across the Amazonian slopes of Colombia, Ecuador, Peru, and Para in, Brazil. Feitosa et al. [83] in a conference presentation that was not peer reviewed, proposed to elevate both subspecies as full species taxa. Valencia et al. [84] followed this proposal, and based on the morphological affinity states that Ecuadorian populations can be allocated to the *M. ortoni* definition without providing a taxonomic assessment.

Currently, there is not available a comprehensive taxonomic assessment available that clearly distinguishes the subspecies of *Micrurus hemprichii* or populations across its whole known geographic distribution. In addition, previous proposals have not included Colombian populations, despite the fact that the type specimen of *M. h. hemprichii* came from an unknown locality of the Orinoquia region; nor have specimens from the type locality of *M. h. ortoni* from the eastern versant of the Andes in Pebas, Loreto, Peru been included. Therefore, we recommend that future publications dealing with the *Micrurus hemprichii* complex refer to it as we suggest (*Micrurus hemprichii*), waiting for a rigorous study that helps to elucidate this species complex.

Despite this, Ayerbe-Gonzalez et al. [85], without proper taxonomic delimitation or review of the specimens involved, reported the first snakebite envenomation caused by *M. ortoni* (=*Micrurus hemprichii*) in Colombia. The cases occurred in two localities of the cis-Andean region of the country. The first case was reported from the eastern slopes of the Cordillera Oriental in the urban area of the municipality of Pajarito, Boyaca. The second case occurred in the urban area of the municipality of Cartagena del Chaira, Caqueta, a settlement located in the floodplain of the Caguan River. Both patients were women who were bitten on the feet, and they presented symptoms such as intense pain in the affected limb that radiated to the lumbar section in the first case. In the second case, the pain radiated to the knee during the first hour of envenoming, reaching the lumbar section after three hours. After 24 hours of envenoming, the patient in the first case exhibited clear neurotoxic symptoms such as mild Rosenfeld's facies, palpebral pto-

sis (drooping upper eyelid), bradylalia (slow speech), and continued intense pain presenting hyperalgesia (increased sensitivity to pain) and hyperesthesia (increased sensitivity to sensory stimuli). In contrast, the patient in the second case lacked neurotoxic symptoms and was discharged after a few hours of observation; she was prescribed acetaminophen and cephalexin.

Leptodeira annulata species complex.— The species of the genus *Leptodeira* are widespread in American. This genus is composed by 16 nominal species that primarily inhabit lowland dry forests, pre-montane forests and gallery forests in most the natural and disrupted habitats of the tropical lowlands, distributed from southern North America to northern Argentina [29,86–88]. The close resemblance in color pattern and antipredator displays (e.g., body like S-coil posture, head posterolaterally expanded simulating a triangular shape) of *Leptodeira* species causes these snakes to be commonly mistaken for *Bothrops* species in tropical regions. All species are nocturnal, with semi-arboreal habits occurring near slow-moving streams or standing water. These snakes have generalist feeding habits, mainly consume mainly small frogs and lizards, frequently anuran eggs and tadpoles, snakes, and occasionally consume small birds and fishes [89–92].

Across the whole distribution of the *Leptodeira* species, the taxonomic status of species populations has historically been incessantly controversial, and Colombian populations are not the exception. According to Duellman [88] there are two species in Colombia of *Leptodeira*: *Leptodeira annulata* with three subspecies (*L a. annulata*, *L a. ashmeadi*, *L a. ashmeadi+rhombiera*) and the species *L. septentrionalis* with a single subspecies *L s. ornata*. The diagnostic characters allowing identification of the two *Leptodeira* species are found in its hemipenial morphology, and differences between subspecies are based on color pattern and some scale counts (e.g., dorsal scale reductions). Duellman [86] also indicates that these species exhibit allopatric distributions in which *Leptodeira annulata* populations inhabit the lowlands of cis-Andean ecosystems, while *L. septentrionalis* is found in the lowlands of trans-Andean ecosystems in Colombia.

Daza et al. [93] tested the hypotheses stated by Duellman [88] using molecular evidence and found paraphyly between *L. annulata* and *L. septentrionalis*, so that genetic distance did not work as a criterion for species delimitation. However, these authors did not provide a comprehensive evaluation of Colombian populations of *Leptodeira* because they focused their sampling on only a few Andean localities (Antioquia, Caldas, Meta [foothills]). Subsequently, Barrio-Amoros [94] provided a comprehensive taxonomic reassessment and taxonomic acts. This author states that *L. annulata*, *L. ornata*, *L. ashmeadi* are full species distributed in Colombia. However, his proposal lacks care, precision, and sufficient information about the methods and datasets used, creating taxonomic instability due to unfounded taxonomic decisions and untidy descriptions. Therefore, most of his conclusions and decisions should be carefully revised and used conservatively.

Torres-Carvajal et al. [88] brought taxonomic stability after Barrio-Amoros [94], by performing a comprehensive and well-supported revision of

Leptodeira snake populations of western Ecuador. They combined molecular and morphological evidence, including the molecular Colombian samples previously utilized by Daza et al. [93], but they did not included morphological or hemipenial assessments of Colombian populations. These authors presented a maximum likelihood tree that retrieved with strong support the *L. annulata/septentrionalis* complex reported by Daza et al. [95], including Colombian populations.

In addition, within deeper nodes of their tree topology, they retrieved some clades considered as moderately to strongly statistically supported. Among these lineages Torres-Carvajal et al. [88] made the following taxonomic decisions: (1) based on molecular and morphological evidence they described a new species (*Leptodeira misinawui*) from a population previously considered as *Leptodeira s. larcorum* [88], and located at moderate to high elevations (950–2,734 m asl) in the southwestern Ecuadorian Andes; (2) based on molecular and morphological evidence solely from Ecuadorian populations, they elevated to full species status the populations previously considered as *Leptodeira s. ornata* from western Ecuador and Colombia, central and eastern Panama, as well as the Darien Mountain slope; (3) based solely on molecular evidence from the same populations previously considered by Daza et al. [93] they elevated to full species *Leptodeira a. ashmeadii.*

Costa et al. [95] reassessing the systematics of *Leptodeira* expanding upon and improved the molecular and morphological sampling and provided a new proposal for the polyphyletic *Leptodeira* species complex for several South American regions. These authors proposed four species distributed in Colombia: *Leptodeira annulata, L. approximans, L. ashmeadii,* and *L. ornata,* and they partially agreed with the taxonomic acts of Torres-Carvajal et al. [90] that recognize *Leptodeira larcorum* and *Leptodeira ornata* as full species, the latter distributed from western Ecuador, through the Pacific Colombian ecoregion to eastern Panama.

Nevertheless, Costa et al. [95] indicate that according to their tree topology results *L. ornata* is composed of three different taxonomic units (*L. ornata* 1 distributed in distributed in Colombia, southern Panama [type locality of *L. ornata* is Isthmus of Darien]; *L. ornata* 2 distributed in Costa Rica and northern Panama; *L. ornata* 3 distributed in Ecuador and Peru).

Costa et al. [95] conclude that the populations morphologically described by Duellman [86] from the Darien region in Panama (*L. ornata* type locality included) and from the Magdalena and Cauca valleys and Choco region in Colombia are combined for redefined *L. ornata*. However, they did not contrast molecular or morphological evidence between specimens from the interandean valleys of Colombia and the Choco region; therefore, they restricted the distribution of *L. ornata* to the Pacific ecoregion of Colombia and southern Panama. However, Costa et al. [95] in their redefinition of *L. ornata* (see the appendix S2 in Costa et al. [95]) depict the older distribution proposed by Duellman [86] for *L. s. ornata* encompassing most of the trans-Andean region despite the several diagnostic charac-

ters employed to distinguish between *L. ornata* and *L. ashmeadii* that are strongly overlapped between these populations (e.g., color pattern of the head dorsal surface, dorsal scale counts, supralabial scale count). Thus, morphological distinctiveness of these two populations remains uncertain and awaits further studies.

Similarly, Costa et al. [95] proposed a taxonomic arrangement for the *Leptodeira annulata ashmeadii/bakeri* group of species, supporting the previous results of Daza et al. [95]. The redefinition of Costa et al. [97] of *L. ashmeadii* locate this species in both the Caribbean coast and cis-Andean region of Colombia. However, morphological distinctiveness within Colombia population remains uncertain requiring further studies. Particularly, because hemipenial morphology of the species in the genus *Leptodeira* proposed by Costa et al. [95] both from trans and cis-Andean have no significant differences between each other (Figure 4; see also the appendix S2 in Costa et al. [95]), and several diagnostic characters employed by these authors to distinguished between them are strongly overlapped.

An ongoing comprehensive study of the hemipenial morphology of the genus *Leptodeira*, with special interest in Colombian populations (Angarita-Sierra, unpublished data), shows that the hemipenial architecture of *Leptodeira ashmeadii* exhibits conspicuous morphological differences between populations that do not match the proposal of Costa et al. [95] (Figure 6 A-D versus H-I), while others apparently do fit what is described by these authors (Figure 6 A-B versus C-D). Similarly, hemipenial morphology of trans-Andean Colombian populations (including western Colombia) mismatches the hemipenial architecture of *L. septentrionalis* (*sensu stricto*) described by Duellman [86], as well as the hemipenial morphology described in *Leptodeira ornata* by Torres-Carvajal et al. [90]. Moreover, the hemipenial architecture of trans-Andean Colombian populations of *Leptodeira* ornata proposed by Costa et al. [97] showed marked variability between sister lineages, as well as within populations of this lineage distributed in Colombia (Figure 6E-G versus K-M).

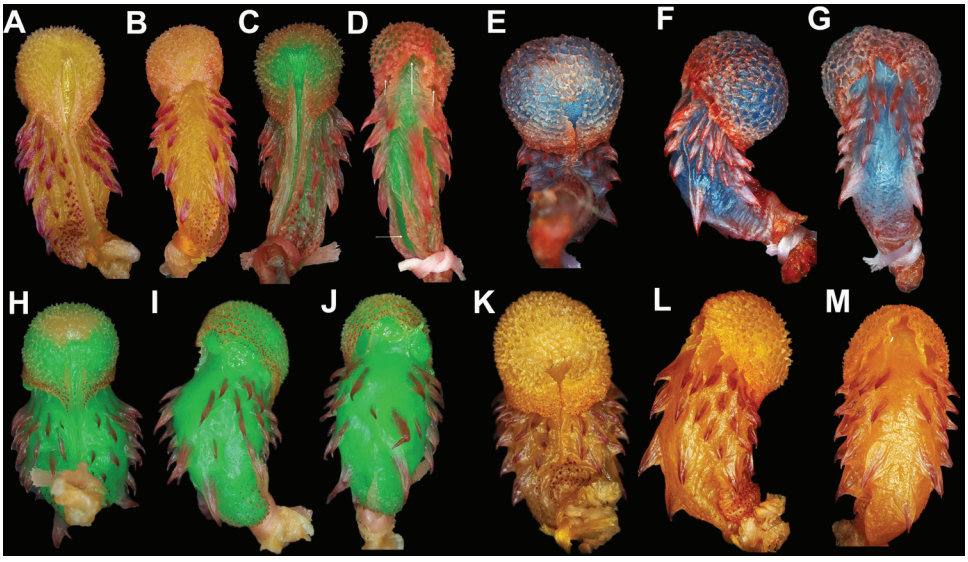


Figure 6. Hemipenial morphology of some populations of *Leptodeira annulata* species complex from cis and trans-Andean Colombia regions. Sensu Costa et al. [95]. (A-B): *L. ashmeadii* (TAS 689) from Finca El porvenir, Vereda La Colombina, Paz de Ariporo, Casanare; (C-D): *L. ashmeadii* (JDL 30721) from Finca El Vogal, Vereda Las Flores de Jaime Botero, El Retén, Magdalena; (E-G) *L. ornata* (JDL 29306) from Tumaco, Nariño; (H-J) *L. ashmeadii* (JDL 30754) from hacienda La Maria, El Retén, Magdalena. (K-M): *L. ornata* (JDL 29872) from Vereda Santa Paola, San Martin, Cesar.

So, despite enormous efforts to disentangle the taxonomy and relationships of *Leptodeira*, as well as the significant advancements made by Costa et al. [95] (including the strongly supported lineages found by them), several problems remain that continue to cause controversy on a fine geographic scale when delimiting the distributional ranges of the genus *Leptodeira*. Future studies require broader molecular sampling and a detailed revision of hemipenial morphology within Colombian populations. Considering the difficulties in determining distribution limits and distinguishing species by external morphology, as well as the limited knowledge about venom variation and snakebites caused by these snakes, we have decided to treat all members of the genus *Leptodeira* in Colombia as a single species, *Leptodeira annulata*, for the purposes of this publication. We hope that future evidence will help clarify the status of Colombian populations of this genus.

Leptophis ahaetulla species complex.— The neotropical genus *Leptophis* encompasses a total of 19 snake species distributed from Central America to northeastern Uruguay and Argentina. Four species inhabit Colombia [29]. Species from this genus are generally diurnal and arboreal, commonly seen in small shrubs and trees, and characterized by their colorful appearance [96]. They feed mainly on hylid frogs, lizards, and young birds [97]. When these snakes are threatened, they display a histrionic and aggressive behavior opening the mouth as much as they can, and following the movements of the object that threatens it [98].

Currently, between the nine *Leptophis* species recognized for Colombia three are not taxonomic conflicts, they are well-delimited and are supported by morphological and molecular evidence allowing distinctions between each other (*L. cupreus*, *L. depressirostris*, *L. riveti*). Nevertheless, within the *Leptophis ahaetulla* species complex significant differences have been detected across its wide distribution from Central America to northeastern Uruguay. Currently the *Leptophis ahaetulla* species complex includes 11 species, six of which were formally recognized in Colombian territory (*L. ahaetulla* sensu stricto*, L. bocourti*, *L. coeruleodorsus, L. nigromarginatus, L. occidentalis,* and *L. urosticus*) [15,42,101,102,150].

Recently, two major efforts were made to understand the phylogenetic relationships and to put in the taxonomy of the *L. ahaetulla* species complex. The first is the most comprehensive phylogenetic study to date [101], where six of the 12 species of the complex were included. Nevertheless, it should be considered that the hypotheses of the relationships within *L. ahaetulla* species group by Torres-Carvajal and Teran [101] was assessed with a limited taxonomic and geographic survey (this study did not incorporate any Colombian specimens) and have many poorly supported nodes (including the node that encompasses the entire species group), so it is likely that the relationships between species will change when more species or genomic regions are incorporated in future analyses. Even so, this study yields interesting results such as the polyphyly of at least three species (*L. ahaetulla*, *L. nigromarginatus* and *L. occidentalis*) and the data suggests that research be conducted soon to resolve the phylogenetic relationships and the taxonomic status of the group.

The second important initiative for consolidating the current taxonomy of the *L. ahaetulla* species group was the revision based on external and hemipenial morphology by Albuquerque and Fernandes [100]. This research establishes the six currently recognized species of the *L. ahaetulla* species complex in Colombia. The research analyzed 71 Colombian specimens of four species (*L. ahaetulla*, *L. nigromarginatus*, *L. occidentalis*, and *L. urostictus*). Likewise, the recognition of *L. bocourti* in Colombian territory is based on the synonymization of *Leptophis occidentalis insularis* from Gorgona Island (Cauca department) with *L. bocourti* by Oliver [104], although the type specimens of *L. o insularis* were not examined by Albuquerque and Fernandes [150]. The recognition of *L. coeruleodorsus* is based on an anecdotal specimen of *L. coeruleodorsus* collected by William W. Lamar and illustrated in Campbell and Lamar [26] in Villavicencio, Meta [101].

Although this article was crucial in establishing the current nomenclature of the *L. ahaetulla* species complex, there are still many issues such as the limits of species distribution at the national scale that need to be resolved. For example, there is no information on the distributional gap of *L. occidentalis* from the tropical dry forest of Guayaquil (Ecuador) from the northern Chocoan Region of Colombia or comments as to whether continental populations of *L. bocourti* exist. Considering such biogeographical issues and the epidemiological interests of this work, we prefer to treat all species of the complex as *L. ahaetulla*.

Oxybelis aeneus and *O. fulgidus*.— The genus *Oxybelis* is composed of ten rear-fanged species native to the subtropical and tropical regions of the Americas often associated with defensive and aggressive behavior. Currently in Colombia have been recognized three species [29]. Herein, we only focused in *Oxybelis aeneus* and *O. fulgidus* species groups that are considered medically important because they have snakebites reports (i.e., in Brazilian socioecosystems) or have the potential to cause snakebites due their behavior (see Chapter 4).

Oxybelis aeneus is a strictly diurnal snake with arboreal habits and an ambushing foraging strategy [103,104]. It feeds mainly on lizards (*Anolis* sp.) and occasionally frogs. However, birds, small mammals, insects, and fish have also been reported as part of its diet [104,105]. Similarly, *Oxybelis fulgidus* is strictly diurnal, spending most of the time on trees or shrubs [106]. This species is more associated with forest environments and sometimes can be found foraging on the ground [108]. Its diet appears to be more varied compared to *O. aeneus*, consisting mainly of lizards and birds at similar rates [107–111].

Historically, *Oxybelis aeneus* has been considered as one species across its wide distribution range from southern Arizona (USA) throughout the Centro and South America into southeastern Brazil. However, recent studies have sorted out the species crypticity of the brown vine snake [112,113]. According to the molecular phylogenetic analysis performed by Jadin et al. [113,114] within the *O. aeneus* complex, the species were found

to represent four clearly differentiated lineages across North and Central America. Moreover, northern South American populations of *O. aeneus* could represent more than one lineage, as well as several undescribed species. For example, *Oxybelis vittatus* has been recently resurrected, and its distribution across the Chocoan region of Colombia is expected [29,113]. Nevertheless, Colombian populations have not been included in the molecular analyses and they lack comprehensive morphological studies. Thus, these populations remain unclear. Due to a lack of new evidence that might help to understand the *O. aeneus* species complex inhabiting Colombia, we retained all the Colombian populations under the name *Oxybelis aeneus*.

Colombian populations of *Oxybelis fulgidus* exhibit similar taxonomic issues as the *O. aeneus* species complex [112], as well as the lack of new lines of evidence that allow us to perform species delimitation analyses to sort its species crypticity. Therefore, we retained all the Colombian populations under the name *Oxybelis fulgidus*.

Wrong or troubled localities, and dubious species determination

Due to the complex topography of Colombia and the lack of accurate knowledge about fine-scale geographic distributions of venomous snakes, several records have been reported with high uncertainty. Over time, these records have been considered 'valid' or probable distributions, without any researcher providing evidence to support their validity. Similarly, edges between ecoregions, in which there might or might not be hybridization zones between widespread species (e.g., *Bothrops asper* and *B. atrox*) provide puzzling distributional records because taxonomic determination is dubious.

Previously, Campbell and Lamar [26] pointed out some of these areas across Colombia, indicating that further studies should be done to elucidate the suitable distribution of Colombian venomous species. Our review detected several problematic localities, as well as dubious species determination of several venomous species in the Colombian area that match the controversial regions indicated by Campbell and Lamar [26], but we were also be able to identify others records and regions of controversy (see https://ofidismo.ins.gov.co). Since the Campbell and Lamar [26] distributional remarks few studies have addressed these concerns [35]. We suggest that, due to the lack of a proper revision of these records, they should be carefully used in future studies that seek to model species distributions, species niches, and ecological traits employed as characters in species diagnosis and delimitation, as well as models that estimated the envenoming snakebite risk. Below, we provide a brief description of the main wrong or troubled localities of several venomous species in Colombian.

Bothrops asper-atrox complex.— Campbell and Lamar [26] indicate that confusion surrounding *Bothrops asper* and *B. atrox* range distributions arose since Garman in 1883 [115] recognized both *B. asper* and *B. atrox* but confused these species both with each other and with other congeners. In

Colombia, the area of controversy encompasses the foothills and uplands of the central and northern eastern slopes of the Cordillera Oriental in the municipalities of Choachi, Fomeque, Ubala, Quetame, Guayabetal, San Antonio del Tequendama, Tena (Cundinamarca), Paya, Pajarito, San Luis de Garagoa (Boyaca), Catatumbo, Pamplona, Sardinata, and Toledo (Norte de Santander). Similarly, there are several problematical localities relating to their elevational ranges. Both *B. asper* and *B. atrox*, throughout their known geographic distributional ranges, occupy the lowlands (<1000 m above sea level [hereafter asl]).

Nevertheless, both *B. asper* and *B. atrox* have records that exceed their known elevational maximum range (Table 1). Problematical records of *Bothrops asper* over 2,200 m asl can be found in municipalities such as Santa Rosa de Osos, San Roque, Yarumal (Antioquia), San Lorenzo (Magdalena), and Calima (Valle del Cauca). Dubious *Bothrops atrox* elevational records have been reported in Quetame (Cundinamarca) and Pamplona (Norte de Santander). These records could result from misidentification, due to confusion with similar congeners (e.g., *Bothrocophias* species), or undescribed species. Recently, studies have provided some evidence that both *B. asper* and *B. atrox* are polyphyletic and could hide unnamed lineages (see Chapter 3) across Colombian ecoregions, but additional efforts must be made to untangle these taxonomic problems and allow the clarification of their accurate range distributions.

Bothrocophias colombianus.— The enigmatic toadheaded pitvipers of the genus *Bothrocophias* are some of the medically important, yet poorly known, South American viperids. These snakes inhabit isolated and difficult-to-access ecosystems in South America and are poorly represented in biological collections [116]. In particular, the known geographic distribution of *B. colombianus* is restricted to the hyperhumid Chocoan rainforest in the departments of Choco, Cauca, and Risaralda. However, there is a problematic locality in Yarumal, Antioquia department. This could likely be a case of species misidentification because it is too far from the historically known geographic distribution. The specimen that supports this record was not reviewed; it is housed in the reptile collection of the Museo de La Salle (Bogota) under catalog number MLS 1832. The geographic record was retrieved from the «Sistema de Información Biológica Colombiano» (SiB Colombia, Spanish acronym).

Crotalus durissus.— This South American rattlesnake, across its known geographic distribution range, mainly occupies lowlands <1,000 m asl (Table 1), but there are some Colombian localities on the western slope of the Sierra Nevada de Santa Marta (Magdalena) that are remarkable elevational confirmed records at 1,700 m asl, exist in localities such as San Lorenzo. However, there are two records from upland that are outside from its historically known range distribution. One is from the vereda El Centro, Villa de Leyva (Boyaca, IAvH 4849), and the second is from Fusagasuga at 1,729 m asl (Cundinamarca, CRODUR00021 INSZ collection). However, both records likely resulted from human activities causing a translocation from the original range distribution area to the reported locality.

Hydrophis platurus.— The Yellowbellied Sea snake is the only sea snake present in Colombia, and probably in the entire Western Hemisphere. Hernandez-Camacho et al. [117] states that there are Colombian populations of *H. platurus* present in four localities of the Caribbean coast [Cienaga de la Virgen (Bolivar); between Punta San Bernardo and San Bernardo archipelago (Sucre); SE Fuerte island (Cordoba); and near to mouth of the Canal del Dique in the Bay of Cartagena (Bolivar)]. However, these authors did not provide collection numbers for the specimens, collector numbers or vouchers that support their findings, neither did they provide pictures or any documentary evidence of the new records. Indeed, they did not provide any information about the origin of the records or how they got them. In contrast, these authors provide a comprehensive speculative analysis of three possible explanatory hypotheses about the expansion of distribution expansion of *H. platurus* into the Caribbean Sea, but this analysis is not supported by evidence that directly links the distributional expansion of *H. platurus*.

Currently, the controversy about the distributional expansion of *H. platurus* into the Caribbean Sea continues because informal communications from fishermen and marine biologists indicate that *H. platurus* could be present in some localities of the Colombian Caribbean Cost, but no evidence has been provided yet. Thus, a comprehensive revision of the specimens housed in biological collections and fieldwork sampling must be conducted to determine whether the distribution for this species has reached the Caribbean Sea.

The most likely route of translocation is through ships' ballast water. Ballast water is one of the major pathways for the introduction of non-indigenous marine species [117,118]. Due to the continuous shipping between the Caribbean Sea and the Pacific Ocean across the Panama Canal, it is possible that *H. platurus* might have expanded its distributional range into some localities of the Colombian Caribbean Coast using ballast water, and this is a possible explanation for the snakebite cases informally reported in this region. Nevertheless, further studies must be developed to obtain reliable information about the epidemiological pattern of this snakebite event, as well as to assess the ships' ballast water hypothesis (see Chapter 9).

Micrurus dumerilii.— This coralsnake is distributed in the South American mainland and restricted trans-Andean ecoregions of Colombia. However, there are two records outside its known historical range distribution. One is from the San Andres Island (Archipelago of San Andres, Providencia, and Santa Catalina) located about 230 kilometers east of mainland Central America and 750 kilometers north of mainland Colombia. The second is from El Encanto (Amazonas). However, both records are likely to result from dubious species determinations, due to confusion with similar congeners (e.g., *Micrurus tikuna* for the Amazonian record).

Micrurus isozonus.— This is a widely distributed in the cis-Andean lowlands of northern South America in the Guiana Shield and Orinoco re-

gions and the northernmost portion of the Amazon Basins [119] Recently, a controversial record of *M. isozonus* was documented from the Caribbean region of Colombia. Tavares-Feitosa et al. [119] reported one specimen housed in the Natural History Museum of Paris, which was collected in Cartagena, Bolivar, Caribbean lowlands. This unexpected record is located approximately 700 km in a straight line from the nearest record, in northwestern Venezuela. Nevertheless, there are no more records of this species along in the Colombian Caribbean, which is one of the best inventoried regions in the country. When reviewing the museum online catalog, the available information states that the specimen is of unknown provenance. Considering that there is no evidence that this specimen comes from Cartagena, this record will not be included in this book.

Micrurus multifasciatus.— This bicolored coralsnake occurs in lower Central America from Nicaragua to Panama, inhabiting lowland moist and wet forests (rainforests), subtropical wet forests, and lower montane wet forests [26]. Castro-Herrera and Vargas-Salinas [120] reported this coralsnake from western Colombia in Valle de Darien, Rio Azul, 7 km from the camp "Campo Alegre" (UVC 6676), extending its geographic distributional range by approximately 419 km (airline) from its known southernmost locality in Panama (Central America). After reviewing this specimen, we noticed that it corresponded to *M. multiscutatus* a snake species endemic to Colombia and restricted to the Pacific region and that had been confused with *M. multifasciatus* (Figure 7), a remarkably similar species. Therefore, although *M. multifasciatus* is expected to be distributed in Colombia in the Chocoan ecoregion, there are currently no confirmed records of it.

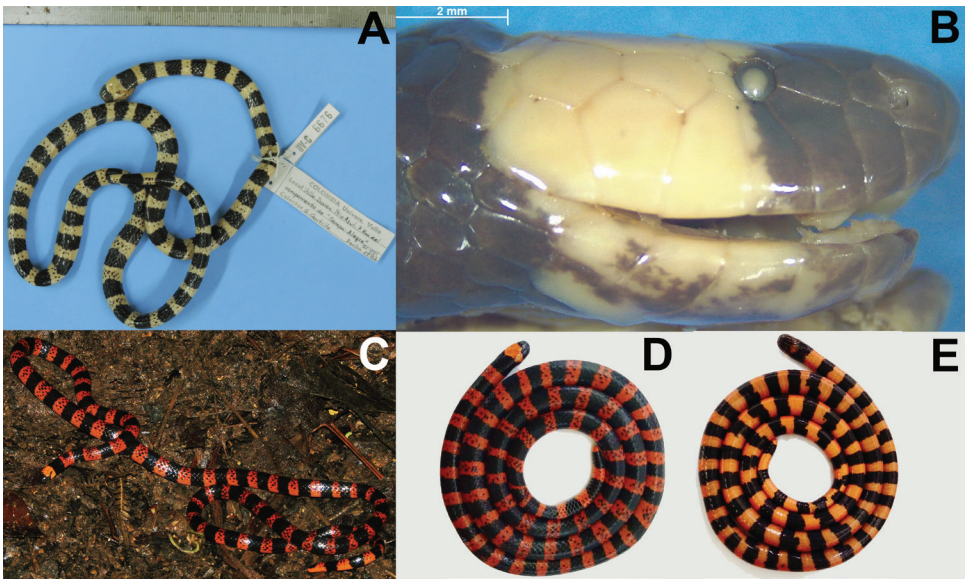


Figure 7. *M. multiscutatus*. (A-B). Specimen the *M. multiscutatus*. from western Colombia in Valle de Darien, Rio Azul, 7 km from the camp "Campo Alegre" (UVC 6676), pictures by Carlos Andres Linares. (C) *M. multiscutatus* in life, and (D-E) fresh euthanized from type locality: El Tambo, Cauca, Colombia. Pictures by Luis Vera-Páez.

Micrurus nigrocinctus.— This widely distributed species occurs from southwestern Mexico to northwestern Colombia, for which a deeply cryptic diversity has already been demonstrated [121]. A controversial record of *M. nigrocinctus* from Old Providence, Colombian Caribbean islands, was

based on one specimen collected by CH Towsend in 1884, but it was lost in 1921 in a shipment from the United States National Museum (Washington, USA) to the Museum of Comparative Zoology (Cambridge, USA) [122–124]. The snake has not been recorded in Old Providence since the late 19th century, so there is some doubt as to whether the locality of the lost specimen was correct. At worst, if the snake did exist in Providence, it is now extinct.

Micrurus putumayensis.— Currently, there are some snake species lists that include *Micrurus putumayensis* among Colombian coralsnakes (i.e., Reptile database [29]), but it appears that this was due to a "broken phone" effect in the interpretation of information over time. Campbell and Lamar [26] report for the first time *M. putumayensis* in Colombia based on the list of Colombian snakes reported by Medem [21]. However, Medem [21] indicates there are not records of *M. putumayensis* in Colombia, but he suggests that likely some specimens and localities of snakes alike *M. steindachneri* (= *Elaps fassli* Werner 1927) could be confused with *M. putumayensis* by Werner [125]. The type locality of *M. putumayensis* is Puerto Socorro (Peru?) 270 Km. NE from Iquitos. However, after looking for it at digital geographic gazetteers (Geonames, Google earth) and Colombian and Peru official maps, this locality is unknown. Thus, the distribution of *M. putumayensis* species does not include Colombia, being restricted to Brazil and Peru.

Porthidium lansbergii-nasutum complex.— In Colombia, *P. nasutum* and *P. lansbergii* are closely related species, characterized by cryptic morphological traits such as a prognathous face or 'hog-nosed snout' character. *Porthidium nasutum* is distributed across the biogeographic Chocoan ecoregion to the Choco-Darien ecoregion, while *P. lansbergii* as well is distributed in the Isthmian-Pacific dry forests in Panama and the Colombian Caribbean, as well as in the Choco-Darien region, where it transitions into the Uraba humid forests and the evergreen forests and dry forest in the Magdalena Valley (see Chapter 3). *Porthidium nasutum* and *P. lansbergii* are sympatric in the Choco-Darien region; thus, in localities such as Apartado, Carepa, Urrao, Segovia (Antioquia) misidentification due to confusion of these species with each other is common.

Both *P. nasutum* and *P. lansbergii* have records that exceed their known elevational maximum range (Table 1). Problematic records of *P. nasutum* over 1,500 m asl can be found in municipalities such as Urrao (Antioquia), and for *P. lansbergii* in localities like Amalfi, Guatape (Antioquia), Cimitarra, Betulia (Santander), and Ocaña (Norte de Santander) with an elevational range between 1,674-1,823 m asl. All these records require a detailed revision to confirm if they represent real distributional records of *Porthidium* species or are misidentifications.

Historically, the presence of *P. lansbergii* in the Orinoquia ecoregion has been based on anecdotal and informal reports, but no evidence has been provided; thus, they have been considered as speculative and spurious records. In this book (see Chapter 3), we confirmed the first record

of *P. lansbergii* from Orinoquia ecoregion. A female (INSV-SR-00288) from Yopal (Casanare) was found by the Fire department during rescue activities in the foothills of the per urban area of Yopal city on July 24, 2021, and was delivered to the Alive Venomous Animal Collection of the National Institute of Health (INSV). Molecular phylogeny analysis highlighted that this specimen was nested within the Caribbean clade, showing low a genetic distance compared to specimens from the Colombian Caribbean coast (see Chapter 3).

3. Endemicity and poorly known venomous snake species in Colombia

Most of Colombia's venomous snakes are by nature cryptic, rarely seen in the wild and apparently having low-density populations. Thus, their endemism or narrow range distributions could be an artifact of our fragmentary understanding of them or might be their true nature. Nevertheless, despite the huge efforts of several researchers conducting fieldwork in their known cryptic habitats and searching through the cabinets of biological collections for uncatalogued or lost specimens, several Colombian venomous snake species remain poorly documented and are represented by few specimens. This circumstance strongly limits our understanding of the essential aspects of their biology or their interaction with human communities. Also, their crypticity has rendered them enigmatic beings, to the point of becoming legendary among herpetologists, who boast of having seen one of these snakes in their lives.



From the total of 49 venomous snake species in Colombia, 16 venomous snake species have ten or fewer known records or localities in Colombia, representing between 0.2-0.02% of the total records available for Colombian venomous snakes (~5,488 records, see https://ofidismo.ins.gov.co), making them the most enigmatic venomous snakes of the country (Table 2). We highlight some of the species here.

For example, *Micrurus oligonellatus* and *M. renjifoi* are known for their type localities and are represented by their type series; indeed, no natural history data is known. *Micrurus spurrelli* is known from five records including the type specimen, no live pictures of this species are available, and no natural historical essential data is known. Since its erection by Boulenger in 1914 its known distribution is restricted to four localities in the Chocoan rainforest of the Colombia [25]. *Micrurus psyches* has a controversial distribution. Campbell and Lamar [26] reported that this species is absent of Colombia, suggesting that records of *M. psyches* from the Orinoquia region are questionable and that these specimens could potentially represent an undescribed bicolored coralsnake species mistaken for *M. psyches*. However, some specimens housed in the INS reptile collection match with the diagnostic characters described by Roze [126]. Nevertheless, due to the small sample size, the evidence they provide is insufficient to test any hypothesis. Thus, for this work, we have decided to treat them as *M. psyches*, hoping that future evidence will help clarify the status of Colombian populations.

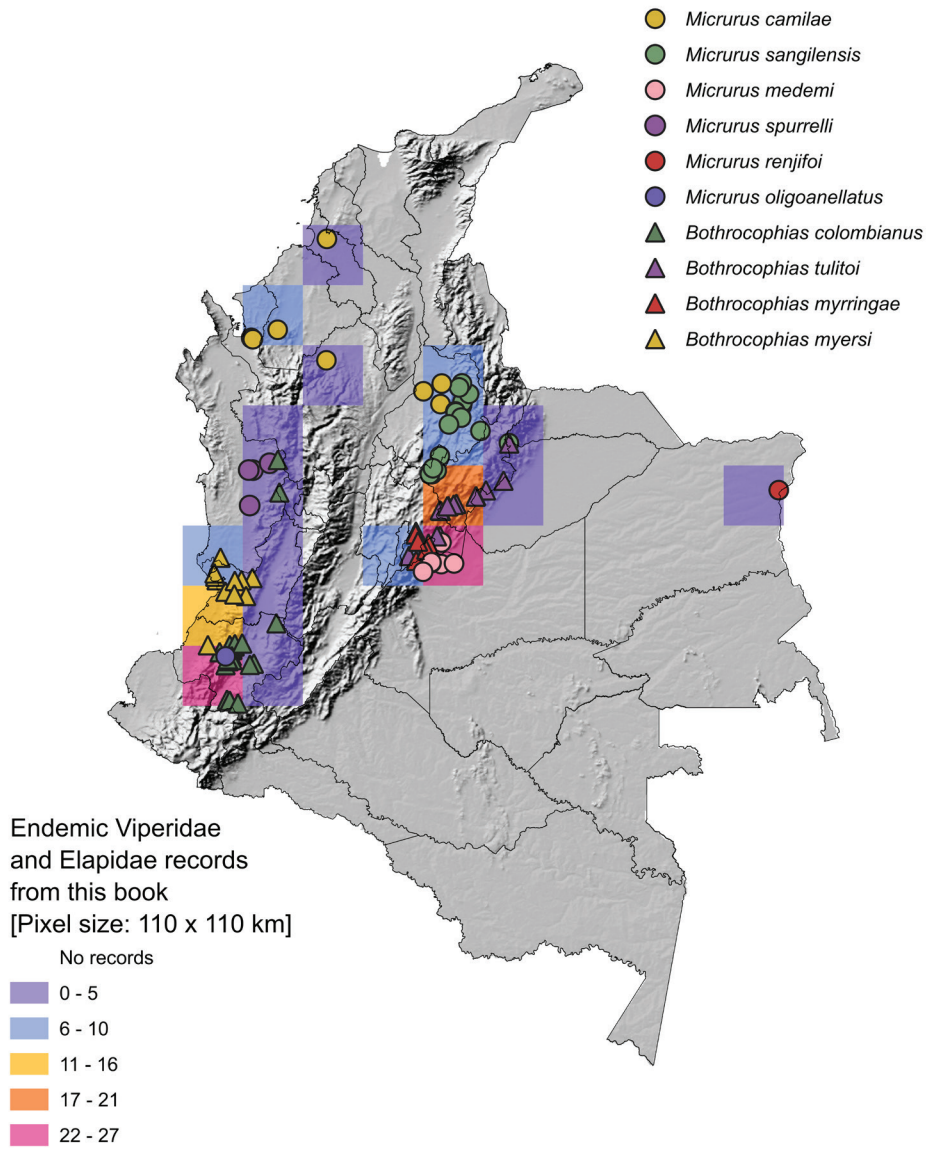


Figure 8. Distribution of endemic venomous snake species in Colombia

Table 2. Poorly known venomous snake species, with unique or restricted records geographically.

Criteria	Species	Geographic distribution
Species with singe record or locality	*Micrurus oligonellatus*	Cauca: El Tambo, (Type locality)
	Micrurus renjifoi	Vichada: Puerto Carreño, Tomo River near its juncture with the Rio Orinoco (Type locality)
Species with between two to five records or localities	*Micrurus nattereri*	Guainia: stream Raya. Vaupes: Yavarate Monfort; Pamopeta, Canoti.
	Bothrops pulcher	Caqueta: Florencia; San Vicente del Caguan, PNN Cordillera de los Picachos
	Micrurus psyches	Arauca: Arauquita. Meta: Villavicencio, Parte superior del Caño El Buque
	Micrurus remotus	Guainia: Negro River. Meta: La Macarena, Piñalito, Cabaña Paujiles. Vaupes: Mitu.
	Micrurus multiscutatus	Cauca: El Tambo (Type locality). Valle del Cauca: Valle de Darien, Rio Azul, 7 km from the camp “Campo Alegre”
	Micrurus spurrelli	Choco: Condoto, Peña Lisa (Type locality); El Carmen de Atrato; Quibdo, Pacurita
	Micrurus tikuna	Amazonas: Leticia, road Leticia-Tarapaca km 7
Species with between six to ten records or localities	*Bothrocophias campbelli*	Choco: Itsmina, Andagoya. Nariño: Barbacoas, Ñambi, Reserva Natural Rio Ñambi, Vereda el Barro, corregimiento de Altaquer. Valle del Cauca: Buenaventura, Bajo Calima
	Micrurus clarki	Cauca: Guapi. Choco: Condoto; Itsmina, Andagoya, upper San Juan River; Riosucio, PNN Los Katios. Valle del Cauca: Dagua, La Elsa, road Cali-Buenaventura.
	Bothrocophias myrringae	Cundinamarca: Choachi, Palo Alto; Fomeque, Vereda de Coasavista; El Calvario; La Calera, Vereda Mundo Nuevo (Type locality); Guayabetal.
	Micrurus nigrocinctus	Antioquia: Carepa; Necocli; Turbo, Currulao River;
	Hydrophis platurus	Cauca: Guapi, PNN Isla de Gorgona. Choco: Bajo Baudo, Cabo corrientes; Jurado, Cabo Marzo; Nuqui, Morros de Jurubida. Nariño: Tumaco
	Micrurus scutiventris	Amazonas: Leticia, Leticia; La Chorrera
	Bothrops taeniatus	Amazonas: Leticia, PNN Amacayacu, Rio Amacayacu; Puerto Rastrojo Miriti-Parana River. Cundinamarca: Medina. Guainia: Puerto Colombia, Macanal, Garagon. Vaupes: Pacoa; Taraira, Estacion biologica Caparu. Vichada: Cumaribo

Venomous snake species with narrow range distribution restricted to Colombia, also known as endemic species, represent 4.9% (10 species) of the total medically important snake species in the country (Figure 8). Except for *Micrurus medemi*, *M. sangilensis*, *Bothrocophias myrringae* and *B. tulitoi,* all of them have in common that they are inhabiting in isolated difficult-to-access habitats, or areas risky for researcher's due to the Colombian armed conflict. Thus, any data or information, robust or anecdotical, gathered for these species represents an important achievement.

Currently, social media platforms such as Facebook, YouTube, Instagram, Twitter, iNaturalist, blogs, and wikis have contributed to obtaining information and knowledge about these enigmatic venomous snakes in a cost-effective and quick manner, rather than relying on traditional, expensive, and challenging expeditions to unvisited, cryptic, or difficult-to-access habitats [3]. Citizen-science initiatives based on local community observers can significantly contribute to an understanding of the poorly known venomous snake species (i.e., *Micrurus ancoralis* geographic range expansion) [3,127,128]. However, biases associated with rainfall, population, and internet penetration must be considered when this type of data is used [3].

In addition, although the quality of the snake records retrieved from social media, the information might be sufficient to determine geographic locations, although the taxonomical identification of species could have several shortcomings. Identification becomes challenging when key characters are not visible. For instance, social media posts often only show conspicuous external traits rather than detailed or internal diagnostic characters such as scutellation or hemipenial morphology [3]. Therefore, we recommend being careful and conservative when using social media records based on human observation as a primary proxy for establishing geographical distributions or assessing ecological niches.

4. Conservation status of the medically important snake species in Colombia

As seen above, previous studies on snakes have focused on public health, taxonomic issues, species lists and descriptions. Since 2007, Colombia has seen the first initiative that focused on snakes as a relevant and important conservation objective. This conservation initiative was led by the Ministry of Environment and Sustainable Development of Colombia (MADS or Minambiente, current Spanish acronym), the National Health Institute of Colombia (INS or Instituto Nacional de Salud, Spanish acronym), the Regional Autonomous Corporation of the Center of Antioquia (CORANTIOQUIA, Spanish acronym), and the Natural Sciences Institute of the Universidad Nacional de Colombia (ICN, Spanish acronym). This initiative generated a workshop named the "*First national Research symposium on snake biology and conservation*," which convened most of the academics, stakeholders, governmental entities concerned with wildlife and environmental heritage, and neophytes.

In 2008, a first draft (not peer reviewed) of the Colombian snake conservation program was organized as a report that summarize the main findings and conclusions of the this workshop [129]. This report was the initial input for ongoing research, initiatives, conservation plans, and social-media initiatives towards snake conservation in Colombia. In 2012, Lynch [6] provided the first general assessment of the potential threats that snake populations face in Colombia, addressing habitat loss, deliberate killing by humans, roadkill, illegal wildlife traffic, and scientific research as possible main threats.

Based on this work, as well as the previous Colombian snake conservation report, and thanks to a research project supported by a cooperation agreement between Minambiente, INS and ICN, Lynch et al. [1] in 2014 presented and updated a peer-reviewed a national program for the conservation of snakes present in Colombia (hereafter PNCS, Spanish acronym). In 2016 the PNCS was adopted by Minambiente as the national public policy guideline for the conservation of snakes in Colombia. This public policy guideline stated that currently in Colombia the three main threats for snake conservation are: habitat loss, deliberate killing by humans, and roadkill.

In 2015, the first snake species conservation assessments were led by the IUCN (International Union for Conservation of Nature) reptile specialist group and the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH, Spanish acronym) [130]. This assessment based on experts' knowledge and judgment (~20 national reptile experts), and IUCN criteria used to evaluate if a taxon belongs to the Red List of Threatened Species categories [131], resulted in the categorization of ten Colombian snakes species, three of them venomous species allocated in two threatened categories: *Micrurus medemi* (Endangered), *M. sangilensis* (Vulnerable), and *Bothrocophias campbelli* (Vulnerable). This assessment has been the main input to public policy resolutions that indicate and rule the state of snake species conservation state of biodiversity in Colombia [132].

For Colombian snakes, besides the experts' knowledge and judgment, the main criterion employed to determine if a species is allocated or not into an IUCN threatened category was the geographic range [31], mainly due to the dearth of information about criteria such as population size reduction, small population size and decline, and quantitative analysis about the probability of extinction in the wild. The geographic range criterion considers two main metrics, the extent of occurrence (EOO) and area of occupancy (AOO), which should be linked together with at least two of the three following conditions: (1) severely fragmented or number of locations; (2) continuing decline of area EOO or AOO (observed, estimated, inferred or projected), and extreme fluctuations in EEO, AOO, number of locations or subpopulations, and number of mature individuals [131].

Therefore, *Micrurus medemi* was categorized as Endangered [133] by having severely fragmented locations, and an EOO <5,000 km² that it is in continuing decline as well as the AOO; while *Micrurus sangilensis* and

Bothrocophias campbelli were categorized as Vulnerable by having severely fragmented locations, and EOO <20,000 km² that it is in continuing decline losing its habitat quality.

Following this criterion, the Colombian threatened venomous species list must be extended to include at least five of the 16 poorly known venomous species listed in Table 2, as well as three endemic species depicted in Figure 8. For example, species such as *Micrurus oligoanellatus*, *M. renjifoi,* and *M. spurrelli* match with Critically Endangered metrics by having EOO <100 km² that a species is in continuing decline losing its habitat quality; *Bothrocophias colombianus, B. myrringae,* and *Micrurus camilae* match the Endangered criteria by having EOO <5,000 km² that the species is continuing loss of its habitat quality. Additionally, species such as *B. tulitoi* match Vulnerable by having severely fragmented locations, and EOO <20,000 km² that it is in continuing decline losing its habitat quality. Therefore, they should also be included.

An updated assessment of the Colombian threatened venomous species is urgently needed because some of the broadly distributed Colombian venomous snake species are facing significant threats. This is the case with *Crotalus durissus*, which beyond facing main threats such as habitat loss and deliberate snake killing by humans, is subject to strong illegal trafficking within Colombia. This species is utilized across the country in magical/religious procedures, as well as ingredients of traditional medicine to treat cancer, erectile dysfunction, and as a sexual enhancer.

Therefore, *C. durissus* populations are constantly under significant pressure due to uncontrolled trafficking, but the species lacks a comprehensive conservation assessment that helps to address actions and strategies to reverse, mitigate, or stop this threat. Currently, *C. durissus* is listed in the appendix III of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) providing it legal protection for trading or traffic when specimens or its parts are exported from Colombia. However, inside Colombia, there are no specific laws or normativity that protect their populations beyond the environmental normative that states the requirements for the licenses for commercial use of wildlife (i.e., Law 84 of 1989; Law 611 of 2000; decree 4688 of 2005; decree 1076 of 2015; decree 1272 of 2016; resolution 1263 of 2006; resolution 1909 of 2017).

Ten years after the publication of the PNCS, the country continues far from the goal of achieving the mission, objectives, and scope proposed in this conservation public policy. This shows that the efforts invested were not enough and we should reinforce them. Despite snakes having the largest number of herpetological studies of non-avian reptile diversity in Colombia [134], there are few studies addressing their main threats [135–141]. However, the major advance in the PNCS implementation was achieved in strategy I: *Increase in the level of knowledge about the snakes present in Colombia*; and strategy IV: *Implement and develop biomedicine and bioprospecting activities with Colombian snake venoms*. Currently, the country has gained a lot of undertesting about Colombian snakes, both in

general and applied biology, which have provided enough merit and motivation to write and edit a book like the one you are reading right now.

Nevertheless, the strategies of the PNCS focused on environmental and conservation education (strategy II), as well as recovery and restoration of the natural snake habitats (strategy III) have been poorly or non-implemented. Similarly, lines of action advocating for legal, administrative and financial strengthening for biological research on snake conservation were never implemented. Thus, the priorities proposed ten years ago remain in force and without being properly implemented, so habitat loss, deliberate snake killing by humans, roadkill, and illegal trafficking continue being the main threats for snake populations in Colombia.

Despite the lack of implementation of the PNCS, the publication of this conservation public policy over the years has helped to increase the number of groups of academics, researchers, zookeepers, and the general public that engage in snake research and conservation. It has also motivated the development of robust initiatives aimed to change the negative perceptions of Colombians towards snakes. For example, a growing Facebook community focused on snake research and conservation advocacy has spontaneously led to a robust network (> 1.5 million members) that contributes important information to the geographic distributions of snakes, valuable natural history observations for several snake species, knowledge about the conditions of snake–human encounters in rural and peri-urban areas, and promotes the education and understanding about the biology of venomous and non-venomous snakes in Colombia [51].

To tackle the main threats for snake populations in Colombia, we recommend promoting the implementation of the lines of action of the PNCS, as well as initiatives that focus on ethnozoological, social, and psychological studies about human attitudes toward snakes, as well as educational actions focused on snake-human encounters in wild or rural areas. Understanding the drivers that provoke negative experiences during these encounters will help develop strategies that promote snake conservation and reduce snakebite incidence.

4.1 Two sides of the same coin: Conservation and ophidism

Conservation biology and ophidism (as a tropical neglected disease) have strong convergences in how these disciplines address their own paradigms. For example, both disciplines share at least eight factors or goals that are assessed and valued: threats, vulnerability, risk, behavior, attitude, prevention, mitigation, and location (Figure 9). Besides, both disciplines address their guiding questions for research and plan actions in a similar manner. Therefore, we propose that actions based on an interdisciplinary approach that integrate knowledge, values, and techniques used by both disciplines could reduce the main snake conservation problems, as well as the incidence of snakebite. The conjunction of these disciplines will help build explicit strategies that are communicable, assessable, and have strong applicability.



Figure 9. The eight common factors or goals that are assessed and valued by conservation and snakebite research.

This conjunction between conservation biology and the study of snakebite can be nested into two comprehensive views of the health sciences, known as the OneHealth and Ecohealth approaches [142,143]. These proposals raised a conceptual framework that could catalyze conservation biology and snakebite as an interdisciplinary approach to face snake conservation and snakebite accidents. Onehealth focused primarily on the interface of humans and domestic animals in specific social and ecological contexts. But it can be extended to wild animals that commonly share rural and peri-urban locations, which snakes share. An Ecohealth approach [143,144] considers the close linkages between ecosystems, society, and health, incorporating multiple types of knowledge from natural and social sciences, and the humanities, with the involvement of stakeholders at many levels within a participatory research frame [144]. EcoHealth has a broader scope compared to OneHealth that focuses specifically on the human-animal health interface. EcoHealth aims to achieve a comprehensive understanding of health and well-being that encompasses the humanities as well as the natural, social, and health sciences [145].

Due to the multifactorial causes of both the decline in snake populations and snakebite incidence, holistic perspectives as shown above could allow better understanding and might aid in building robust lines of action. Currently in Colombia, there are no initiative for snake conservation or snakebite prevention that have used Onehealth or Ecohealth approaches, but interest in these subjects is growing. Despite the broad scope of PNCS, which includes an interdisciplinary approach, its low implementation of their action lines has limited achievements and perspectives for addressing snake conservation and snakebite incidents. Therefore, it is expected that in the near future the integration of conservation biology and snakebite could occur under these conceptual frameworks.

5. Future perspectives

Colombia holds a special spot in the world due to its geopolitical location, historical ecological significance as a tropical country, and evolutionary role as a pathway for interchanges of South and North American biological elements, which facilitated the great immigration and emigration of snake lineages. Additionally, the convergence of three of the most diverse terrestrial ecoregions on the planet—the Andes, Choco, and Amazon—has promoted the evolution of astonishing species richness, with several diversification patterns and lineages observed in a relatively limited area (see Chapter 2 and 3). As a result, Colombia is one of the 25 most important biodiversity hotspots worldwide [146], and is classified as a megadiverse country.

This fact presents significant challenges for snake research and the reduction of snakebite accidents. In particular, Colombia faces strong constraints related to poverty, food insecurity, limited access to medical assistance in rural areas, armed conflict, poor implementation of environmental policies, and low state and private investment in science and technology research that addresses the unmet needs of the general population.

Therefore, to achieve high sampling completeness of Colombian snake assemblages and gain the understanding needed to address snakebites as a neglected tropical disease, an interdisciplinary approach is required to tackle the eight factors described above that underlie the relationship between humans and snakes (Figure 9).

This interdisciplinary approach should be addressed by transforming our relationship with nature, as well as our production and consumption practices based on collective actions with the participation of all members Colombia society. The guidelines to undertake this task were defined ten years ago in the mission, goals, and scope proposed in this PNCS conservation public policy [1]. Particularly, to reinforce the environmental and conservation education (strategy II), as well as a recovery and restoration of the snakes' natural habitats (strategy III) a reduction of the threats for snake populations in Colombia is urgently needed, as well as to mitigate and properly attend snakebites accidents in Colombia (see Chapter 9).

Particularly, the research presented in this chapter highlights the urgent need to establish a research grant program to support field surveys and studies across various disciplines, aiming to enhance our knowledge of medically important snake species and to train researchers to address the challenges associated with venomous and non-venomous snake species. Taxonomic studies and species descriptions in a megadiverse country like Colombia play a substantial role in the conservation of our natural heritage and contribute to the growing emphasis on caring for the ecosystems that sustain us. Encouraging these activities will also enable us to explore and expand our understanding of how snakes can contribute to the development of new medicines and markets based on venoms, as well as the ecosystem services provided by snakes (see Chapter 6 and 10).

Appendix: Material and Methods

Revision of the available lines of evidence
To achieve the objective of providing taxonomic treatment and criteria that help assign problematic snake species to a taxonomic entity based on the available evidence, we employed three approaches. First, we followed the PRISMA methodology [147] to retrieve from Google academic and Web of science papers about taxonomic or systematic revisions, as well as species descriptions of the venomous snakes distributed in Colombia according to the Reptile Database Web site [29]. Second, we consulted, as far as possible, the taxa-related references included in the Reptile Database Web site [29]. Third, in the cases in which we were aware that some researcher was currently working on taxonomic or systematic revisions of snakes of medical importance in Colombia, we enquired about his/her expert-opinion in order to incorporate it into the taxonomic treatment proposed [148].

Search methods for identification of studies: Electronic searches
We conducted monthly searches from April 2021 to June 2024 in the following electronic repositories and Web engines: MEDLINE, Scielo, Scopus, Google Scholar and Pubmed. The search strategies contained the following keywords and Mesh were combined in different ways using the boolean connectors AND OR: snake venoms, *Bothrops*, *Crotalus*, *Bothriechis*, *Porthidium*, *Lachesis*, *Micrurus*, *Xenodon*, *Leptophis*, *Erythrolamprus*, *Oxybelis*, *Helicops*, *Bothrocophias*, *Thamnodynastes*, *Leptodeira*, *Philodryas*, taxonomy, distribution, Neotropics, morphology, species delimitation, integrative taxonomy.

Searching for other resources
We checked relevant cited studies while reviewing the reports identified by the electronic searches, as well as reference lists from any directly relevant reviews identified. We did not apply language or date restrictions and included studies regardless of the type of publication (e.g., conference abstract, trial registry entry, journal article, book).

Contrasting lines of evidence with previous taxonomic proposal
We identified 2,234 publications with potential for inclusion. Of these, we identified a total of 320 articles that underwent title and abstract screening; of these, 36 studies were duplicates and 40 did not meet the search criteria, thus all were excluded. A total of 244 articles were included.

We took as baseline the taxonomic treatment proposed by Campbell and Lamar [26] for snakes of medical importance in Colombia. Then, we contrasted it with new evidence (after 2004) retrieved from searches done aforesaid available from taxonomic or systematic revisions, and finally, we made a taxonomic decision.

References

1. Lynch, J.D.; Angarita-Sierra, T.; Ruiz, F.J. *Programa Nacional Para La Conservación de Las Serpientes Presentes En Colombia*; Universidad Nacional de Colombia, Instituto Nacional de Salud, Ministerio de Medio ambiente y Desarrollo: Bogotá, **2014**; ISBN 9789588901183.
2. Bravo-Vega, C.; Renjifo-Ibañez, C.; Santos-Vega, M.; Nuñez, L.J.L.; Angarita-Sierra, T.; Cordovez, J.M. Estimating Real Snakebite Incidence in Colombia by Using Mathematical Modelling and Statistical Inference. *bioRxiv* **2021**, 2021.12.09.472006, doi:10.1101/2021.12.09.472006.
3. Angarita-Sierra, T.; Montaño-Londoño, L.F.; Bravo-Vega, C.A. ID Please: Evaluating the Utility of Facebook as a Source of Data for Snake Research and Conservation. *An. Acad. Bras. Cienc.* **2022**, *94*, doi:10.1590/0001-3765202220211043.
4. Ospina-Sarria, J.J.; Angarita-Sierra, T. A New Species of *Pristimantis* (Anura: Strabomantidae) from the Eastern Slope of the Cordillera Oriental, Arauca, Colombia. *Herpetologica* **2020**, *76*, 83–92.
5. Angarita-Sierra, T.; Cubides-Cubillos, S.D.; Hurtado-Gómez, J.P. Hidden in the Highs: Two New Species of the Enigmatic Toadheaded Pitvipers of the Genus Bothrocophias. *Vertebr. Zool.* **2022**, *72*, doi:10.3897/vz.72.e87313.
6. Lynch, J.D. El Contexto de Las Serpientes de Colombia Con Un Análisis de Las Amenazas En Contra de Su Conservación. *Rev. la Acad. Colomb. Ciencias Exactas, Físicas y Nat.* **2012**, *36*, 435–449.
7. Pinto-Sánchez, N.R.; Calderón-Espinosa, M.L.; Miralles, A.; Crawford, A.J.; Ramírez-Pinilla, M.P. Molecular Phylogenetics and Biogeography of the Neotropical Skink Genus *Mabuya* Fitzinger (Squamata: Scincidae) with Emphasis on Colombian Populations. *Mol. Phylogenet. Evol.* **2015**, *93*, 188–211, doi:10.1016/j.ympev.2015.07.016.
8. Lozano, J.T. Sobre Las Serpientes. *Sem. del Nuevo Reino Granada* **1808**, 117–182.
9. Universidad Jorge Tadeo Lozano *Jorge Tadeo Lozano: Estudios Científicos*; **2021**.
10. Cañas-Dávila, C.A. Capítulo 12. Aspaectos Históricos de La Ofidiología Colombiana. In *Serpientes venenosas: lecciones aprendidas desde Colombia*; Cañas-Dávila, C.A., Castro-Herrera, F., Castaño-Valencia, R., Eds.; Fundación Valle de Lili: Santiago de Cali, **2016**; pp. 213–240 ISBN 9789585721722.
11. Calvete, J.J. Snake Venomics at the Crossroads between Ecological and Clinical Toxinology. *Biochem. (Lond).* **2019**, *41*, 28–33, doi:10.1042/bio04106028.
12. Bektimirov, T.; Furesz, J.; Hardegree, M.C.; Hollan, S.R.; Mirchamsy, H.; Netter, R.; Oya, A.; Reichert, L.E.; Sjodin, L.; Pang-chu, T. *WHO Expert Committee on Biological Standardization*; **1987**; ISBN 9789241210133.
13. Bhaumik, S.; Jagadesh, S.; Lassi, Z. Quality of WHO Guidelines on Snakebite: The Neglect Continues. *BMJ Glob. Heal.* **2018**, *3*, doi:10.1136/bmjgh-2018-000783.
14. McDiarmid, R.; Campbell, J.A.; Touré, T. *Snake Species of the World: A Taxonomic and Geographic Reference, Volume 1*; Herpetologists' League: Washington, **1999**.

15. Peters, J.; Orejas-Miranda, B. Catalogue of the Neotropical Squamata Snakes. *United States Natl. Museum Bull.* **1970**, *297*, 1–347.
16. Arteaga, A.; Pyron, R.A.; Batista, A.; Vieira, J.; Pelayo, E.M.; Smith, E.N.; Barrio, C.L.; Koch, C.; Agne, S.; Valencia, J.H.; et al. Systematic Revision of the Eyelash Palm-Pitviper *Bothriechis Schlegelii* (Serpentes, Viperidae), with the Description of Five New Species and Revalidation of Three. *Evol. Syst.* **2024**, *8*, 15–64, doi:10.3897/evolsyst.8.114527.
17. Garcia Piedrahita, E. *Ofidios Venenosos Del Cauca: Métodos Emperico y Racionales Empleados Contra Los Accidentes Producidos Por La Mordedura de Esos Reptiles*; Librería e Imprenta de la Vda. de Ch. Bouret.: Cali, **1896**.
18. Amaral, A. Studies of Neotropical Ophidia. VII An Interesting Collection of Snakes from West Colombia. *Mem. Inst. Butantan* **1927**, *I*, 44–47.
19. Amaral, A. Studies of Neotropical Ophidia. XI Snakes from The Santa Marta Region, Colombia. *Mem. Inst. Butantan* **1928**, *2*, 7–8.
20. Nicéforo-María Los Ofidios de Colombia. *Rev. la Acad. Colomb. Ciencias Exactas Físicas y Nat.* **1942**, 89–101.
21. Medem, F. El Desarrollo de La Herpetología En Colombia. *Rev. la Acad. Colomb. Ciencias Exactas Fis. y Nat.* **1968**, *13*, 149–200.
22. Angel, R. *Serpientes de Colombia*; **1982**; Vol. 36; ISBN 64001000072288.
23. Cadle, J. On Colombian Snakes. *Herpetologica* **1992**, *48*, 134–143.
24. Sánchez, C.H.; Castaño-Moreno, O.; Cárdenas, G. Diversidad de Reptiles de Colombia. In *Diversidad Biótica I, Clima, Centros de concentración de especies, Fauna, Reptiles Arácnidos, Himenópteros*; Rangel-Ch, O.J., Ed.; Instituto de Ciencias Natirales, Universidad Nacional de Colombia: Bogotá D.C, **1995**; pp. 227–325.
25. Campbell, J.A.; Lamar, W.W. *The Venomous Reptiles of Latin America*; Campbell, J.A., Lamar, W.W., Eds.; Comstock Pub. Associates, **1989**; ISBN 9780801420597.
26. Campbell, J.A.; Lamar, W.W. *The Venomous Reptiles of the Western Hemisphere (Vol. 1)*; Comstock Publishing: New York, **2004**; ISBN 978-0801441417.
27. Cañas-Dávila, C.A.; Castro-Herrera, F.; Castaño-Valencia, R. *Serpientes Venenosas: Lecciones Aprendidas Desde Colombia*; Fundación Valle de Lili: Santiago de Cali, **2016**; ISBN 9789585721722.
28. Ayerbe González, S.A. *SERPIENTES | Accidentes & Soluciones*; Ayerbe doc serpientes: Popayán, **2021**; ISBN 9789584924872.
29. Uetz, P.; Freed, P.; Hošek, J. The Reptile Database Available online: https://reptile-database.reptarium.cz/ (accessed on 23 January **2024**).
30. da Silva-Jr, N.J.; Buononato, M.A.; Godoy-Pires, M.; Tavares-Feitosa, D. Chapter 4 New World Coralsnakes: An Overview. In *Advances in Coralsnake Biology: with Emphasis on South America*; da Silva-Jr, N.J., Porras, L.W., Aird, S.D., da Costa Prudente, A., Eds.; Eagle Mountain Publishing: Eagle Mountain, **2021**; pp. 115–140 ISBN 9780972015462.
31. WHO Management of Snakebites (WHO 2nd Ed) 2016. **2016**.
32. Pineda, D. *Accidentes Por Animales Venenosos*; Pineda, D., Hernández, C.A., Eds.; Instituto Nacional de Salud: Bogotá, **2002**; ISBN 9581301305.
33. Otero-Patiño, R. Snake Bites in Colombia. In *Clinical Toxinology in Australia, Europe, and Americas*; Gopalakrishnakone, P., V, C.-W.,

AS, S., VT, D., Eds.; Springer Science: Adelaide, **2018**; pp. 3–50 ISBN 9789401774383.
34. Naomi, S.I. Proposal of an Integrated Framework of Biological Taxonomy: A Phylogenetic Taxonomy, with the Method of Using Names with Standard Endings in Clade Nomenclature. *Bionomina* **2014**, *7*, 1–44, doi:10.11646/bionomina.7.1.1.
35. Hurtado-Gómez, J.P.; Vargas-Ramírez, M.; Ruíz Gómez, F.J.; Fouquet, A.; Fritz, U. Multilocus Phylogeny Clarifies Relationships and Diversity within the *Micrurus lemniscatus*. *Salamandra* **2021**, *57*, 229–239.
36. Mora-Obando, D.; Salazar-Valenzuela, D.; Pla, D.; Lomonte, B.; Guerrero-Vargas, J.A.; Ayerbe, S.; Gibbs, H.L.; Calvete, J.J. Venom Variation in *Bothrops asper* Lineages from North-Western South America. *J. Proteomics* **2020**, *229*, 103945, doi:10.1016/j.jprot.2020.103945.
37. Passos, P.; Fernandes, D.S. Variation and Taxonomic Status of the Aquatic Coral Snake *Micrurus surinamensis* (Cuvier, 1817) (Serpentes: Elapidae). *Zootaxa* **2005**, 1–14, doi:10.11646/zootaxa.953.1.1.
38. Feitosa, D.T.; Da Silva, N.J.; Pires, M.G.; Zaher, H.; Prudente, A.L.D.C. A New Species of Monadal Coral Snake of the Genus *Micrurus* (Serpentes, Elapidae) from Western Amazon. *Zootaxa* **2015**, *3974*, 538–554, doi:10.11646/zootaxa.3974.4.5.
39. Köhler *Reptiles of Central America*; Köhler, E., Ed.; Herpeton, Verlag: Offenbach, **2003**; ISBN 3-936180-02-4.
40. Cadle, J.E.; Myers, C.W. Systematics of Snakes Referred to *Dipsas variegata* in Panama and Western South America, with Revalidation of Two Species and Notes on Defensive Behaviors in the Dipsadini (Colubridae). *Am. Museum Novit.* **2003**, *3409*, 1–47, doi:10.1206/0003-0082(2003)409<0001:sosrtd>2.0.co;2.
41. Myers, C.W.; McDowell, S.B. New Taxa and Cryptic Species of Neotropical Snakes (Xenodontinae), with Commentary on Hemipnes as Generic and Specific Characters. *Am. Museum Novit.* **2014**, 1–112.
42. De Albuquerque, N.R.; Passos, P.; Gotte, S.W. *Leptophis santamartensis* (Serpentes, Colubridae), a Junior Synonym of *Leptophis ahaetulla* Occidentalis. *J. Herpetol.* **2012**, *46*, 248–252, doi:10.1670/10-263.
43. Passos, P.; Lynch, J.D. Revision of *Atractus* (Serpentes: Dipsadidae) from Middle and Upper Magdalena Drainage of Colombia. *Herpetol. Monogr.* **2010**, *24*, 149–173, doi:10.1655/09-041.1.
44. Auerbach, P.S. The Venomous Reptiles of the Western Hemisphere. *Wilderness Environ. Med.* **2005**, *16*, e13–e13, doi:10.1580/1080-6032(2005)16[e13a:br]2.0.co;2.
45. Roze, J. *Coral Snakes of the Americas: Biology, Identification, and Venoms*; Krieger Publishing Company, **1996**; ISBN 0894648470.
46. Bailey, J.R.; Thomas, R.A.; Silva-Jr, N.J. da A Revision of the South American Snake Genus *Thamnodynastes* Wagler, 1830 (Serpentes, Colubridae, Tachymenini). I. Two New Species of *Thamnodynastes* from Central Brazil and Adjacent Areas, with a Redefinition of and Neotype Designation for *Thamnodynastes* P. *Phyllomedusa J. Herpetol.* **2005**, *4*, 83–101.
47. Silva, J.J. Los *Micrurus* de La Amazonia Colombiana . Biología y Toxicología Experimental de Sus Venenos. *Colomb. Amaz.* **2016**, *7*, 1–77.
48. Nogueira, C.C.; Argôlo, A.J.S.; Arzamendia, V.; Azevedo, J.A.; Barbo, F.E.; Bérnils, R.S.; Bolochio, B.E.; Borges-Martins, M.; Brasil-Godinho,

M.; Braz, H.; et al. Atlas of Brazilian Snakes: Verified Point-Locality Maps to Mitigate the Wallacean Shortfall in a Megadiverse Snake Fauna. *South Am. J. Herpetol.* **2020**, *14*, 1, doi:10.2994/sajh-d-19-00120.1.
49. Wüster, W.; Thomson, S.A.; O'Shea, M.; Kaiser, H. Confronting Taxonomic Vandalism in Biology: Conscientious Community Self-Organization Can Preserve Nomenclatural Stability. *Biol. J. Linn. Soc.* **2021**, *133*, 645–670, doi:10.1093/biolinnean/blab009.
50. Kaiser, H.; Crother, B.I.; Kelly, C.M.R.; Luiselli, L.; Marko'shea; Hidetoshiota; Passos, P.; Schleip, W.D.; Wüster, W. Best Practices: In the 21st Century, Taxonomie Decisions in Herpetology Are Acceptable Only When Supported by a Body of Evidence and Published via Peer Review. *Herpetol. Rev.* **2013**, *44*, 8–23.
51. Hoser, R. A Review of the South American Snake Genera *Leptodeira* and *Imantodes* Including Three New Genera and Two New Subgenera (Serpentes: Dipsadidae: Imantodini). *Australas. J. Herpetol.* **2012**, 40–47.
52. Hoser, R. A New Genus of Pitviper (Serpentes: Viperidae) from South America. *Australas. J. Herpetol. Joura* **2012**, 25–27.
53. Habib, A.G.; Kuznik, A.; Hamza, M.; Abdullahi, M.I.; Chedi, B.A.; Chippaux, J.-P.; Warrell, D.A. Snakebite Is Under Appreciated: Appraisal of Burden from West Africa. *PLoS Negl. Trop. Dis.* **2015**, *9*, 1–8, doi:10.1371/journal.pntd.0004088.
54. Chippaux, J.P.; Williams, V.; White, J. Snake Venom Variability: Methods of Study, Results and Interpretation. *Toxicon* **1991**, *29*, 1279–1303, doi:10.1016/0041-0101(91)90116-9.
55. Bravo-Vega, C.A.; Cordovez, J.M.; Renjifo-Ibáñez, C.; Santos-Vega, M.; Sasa, M. Estimating Snakebite Incidence from Mathematical Models: A Test in Costa Rica. *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007914, doi:10.1371/journal.pntd.0007914.
56. Tan, K.Y.; Tan, C.H.; Chanhome, L.; Tan, N.H. Comparative Venom Gland Transcriptomics of *Naja kaouthia* (Monocled Cobra) from Malaysia and Thailand: Elucidating Geographical Venom Variation and Insights into Sequence Novelty. *PeerJ* **2017**, *2017*, doi:10.7717/peerj.3142.
57. Sevilla-Sánchez, M.J.; Mora-Obando, D.; Calderón, J.J.; Guerrero-Vargas, J.A.; Ayerbe-González, S. Accidente Ofídico En El Departamento de Nariño, Colombia: Análisis Retrospectivo, 2008-2017. *Biomedica* **2019**, *39*, 715–736, doi:10.7705/biomedica.4830.
58. De Queiroz, K. Toward an Integrated System of Clade Names. *Syst. Biol.* **2007**, *56*, 956–974, doi:10.1080/10635150701656378.
59. Wolff, J.; Krebs, C.J. Hypothesis Testing and Scientific Method Revised. *Acta Zool. Sin.* **2008**, *54*, 383–386.
60. Mayden, R. A Hierarchy of Species Concepts: The Denouement in the Saga of the Species Problem. In *Species: The Units of Driversity*; Claridge, M., Dawah, H., Wilson, M., Eds.; Chapman and Hall: London, **1997**; pp. 381–423.
61. Naomi, S.I. On the Integrated Frameworks of Species Concepts: Mayden's Hierarchy of Species Concepts and de Queiroz's Unified Concept of Species. *J. Zool. Syst. Evol. Res.* **2011**, *49*, 177–184, doi:10.1111/j.1439-0469.2011.00618.x.
62. Sites Jr, J.; Myers, E.; Ruane, S. Species Delemitation and Integrative Taxonomy: Challenges and Opportunies in Snake Systematics.

In *Advances in Coralsnake Biology: with Emphasis on South America*; da Silva Jr, N., Porras, L., Aird, S., da Costa Prudente, A.L., Eds.; PUC Goia´s, Eagle montain Publishing: Goiás, **2021**; pp. 1–36 ISBN 9780972015462.

63. Bravo-Vega, C.I.; Santos-Vega, M.I.; Manuel Cordovez, J.I. Disentangling Snakebite Dynamics in Colombia: How Does Rainfall and Temperature Drive Snakebite Temporal Patterns? *PLoS Negl. Trop. Dis.* **2022**, *16*, e0010270, doi:10.1371/JOURNAL.PNTD.0010270.
64. Pesantes, O. A Method for Preparing the Hemipenis of Preserved Snakes. *J. Herpetol.* **1994**, *28*, 93–95.
65. Carrasco, P.A.; Mattoni, C.I.; Leynaud, G.C.; Scrocchi, G.J. Morphology, Phylogeny and Taxonomy of South American Bothropoid Pitvipers (Serpentes, Viperidae). *Zool. Scr.* **2012**, *41*, 109–124, doi:10.1111/j.1463-6409.2011.00511.x.
66. Porto, M.; de Oliveira, M.A.; Pissinatti, L.; Rodrigues, R.L.; Rojas-Moscoso, J.A.; Cogo, J.C.; Metze, K.; Antunes, E.; Nahoum, C.; Mónica, F.Z.; et al. The Evolutionary Implications of Hemipenial Morphology of Rattlesnake *Crotalus durissus terrificus* (Laurent, 1768) (Serpentes: Viperidae: Crotalinae). *PLoS One* **2013**, *8*, doi:10.1371/journal.pone.0066903.
67. Fenwick, A.M.; Gutberlet, R.L.; Evans, J.A.; Parkinson, C.L. Morphological and Molecular Evidence for Phylogeny and Classification of South American Pitvipers, Genera *Bothrops*, *Bothriopsis*, and *Bothrocophias* (Serpentes: Viperidae). *Zool. J. Linn. Soc.* **2009**, *156*, 617–640, doi:10.1111/j.1096-3642.2008.00495.x.
68. Barbo, F.E.; Gasparini, J.L.; Almeida, A.P.; Zaher, H.; Grazziotin, F.G.; Gusmão, R.B.; Ferrarini, J.M.G.; Sawaya, R.J. Another New and Threatened Species of Lancehead Genus *Bothrops* (Serpentes, Viperidae) from Ilha Dos Franceses, Southeastern. *Zootaxa* **2016**, *4097*, 511–529, doi:10.11646/zootaxa.4105.5.7.
69. Timms, J.; Chaparro, J.C.; Venegas, P.J.; Salazar-Valenzuela, D.; Scrocchi, G.; Cuevas, J.; Leynaud, G.; Carrasco, P.A. A New Species of Pitviper of the Genus *Bothrops* (Serpentes: Viperidae: Crotalinae) from the Central Andes of South America. *Zootaxa* **2019**, *4656*, 99–120, doi:10.11646/zootaxa.4656.1.4.
70. Carrasco, P.A.; Grazziotin, F.G.; Cruz Farfán, R.S.; Koch, C.; Antonio Ochoa, J.; Scrocchi, G.J.; Leynaud, G.C.; Chaparro, J.C. A New Species of *Bothrops* (Serpentes: Viperidae: Crotalinae) from Pampas Del Heath, Southeastern Peru, with Comments on the Systematics of the Bothrops Neuwiedi Species Group. *Zootaxa* **2019**, *4565*, 301–344, doi:10.11646/zootaxa.4565.3.1.
71. Dal Vechio, F.; Prates, I.; Grazziotin, F.G.; Graboski, R.; Rodrigues, M.T. Molecular and Phenotypic Data Reveal a New Amazonian Species of Pit Vipers (Serpentes: Viperidae: *Bothrops*). *J. Nat. Hist.* **2020**, *54*, 2415–2437, doi:10.1080/00222933.2020.1845835.
72. Fujita, M.K.; Leaché, A.D.; Burbrink, F.T.; McGuire, J.A.; Moritz, C. Coalescent-Based Species Delimitation in an Integrative Taxonomy. *Trends Ecol. Evol.* **2012**, *27*, 480–488, doi:10.1016/j.tree.2012.04.012.
73. Struck, T.H.; Feder, J.L.; Bendiksby, M.; Birkeland, S.; Cerca, J.; Gusarov, V.I.; Kistenich, S.; Larsson, K.H.; Liow, L.H.; Nowak, M.D.; et al.

Finding Evolutionary Processes Hidden in Cryptic Species. *Trends Ecol. Evol.* **2018**, *33*, 153–163, doi:10.1016/j.tree.2017.11.007.
74. Mones, A. Nomen Dubium vs. Nomen Vanum. *J. Vertebr. Paleontol.* **1989**, *9*, 232–234, doi:10.1080/02724634.1989.10011757.
75. Folleco-Fernández, A.J. Taxonomía del complejo *Bothrops* asper (Serpentes: Viperidæ) en el sudoeste de Colombia. Revalidación de la especie *Bothrops rhombeatus* (García 1896) y descripción de una nueva especie. *Revi i sta Noved. Col l ombi i anas* **2010**, *10*, 33–70.
76. Ramírez-Chaves, H.E.; Solari, S. *Bothrops* ayerbei Folleco-Fernández, 2010 y *Bothrops rhomboatus* García, 1896 (Serpentes: Viperidae) son un nombre no Disponible y un nomen dubium, Respectivamente. *Bol. Cient. del Cent. Museos* **2014**, *18*, 138–141.
77. Mora-Obandoi, D.; Pla, D.; Lomonte, B.; Guerrero-Vargas, J.A.; Ayerbe, S.; Calvete, J.J. Antivenomics and in Vivo Preclinical Efficacy of Six Latin American Antivenoms towards South-Western Colombian *Bothrops asper* Lineage Venoms. *PLoS Negl. Trop. Dis.* **2021**, *15*, e0009073, doi:10.1371/JOURNAL.PNTD.0009073.
78. Mora-Obando, D.; Guerrero-Vargas, J.A.; Prieto-Sánchez, R.; Beltrán, J.; Rucavado, A.; Sasa, M.; Gutiérrez, J.M.; Ayerbe, S.; Lomonte, B. Proteomic and Functional Profiling of the Venom of *Bothrops ayerbei* from Cauca, Colombia, Reveals Striking Interspecific Variation with *Bothrops asper* Venom. *J. Proteomics* **2014**, *96*, 159–172, doi:10.1016/j.jprot.2013.11.005.
79. Salazar-Valenzuela, D.; Kuch, U.; Torres-Carvajal, O.; Valencia, J.H.; Gibbs, H.L. Divergence of Tropical Pitvipers Promoted by Independent Colonization Events of Dry Montane Andean Habitats. *J. Biogeogr.* **2019**, *46*, 1826–1840, doi:10.1111/jbi.13661.
80. Bernarde, P.S.; Turci, L.C.B.; Abegg, A.D.; Franco, F.L. A Remarkable New Species of Coralsnake of the *Micrurus hemprichii* species Group from the Brazilian Amazon. *Salamandra* **2018**, *54*, 249–258.
81. Schmidt, K.P. Hemprich's Coral Snake, *Micrurus hemprichii*. *Fieldiana Zool.* **1953**, *34*, 165–170.
82. Feitosa, D.; Prudente, A.L.D.C.; da Silva-Jr, N.J.; Pires, M.G. Análise Taxonômica Do Com- Plexo *Micrurus hemprichii* (Jan, 1858) (Serpentes, Elapidae). In Proceedings of the IX Congresso Latinoamericano de herpetologia,; Curitiba, **2011**; p. 23.
83. Valencia, J.; Garzón-Tello, K.; Barragán-Paladines, M. *Serpientes Venenosas Del Ecuador*; Fundación Herpetológica Gustavo Orcés: Quito, **2016**; ISBN 9789942216632.
84. Ayerbe-González, S.; Condiza-Benavides, G.E.; Sevilla-Sánchez, M.J. Primer Registro de Accidentes Ofídicos Por Mordedura de *Micrurus ortoni* y *Micrurus hemprichii* (Serpentes: Elapidae) En Colombia y Perú. *Biomédica* **2021**, *41*, 631–642.
85. Duellman, W.E. A Monographic Study of the Colubrid Snake Genus *Leptodeira*. Bulletin of American Museum Natural History. *Bull. Am. museum Nat. Hist.* **1958**, *114*, 1–152.
86. Daza, J.M.; Smith, E.N.; Paez, V.P.; Parkinson, C.L. The Origen and Diversificatión of the Widespread Genus *Leptodeira* (Serpentes:Colubridae). *Molecualr Phylogenetics Evol.* **2009**, *53*, 653–667, doi:https://doi.org/10.1016/j.ympev.2009.07.022.

87. Torres-Carvajal, O.; Sánchez-Nivicela, J.C.; Posse, V.; Celi, E.; Koch, C. A New Species of Cat-Eyed Snake (Serpentes: Dipsadinae: Leptodeirini) from the Andes of Southern Ecuador. *Zootaxa* **2020**, *4895*, 357–380, doi:10.11646/zootaxa.4895.3.3.
88. Mendoza, I. Defensive Behavior in *Leptodeira annulata ashmeadii* (Hallowell, 1845). *Herpetotropicos* **2008**, *5*, 67.
89. Lopes, D.; Nomura, F. *Leptodeira annulata* (Banded Cat-Eyed Snake). Diet. *Herpetol. Rev.* **2014**, *45*, 145.
90. Costa, W.P.; de Andrade, F.S. Predation Behaviour of *Leptodeira annulata* Linnaeus, 1758 (Serpentes: Dipsadidae) on Physalaemus Cuvieri Fitzinger, 1826 (Anura, Leptodactylidae). *Herpetol. Notes* **2020**, *13*, 457–459.
91. Yánez, G.; Chuquimarca, E. Dieta de *Leptodeira* (Colubridae: Serpentes) (Fitzinger 1843) En Ecuador y Notas Ecológicas de Una Población de *L. septentrionalis larcorum* (Kennicott 1859) En Zapotillo-Loja, Ecuador, Universidad Central Del Ecuador, **2020**.
92. Daza, J.M.; Smith, E.N.; Páez, V.P.; Parkinson, C.L. Complex Evolution in the Neotropics: The Origin and Diversification of the Widespread Genus *Leptodeira* (Serpentes: Colubridae). *Mol. Phylogenet. Evol.* **2009**, *53*, 653–667, doi:10.1016/j.ympev.2009.07.022.
93. Barrio-Amorós, C.L. On the Taxonomy of Snakes in the Genus *Leptodeira*, with an Emphasis on Costa Rican Species. *Reptil. Amphib.* **2019**, *26*, 1–15, doi:10.17161/RANDA.V26I1.14321.
94. Costa, J.C.L.; Graboski, R.; Grazziotin, F.G.; Zaher, H.; Rodrigues, M.T.; Prudente, A.L. da C. Reassessing the Systematics of *Leptodeira* (Serpentes, Dipsadidae) with Emphasis in the South American Species. *Zool. Scr.* **2022**, *51*, 415–433, doi:https://doi.org/10.1111/zsc.12534.
95. de Albuquerque, N.R.; Galatti, U.; Di-Bernardo†, M. Diet and Feeding Behaviour of the Neotropical Parrot Snake (*Leptophis ahaetulla*) in Northern Brazil. *https://doi.org/10.1080/00222930701400954* **2010**, *41*, 1237–1243, doi:10.1080/00222930701400954.
96. Clegg, J.R. *Leptophis ahaetulla marginatus* (Southern Green Parrot Snake): Behaviour. *Herpetol. Bull.* **2015**, 26–27.
97. Sánchez, M.N.; Teibler, G.P.; López, C.A.; Mackessy, S.P.; Peichoto, M.E. Assessment of the Potential Toxicological Hazard of the Green Parrot Snake (*Leptophis ahaetulla marginatus*): Characterization of Its Venom and Venom-Delivery System. *Toxicon* **2018**, *148*, 202–212, doi:10.1016/j.toxicon.2018.04.027.
98. de Albuquerque, N.R. New Records of *Leptophis ahaetulla ahaetulla* (Serpentes, Colubridae) for Venezuela, Colombia and the Placement of L. a. Copei into the Synonymy of *L. a. ahaetulla*. *Biota Neotrop.* **2009**, *9*, 293–296, doi:10.1590/s1676-06032009000400035.
99. Albuquerque, N.R. De Revisão Taxonômica Das Subespécies de *Leptophis ahaetulla* (Linnaeus, 1758) (Serpentes, Colubridae), Pontifícia Universidade Católica do Rio Grande do Sul, **2008**.
100. Torres-Carvajal, O.; Terán, C. Molecular Phylogeny of Neotropical Parrot Snakes (Serpentes: Colubrinae: *Leptophis*) Supports Underestimated Species Richness. *Mol. Phylogenet. Evol.* **2021**, *164*, 107267, doi:10.1016/j.ympev.2021.107267.

101. Oliver, J.A. A Check List of the Snakes of the Genus *Leptophis*, with Descriptions of New Forms. *Occas. Pap. Museum Zool. Univ. Michigan* **1942**, *1942*, 1–19.
102. Goldberg, S.R. *Oxybelis aeneus* (Brown Vine Snake). Reproduction. *Oxybelis aeneus* (Bejuquilla Café). Reproducción. *Herpetol. Rev.* **2005**, *36*, 70.
103. Mesquita, P.C.M.D.; Borges-Nojosa, D.M.; Passos, D.C.; Bezerra, C.H. Activity Patterns of the Brown Vine Snake *Oxybelis aeneus* (Wagler, 1824) (Serpentes, Colubridae) in the Brazilian Semiarid. *Anim. Biol.* **2012**, *62*, 289–299, doi:10.1163/157075611X618228.
104. Almeida, L. de S.; da Silva, A.W.O.; Trevine, V.C. Two New Records of Predation by *Oxybelis fulgidus* (Squamata: Colubridae) in the Northern Region of Brazil. *Herpetol. Notes* **2020**, *13*, 283–289.
105. Toro-Orozco, W. Del; Montanarin, A.; Ramalho, E.E.; Kaefer, I.L. Predation by the Green Vinesnake *Oxybelis fulgidus* on the Diving Lizard *Uranoscodon superciliosus*. *Herpetol. Notes* **2021**, *14*, 591–592.
106. Ramalho, W.P.; Batista, V.G.; Lozi, L.R.P. Anfíbios e Répteis Do Médio Rio Aporé, Estados de Mato Grosso Do Sul e Goiás, Brasil. *Neotrop. Biol. Conserv.* **2014**, *9*, 147–160, doi:10.4013/nbc.2014.93.04.
107. Pinheiro Miranda, J.; Lopes Costa, J.C.; Micheletto Scarpa, F.; Rocha, C.F.D. Predation on Columbina Squammata (Aves: Columbidae) by the Green Vine Snake Oxybelis Fulgidus (Serpentes: Colubridae) at the Lençóis Maranhenses National Park, Maranhão, Brazil. *Herpetol. Notes* **2013**, *6*, 187–188.
108. Norris, J.L.; Burtt, E.H. *Oxybelis fulgidus* (Green Vine Snake or Bejucillo). Feeding. *Herpetol. Rev.* **1998**, *29*, 243.
109. Roveri Scartozzoni, R.; Da Graça Salomão, M.; De Almeida-Santos, S.M. Natural History of the Vine Snake *Oxybelis fulgidus* (Serpentes, Colubridae) From Brazil. *South Am. J. Herpetol.* **2009**, *4*, 81–89, doi:10.2994/057.004.0111.
110. Jadin, R.C.; Jowers, M.J.; Orlofske, S.A.; Duellman, W.E.; Blair, C.; Murphy, J.C. A New Vine Snake (Reptilia, Colubridae, *Oxybelis*) from Peru and Redescription of *O. acuminatus*. *Evol. Syst.* **2021**, *5*, 1–12, doi:10.3897/evolsyst.5.60626.
111. Jadin, R.C.; Blair, C.; Orlofske, S.A.; Jowers, M.J.; Rivas, G.A.; Vitt, L.J.; Ray, J.M.; Smith, E.N.; Murphy, J.C. Not Withering on the Evolutionary Vine: Systematic Revision of the Brown Vine Snake (Reptilia: Squamata: *Oxybelis*) from Its Northern Distribution. *Org. Divers. Evol.* **2020**, *20*, 723–746, doi:10.1007/s13127-020-00461-0.
112. Jadin, R.C.; Blair, C.; Jowers, M.J.; Carmona, A.; Murphy, J.C. Hiding in the Lianas of the Tree of Life: Molecular Phylogenetics and Species Delimitation Reveal Considerable Cryptic Diversity of New World Vine Snakes. *Mol. Phylogenet. Evol.* **2019**, *134*, 61–65, doi:10.1016/j.ympev.2019.01.022.
113. Garman, S. The Reptiles and Batrachians of North America. *Mem. Museum Comp. Zoölogy* **1883**, *8*, 184.
114. Angarita-Sierra, T.; Cubides-Cubillos, S.D.; Hurtado-Gómez, J.P. Hidden in the Highs: Two New Species of the Enigmatic Toadheaded Pitvipers of the Genus *Bothrocophias*. *Vertebr. Zool.* **2022**, *72*, 971–996, doi:10.3897/vz.72.e87313.

115. Hernández-Camacho, J.I.; Alvarez-León, R.; Renjifo-Rey, J.M. Pelagic Sea Snake *Pelamis platurus* (Linnaeus, 1766) (Reptilia: Serpentes: Hydrophidae) Is Found on the Caribbean Coast of Colombia. *Mem. la Fund. La Salle Ciencias Nat.* **2006**, *164*, 7–16.
116. Briski, E.; Gollasch, S.; David, M.; Linley, R.D.; Casas-Monroy, O.; Rajakaruna, H.; Bailey, S.A. Combining Ballast Water Exchange and Treatment To Maximize Prevention of Species Introductions to Freshwater Ecosystems. *Environ. Sci. Technol.* **2015**, *49*, 9566–9573, doi:10.1021/ACS.EST.5B01795/SUPPL_FILE/ES5B01795_SI_001.PDF.
117. Tavares Feitosa, D.; Godoy-Pires, M.; da Costa Prudente, A.; da Silva Jr., N. Distribution Extension in Colombia and New Records for Brazil of *Micrurus isozonus* (Cope, 1860) (Squamata, Serpentes, Elapidae). *Check List* **2013**, *9*, 1108–1112.
118. Castro-Herrera, F.; Vargas-Salinas, F. Anfibios y Reptiles En El Departamento Del Valle Del Cauca, Colombia. *Biota Colomb.* **2008**, *9*, 251–277.
119. Jowers, M.J.; Smart, U.; Sánchez-Ramírez, S.; Murphy, J.C.; Gómez, A.; Bosque, R.J.; Sarker, G.C.; Noonan, B.P.; Faria, J.F.; Harris, D.J.; et al. Unveiling Underestimated Species Diversity within the Central American Coralsnake, a Medically Important Complex of Venomous Taxa. *Sci. Rep.* **2023**, *13*, 1–12, doi:10.1038/s41598-023-37734-5.
120. Dunn, E. The Amphibians and Reptiles of the Caribbean Islands of San Andres and Providencia. *Caldasia* **1945**, *3*, 363–365.
121. Dunn, E.; Saxe, H. Results of the Catherwood-Chaplin West Indies Expedition. Part V. Amphibians and Reptiles of San Andres and Providencia. *Proc. Acad. Nat. Sci. Philadelphia* **1948**, 141–165.
122. McNish, T. *La Fauna Del Archipiélago de San Andres, Providencia y Santa Catalina, Colombia, Sudamérica*; M&B Producciones y Servicios Ltda: Bogotá, **2011**; ISBN 978-958-99518-1-1.
123. Werner, F. Ueber Reptilien Und Batrachier Aus Ecuador Und Neu-Guinea. *Verhandlungen der Kais. Zool. Gesellschaft Wien* **1901**, *51*, 593–614.
124. Roze, J. Notes on Taxonomy of Venomous Coral Snakes (Elapidae) of South America. *Bull. Maryl. Herpetol. Soc.* **1994**, *30*, 177–1985.
125. Maritz, R.A.; Maritz, B. Sharing for Science: High-Resolution Trophic Interactions Revealed Rapidly by Social Media. *PeerJ* **2020**, *2020*, doi:10.7717/peerj.9485.
126. Barrera Ocampo, F.; Renjifo, J.M. Occurrence of Anchor Coralsnake, Micrurus ancoralis (Jan, 1872) (Squamata: Elapidae) Confirmed in the Magdalena River Valley of Colombia, with Novel Citizen Science Distribution Records. *Anartia* **2024**, *38*, 11–22.
127. CORANTIOQUIA *Programa Nacional Para La Conservación de Serpientes En Colombia*; **2008**.
128. Morales-Betancourt, M.; Lasso, C.A.; Paez, V.P.; Bock, B.C. *Libro Rojo de Reptiles de Colombia*; Morales-Betancourt, M., Lasso, C.A., Paez, V.P., Bock, B.C., Eds.; Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Universidad de Antioquia: Bogotá, **2015**; ISBN 9789588889795.
129. IUCN *Summary of the Five Criteria Use to Evaluate If a Taxonin an IUCN Red List Threatened Category*; **2012**.
130. MADS [Ministerio de Ambiente y Desarrollo Sostenible] *Resolución No. 0126 de 2024*; Colombia, **2024**; p. 69.

131. Caicedo-Portilla, J.R.; Lynch, J.D. *Micrurus medemi*. In *Libro rojo de reptiles de Colombia (2015).*; Orales-Betancourt, M.A., Lasso, C.A., Páez, V.P., Bock, B.C., Eds.; Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH), Universidad de Antioquia: Bogotá, **2015**; pp. 98–100 ISBN 978-958-888-979-5.
132. Urbina-Cardona, N.; Acosta, L.S.; Camacho-Rozo, C.P.; Peña, A.R.A.; Arenas-Rodríguez, A.; Albarracín-Caro, J.F.; Moreno-Cabal, A.M.; Novoa-Salamanca, N.M.; Camacho-Durán, M.J.; Echeverry, N.G.; et al. Scientific Production on Herpetology in Colombia: Perspectives from Research Topics towards Biological Conservation. *Caldasia* **2023**, *45*, 1–20, doi:10.15446/caldasia.v45n1.97216.
133. Angarita-Sierra, T. Demographic Analysis of Snake Killing as a Conservation Threat: Study Case for a Population of *Ninia atrata*, Universidad Nacional de Colombia, sede Bogotá, **2017**.
134. Rincón-Aranguri, M.; Toro-Cardona, F.A.; Galeano, S.P.; Roa-Fuentes, L.; Urbina-Cardona, N. Functional Diversity of Snakes Is Explained by the Landscape Composition at Multiple Areas of Influence. *Ecol. Evol.* **2023**, *13*, e10352, doi:10.1002/ece3.10352.
135. López-Herrera, D.F.; León-Yusti, M.; Guevara-Molina, S.C.; Vargas-Salinas, F. Reptiles in Biological Corridors and Roadkills in Barbas-Bremen, Quindio, Colombia. *Rev. la Acad. Colomb. Ciencias Exactas, Fis. y Nat.* **2016**, *40*, 484–493, doi:10.18257/raccefyn.334.
136. Vera-Pérez, L.E.; Zúñiga-Baos, J.A.; Ayerbe-González, S. *Reptiles Del Parque Nacional Natural Munchique, Colombia*; **2018**.
137. Zúñiga-Baos, J.A.; Vera-Pérez, L.E. Mortalidad de Serpientes En La Vía El Valle de Toledo-Toledo, Antioquia –Colombia. *Rev. Colomb. Cienc. Anim. - RECIA* **2020**, *12*, 745, doi:10.24188/recia.v12.n1.2020.745.
138. Zúñiga-Baos, J.A. Mortalidad de Serpientes En El Área Urbana Del Municipio Neira, Caldas, Colombia. *Rev. Colomb. Cienc. Anim. - RECIA* **2023**, *15*, e968, doi:10.24188/recia.v15.n1.2023.968.
139. Muñoz-Avila, J.A.; Bedoya-Cañon, M.A.; Díaz-Pérez, C.N.; Vargas-Salinas, F. Roadkills Give Us Information about the Reproductive Biology of the Snake *Boa constrictor* (Linnaeus 1758). *Herpetol. Notes* **2014**, *7*, 761–762.
140. Angarita-Sierra, T.; Montaño-Londoño, L.F.; Bravo-Vega, C.A. ID Please: Evaluating the Utility of Facebook as a Source of Data for Snake Research and Conservation. *An. Acad. Bras. Cienc.* **2022**, *94*, 1–25, doi:10.1590/0001-3765202220211043.
141. Zinsstag, J.; Waltner-Toews, D.; Tanner, M. Theoretical Issues of One Health. In *One Health. The Theory and Practice of Integrated Health Approaches*; Zinsstag, J., Schelling, E., Waltner-Toews, D., Whittaker, M., Tanner, M., Eds.; CABI: Croydon, **2015**; pp. 16–25 ISBN 9781780643410.
142. Machalaba, C.C.; Daszak, P.; Karesh, W.B.; Shrivastava, P. Future Earth and EcoHealth: A New Paradigm Toward Global Sustainability and Health. *Ecohealth* **2015**, *12*, 553–554, doi:10.1007/s10393-015-1076-6.
143. Gutiérrez, J.M. Snakebite Envenoming from an Ecohealth Perspective. *Toxicon X* **2020**, *7*, doi:10.1016/j.toxcx.2020.100043.
144. Harrison, S.; Kivuti-Bitok, L.; Macmillan, A.; Priest, P. EcoHealth and One Health: A Theory-Focused Review in Response to Calls for Convergence. *Environ. Int.* **2019**, *132*, 105058, doi:10.1016/j.envint.2019.105058.

145. Martínez-López, O. La Taxonomía Integral y Su Importancia Para La Conservación. *Cienc. Conserv.* **2015**, *6*, 54–65.
146. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews. *BMJ* **2021**, *372*, 1–9, doi:10.1136/bmj.n71.
147. Rowe, G.; Wright, G. Expert Opinions in Forecasting: The Role of the Delphi Technique. In *Principles of Forecasting*; Armstrong, J., Ed.; Kluwer Academic: Boston, **2001**; pp. 125–144.
148. Reyes-Velasco, J. A revision of recent taxonomic changes to the eyelash palm pitviper (Serpentes , Viperidae , *Bothriechis schlegelii*). *Herpetozooa* **2024**, *37*, 1–14, doi:10.3897/herpetozoa



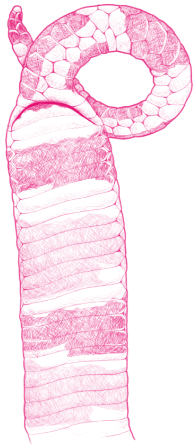
Chapter 2

Lineages, venom variability, and warning signals in New World Coralsnakes

Juan David Bayona-Serrano, Chris Akcali, Juan Pablo Hurtado-Gomez, Teddy Angarita-Sierra

Abstract: Coralsnakes of the genus *Micrurus* are the most diverse group of elapids in the Americas. Their potent neurotoxic venoms have been extensively studied, and various toxins, especially neurotoxins, have been identified. However, data on the composition and variability of the toxins of most *Micrurus* species are lacking. We compile available information on *Micrurus* venoms to show that we still lack a robust understanding of the factors driving variation in venom composition. Sampling of the venoms of a broader range of species is needed given that various species have venom with unique properties. Coralsnakes are also known for their brightly colored rings, and their color patterns have long been assumed to have an aposematic function (i.e., serve as warning signals of their danger) against mammalian and avian predators. However, recent work has suggested that there are some serious problems with the aposematic framework. We discuss the evidence for and against this framework from a field herpetologist perspective and consider alternative explanations for the defensive function of coralsnake color patterns.

Keywords: Elapidae, Three finger toxin, Venom, Evolution, Aposematism.



Citation: Bayona-Serrano JD; Akcali C; Hurtado-Gómez JP; Angarita-Sierra T. Chapter 2. Lineages, venom variability, and warning signals in New World Coral snakes. In Book: *Bites, venoms, and venomous snakes of Colombia*; Angarita-Sierra, T., Ruiz-Gomez, F.J, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 83–114.
doi: 10.33610/664742zbhahc

Copyright: © 2024 by the authors. Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0) license (http://creativecommons.org/licenses/by/4.0/).

Illustrations by:
Oscar A. Ramirez Ruiz

1. Introduction

The family Elapidae is a diverse group of venomous snakes, with nearly 390 species, and most elapids are distributed in the tropics [1–4]. In the Americas, the family is represented by a monophyletic clade containing the coralsnakes (genera *Micruroides* and *Micrurus*) and the marine species *Hydrophis platurus* [1–4]. Coralsnakes comprise almost 90 species and range from the United States to Argentina, but they are absent in Chile and most Antillean islands [1,2].

The genus *Micruroides* is the most northerly distributed coral snake clade, and the only recognized species in this genus, *M. euryxanthus*, occurs in Mexico and the United States [1,2]. The genus *Micrurus* is diverse [1,2]; most species occur in South America, being Brazil and Colombia the countries with the highest species diversity (~ 30 species each) [1,2,5].

Coralsnakes are widely recognized for their bright coloration and for being highly venomous. Their color patterns consist of rings arranged in three colors, which normally include a light color (white or yellow), a bright color (red or orange), and black; however, some species have atypical color patterns [5–8]. Four types of ring arrangements are recognized among coral snakes of the genus *Micrurus*, and this corresponds to clades in their phylogenetic tree [2,5,6]. The ring arrangements described below correspond to their body coloration and may or may not occur on the head and tail (Figure 1).

1. *Bicolor*: rings in the body arranged in two colors, black alternating with a light or bright color. The head and tail in these species normally have black rings arranged between a bright color (red or orange).
2. *Monads:* rings in the body in three colors, with one black ring between the brightly colored rings, and separated from these by light colored rings. The head and tail in these species normally have rings with a light color and black. The name of this color pattern refers to the number of black rings (one) between the brightly colored ones. This color pattern occurs independently in one large South American clade, in two Central American species and two south American species.
3. *Triads:* rings in the body arranged in three colors, with three black rings, separated by light colored rings, which are together between the bright rings. The head and tail ring arrangement are variable but normally is similar to that of the body. The name of this color pattern refers to the number of black rings (three) between the brightly colored ones. This color pattern occurs independently in one large South American clade and in two Central American species.
4. *Incomplete rings:* this pattern is almost identical to the bicolored pattern, but features light rings along the body, which shorten down towards the dorsum and normally are not complete; the dorsal coloration is mainly black. This pattern occurs in a group of species known as pygmy coral snakes, which were previously grouped in a different genus, *Leptomicrurus*.

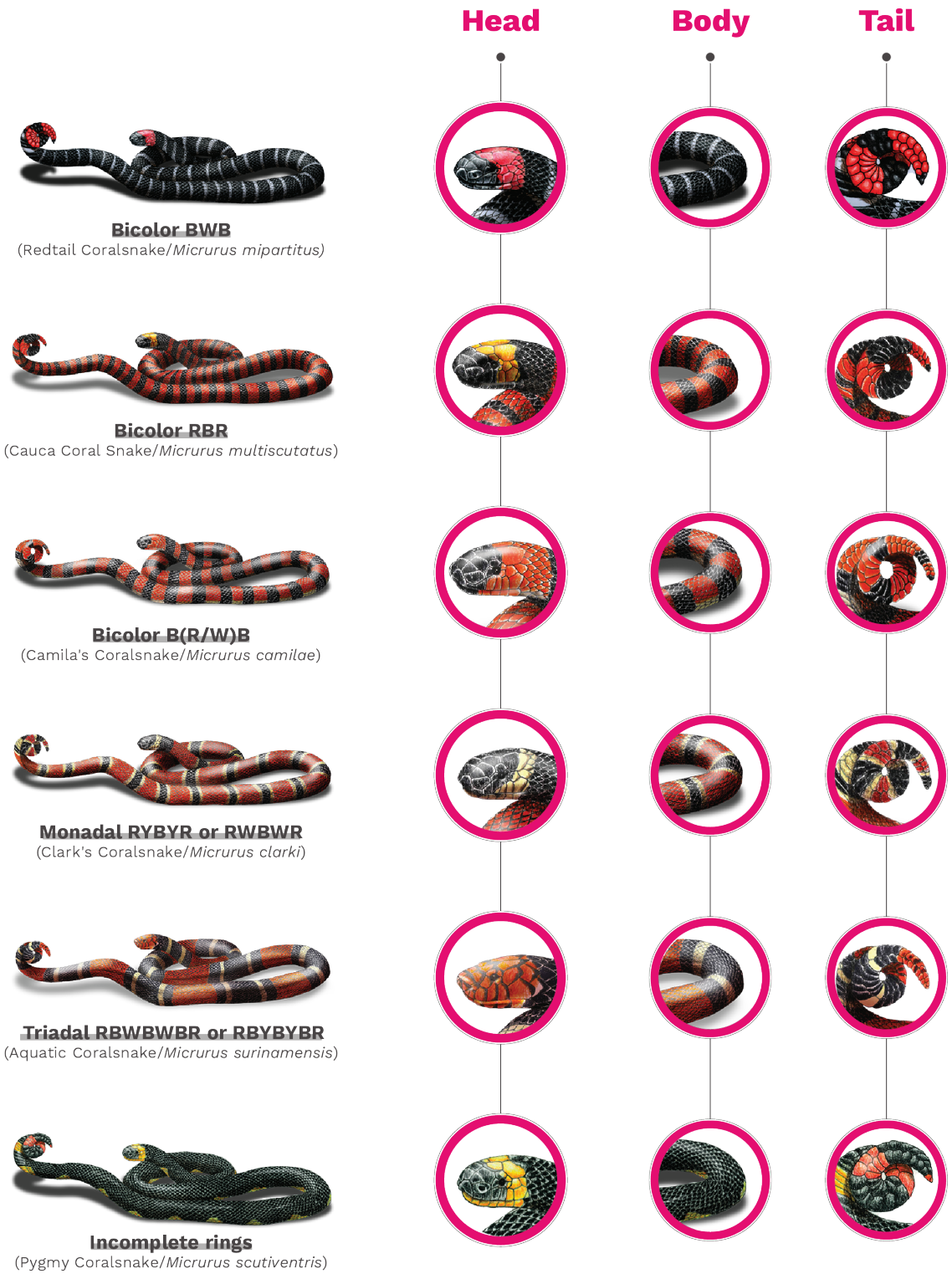


Figure 1. General patterns of the body coloration in Colombian Coral snakes. Bicolor BWB (*Micrurus mipartitus*): Bicolor pattern with alternating black and white rings. Bicolor RBR (*Micrurus multiscutatus*): Bicolor with alternating red and black rings. Bicolor B(R/W)B (*Micrurus camilae*): Bicolor pattern alternating red and black dorsally and alternating white and black ventrolaterally; Monadal RYBYR or RWBWR: Monadal pattern with red rings separated from black rings, alternating with white or yellow rings; Triadial RBWBWBR (*Micrurus surinamensis*): Triadal with red rings separated by three black rings and two white/yellow rings; Incomplete rings (*Micrurus scutiventris*): incomplete light rings along the body, which narrow down towards the dorsum and normally are not complete; the dorsal coloration is mainly black. Acronyms: BWB= Black/White/Black. RBR=Red/Black/Red. B(R/W)B= Black/half red and half white/Black. RYBYR= Red/Yellow/Black/Yellow/Red. RWBWR=Red/White/Black/White/Red. RBWBWBR= Red/Black/White/Black/White/Black/Red. RBYBYBR=Red/Black/Yellow/Black/Yellow/Black/Red.

Coralsnake venom is primarily neurotoxic, inducing a neuromuscular blockade that can result in the prey's death through respiratory arrest [9]. Their venoms exhibit moderate heterogeneity compared to other groups, such as viperids or colubrids, but contain a diverse range of toxin components, with each species having between 4 to 24 different protein families [2,4,5,9,10]. The main components of these venoms are phospholipases A_2 (PLA_2), which predominantly mediate presynaptic neurotoxicity, and three finger toxins (3FTx), which are mostly associated with postsynaptic effects [2]. One of these toxin families is usually dominant over the other in almost all species of coralsnakes, and this creates a phenotypic dichotomy across species [2,9,11].

The dominance of these two proteins has been suggested to respond to a geographical gradient, with species in the northern range of the genus *Micrurus* having PLA_2-dominated venoms while those in the southern range having a predominance of 3FTx [9,11]. This hypothesis has been subject to recent debate, given that some studies have provided contradicting evidence [2,12]. Likewise, as we will show in this chapter, the geographical pattern of the ratio PLA_2/3FTx is blurred when it is confronted to a larger population sampling and increase of species richness. However, an additional evolutionary hypothesis for the observed phenotypic dichotomy has not been proposed.

The phylogenetic relationships of the genus *Micrurus* have been discussed in several studies [3,6,13–16], but advances come at a low pace due to the challenges associated with the high diversity of the group and the cryptic nature of most species [6,14,15]. In general, most studies have suggested that species with the monadal pattern form a monophyletic group and South American triadal snakes plus the bicolored species also form a clade [13–15]. The phylogenetic position and monophyly of the pygmy coralsnakes (which have incomplete rings) and the Central American species with triadal patterns (*M. elegans*) have not yet been elucidated, mainly due to a scarcity of samples and poor genetic resolution [15,17].

In this chapter, we propose a phylogenetic hypothesis for the coralsnakes of the genus *Micrurus* using published sequences and test whether venom composition is associated with phylogenetic and geographic patterns. We also review evidence for and against the longstanding hypothesis that the color patterns of coral snake's function as warning signals and discuss alternative explanations for the function of coral snake color patterns.

2. Phylogenetic relationships of the New World coralsnakes in the genus *Micrurus*

Our maximum likelihood phylogenetic tree recovered New World coralsnakes (*Micruroides* + *Micrurus*) and the genus *Micrurus* as monophyletic with high support (Figure 2). Within *Micrurus*, two highly supported clades were recovered, one including all the species with monads plus *M. elegans*, one of the Central American species with triads; and the second contained all the South American species with triads, *M. mipartitus*, the only bicol-

ored species, and *M. narducci*, the only species from the old-fashioned genus *Leptomicrurus* [8] (also known as pygmy coralsnakes, which are herein classified as coralsnakes with a color pattern of incomplete rings).

Three main subclades can be recognized within the clade containing the triadal species: one highly supported subclade containing species in the *M. lemniscatus* complex (*M. diutius*, *M. carvalhoi*, *M. filiformis*, *M. helleri*, and *M. lemniscatus*), *M. surinamensis*, and *M. ortonii* (clade T1, Figure 2), which is sister to *M. narduccii*, forming a poorly supported clade. A second highly supported subclade that includes *M. dissoleucus* and *M. mipartitus* (clade T2, Figure 2). A third weakly supported clade that includes species of the *M. frontalis* group (*M. altirostris*, *M. baliocoryphus*, *M. brasiliensis*, *M. frontalis*, *M. ibiboboca*, *M.* cf. *ibiboboca*, and *M. pyrrhocryptus*), plus *M. decoratus* and *M. obscurus* (clade T3, Figure 2).

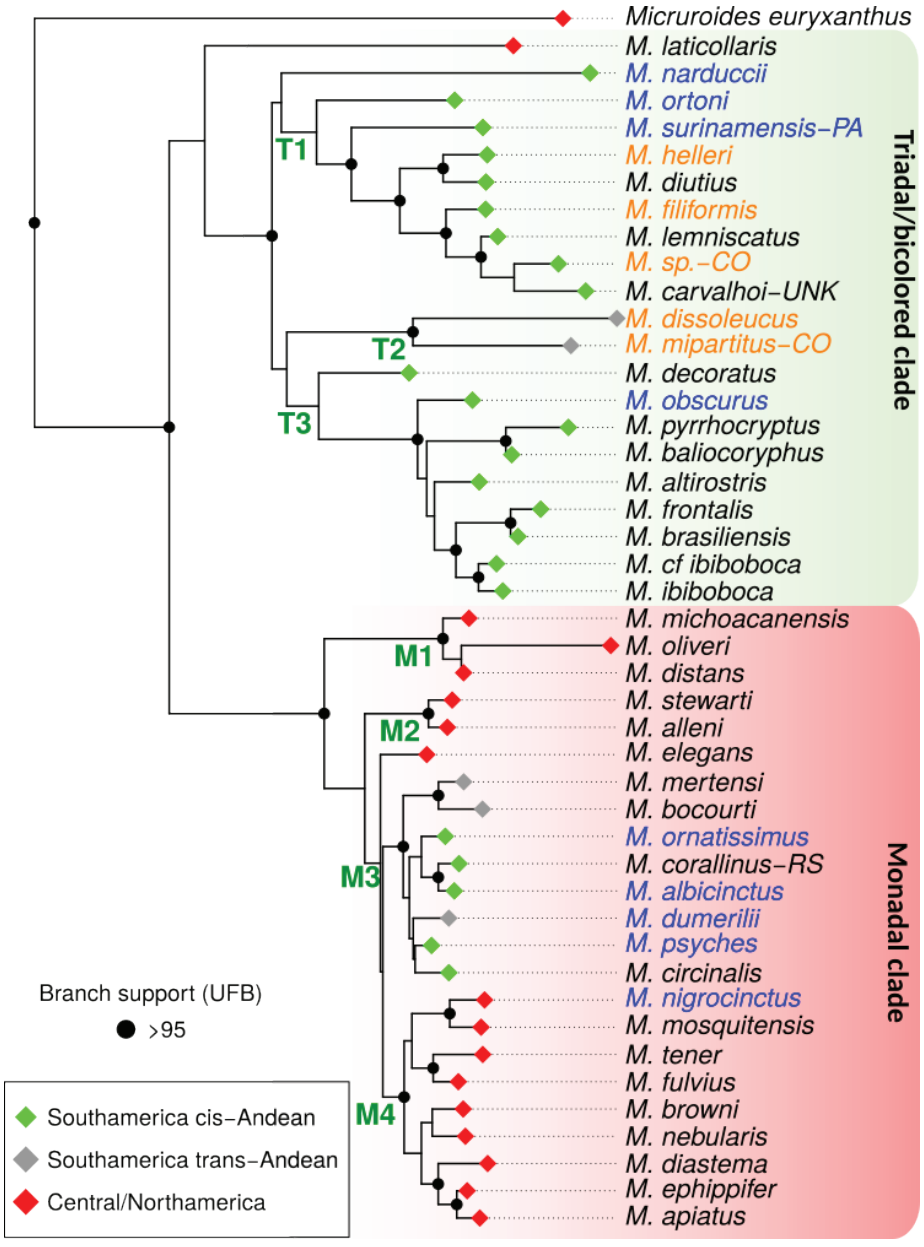


Figure 2. Maximum likelihood tree obtained via IQ-Tree, based on the concatenated alignment of four mitochondrial (12S, 16S, cytb and ND4) and one nuclear (Cmos) gene (3,175 bp). The colors of the node circles indicate ultrafast bootstrap support values, red: >95, black: <95. Color at tips indicates species that occur in Colombia; orange names indicate sequences from Colombian specimens, and blue names indicate sequences from non-Colombian samples. Notations for some samples (CO, PA, RS, etc) are specified in the appendix section.

A total of four main subclades were recovered with high support for the clade containing the monadal species: the first contained taxa in the *M. distans* group (*M. distans*, *M. michoacanensis*, and *M. oliveri*; clade M1, Figure 2); the second contained *M. stewarti* and *M. alleni* (clade M2), two central American species; the third contained all the South American monadal species (*M. albicinctus*, *M. bocourti*, *M. circinalis*, *M. corallinus*, *M. dumerilii*, *M. mertensi*, *M. ornatissimus*, and *M. psyches*; clade M3, Figure 2); and the fourth contained the remaining Central and North American species (clade M4, Figure 2). In our phylogenetic tree, 14 of the 46 species sampled occur in Colombia, and six of these sequences were derived from Colombian specimens (Figure 2).

In general, our phylogenetic hypothesis is in line with most recent revisions, finding as monophyletic the monads clade with the bicolored and the triadal clades forming a monophyletic clade [14-17,19–23]. The relationships of *Micrurus narducii*, the only representative of the old-fashioned genus *Leptomicrurus*, remain ambiguous among studies, while in our tree it falls within the triads clade, as in Jowers et al. [14], other recent studies found it as sister to the triadial clade [20,21,23].

The phylogenetic hypothesis presented herein (Figure 2), represents the largest phylogenetic proposal for the new world coralsnakes in the genus *Micrurus*, including 44 (plus two unclassified taxa, *M.* sp., and *M.* cf. *ibiboboca*), out of the 83 currently recognized species on the genus (53%) [1]. With this sampling and the phylogenetic context some biogeographic patterns arise.

The clade grouping the triadal + bicolored + incomplete rings is composed almost exclusively of South American species (Figure 2), except for the basal species, *M. laticollaris*, which is distributed in Mexico [24,25]. Nevertheless, the position of *M. laticollaris* is poorly supported and its position might change with more evidence. Within the South American cluster, all species are strictly distributed in the cis-Andean region (i.e. Orinoquia ecoregion and Amazon ecoregion, Figure 3), except for two taxa which form a highly supported clade, *M. dissoleucus* and *M. mipartitus*, both distributed in the trans-Andean region (i.e. across or west of the Andes, Figure 3) [24,25]. This suggests that this clade diversified in the cis-Andean region and most likely colonized the trans-Andean region a single time.

On the other hand, the bicolored clade groups taxa from the whole American continent, but with most taxa distributed in Central America (Figure 2). Within this clade, there is a lineage that groups all South American species, which might be suggestive of a single colonization event to South America for this clade (Figure 2). Nevertheless, some other species in the monadal clade, also enter northern South America, like *M. nigrocinctus* and *M. clarki* [24,25]. Despite the internal relationships that are not well supported within the South American monadal lineage, the retrieved topology suggests at least two crosses of the Andes (e.g. from the trans to the cis-Andean region, or vice versa, Figure 2).

Even though it is widely known in the literature that the monadal clade is mainly comprised of Central American species, and the triadal clade is composed mainly of South American/cis-Andean taxa [24,25], no previous study has shown or discussed the biogeographic patterns of diversification within *Micrurus*, based on a phylogenetic framework. Also, the fact that the main clades have most of their diversity grouped in specific regions (e.g. Central or South America), might have led to some authors (i.e. Lomonte et al. [9]) to suggest latitudinal variation in the venom composition within *Micrurus*, which is further discussed in the next section.

3. Compositional diversity across *Micrurus* venoms

We compiled literature data of 34 proteomes belonging to 28 *Micrurus* species (Table 1). *Micrurus corallinus, M. mipartitus*, *M. helleri*, and *M. surinamensis* had two published proteomes, and *M. carvalhoi* had three published proteomes. Only six of the available proteomes came for Colombian specimens (*M. dumerilii, M. mipartitus-CO, M. sangilensis, M. helleri-CO, M. medemi*, and *M. sp.-CO*).

Based on the 34 proteomes, a total of 24 protein families was reported among *Micrurus* venoms, and PLA_2 and 3FTx were the dominant venom components in all proteomes, as has been previously found in multiple studies [26]. Fifteen toxin-like protein classes were identified in the proteomes of *M. carvalhoi-UNK* and *M. pyrrhocryptus*, which was the highest among all proteomes in the triadal clade. Twelve toxins were identified in the proteomes of *M. corallinus*-RJ and *M. paraensis*, which were the most diverse among all proteomes in the monadal clade (Table 1). In general, PLA_2-dominated venoms were more common in the monadal clade, whereas 3FTx-dominated venoms were more common within the triadal clade (Figures 3 and 4).

Our comparisons of available compositional data for *Micrurus* species revealed intraspecific variation in both the occurrence and relative abundance of toxins in *M. carvalhoi, M. corallinus, M. helleri, M. mipartitus*, and *M. surinamensis*, which had available data for multiple populations or individuals (Table 1). In these species, we noted that the dominant toxin, either 3FTx or PLA_2, varied between individuals across their geographic distribution, without any clear pattern (Figure 2). Differences in the relative abundance of 3FTx and PLA_2 (|%3FTx-%PLA_2|) were more pronounced in the triadal + bicolored clade (~44%), than in the monadal clade (~29%; Table 1). This indicates that proteomes from the triadal + bicolored clade tend to exhibit more extreme phenotypes, favoring a marked dominance of either PLA_2 or 3FTx, whereas the monadal groups possess more balanced PLA_2 or 3FTx proportions. Moreover, the genus *Micruroides* shows a higher proportion of 3FTx, suggesting that the emergence of PLA_2-dominated venoms could be a derived phenotype within coralsnakes.

Table 1. Venom proteome composition from available proteomes in the literature. Values are shown in percentages of total spectra to allow for compositional comparisons between species. Red and orange cells indicate higher proportions of each toxin, and yellowish and green cells indicate lower proportions of each toxin. Species are catalogued by their clade (monadal, triadal/bicolored, or the genus *Micruroides*). Only major toxins are shown. SVMP: Snake venom metalloproteinases, LAO: L-amino acid oxidases, Kun: Kunitz inhibitors, CTL: C-type lectins, SP: Serineproteases, Oha: Ohanin.

Species	Clade	PLA_2/3FTx delta	3FTx	PLA_2	SVMP	LAO	Kun	CTL	SP	Oha	Minor toxins
Micruroides euryxanthus	*Micruroides*	48.1	62.3	14.2							0
Micrurus alleni	Monadal	66.4	77.3	10.9	1.2	3		1.8		0.2	1
Micrurus browni	Monadal	12.2	50.4	38.2	1.4	0.4	4.2	1.1	0.3	0.2	2.9
Micrurus clarki	Monadal	11.7	48.2	36.5	1.6	3.8	0.9	1.1	1		0.4
Micrurus corallinus-RJ	Monadal	3.71	34.83	38.54	10.38	5.42	0.46				10.37
Micrurus corallinus-UNK	Monadal	69.8	81.7	11.9	2.4	2.3		0.9	0.8		0
Micrurus dumerilii	Monadal	23.9	28.1	52	1.8	3.1	9	1.1	1.9		0.4
Micrurus fulvius	Monadal	33.4	24.9	58.3							0
Micrurus mosquitensis	Monadal	33.1	22.5	55.6	2.6	2.8	9.8	1.3	0.5		1
Micrurus nigrocinctus	Monadal	10	38	48	4.3	2.3		2.2	0.7	3.8	0.5
Micrurus paraensis	Monadal	53.58	12.31	65.89	7.97	3.13	3.43	4.95			2.3
Micrurus sangilensis	Monadal	12.71	17.69	30.4	11.81	9.17	2.97	6.27	5.79	2.27	13.63
Micrurus tener	Monadal	8.1	37.5	45.6							0
Micrurus altirostris	Triadal + Bicolored	65.8	79.5	13.7	0.9	1.2	2.1	0.1			2.5
Micrurus carvalhoi-GO	Triadal + Bicolored	46.31	2.25	48.56	6.29	2.01	12.61	2.64	2.12	21.51	1.99
Micrurus carvalhoi-SP-B	Triadal + Bicolored	49.2	71.3	22.1	0.8	1.5	0.03				0.01
Micrurus carvalhoi-SP-J	Triadal + Bicolored	56.5	76.7	20.2	1.13	1.3	0.03				0.175
Micrurus frontalis-BR-SP	Triadal + Bicolored	6.8	42.4	49.2	0.96	0.4	1.3	0.06			
Micrurus helleri-CO	Triadal + Bicolored	26.53	14.1	40.63	13.1	8.64	2.2	0.58	5.24		15.52
Micrurus helleri-EC	Triadal + Bicolored	54.3	17.8	72.1		5.1	0.5				4.5

Micrurus ibiboboca	Triadal + Bicolored	53	13.5	66.5	2.5	2.8	9.4	0.8			3.4
Micrurus lemniscatus	Triadal+ Bicolored	14.95	34.33	19.38	4.93	3.48	11.69	2.59	11.43	9.32	2.86
Micrurus medemi	Triadal + Bicolored	25.45	17.69	43.14	9.63	7.48	5.35	7.83	0.39		8.5
Micrurus mipartitus-CO	Triadal + Bicolored	32.1	61.1	29	1.6	4	1.9	1.1	1.3		0
Micrurus mipartitus-EC	Triadal + Bicolored	44.3	63.4	19.1	6	8.4					3.1
Micrurus multifasciatus	Triadal + Bicolored	74.8	83	8.2	3.6	3.2	1.9				0.3
Micrurus pyrrhocryptus	Triadal + Bicolored	10	27	17	12	5	5	7	7	2	10
Micrurus ruatanus	Triadal + Bicolored	16.6	46.4	29.8	2.7	3.4	10.6	2	1.2	0.4	0.5
Micrurus sp-AL	Triadal + Bicolored	31.7	26.1	57.8	1.8	2	5.6	2		0.38	3.0
Micrurus sp-CO	Triadal + Bicolored	41.4	21.1	62.5			5.9	0.5			0
Micrurus spixii	Triadal + Bicolored	54.56	9.48	64.04	6.57	4.78	0.74	7.37		3.73	3.27
Micrurus spixii-BR-PA	Triadal + Bicolored	19.1	56.5	37.4		5.1	1				
Micrurus sp-RJ	Triadal + Bicolored	94.1	94.1	0		0.07	0.98				0.97
Micrurus surinamensis-BR-RO	Triadal + Bicolored	91.2	95.4	4.2		0.4					
Micrurus surinamensis-PA	Triadal + Bicolored	83.54	90.1	6.56	0.11	0.19	2.55	0.32			0.19
Micrurus surinamensis-TO	Triadal + Bicolored	3.86	36.84	32.98	1.23	0.32	25.26	1.66			1.7
Micrurus tschudii	Triadal + Bicolored	91.1	95.2	4.1		0.7	1.6				0

AL (Alagoas, Brazil), B (Bauru, Brazil), BR (Brazil), CO (Colombia), EC (Ecuador), GO (Goias, Brazil), J (Jau, Brazil), PA (Para, Brazil), RJ (Rio de Janeiro, Brazil), RO (Roraima, Brazil), SP (São Paulo, Brazil), TO (Tocantins, Brazil), UNK (Unknown exact location). *M. sp.*-CO: A specimen of *Micrurus aff. lemniscatus,* but with a significant genetic distance from its congeners; see Hurtado-Gomez [13] for details.

Several studies have suggested a compositional dichotomy in the venom of *Micrurus*, in which the dominant toxin (either PLA_2 or 3FTx) alternates between species across a geographic spectrum [9,11]. However, such a dichotomy is not evident when we map the variation of venom composition across the geographic range of *Micrurus* (Figure 3).

Latitudinal geographic location alone thus appears to be a poor predictor of a predominance of 3FTx or PLA_2 in their venom proteomes. However, some species have consistently higher levels of 3FTx compared with PLA_2 and vice versa, and this might be explained by their phylogenetic relationships or ecological convergences. To assess whether the predominance of 3FTx/PLA_2 is determined by phylogenetic signal, we mapped the proportions of each toxin in the venom proteome to the species phylogeny (Figure 4). As a result, we did not observe any clear pattern of 3FTx/PLA_2 predominance across the phylogeny of coralsnakes. Both the triadal and the monadal clades have species with venom proteomes dominated by either 3FTx or PLA_2. However, most species possess a balanced ratio between both toxins, with medium proportions of each one. This balanced phenotype is the most common in both clades, and extreme phenotypes, in which one toxin dominates, have emerged in specific groups within both clades, regardless their geographical origin.

This might indicate that ecological factors, perhaps related to diet, microhabitat use, abiotic features, climate, or other environmental variables could be determining the dominant toxins in these snakes. Additional ecological studies could help clarify the evolutionary origins of the observed variation in the venoms of *Micrurus*. Moreover, a recent work reported elevated proportions of Snake Venom Metalloproteinases (SVMPs) in two Colombian species of the genus [27]. These toxins are commonly associated with viper venoms and are linked with local effects (e.g., bleeding, edema, necrosis) not normally observed with coral snake envenomations [2,10,28]. These findings are particularly relevant to the diagnosis of coral snake envenoming, given that their bites may occasionally present with mild bleeding or local manifestations that could be mistaken for other types of envenomation (e.g., bothropic; see Chapter 9), which may lead to adverse clinical consequences.

In summary, available proteomic information for *Micrurus* venoms reveals that our knowledge of the factors driving their compositional variance remains scarce and much compositional variation is likely yet to be discovered (e.g., some coralsnakes might have higher than expected proportions of SVMPs in their venoms). Studies with increased sampling of coralsnakes that occupy diverse environments are needed to improve our understanding of the compositional range of coral snake venoms and their local medical implications (see Chapters 5, 6, and 9).

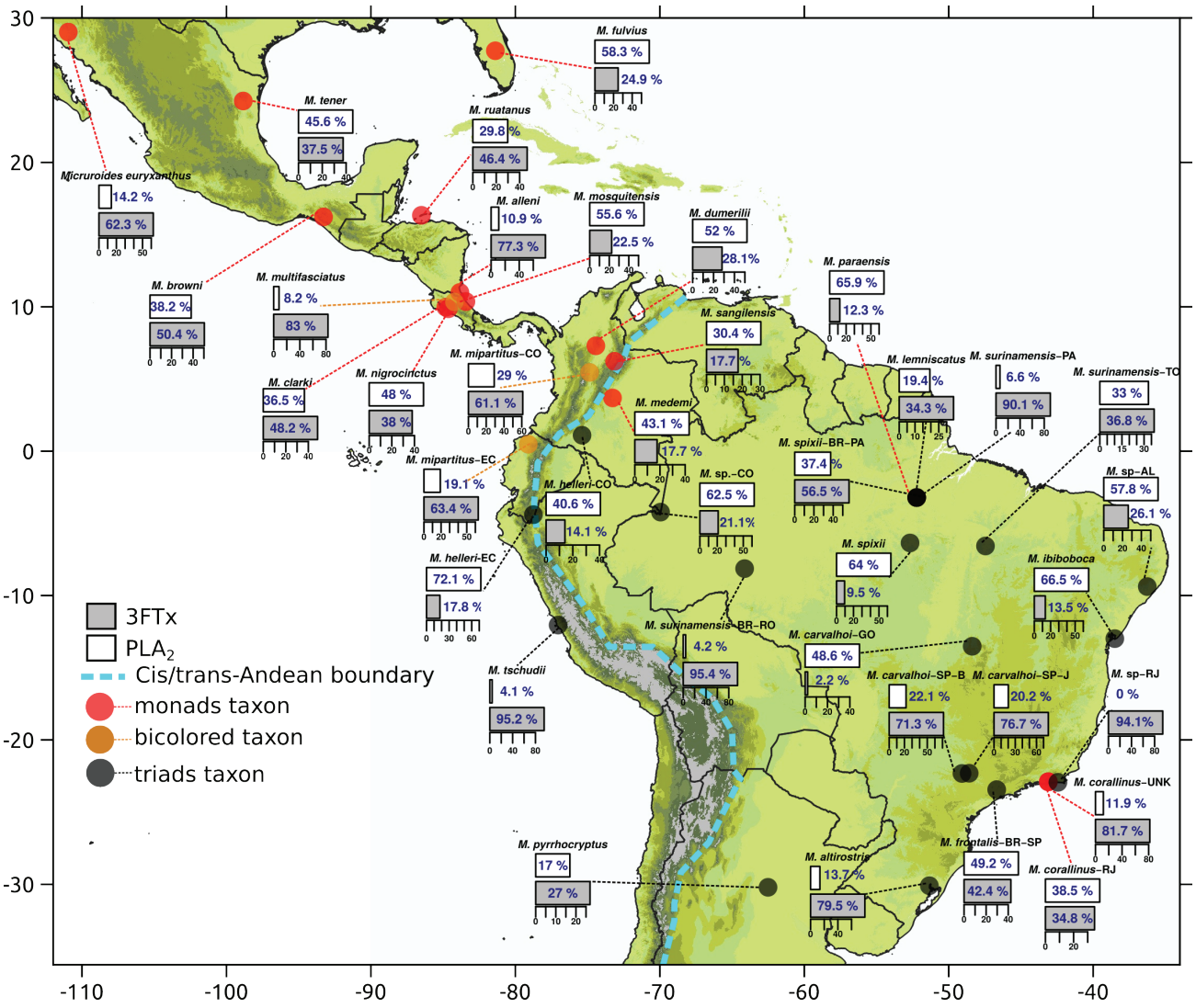
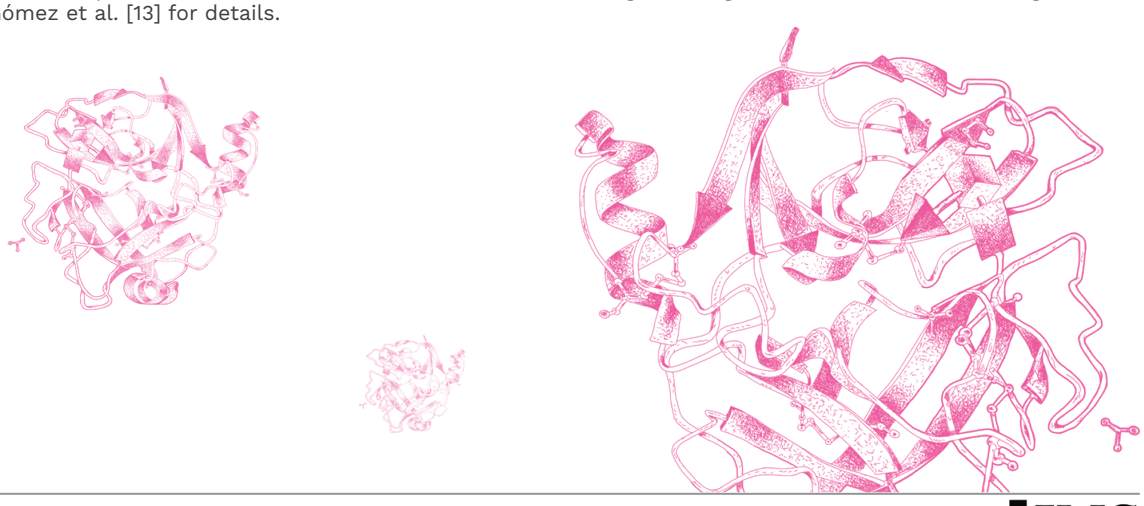


Figure 3. Geographic distribution of *Micrurus* samples with available venom proteomes. 3FTx and PLA_2 proportions are shown for each sample. Color patterns are indicated by colored points above the geographic location of each sample. AL (Alagoas, Brazil), B (Baurú, Brazil), BR (Brazil), CO (Colombia), EC (Ecuador), GO (Goiás, Brazil), J (Jaú, Brazil), PA (Pará, Brazil), RJ (Rio de Janeiro, Brazil), RO (Roraima, Brazil), SP (São Paulo, Brazil), TO (Tocantins, Brazil), UNK (Unknown exact location). *M. sp.*-CO: A specimen of *Micrurus aff. lemniscatus,* but with a significant genetic distance from its congeners; see Hurtado-Gómez et al. [13] for details.



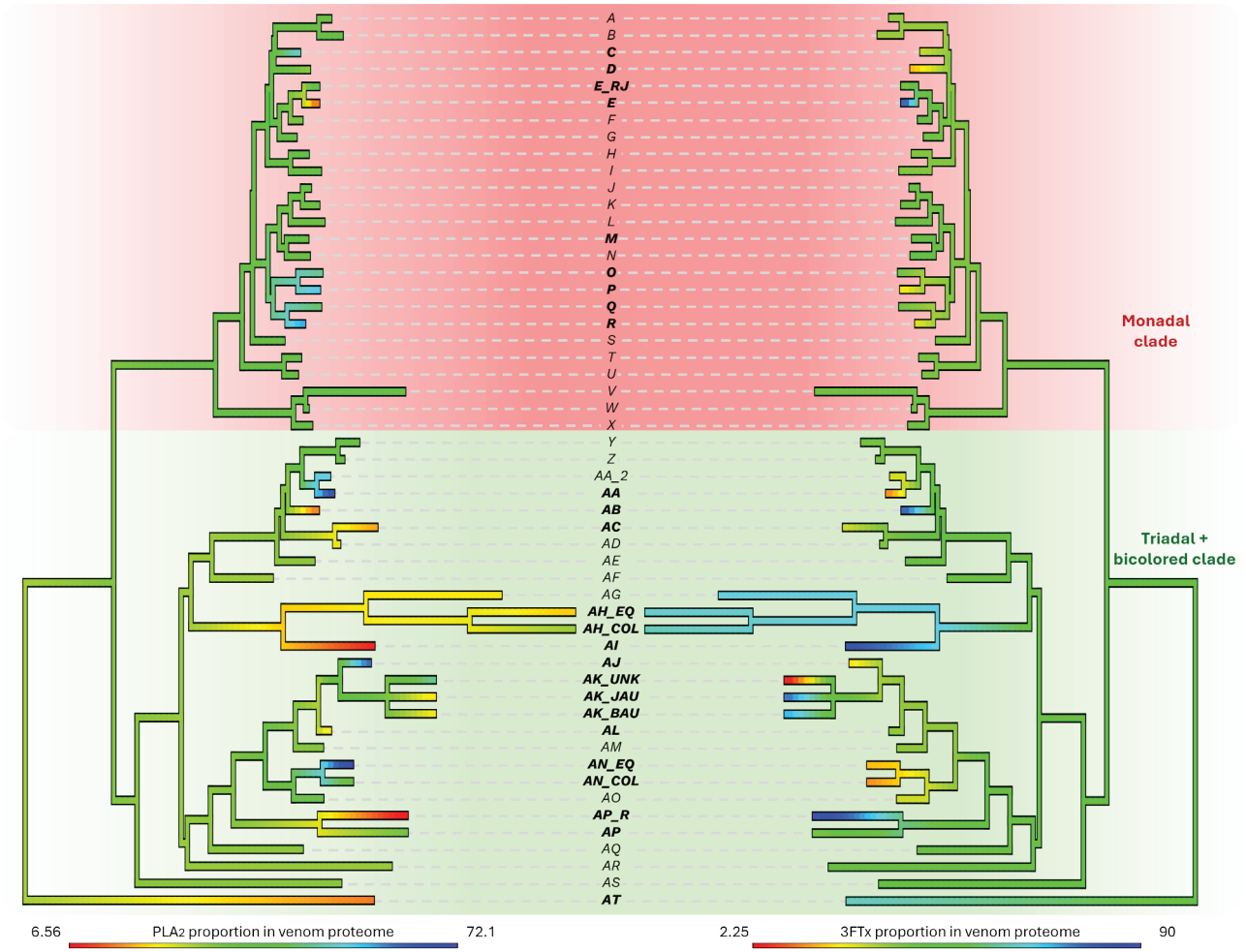


Figure 4. The phylogenetic distribution of the proportions of 3FTx/PLA_2 in the venom proteomes of coral snakes. Terminal branches with names in bold font correspond to species with published proteomic data. All others were estimated using the anc - ML function of the phytools package in R (see methods). Blue branches indicate higher proportions of each of the toxins (PLA_2 on the left and 3FTx on the right). AL (Alagoas, Brazil), B (Bauru, Brazil), BR (Brazil), CO (Colombia), EC (Ecuador), GO (Goias, Brazil), J (Jau, Brazil), PA (Para, Brazil), RJ (Rio de Janeiro, Brazil), RO (Roraima, Brazil), SP (São Paulo, Brazil), TO (Tocantins, Brazil), UNK (Unknown exact location). M. sp.-CO: A specimen of *Micrurus aff. lemniscatus* but with a significant genetic distance from its congeners see Hurtado-Gomez et al. [13]. Codes for species are as follow: *M. psyches* (A), *M. circinalis* (B), *M. dumerilii* (C), *M. sangilensis* (D), *M. corallinus* (E), *M. albicintus* (F), *M. ornatissimus* (G), *M. mertensi* (H), *M. bocourti* (I), *M. ephippifer* (J), *M. apiatus* (K), *M. diastema* (L), *M. borwni* (M), *M. nebularis* (N), *M. nigrocinctus* (O), *M. mosquitensis* (P), *M. tener* (Q), *M. fulvius* (R), *M. eleganselegans* (S), *M. stewarti* (T), *M. alleni* (U), *M. oliveni* (V), *M. distans* (W), *M. michoacanensis* (X), *M. frontalis* (Y), *M. brasiliensis* (Z), *M. ibiboboca* (AA), *M.altirostris* (AB), *M. pyrrhocryptus* (AC), *M. baliocoryphus* (AD), *M. obscurus* (AE), *M. decoratus* (AF), *M. dissoleucus* (AG), *M. mipartitus* (AH), *M. multifasciatus* (AI), *M.* sp (AJ), *M. carvalhoi* (AK), *M. lemniscatus* (AL), *M. filiformis* (AM), *M. helleri* (AN), *M. diutius* (AO), *M. surinamensis* (AP), *M. ortoni* (AQ), *M. narducci* (AR), *M. laticollaris* (AS), *M. euryxanthus* (AT).

4. Aposematism and coralsnake mimicry

Coralsnakes are often brightly colored and seem to defy expectations that camouflage is the main driver of the coloration of non-social animals. The function (i.e., the effect that selected for the evolution and maintenance of a trait) of these unusual colors has been the subject of scientific inquiry for over 150 years [18,30]. The function of the color patterns of coralsnakes has long been assumed to be aposematic, meaning that their color patterns are thought to serve as warning signals that advertise their danger to predators [43,44]. False coralsnakes have also been assumed to have similar color patterns because a resemblance to highly venomous coralsnakes is thought to protect them from predators, which confuse false coralsnakes with more dangerous coralsnakes [35,45,50]. These assumptions are widespread among biologists, especially those engaged in evolutionary ecology studies of coralsnakes and false coralsnakes [29].

A recent meta-analysis has shown that aposematism and mimicry in coralsnakes are supported by most studies since the 1980s despite gaps in evidence [30], and a recent meticulous analysis of aposematism and mimicry in coralsnakes and false coralsnakes has shown that the theoretical and empirical basis of these ideas has serious problems [31], suggesting that these longstanding assumptions merit reconsideration.

In light of this lack of consensus, we provide a field herpetologist perspective on this controversy. We highlight some of the main unresolved problems and possible alternative explanations for the functions of coral snake color patterns. In contrast to a recent review by Almeida et al. [29], we take a more critical perspective on past work and concur with Mouy [31] that there is a need for increased focus on alternative explanations for the functions of coral snake color patterns.

4.1 Summary of the evidence for aposematism and coral snake mimicry

Observations of avoidance of coralsnake color patterns by predators are considered by proponents of aposematism and coral snake mimicry to provide some of the main evidence [29,30]. Predator avoidance has been inferred experimentally using field experiments with artificial prey. In these experiments, replicas with coral snake color patterns and non-coral snake color patterns are placed in natural habitats, where they are exposed to free-ranging predators [32-42]. After a certain period of time, the replicas are scored as attacked or not based on the presence of tooth or beak marks, and these data are then used to infer the relative attack rates of the predator community on coral snake color patterns and thus the degree to which these patterns are protected from predation [32-42].

These experiments have been conducted in various regions of the Americas and have often shown that the frequency of tooth and beak marks on replicas with coralsnake color patterns is much lower than that on replicas with non-coralsnake color patterns, and this has been interpreted as evidence that coralsnake color patterns provide protection from predation by

avian and mammalian predators [32-38]. Studies of coralsnake color patterns using artificial prey have not yet been conducted in Colombia.

Avoidance of coralsnake color patterns has also been inferred through laboratory experiments. Young turquoise-browed motmots (*Eumomota superciliosa*) and great kiskadees (*Pitangus sulphuratus*) from Costa Rica raised in captivity were presented with dowels painted with coralsnake color patterns, and they were reported to display strong negative reactions to dowels painted with coralsnake color patterns [43,44]. However, these negative reactions were not observed when these birds were presented with dowels painted with single colors, rings with non-coralsnake colors, and stripes with coral snake colors. As these birds had no prior exposure to coral snake color patterns, the aversive reactions observed have been widely cited as evidence of innate avoidance of coralsnake color patterns [45,46].

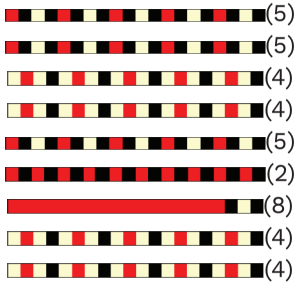
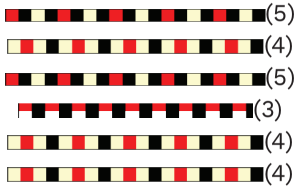
Correlations in color patterns between coralsnakes and false coralsnakes have also been suggested to provide support for aposematism and mimicry hypotheses. The color patterns of various coralsnake and false coralsnake species have been shown to vary geographically in parallel with each other. For example, *Erythrolamprus guentheri* in southeastern Ecuador and Peru have color patterns that resemble *Micrurus langsdorffi* and *Micrurus steindachneri*, which have monadal patterns with red and black rings of the same width separated by white rings [45]. However, at localities in Peru where it co-occurs with the bicolor *Micrurus margaritiferus*, which have bicolor patterns with speckled white rings, some individuals of *E. guentheri* bear this same pattern [45].

Similarities in the color pattern of *Oxyrhopus vanidicus* with local coralsnakes (i.e., *Micrurus hemprichii*) have been observed in Colombia on the eastern slopes of the Cordillera Oriental as well as on high savannah plateaus of the Orinoquia ecoregion (pers. obs. J.D. Lynch and Teddy Angarita-Sierra). No studies examining correlations in color patterns between false coralsnakes and coralsnakes have been conducted in Colombia; the high diversity in shared color patterns among several coralsnakes and false coralsnakes in the cis-Andean and trans-Andean regions suggests that their color patterns might be correlated in Colombia (Table 2). The study of Bosque et al. [47] showed that the color patterns and geographic distributions of red and black banded *Oxyrhopus* species and coralsnakes in South America is associated with the presence and richness of *Micrurus* species with the same color patterns.

Variation in the color patterns of *Atractus latifrons* and *Erythrolamprus aesculapii* has been shown to be associated with the distributions of local *Micrurus* species [48], and the color patterns of various triadal *Micrurus* species in Brazil have been shown to be correlated [49]. Another study integrating distribution and phylogenetic data showed that the evolution of coral snake color patterns among all New World coralsnakes and false coralsnakes is correlated in space and time; specifically, the evolution of coral snake color patterns in false coralsnakes only occurred after the arrival of coralsnakes to the New World [50].

Table 2. Coralsnakes of Colombia with cis-Andean and trans-Andean distributions and their co-occurring false coralsnakes sharing similar patterns. Color pattern types (following scheme adapted from Mouy [31]) are indicated in parentheses.

Coralsnakes	Color pattern type	False coralsnakes	Color pattern type
Cis-Andean distribution			
Micrurus hemprichii	(6)	*Oxyrhopus vanidicus*	(6)
Micrurus ortoni	(6)	*Rhinobothryum lentiginosum*	(4)
Micrurus surinamensis	(6)	*Hydrodynastes bicinctus*	(5)
Micrurus nattereri	(6)	*Hydrops triangularis*	(3)
		Oxyrhopus melanogenys	(6)
Micrurus psyches	(1)	*Hydrops martii*	(4)
	(4)	*Oxyrhopus petolarius*	(2)
Micrurus langsdorffi	(4)	*Oxyrhopus occipitalis* (juvenile)	(1)
Micrurus narduccii	(9)	*Ninia atrata*	(9)
Micrurus renjifoi	(9)	*Ninia hudsoni*	(9)
Micrurus scutiventris	(9)		
Micrurus medemi	(1)	*Oxyrhopus petolarius*	(2)
	(2)	*Atractus elaps*	(5)
	(4)		(6)
			(7)
Micrurus filiformis	(6)	*Atractus elaps*	(5)
Micrurus helleri	(6)		(6)
Micrurus isozonus	(6)		(7)
Micrurus lemniscatus	(6)	*Atractus latifrons*	(5)
Micrurus obscurus	(6)	*Erythrolamprus bizona*	(5)
		Erythrolamprus aesculapii	(5)
Micrurus tikuna	(4)	*Atractus elaps*	(5)
			(6)
			(7)
		Anilius scytale	(2)
		Erythrolamprus aesculapii	(5)
		Oxyrhopus petolarius	(2)
		Pseudoboa coronata	(8)

Trans-Andean distribution			
Micrurus dumerillii	 (4) (6)	*Atractus obesus* *Erythrolamprus bizona* *Erythrolamprus pseudocorallus* *Erythrolamprus mimus* *Lampropeltis micropholis* *Oxyrhopus leucomelas* *Pseudoboa neuwiedii* *Scaphiodontophis annulatus* *Scaphiodontophis venustissimus*	 (5) (5) (4) (4) (5) (2) (8) (4) (4)
Micrurus camilae	 (3)	*Erythrolamprus bizona* *Oxyrhopus petolarius* *Pseudoboa neuwiedii*	 (5) (2) (8)
Micrurus clarki *Micrurus nigrocinctus*	 (4) (4)	*Erythrolamprus bizona* *Oxyrhopus petolarius* *Pseudoboa neuwiedii*	 (5) (2) (8)
Micrurus dissoleucus	 (6)	*Erythrolamprus bizona* *Erythrolamprus pseudocorallus* *Psuedoboa neuwiedi*	 (5) (4) (8)
Micrurus mipartitus	(1)	*Atractus erythromelas* *Atractus multicinctus* *Oxyrhopus leucomelas* *Urotheca euryzona*	 (2) (2) (1) (3)
Micrurus multiscutatus *Micrurus oligoanellatus* *Micrurus spurrelli*	 (2) (2) (2)	*Atractus multicinctus*	(2)
Micrurus sangilensis	(4)	*Erythrolamprus bizona* *Erythrolamprus pseudocorallus* *Lampropeltis micropholis* *Oxyrhopus leucomelas* *Scaphiodontophis annulatus* *Scaphiodontophis venustissimus*	 (5) (4) (5) (3) (4) (4)

Pattern 1: Bicolor with alternating black and white rings; Pattern 2: Bicolor with alternating red and black rings; Pattern 3: Alternating red and black dorsally and alternating white and black ventrolaterally; Pattern 4: Monadal with red rings separated from black rings; Pattern 5: Monadal with red rings separated from white/yellow rings; Pattern 6: Triadal with red rings separated by three black rings and two white/yellow rings; Pattern 7: Tetradal with red rings separated by four black rings and three white/yellow rings; Pattern 8: Red body with white/yellow nuchal collar between two black rings; Pattern 9: Black body with white/yellow nuchal collar.

4.2 Outstanding issues

A robust, unified framework with clear criteria for determining whether coral snake color patterns have aposematic and mimetic functions is lacking. Consequently, aposematic and mimetic functions of coral snake patterns are assumed a priori in many studies. Although the aforementioned support for aposematism and coralsnake mimicry comprises evidence that is consistent with aposematic and mimetic functions, this evidence is not a perfect match with theory.

What we consider to be the main problems with the aposematism and mimicry hypotheses in coralsnakes and false coralsnakes are the inconsistencies between the putative characteristics of prototypical aposematic and mimetic species [46], and the natural history and behavior of these snakes [24]. For aposematism and mimicry to be effective defenses in coralsnakes and false coralsnakes, the color patterns of coralsnakes must be perceived by their avian and mammalian predators (i.e., predators can actually see the colors) and they should be well defended (i.e., coralsnakes are not profitable prey). In the case of learned avoidance, interactions of these predators with coralsnakes should be sufficiently frequent for predators to learn that coralsnakes are unprofitable and for the learning to be reinforced [46], which means that coralsnakes should occur in microhabitats/time periods in which they would be frequently exposed to their predators to advertise their danger. If these assumptions are not met, aposematism as an explanation for warning coloration becomes untenable. Our own observations suggest that these assumptions are not all met for coralsnakes

We find that coralsnakes are not frequently active or not readily observable when active on the surface and seem to spend much of their time in microhabitats that are inaccessible to mammalian and avian predators (e.g., under fallen objects or underground). We also find that most coralsnakes are highly inoffensive and reluctant to bite, and this is consistent with observations made by other coralsnake researchers [6,11,51]. This might contribute, at least in part, to explaining the very low incidence of snakebite accidents caused by coralsnakes in Colombia (<1%) and several other countries in the Americas (see Chapter 9). Although some coralsnakes are active by day, many are active at night or twilight conditions when the perception of their warning color patterns by avian predators would be compromised [6]. Most coralsnakes attempt to flee after we first find them, and many engage in erratic movements after being handled; such behaviors would not be expected for well-defended, aposematic species.

Another serious problem with aposematism and mimicry as explanations of coralsnake color patterns is the frequent occurrence of false coralsnakes in allopatry with coralsnakes, including some “mimic” species that occur far outside the range of any coralsnake [52]. Several explanations have been proposed to explain the presence of allopatric mimics [52]. These basically either propose that allopatric mimics are not really mimics (e.g., the functions of the color patterns of allopatric mimics differ in allopatry and sympatry with coralsnakes) or that mimicry can occur but via highly unreasonable means (e.g., generalized avoidance).

We have observed no differences in the defensive behavior nor activity patterns of false coralsnakes in sympatry and allopatry with coralsnakes that would suggest any difference in the defensive function of their color patterns. We are also not aware of any studies that have demonstrated a generalized avoidance of coral snake color patterns by any predator, much less how such an avoidance could be genetically programmed (see discussion in Mouy [31]). The existence of allopatric coralsnakes and false coralsnakes with the same color patterns is also difficult to explain by aposematism and mimicry. Perhaps the most striking case is that of *Micrurus camilae* (endemic to Colombia), *Scolecophis atrocinctus* (Central America: Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica), *Siphlophis cervinus* (trans and cis-Andean regions of tropical South America), *Lygophis elegantissimus* (endemic to Argentina), and *Sonora semiannulata* (United States and Mexico) [31].

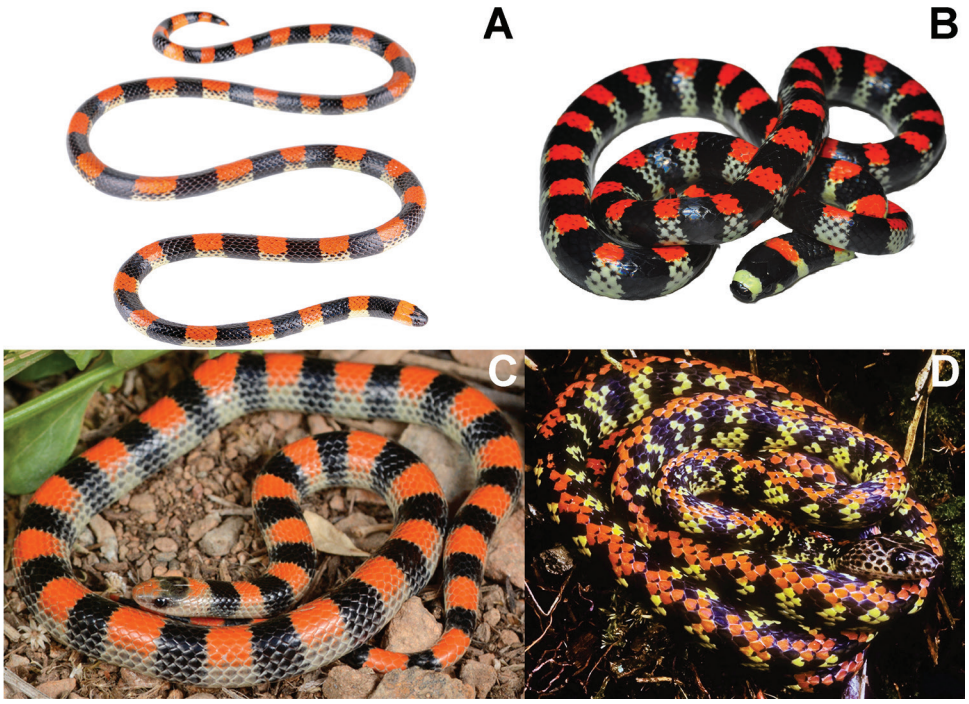


Figure 5. Geographically distant coralsnakes and false coralsnakes with a similar color pattern (pattern 3 in Figure 1). (A) *Micrurus camilae*, photo by José Viera | Ex-situ project. (B) *Scolecophis atrocinctus*, photo by Antonia Pachmann. (C) *Sonora semiannulata* photo by Ben Thesing. (D) *Siphlophis cervinus*, photo by William W. Lamar.

The extensive variation and polymorphism in coralsnake color patterns are difficult to explain under aposematism and mimicry, as diversity in color patterns would only increase the difficulty for predators to learn or acquire aversion of any particular color pattern. This, coupled with the low frequency with which coralsnakes and false coralsnakes are likely encountered by avian and mammalian predators, indicates that the color pattern diversity has an alternative explanation.

The evolution of highly complex color patterns is also problematic to explain via aposematism and mimicry, which would seem to favor the evolution of simple color patterns that would facilitate learning or the evolution of innate recognition. Curiously, some of the most conspicuously colored coralsnakes (e.g., *Micrurus distans*, *Micrurus averyi*) and false coralsnakes (e.g., *Drepanoides anomalus*, *Oxyrhopus occipitalis*) with per-

haps the simplest and easily learnable signals are chiefly nocturnal when their signals would be least visible to mammalian and avian predators.

Several studies have not found any evidence of avoidance of coralsnake color patterns compared with non-coralsnake color patterns. For example, some artificial prey studies employing plasticine replicas have found no evidence of a significant reduction in the number of tooth and beak marks on replicas with coral snake color patterns compared with replicas without coral snake color patterns [39-42]. Coatis (*Nasua narica*) exposed to live *Micrurus nigrocinctus* in a laboratory experiment exhibited no negative responses or aversion to coral snake color patterns [53]. A recent study in which plasticine models of various snake patterns, including coral snake color patterns, were monitored with camera traps showed that most of the predators that left marks on the replicas were not vertebrate predators; furthermore, the few vertebrate predators that attacked the repli-cas did not behave in a manner consistent with observations of interactions with natural prey, which suggests that the conclusions of previous studies employing artificial prey techniques should be called into question [54].

Although coralsnakes have been documented to be preyed upon by various mammalian and avian predators [55-58], none of these documented predators would likely be strong agents of selection for coralsnake color patterns. For example, some predators, such as *Herpetotheres cachinnans* (laughing falcon), can consume any snake, even more well-defended ones such as *Bothrops* and *Crotalus* species [59]. Other predators that are not considered snake specialists, such as *Buteo jamaicensis* (red-tailed hawk), are also capable of consuming well-defended snakes, such as rattlesnakes of the genus *Crotalus* [60]. The overall rarity of observations of predation of coralsnakes by their widely assumed selective agents (mammals and birds) for aposematism and mimicry over the last 100 years further suggests that they are not major selective agents for coral snake color patterns. Rather than dismissing these findings and observations as innocent exceptions, the possibility that herpetologists have long been mistaken about the function of coral snake color patterns merits serious consideration.

4.3 Aposematism and coral snake mimicry alternatives

Several alternative hypotheses to aposematism and coral snake mimicry have been proposed, but most have not been widely cited nor critically examined. One proposal is that the color patterns of coralsnakes mimic other organisms, such as millipedes, or that coralsnakes are mimicking mildly venomous false coralsnakes, such as *Erythrolamprus* species [45,61]. A mimetic relationship between snakes and millipedes seems unlikely given that most vertebrate predators could probably easily distinguish between them. Moreover, a resemblance between millipedes and snakes with coral snake color patterns is not a widespread phenomenon in the Americas. Coralsnakes are also unlikely mimics of mildly venomous snakes given that coralsnakes can be equally “mild” to predators in the sense that they are incapable of reliably delivering a deadly bite (see Chapter 5). Coralsnakes also occur in many areas without mildly venomous “models.”

The flicker-fusion hypothesis proposes that the rings of snakes' aid escape from predators by forming a motion blur when the rate of transition of their rings exceeds the flicker-fusion rate of predator eyes; this can cause snakes to appear stationary while moving, which can reduce the success of capture of snake prey by predators [62]. Although the flicker-fusion hypothesis sounds plausible in that it could potentially provide protection against certain predators, it provides no explanation for the complexity and bright colors of snakes with coral snake color patterns.

Another alternative is that the color patterns serve a direct protective function [63]. How these color patterns might function in this manner against mammalian and avian predators remains unclear. Mouy [64] proposed a novel hypothesis for explaining the evolution of coral snake color patterns, the "ophiophagy hypothesis," which proposes that the rings and colors of coralsnakes and false coralsnakes serve a direct protective function against snake predators. In support of this idea, Mouy [64] suggested that predatory pressure on snakes in the Americas by ophiophagous snakes might be disproportionately high compared with ophiophagous non-snake predators (i.e., birds and mammals). He then conducted an analysis of snakes with specialized diets of elongated vertebrates and snakes with red and rings and found that there was an association between ophiophagy, red coloration, and ringed patterns, suggesting that the evolution of coralsnake color patterns as a defense against ophiophagy is plausible. Another recent study using a different analytical approach also demonstrated an association between foraging strategy and contrasting colorations in elapid and dipsadine snakes [65], which provides additional support for the ophiophagy hypothesis.

Mouy [66] also described a possible mechanism for how coral snake color patterns could thwart predation by ophiophagous snakes. He proposed that rings interfere with the focusing ability of snakes, which have rigid ball lenses, and used a series of simulations to show that spherical aberrations generated by ball lenses can produce local maxima in contrast that would impede the lens from bringing the rings into focus. He further proposed that the function of red in coral snake color patterns is to restrict the vision of snakes in low light environments to rods (photoreceptor cells), which provide a less temporally and spatially accurate vision.

If these functions of rings and red are assumed, the diversity and complexity of coral snake color patterns becomes relatively straightforward to explain. Snakes with lots of red (e.g., *Pseudoboa neuwiedii*, juvenile *Clelia clelia, Drepanoides anomalus,* and *Oxyrhopus occipitalis*) would likely be preyed upon by nocturnal ophiophagous snakes that rely heavily on rod vision. This is consistent with our observations that these snakes are exclusively nocturnal and relatively fast-moving, which would be particularly important for evading predators with a slow rod-based vision vulnerable to saturation (i.e., the failure of rods to respond beyond certain levels of illumination).

These snakes also appear to avoid moving in open areas under moonlight, which makes sense given that greater light levels could potentially reduce the efficacy of red as a defense. By contrast, snakes with lots of

rings (e.g., triadal snakes) are likely preyed upon by ophiophagous snakes with various types of vision, as their patterns would provide protection both through the effect of rings and red. Consistent with this, many of the triadal snakes (e.g., *Micrurus elegans*) have more variable activity patterns and have less red at high elevations (>1000 m above sea level) when temperatures are only conducive to diurnal activity (C.K. Akcali pers. obs.).

To provide preliminary insights into whether coralsnakes would be vulnerable to spherical aberrations, we scanned the eye of a coral snake specimen (*Micrurus mipartitus*) using an optical coherence tomography system (Figure 6).

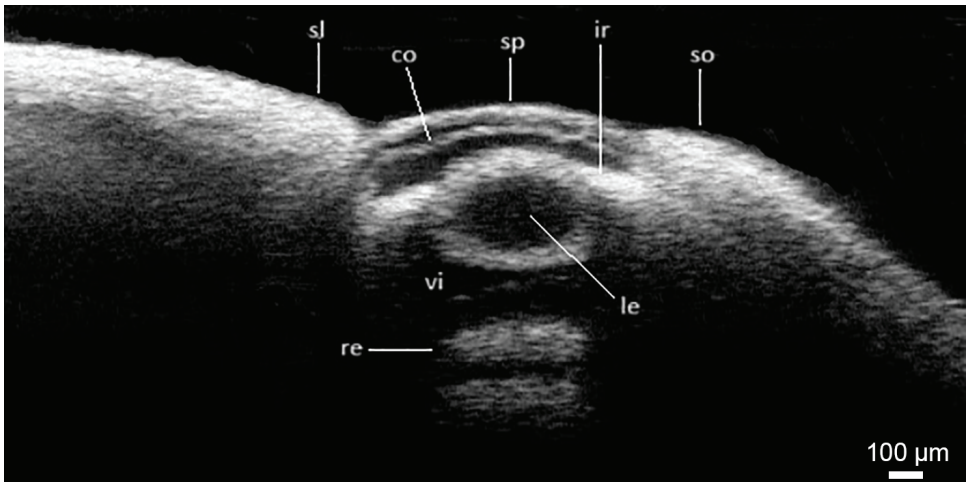


Figure 6. Averaged optical coherence tomography B-scan of the left eye of *Micrurus mipartitus* (Panama, Bocas del Toro, Isla San Cristobal; USNM 348250) along the dorsal–ventral axis. sl: supralabial scale; so: supraocular scale; sp: spectacle; co: cornea; ir: iris; le: lens re: retina, vi: vitreous. Horizontal spectacle diameter: 1.38 mm; Equatorial lens diameter: 0.765 mm.

The scan revealed that the anterior surface of the lens of *M. mipartitus* was subspherical as has been observed in other snakes [67], which indicates that the eyes of *M. mipartitus* would in fact be vulnerable to experiencing spherical aberrations. Detailed studies of the eye morphology and visual systems of coralsnakes and false coralsnakes are needed to determine the extent to which coralsnake color patterns could prove effective as direct defenses against snake predators.

Although much work is needed to evaluate the ophiophagous hypothesis and possible mechanisms for how coralsnake color patterns could provide a protective function, the explanatory power of the framework proposed by Mouy [64,66] is immensely greater than that of all other explanations for the evolution of coralsnake color patterns proposed to date, including aposematism and mimicry. It provides compelling explanations for the diversity and complexity of coral snake color patterns and is free of many of the traditional problems of aposematism and mimicry.

It can also explain various curiosities that alternatives provide no explanation for, including: (1) why certain color patterns, such as red and white rings without any black rings, have never evolved (the proposed function requires contrasting rings, and red and white rings might not provide sufficient contrast under most lighting conditions); (2) why white/yellow rings are rarely more than 50% of the body unlike red (the exclusive

purpose of white/yellow is to contrast with black, whereas that of red is to target rods; thus, the function of red is decoupled from that of black rings unlike white/yellow rings); and (3) why false coralsnakes show more pattern irregularities than coralsnakes and have lost coralsnake color patterns more frequently (greater variation in the strength of ophiophagy as a selective force likely stemming from their more diverse foraging habits). Moreover, the clear predictions proposed by Mouy [66] are testable and permit a direct protective function to be diagnosed.

One final possibility is the proposition that coralsnake colors do not have a single function but perform many functions; that is, the aforementioned explanations for the functions of coralsnake colors are not necessarily mutually exclusive. We think that this is unparsimonious given that it is improbable that the evolution and maintenance of coralsnake colors are driven by several factors. As defined previously, the function of a trait is the effect that selected for the evolution and maintenance of that trait.

Animal colorations can have many effects, like the red coloration of coralsnakes. However, the function of red coloration is only the effect that selected for red, that contributes to increases in redness, and that maintains redness despite selection against it. Although red coloration has several effects, it is unlikely to have several functions; red coloration is a relatively unusual trait, and it is not parsimonious to expect that several effects select for increases in redness. Rather than casting the problem of determining the function of coralsnake color patterns as undecipherably complex because they have many functions, we suggest that biologists focus on identifying the main effect selecting for coralsnake color patterns.

4.4 Conclusion

Aposematism and mimicry are highly complex solutions to the simple problem of avoiding being eaten. The solutions that organisms are prone to evolve in response to this problem in nature tend to be fairly simple (e.g., mammalian herbivores evolve faster flight capabilities to outrun their carnivorous predators, snakes evolve increased venom toxicity to incapacitate their prey more rapidly and prevent counterattacks). The same is likely to be the case for snakes with red and black rings. Over 150 years have passed since the possibility of aposematism and mimicry in coralsnakes was first proposed, yet a single clear case of either of these phenomena in coralsnakes and false coralsnakes is lacking.

Our own field observations and other published observations indicate that coralsnakes and false coralsnakes do not meet many of the putative characteristics of prototypical aposematic and mimetic species, and the existing evidence for aposematism and mimicry in coralsnakes is far from convincing. Rather than assuming an aposematic function for coral snake color patterns, we encourage more research aimed at exploring simpler, alternative solutions with clear testable predictions for the defensive function of coral snake color patterns.

Appendix: Material and Methods

Studies were obtained via weekly searches from January 2021 to March 2024. We performed the searches as follows:

1. Weekly searches of Google scholar
2. Weekly searches of Scielo;
3. Weekly searches of Scopus.

Searches were conducted using the following keywords in different combinations using the Boolean connectors AND OR: snake venoms, *Micrurus*, *Leptomicrurus*, *Micruroides*, transcriptome, proteome, evolution.

Genetic data were retrieved from Genbank for four mitochondrial markers (12s, 16s, cyt b and ND4) and one nuclear marker (C-mos), for 44 species of *Micrurus* (one specimen per species), plus *Micruroides euryxanthus* (1 specimen), and with *Ophiophagus hannah* and *Sinomicrurus macclellandi* as outgroups (Table S1). Sequences for each genetic marker were aligned using the MUSCLE algorithm [68] as implemented in GENEIOUS 9.1.8 [69] with the default parameters.

In IQTree 2.2.0 [70], we calculated simultaneously the best partition scheme (by gene or codon) and evolutionary models (using ModelFinder, [71]); the Maximum Likelihood tree, and node support (using 5000 replicates of UltraFast Bootstrap, [72]). For tree annotation and plotting, we used the R packages 'ape' [73], 'phangorn' [74], and 'phytools' [75] with some posterior edits made using Inkscape 1.3 (www.inkscape.org).

Table S1. Voucher and Genbank accession numbers for the sequences used herein.

Species	Voucher	12S	16S	cyt b	ND4	Cmos
Micrurus albicinctus	MPEG19548				JF308714	
Micrurus alleni	ADM272		KX660164	KX660439	KX660567	KX660303
Micrurus altirostris	MCP5035				AF228431	
Micrurus apiatus	RH52446/M50	Z46454	Z46484	KU754325	KU754434	
Micrurus baliocoryphus	MZUSP10808				AF228433	
Micrurus bocourti	MECN2608				KP998031	
Micrurus brasiliensis	CEPB2301				AF228427	
Micrurus browni	M223			MG947754	MG947664	
Micrurus carvalhoi	CEPB2687				AF228438	

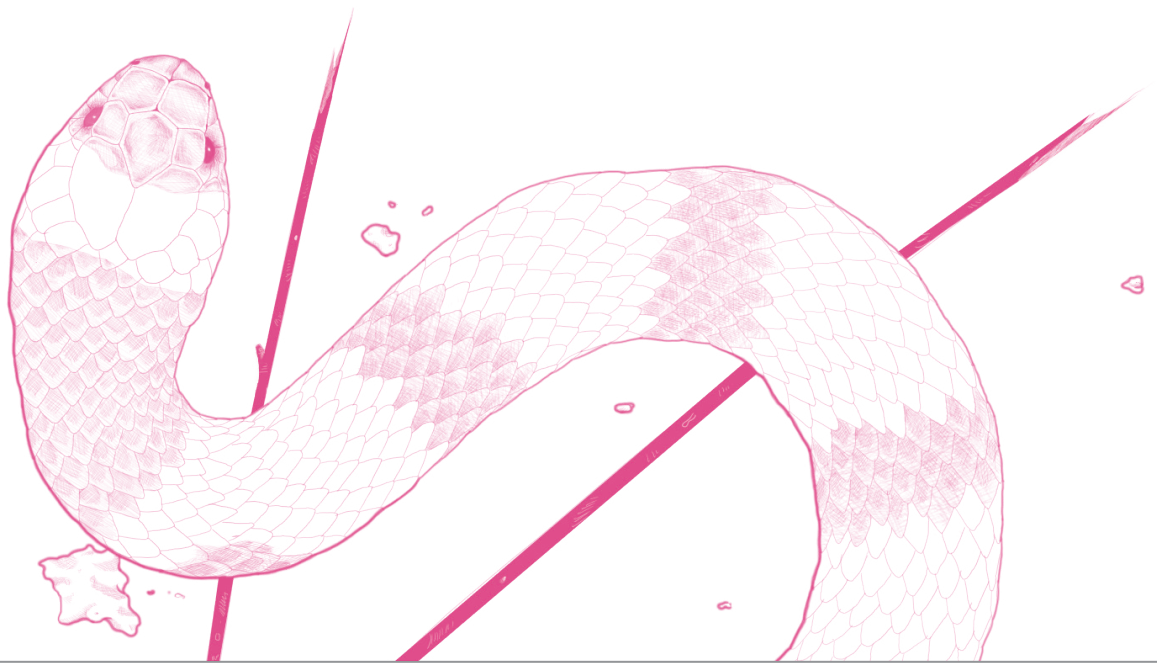
Micrurus cf ibiboboca	IVB1757				AF228436	
Micrurus circinalis	UWIZM2011.19.7	MK534131	MK534141	MK534160	MK534170	MK534151
Micrurus corallinus	USNM253597				JF308715	
Micrurus decoratus	IVB 2453				AF228441	
Micrurus diastema	M299			MG947767	MG947683	
Micrurus dissoleucus	UTAM673				JF308717	
Micrurus distans	M301			MG947768	MG947685	
Micrurus diutius	CAS231787	MK534136	MK534149	MK534168	K534178	MK534158
Micrurus dumerilii	MECN2881				KP998035	
Micrurus elegans	M48			MG947782	MG947710	
Micrurus ephippifer	M336				MG947697	
Micrurus filiformis	ICN11380	MW665121	MT862136		MW662060	MW662077
Micrurus frontalis	CEPB1587				AF228425	
Micrurus fulvius	Mitohondrion	GU045453	GU045453	GU045453	GU045453	
Micrurus helleri	AF4455	MW665123	MW672259	MW662069	MW662067	MW662081
Micrurus ibiboboca	YPX161	YPX161			YPX161	
Micrurus laticollaris	UTAM673				JF308717	
Micrurus lemniscatus	CAS257629	MK534136	MK534150	MK534169	MK534179	MK534159
Micrurus mertensi	CORBIDI14775				KP998037	
Micrurus michoacanensis	M331			MG947774	MG947693	
Micrurus mipartitus	CH5377/ USNM348250		MH140862	MH140283	EF137406	
Micrurus mosquitensis	M12			MG947741	MG947650	
Micrurus narduccii	WED54125			EF137412	EF137404	EF137420

Micrurus nebularis	M341			MG947778	MG947701	
Micrurus nigrocinctus	M120			MG947742	MG947651	
Micrurus obscurus	KU222205				JF308708	
Micrurus oliveri	M632				MG947722	
Micrurus ornatissimus	QCAZ2584				AF228442	
Micrurus ortoni	QCAZ2584				AF228442	
Micrurus psyches	ROM28378				JF308713	
Micrurus pyrrhocryptus	MZUSP10809				AF228434	
Micrurus sp	CEPB2289				AF228439	
Micrurus stewarti	CH5408		MH140870		KX090915	
Micrurus surinamensis	CEPB8984	MN587874	MN587874	MN587874	MN587874	MN587874
Micrurus tener	M200			KU754314	KU754411	
Ophiophagus hannah		NC_011394	NC_011394	NC_011394	NC_011394	KX694798
Micruroides euryxanthus				EF137416	EF137408	EF137423
Sinomicrurus macclellandi		NC_054255	NC_054255	NC_054255	NC_054255	MG653595

The map was produced using QGIS 3.34 [76], and edited using Inkscape 1.3 (www.inkscape.org). Samples were names according to their species, notating individuals from different populations with a an abbreviated code at the end of the sample name as follows: AL (Alagoas, Brazil), B (Bauru, Brazil), BR (Brazil), CO (Colombia), EC/EQ (Equador), GO (Goias, Brazil), J (Jau, Brazil), PA (Para, Brazil), RJ (Rio de Janeiro, Brazil), RO (Roraima, Brazil), SP (São Paulo, Brazil), TO (Tocantins, Brazil), UNK (Unknown exact location).

To determine the relationships between different venom toxin classes, we recovered proteomic data of relevant toxins from published works with coral snake species [9,11,12,77–81]. We transformed the obtained data into percentages to obtain comparable values of the proportion of each toxin class. We then performed an ancestral state reconstruction analysis using the default parameters of the *fastAnc* function implemented in the Phytools package [75] in R software using the phylogeny assembled herein to estimate branch lengths. We built a tree using the *contMap* function in Phytools to display continuous variation in toxin proportions for each node using a color gradient.

The left eye of the *Micrurus mipartitus* specimen (Panama, Bocas del Toro, Isla San Cristobal; USNM 348250) was scanned using an optical coherence tomography scanner (OQ StrataScope, Lumedica Inc., Durham, NC, USA). A total of 30 B-scans were taken of the eye along both the dorsal–ventral and anterior–posterior axes, and both sets of these 30 B-scans were averaged. The equatorial diameter of the lens was measured by taking the average of measurements from the two averaged B-scans using electronic calipers in Lumedica software. Horizontal spectacle diameter of the left eye was measured using handheld digital calipers with the aid of a stereo microscope.



References

1. Uetz, P. The Original Descriptions of Reptiles. *Zootaxa* **2010**, 59–68, doi:10.11646/zootaxa.2334.1.3.
2. Mackessy, S.P. *Handbook of Venoms and Toxins of Reptiles.*; 1st ed.; CRC Press: Boca Raton, **2021**.
3. Zaher H, Murphy RW, Arredondo JC, Graboski R, Machado-Filho PR, Mahlow K, et al. (2019) Correction: *Large-scale molecular phylogeny, morphology, divergence-time estimation, and the fossil record of advanced caenophidian snakes (Squamata: Serpentes)*. PLoS ONE 14(5): e0217959. https://doi.org/10.1371/journal.pone.0217959
4. Tanaka, G.D.; Furtado, M.D.F.D.; Portaro, F.C.V.; Sant'Anna, O.A.; Tambourgi, D. V. Diversity of Micrurus Snake Species Related to Their Venom Toxic Effects and the Prospective of Antivenom Neutralization. *PLoS Negl Trop Dis* **2010**, *4*, e622, doi:10.1371/JOURNAL.PNTD.0000622.
5. Silva Jr., N.J. As Cobras-Corais Do Brasil: Biologia, Taxonomia, Venenos, Envenenamentos. **2016**, pp.415.
6. Roze, J.A. Coralsnakes of the Americas: Biology, Identification, and Venoms. *Coralsnakes of the Americas: biology, identification, and venoms.* **1996**.
7. Bucaretchi, F.; Capitani, E.M. De; Vieira, R.J.; Rodrigues, C.K.; Zannin, M.; Jr, N.J.D.S.; Casais-e-Silva, L.L.; Hyslop, S. Coral Snake Bites (Micrurus Spp.) in Brazil: A Review of Literature Reports. *https://doi.org/10.3109/15563650.2015.1135337* **2016**, *54*, 222–234, doi:10.3109/15563650.2015.1135337.
8. Campbell, J.A.; Lamar, W.W.; Brodie, E.D.; others *The Venomous Reptiles of the Western Hemisphere*; Comstock Pub. Associates Ithaca [NY], **2004**; Vol. 1.
9. Lomonte, B.; Rey-Suárez, P.; Fernández, J.; Sasa, M.; Pla, D.; Vargas, N.; Bénard-Valle, M.; Sanz, L.; Corrêa-Netto, C.; Núñez, V.; et al. Venoms of Micrurus Coralsnakes: Evolutionary Trends in Compositional Patterns Emerging from Proteomic Analyses. *Toxicon* **2016**, *122*, 7–25.
10. Bucaretchi, F.; De Capitani, E.M.; Vieira, R.J.; Rodrigues, C.K.; Zannin, M.; Da Silva, N.J.; Casais-E-Silva, L.L.; Hyslop, S. Coral Snake Bites (*Micrurus* Spp.) in Brazil: A Review of Literature Reports. *Clin Toxicol (Phila)* **2016**, *54*, 222–234, doi:10.3109/15563650.2015.1135337.
11. Sanz, L.; Pla, D.; Pérez, A.; Rodríguez, Y.; Zavaleta, A.; Salas, M.; Lomonte, B.; Calvete, J.J. Venomic Analysis of the Poorly Studied Desert Coral Snake, *Micrurus tschudii tschudii,* Supports the 3FTx/PLA_2 Dichotomy across *Micrurus* Venoms. *Toxins 2016, Vol. 8, Page 178* **2016**, *8*, 178, doi:10.3390/TOXINS8060178.
12. Sanz, L.; Quesada-Bernat, S.; Ramos, T.; Casais-e-Silva, L.L.; Corrêa-Netto, C.; Silva-Haad, J.J.; Sasa, M.; Lomonte, B.; Calvete, J.J. New Insights into the Phylogeographic Distribution of the 3FTx/PLA_2 Venom Dichotomy across Genus *Micrurus* in South America. *J Proteomics* **2019**, *200*, 90–101, doi:10.1016/J.JPROT.2019.03.014.
13. Hurtado-Gómez, J.; Vargas-Ramírez, M.; Ruiz-Gómez, Fouquet, A.; Fritz. U. Multilocus phylogeny clarifies relationships and diversity within the *Salamandra* **2021***, 57, 229-239*.
14. Jowers, M.J.; Smart, U.; Sánchez-Ramírez, S.; Murphy, J.C.; Gómez, A.; Bosque, R.J.; Sarker, G.C.; Noonan, B.P.; Faria, J.F.; Harris, D.J.; et al.

Unveiling Underestimated Species Diversity within the Central American Coralsnake, a Medically Important Complex of Venomous Taxa. *Sci Rep* **2023**, *13*, doi:10.1038/S41598-023-37734-5.

15. Zaher, H.; Grazziotin, F.G.; Prudente, A.L. da C.; Quadros, A.B.A.; Trevine, V.C.; Silva, Jr., N.J. Origin and Evolution of Elapids and New World Coralsnakes. In *Advances in coralsnake biology: With emphasis on south america*; Silva, Jr., N.J., Porras, L.W., Aird, S.D., Prudente, A.L. da C., Eds.; Eagle Mountain Publishing: Goiás, **2021**; pp. 97–113.
16. Silva, Jr., N.J.; Buononato, Marcus, A.; Pires, M.G.; Feitosa, D.T. New World Coralsnakes: An Overview. In *Advances in coralsnake biology: With emphasis on south america*; Silva, Jr.N.J., Porras, L.W., Aird, S.D., Prudente, A.L. da C., Eds.; Eagle Mountain Publishing: Goiás, **2021**; pp. 115–139.
17. Reyes-Velasco, J.; Adams, R.H.; Boissinot, S.; Parkinson, C.L.; Campbell, J.A.; Castoe, T.A.; Smith, E.N. Genome-Wide SNPs Clarify Lineage Diversity Confused by Coloration in Coralsnakes of the *Micrurus diastema* Species Complex (Serpentes: Elapidae). *Mol Phylogenet Evol* **2020**, *147*, 106770, doi:10.1016/J.YMPEV.2020.106770.
18. Wallace, A.R. Mimicry and other protective resemblances among animals. Alfred Russel Wallace Classic Writings **1867**, 8, 1–27
19. Jowers, M.J.; Garcia Mudarra, J.L.; Charles, S.P.; Murphy, J.C. Phylogeography of West Indies Coralsnakes (*Micrurus*): Island Colonisation and Banding Patterns. *Zool Scr* **2019**, *48*, 263–276, doi:10.1111/ZSC.12346.
20. Nascimento, L.R.S.; Graboski, R.; Silva J.R., N.J.; Prudente, A.L.C. Integrative taxonomy of *Micrurus ibiboboca* (Merrem, 1820) (Serpentes, Elapidae) reveals three new species of coral snake. Systematics and Biodiversity **2024**, 22(1). https://doi.org/10.1080/14772000.2024.2315958
21. Zaher, H.; Grazziotin, F.; Prudente, A.L.C.; Siva Jr, N.J. Origem e Evolução Dos Elapideos e Das Cobras-Corais Do Novo Mundo. *As Cobras-corais do Brasil* **2016**, *1*, 25–45.
22. Lee, M.S.Y.; Sanders, K.L.; King, B.; Palci, A. Diversification Rates and Phenotypic Evolution in Venomous Snakes (Elapidae). *R Soc Open Sci* **2015**, *3*, doi:10.1098/RSOS.150277.
23. Zaher, H.; Grazziotin, F.G.; Prudente, A.L. da C.; Quadros, A.B.A.; Trevine, V.C.; Silva, Jr., N.J. Origin and Evolution of Elapids and New World Coralsnakes. In *Advances in coralsnake biology: With emphasis on south america*; Silva, Jr., N.J., Porras, L.W., Aird, S.D., Prudente, A.L. da C., Eds.; Eagle Mountain Publishing: Goiás, **2021**; pp. 97–113.
24. Roze, J.A. Coralsnakes of the Americas: Biology, Identification, and Venoms. *Coralsnakes of the Americas: biology, identification, and venoms.* **1996**.
25. Campbell, J.A.; Lamar, W.W.; Brodie, E.D.; others *The Venomous Reptiles of the Western Hemisphere*; Comstock Pub. Associates Ithaca [NY], 2004; Vol. 1.
26. Lomonte, B.; Rey-Suárez, P.; Fernández, J.; Sasa, M.; Pla, D.; Vargas, N.; Bénard-Valle, M.; Sanz, L.; Corrêa-Netto, C.; Núñez, V.; et al. Venoms of *Micrurus* Coralsnakes: Evolutionary Trends in Compositional Patterns Emerging from Proteomic Analyses. *Toxicon* **2016**, *122*, 7–25, doi:10.1016/J.TOXICON.2016.09.008.

27. Rodríguez-Vargas, A.; Franco-Vásquez, A.M.; Bolivar-Barbosa, J.A.; Vega, N.; Reyes-Montaño, E.; Arreguín-Espinosa, R.; Carbajal-Saucedo, A.; Angarita-Sierra, T.; Ruiz-Gómez, F. Unveiling the Venom Composition of the Colombian Coralsnakes *Micrurus helleri, M. medemi,* and *M. sangilensis*. *Toxins (Basel)* **2023**, *15*, 622, doi:10.3390/toxins15110622.
28. Jorge Da Silva, N.; D. Aird, S. Prey Specificity, Comparative Lethality and Compositional Differences of Coral Snake Venoms. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **2001**, *128*, 425–456, doi:10.1016/S1532-0456(00)00215-5.
29. Almeida et al. In *Advances in Coralsnake Biology: With an Emphasis on South America*; Silva Jr., N.J., Porras, L.W., Aird, S.D., Prudente, A.L.C., Eds.; Eagle Mountain Publishing, LC: Eagle Mountain, USA, **2021**; pp. 291–314.
30. Sena II, A.T.; Ruane, S. Concepts and contentions of coral snake resemblance: Batesian mimicry and its alternatives. *Biological Journal of the Linnean Society* **2022**, *135*, 631–644, doi:10.1093/biolinnean/blab171.
31. Mouy, H. The function of red and banded patterns in snakes: A review of hypotheses and evidence, and of the appearance of a similar selective force outside of the Americas. *Biological Journal of the Linnean Society* **2024**, 142, 351–374, *in press*. doi:10.1093/biolinnean/blad151.
32. Brodie III, E.D. Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. *Evolution* **1993**, , doi:10.1111/j.1558-5646.1993.tb01212.x.
33. Brodie III, E.D.; Janzen, F.J. Experimental studies of coral snake mimicry: Generalized avoidance of ringed snake patterns by free-ranging avian predators. *Functional Ecology* **1995**, *9*, 188–190, doi:10.2307/2390563.
34. Hinman, K.E.; Throop, H.L.; Adams, K.L.; Dake, A.J.; McLauchlan, K.K.; McKone, M.J. Predation by free-ranging birds on partial coral snake mimics: The importance of ring width and color. *Evolution* **1997**, *51*, 1011–1014, doi:10.1111/j.1558-5646.1997.tb03684.x.
35. Pfennig, D.W.; Harcombe, W.R.; Pfennig, K.S. Frequency-dependent Batesian mimicry. *Nature* **2001**, *410*, 323, doi:10.1038/35066628.
36. Buasso, C.M.; Leynaud, G.C.; Cruz, F.B. Predation on snakes of Argentina: Effects of coloration and ring pattern on coral and false coralsnakes. *Studies on Neotropical Fauna and Environment* **2006**, *41*, 183–188, doi:10.1080/01650520600630725.
37. Pfennig, D.W.; Harper, G.R. Jr.; Brumo, A.F.; Harcombe, W.R.; Pfennig, K.S. Population differences in predation on Batesian mimics in allopatry with their model: Selection against mimics is strongest when they are common. *Behavioral Ecology and Sociobiology* **2007**, *61*, 505–511, doi:10.1007/s00265-006-0278-x.
38. Kikuchi, D.W.; Pfennig, D.W. Predator cognition permits imperfect coral snake mimicry. *The American Naturalist* **2010**, *176*, 830–834, doi:10.1086/657041.
39. Akcali, C.K.; Perez-Mendoza, H.A.; Kikuchi, D.W.; Pfennig, D.W. Multiple models generate a geographical mosaic of resemblance in a Batesian mimicry complex. *Proceedings of the Royal Society B: Biological Sciences* **2019**, *286*, 20191519, doi:10.1098/rspb.2019.1519.

40. Sanches, V.Q.A.; Gomes, C.A.B. Aposematic and cryptic snakes are equally attacked at occidental Amazonian Forest. *Herpetology Notes* **2019**, *12*, 1105–1111.
41. Banci, K.R.S.; Eterovic, A.; Marinho, P.S.; Marques, O.A.V. Being a bright snake: Testing aposematism and mimicry in a neotropical forest. *Biotropica* **2020**, *52*, 1229–1241, doi:10.1111/btp.12831.
42. Wilson, L.; Lonsdale, G.; Curlis, J.D.; Hunter, E.; Cox, C.L. Predator-based selection and the impact of edge sympatry on components of coral snake mimicry. *Evolutionary Ecology* **2022**, *36*, 135–149, doi:10.21203/rs.3.rs-493560/v1.
43. Smith, S. Innate recognition of coral snake pattern by a possible avian predator. *Science* **1975**, *187*, 759–760, doi:10.1126/science.187.4178.759.
44. Smith, S. Coral-snake pattern recognition and stimulus generalisation by naive great kiskadees (Aves: Tyrannidae). *Nature* **1977**, *265*, 535–536, doi:10.1038/265535a0.
45. Greene, H.W.; McDiarmid, R. Coral snake mimicry: does it occur? *Science* **1981**, *213*, 1207–1212, doi:10.1126/science.213.4513.1207.
46. Mappes, J.M.; Endler, J. The complex business of survival by aposematism. *Trends in Ecology & Evolution* **2005**, *20*, 598–603, doi:10.1016/j.tree.2005.07.011.
47. Bosque, R.J.; Noonan, B.P.; Colli, G.R. Geographical concordance and mimicry between harmless snakes (Colubridae: *Oxyrhopus*) and harmful models (Elapidae: *Micrurus*). *Global Ecoogy and Biogeography* **2016**, *25*, 218–226, doi:10.1111/geb.12401.
48. Almeida, P.C.; Feitosa, D.T.; Passos, P.; Prudente, A.L.C. Morphological variation and taxonomy of Atractus latifrons (Günther, 1868) (Serpentes: Dipsadidae). *Zootaxa* **2014**, *3860*, 64–80, doi:10.11646/zootaxa.3860.1.3.
49. Bosque, R.J.; Hyseni, C.; Santos, M.L.G.; Rangel, E.; Da Silva Dias, C.J.; Hearin, J.B.; Da Silva Jr., N.J.; Bicalho Domingos, F.M.C.; Colli, G.R.; Noonan, B.P. Müllerian mimicry and the coloration patterns of sympatric coralsnakes. *Biological Journal of the Linnean Society* **2022**, *135*, 645–651, doi:10.1093/biolinnean/blab155.
50. Davis Rabosky, A.R.; Cox, C.L.; Rabosky, D.L.; Title, P.O.; Holmes, I.A.; Feldman, A.; McGuire, J.A. Coralsnakes predict the evolution of mimicry across New World snakes. *Nature Communications* **2016**, *7*, 1–9, doi:10.1038/ncomms11484.
51. Strauch, M.A.; Souza, G.J.; Pereira, J.N.; Ramos, T.S.; Cesar, M.O.; Tomaz, M.A.; Monteiro-Machado, M.; Patrão-Neto, F.C.; Melo, P.A. True or false coral snake: is it worth the risk? A *Micrurus corallinus* case report. *Journal of Venomous Animals and Toxins including Tropical Diseases* **2018**, *24*, 10, doi:10.1186/s40409-018-0148-9.
52. Pfennig, D.W.; Mullen, S.P. Mimics without models: causes and consequences of allopatry in Batesian mimicry. *Proceedings of the Royal Socieyt of London, Series B* **2010**, *277*, 2577–2585, doi:10.1098/rspb.2010.0586.
53. Beckers, G.J.L., Leenders, T.A.A.M.; Strijbosch, H. Coral snake mimicry: live snakes not avoided by a mammalian predator. *Oecologia* **1996**, *106*, 461–463.
54. Muscat, E., de Toledo Moroti, M., Sazima, I., Toledo, L. F., & Rebouças, R. Are plasticine models efficient to test defensive colou-

ration of snakes?. Herpetological Journal, **2024** 34(2), 75-83, doi: 10.33256/34.2.7583
55. Smith, N.G. Avian predation of coralsnakes. *Copeia* **1969**, *2,* 402–404, doi:10.2307/1442098.
56. Brugger, K.E. Red-tailed hawk dies with coral snake in talons. *Copeia* **1989**, *2*, 508–510, doi:10.2307/1445456.
57. DuVal, E.H.; Greene, H.W.; Manno, K.L. Laughing falcon (*Herpetotheres cachinnans*) predation on coralsnakes (*Micrurus nigrocinctus*). *Biotropica* **2006**, *38*, 566–568, doi:10.1111/j.1744-7429.2006.00162.x.
58. Gómez-Martínez, M.J.; Gutierrez, A.; DeClerck, F. Four-eyed opossum (*Philander opossum*) predation on a coral snake (*Micrurus nigrocinctus*). *Mammalia* **2008**, *72*, 350–351, doi:10.1515/MAMM.2008.031.
59. Costa, H.C.; Lopes, L.E.; Marçal, B.F.; Zorzin, G. The reptile hunter's menu: A review of the prey species of Laughing Falcons, Herpetotheres cachinnans (Aves: Falconiformes). *North-Western Journal of Zoology* **2014**, *10*, 445–453.
60. Knight, R.L.; Erickson, A.W. High incidence of snakes in the diet of nestling red-tailed hawks. *Raptor Research* **1976**, *10*, 108–111.
61. Brodie III, E.D.; Moore, A. Experimental studies of coral snake mimicry: do snakes mimic millipedes? *Animal Behaviour* **1995**, *49*, 534–536, doi:10.1006/anbe.1995.0072.
62. Titcomb, G.C.; Kikuchi, D.W.; Pfennig, D.W. More than mimicry? Evaluating scope for flicker-fusion as a defensive strategy in coral snake mimics. *Current Zoology* **2014**, *60*, 123–130, doi:10.1093/czoolo/60.1.123.
63. Brattstrom, B.H. The coral snake 'mimic' problem and protective coloration. *Evolution* **1955**, *9*, 217–219, doi:10.2307/2405591.
64. Mouy, H. The function of red and banded patterns in snakes: The ophiophagy hypothesis. *Biological Journal of the Linnean Society* **2024**, 142, 375–396, *in press*. doi:10.1093/biolinnean/blad153.
65. Kojima, Y.; Ito, R.K.; Fukuyama, I.; Ohkubo, Y.; Durso, A.M. Foraging predicts the evolution of warning coloration and mimicry in snakes. *Proc. Natl. Acad. Sci.* **2024**, *121*, e2318857121, doi:10.1073/pnas.2318857121.
66. Mouy, H. The function of red and banded patterns in snakes: The eyes of the beholders. Also, the function of bands in fish. *Biological Journal of the Linnean Society* **2024**, 142, 452–467, *in press*. doi:10.1093/biolinnean/blad154.
67. Caprette, C. Conquering the cold shudder: the origin and evolution of snake eyes. PhD Thesis **2005**, The Ohio State University.
68. Edgar, R.C. MUSCLE: Multiple Sequence Alignment with High Accuracy and High. *Nucleic Acids Res.* **2004**, *32*, 1792–1797.
69. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; et al. Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data. *Bioinformatics* **2012**, *28*, 1647–1649.
70. Minh, B.Q.; Schmidt, H.A.; Chernomor, O.; Schrempf, D.; Woodhams, M.D.; Von Haeseler, A.; Lanfear, R.; Teeling, E. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol Biol Evol* **2020**, *37*, 1530–1534, doi:10.1093/molbev/msaa015.
71. Kalyaanamoorthy, S.; Minh, B.Q.; Wong Thomas K F and von Haeseler,

A.; Jermiin, L.S. ModelFinder: Fast Model Selection for Accurate Phylogenetic. *Nat. Methods* **2017**, *14*, 587–589.
72. Hoang, D.T.; Chernomor, O.; von Haeseler, A.; Minh, B.Q.; Vinh, L.S. UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Mol. Biol. Evol.* **2018**, *35*, 518–522.
73. Paradis, E.; Schliep, K. Ape 5.0: An Environment for Modern Phylogenetics and Evolutionary in R. *Bioinformatics* **2019**, *35*, 526–528.
74. Schliep, K.P. Phangorn: Phylogenetic Analysis in R. *Bioinformatics* **2011**, *27*, 592–593.
75. Revell, L.J. Phytools: An R Package for Phylogenetic Comparative Biology (and Other Things). *Methods Ecol Evol* **2012**, *3*, 217–223, doi:10.1111/j.2041-210X.2011.00169.x.
76. QGIS Development Team QGIS Geographic Information System **2024**.
77. Lippa, E.; Török, F.; Gómez, A.; Corrales, G.; Chacón, D.; Sasa, M.; Gutiérrez, J.M.; Lomonte, B.; Fernández, J. First Look into the Venom of Roatan Island's Critically Endangered Coral Snake *Micrurus ruatanus*: Proteomic Characterization, Toxicity, Immunorecognition and Neutralization by an Antivenom. *J Proteomics* **2019**, *198*, 177–185, doi:10.1016/J.JPROT.2019.01.007.
78. Bénard-Valle, M.; Neri-Castro, E.; Elizalde-Morales, N.; Olvera-Rodríguez, A.; Strickland, J.; Acosta, G.; Alagón, A. Protein Composition and Biochemical Characterization of Venom from Sonoran Coralsnakes (*Micruroides euryxanthus*). *Biochimie* **2021**, *182*, 206–216, doi:10.1016/J.BIOCHI.2021.01.003.
79. Olamendi-Portugal, T.; Batista, C.V.F.; Pedraza-Escalona, M.; Restano-Cassulini, R.; Zamudio, F.Z.; Benard-Valle, M.; Rafael de Roodt, A.; Possani, L.D. New Insights into the Proteomic Characterization of the Coral Snake *Micrurus pyrrhocryptus* Venom. *Toxicon* **2018**, *153*, 23–31, doi:10.1016/J.TOXICON.2018.08.003.
81. Sanz, L.; de Freitas-Lima, L.N.; Quesada-Bernat, S.; Graça-de-Souza, V.K.; Soares, A.M.; Calderón, L. de A.; Calvete, J.J.; Caldeira, C.A.S. Comparative Venomics of Brazilian Coralsnakes: *Micrurus frontalis, Micrurus spixii spixii*, and *Micrurus surinamensis*. *Toxicon* **2019**, *166*, 39–45, doi:10.1016/J.TOXICON.2019.05.001.
81. Hernández-Altamirano, J.A.; Salazar-Valenzuela, D.; Medina-Villamizar, E.J.; Quirola, D.R.; Patel, K.; Vaiyapuri, S.; Lomonte, B.; Almeida, J.R. First Insights into the Venom Composition of Two Ecuadorian Coralsnakes. *International Journal of Molecular Sciences* **2022**, *23*, 14686, doi:10.3390/IJMS232314686.

Chapter 3

Natural-born killers: Understanding the evolutionary paths of Colombian pitvipers

Sergio D. Cubides-Cubillos, Monica M. Saldarriaga-Cordoba, Jaime A. Pereañez, Teddy Angarita-Sierra

Abstract: Historically, lanceheads, rattlesnakes, bushmasters, and hog-nosed pitvipers have received significant attention due to their medical importance and their remarkable diverse biology and venom (e.g., infrared-sensing loreal pits, rattles, lethal toxins). Hundreds of papers and books published about pitvipers from the Western Hemisphere have established a broad and robust conceptual framework that has allowed for their understanding. However, most of these contributions have focused on temperate-zone taxa or on specific tropical countries like Brazil, Costa Rica, and Mexico. This disparity has limited our understanding of the diversity and endemism of vipers that live in geographically complex regions of tropical America, such as Colombia. Colombian pitvipers boast fascinating evolutionary histories, astonishing morphological and species diversity, and intricate geographical distribution patterns, that still await in-depth comprehension. Currently, the venom of Colombian pitvipers and its various forms hold considerable scientific importance, becoming model systems that allow for the exploration of evolutionary biology principles, from genes, individuals, and populations to macroevolutionary contexts. Based on a detailed review of available information, as well as the generation of new evidence, in this chapter we review and discuss the evolutionary lineages, venomics, and biological activities of the venoms of vipers present in Colombia.

Keywords: Diversity, fangs, venomous snakes, snakebite, tropics.



Citation: Cubides-Cubillos, SD; Saldarriaga-Cordoba, MM; Pereañez, JA; Angarita-Sierra, T. Chapter 3. Natural born killers: Understanding the evolutionary paths of the Colombian pitvipers. In Book: *Bites, venoms and venomous snakes of Colombia*; Angarita-Sierra, T., Ruiz-Gomez, FJ, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 115–164.
doi: 10.33610/866780odgbqc

Copyright: © 2024 by the authors. Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0) license (http://creativecommons.org/licenses/by/4.0/).

Illustrations by:
Oscar A. Ramirez Ruiz

1. Ambush predators: Pitvipers around the world

Venom is a remarkable biological trait that has evolved independently in numerous organisms on our planet. However, this trait, which characterizes some groups of snakes, culturally generates mixed reactions and attitudes, evoking fear, fascination, and respect at the same time, making snakes stand out among all species in the animal kingdom as creatures of power [1]. Nevertheless, in the entire world only a handful of snake species exhibit exceptional venom potency or pose a significant threat owing to their sophisticated venom delivery mechanisms and specialized venom composition (see Chapter 5). Approximately 10% of all living snakes belong to the Viperidae family, encompassing vipers, adders, and pitvipers [2,3]. This family is phylogenetically divided into three subfamilies: Azemiopinae (Fea vipers), Crotalinae (pitvipers), and Viperinae ("true vipers" or "vipers without pits").

Currently, about 383 species of viperids are recognized, grouped into 37 genera [4]. Thirteen of these genera are distributed across America: *Agkistrodon* [5], *Atropoides* [6], *Bothriechis* [7], *Bothrocophias* [8], *Bothrops* [9], *Cerrophidion* [10], *Crotalus* [11], *Lachesis* [12], *Metlapilcoatlus* [13], *Mixcoatlus* [14], *Ophryacus* [15], *Porthidium* [16], *Sistrurus* [17]; and 24 genera are distributed among the Asian, European and African continents: *Atheris* [18], *Azemiops* [19], *Bitis* [20], *Calloselasma* [21], *Causus* [22], *Cerastes* [11], *Craspedocephalus* [23], *Daboia* [20], *Deinagkistrodon* [24], *Echis* [25], *Eristicophis* [26], *Garthius* [27], *Gloydius* [28], *Hypnale* [29], *Macropvipera* [30], *Montatheris* [31], *Montivipera* [32], *Ovophis* [33], *Proatheris* [31], *Protobothrops* [34], *Pseudocerastes* [35], *Trimeresurus* [36], *Tropidolaemus* [22], and *Vipera* [37].

Among all venomous snakes, the highly specialized venom delivery system of viperids is characterized by tubular front fangs enclosed in venom-conducting canals positioned on a mobile maxillary bone [38] (see Chapter 5). The specialized long teeth developed by viperids represent one of the most remarkable functional traits linked to their life history. They are primarily sedentary ambush predators that consume mostly hot-blood prey during their adulthood [38,39]. Consequently, the efficacy of this sophisticated venom release mechanism in hunting, defense, and deterrent functions has resulted in the designation of several viper genera as medically important due to the elevated annual incidence of snakebite (see Chapter 9).

In these ambush hunters, venom composition limits prey selection; hence, feeding has notorious adaptations and specificities. For example, newborn or young snakes exhibit greater lethal activity than those of their adult counterparts [40], indicating that these biochemical differences are linked with the diet changes and the evolution of the prey. Indeed, proteomic studies have revealed that ontogenetic variation in venom composition is a significant indicator for understanding toxin dynamics and actions for the two most significant Colombian pitvipers: *Bothrops atrox* [41], and *B. asper* [42,43].

To understand the evolution of snakes from a mechanical means of dominating prey (constriction) to a chemical means (venom injection [44]),

it is imperative to first comprehend the many processes through which natural selection has favored the harmony between divergence and adaptation. This balance has allowed the emergence of vast heterogeneity in the composition, function, and action of snake venom toxins. This chapter provides a brief overview of the past 50 years of research on the evolutionary and natural history of pitviper species that inhabit the tropical ecosystems of Colombia, focusing on the medically important snake species.

2. The evolutionary and geographic perspectives of pitviper colonization and diversification in the South American tropics

One of the great and pioneering contributions in the systematic and biology of pitvipers corresponds to the work of William L. Burger [45], who clarified the delimitation of several groups and the description of new characters of taxonomic use for the Viperidae family. His pioneering work suggested the division of the large *Bothrops* group into five morphologically divergent genera: *Bothriechis, Bothriopsis, Bothrops, Ophryacus* and *Porthidium*. Nowadays, the validity of *Bothriopsis* as a separate genus of snakes is contentious. Historically, *Bothriopsis* was considered a valid genus, but phylogenetic and genetic studies have questioned its distinction from the genus *Bothrops*. In fact, Burger's work was crucial because it triggered a series of rearrangements that allowed the description of genera derived from *Porthidium*, such as *Atropoides* [6], and *Cerrophidion* [10].

Later, Avise was one of the pioneers [46] to examine hypotheses of phylogenetic and phylogeographic inference in pitvipers from a genetic perspective used molecular markers. He studied the ancestral relationships that are unaffected by convergent selection in similar natural history traits by assessing molecular markers as independent evidence, assuming that these traits were unlikely to be influenced by the same selective pressures that act on morphological traits [47]. Since the 1990s, DNA sequences have been used to calculate the historical timelines in which different viperid clades diverged, helping to understand morphological diversification in a biogeographical context [48]. However, these initial studies examined the evolutionary value of mitochondrial genes, revealing that genes such as cytochrome *b* and NADH are the suitable predictors of phylogenetic relationships, largely due to the slower evolution of ribosomal unities [49].

Initially, Parkinson et al. [50], and Gutberlet Jr et al. [51] identified and suggested the existence of two monophyletic groups of pitvipers in America: A clade of North American species that groups *Agkistrodon*, *Atropoides, Cerrophidion Crotalus, Metlapilcoatlus, Ophryacus* and *Sistrurus*, and a second clade that groups the other genera located in the Neotropical region. One analysis [50] recovered Americas viperids as a monophyletic group but without a suitable identification of their sister group: a single invasion of North America with a subsequent divergence between the temperate and tropical north and at least three invasions of South America by the pitviper historical ancestors. Furthermore, one

study [50] reported that the species at the time considered as *Porthidium hyoprora* corresponded to a lineage more closely related to the *Bothrops* group (coinciding with the first description [52], naming it *Bothrops hyoprora*), resulting years later in the description of a new genus, *Bothrocophias* that would include other species also included within the *Bothrops* group, such as: *B. campbelli*, *B. hyoprora, B. microphthalmus,* and *B. myersi* [8].

In Colombia, the significant elevation of the three Andes Cordilleras played a pivotal role in the cladogenesis of numerous pitviper species, such as the *Bothrops* species group, currently distributed allopathically [53]. The Andes Cordillera did not reach a maximum elevation of more than 40% until the Neogene era. This resulted in significant mountain formation only during the late Miocene and especially during the Pliocene, when the orogenic process accelerated quickly [54]. Hence, it is posited that the initial division among the inter-Andean pitviper groups in northern South America is attributed to a dispersal event within the Caribbean of Mesoamerica that influenced the biogeography of the region.

Molecular studies of pitvipers have primarily focused on the Neotropical region, examining various hypotheses that could explain their astonishing diversity [55–57]. Phylogenies have been used to explain the evolutionary trajectories that give origin to the types of plants and animals that currently live with us, helping to postulate theories that try to explain the diversity of organisms during geological time scales (e.g., during the Pliocene and Miocene periods) and after milestone geological events such as the Andean orogeny or the uplift of the Isthmus of Panama.

The uplift of the Andes in South America was, in fact, one of the most significant geological developments in the evolution of numerous vertebrate species [58,59]. For instance, some authors utilize historical migration analysis in populations of the lancehead (*B. asper*) to examine its recent diversification events in South America [60]. Their methodology includes the incorporation of multiple demographic processes into the inferences about patterns of divergence that are not feasible to infer or comprehend using methods based on phylogenetic trees [61,62].

Indeed, the lancehead pitviper species have demonstrated that the impact of habitat diversity is correlated with phenotypic variability, a biological phenomenon inherent to the different *Bothrops* species [63,64]. The absence of any other potential adversaries at the onset of the expansion of distinct regions in northern South America may have contributed to the spread of the lanceheads across most of the geographical regions of Colombia. Nonetheless, it is imperative to emphasize that certain genera such as *Porthidium* (*P. nasutum* and *P. lansbergii*) and *Lachesis* (*L. acrochorda* and *L. muta*) exhibit convergence with *B. asper* and *B. atrox* in some ecoregions and localities. Nevertheless, *Porthidium* species exhibit a widespread distribution in the southwestern zone of the Pacific region [65], and in the north-Caribbean region [66], being a species that are sympatric with *B. asper*.

Nowadays, the bioinformatics methods have enabled the analysis of extensive datasets, such as genomic data, allowing the evaluation of intricate historical processes with high resolution [67–69]. Furthermore, the genome has proven to be especially useful due to the accuracy and precision with which the different parameters are estimated [70–72]. In summary, systematic deficiencies in certain pitviper studies have been addressed by utilizing integrative taxonomy and various lines of evidence for species delimitation, including morphological characteristics, genetic distance analysis, molecular phylogeny, and coalescent species delimitations that can encompass geographic and ecological isolation [73–75].

2.1 Understanding how diversification events affect the diversity of pitviper species

Numerous hypotheses on Neotropical diversification have been proposed, including allopatric speciation and ecological displacement through mountain ranges [58], rivers that functioned as prezygotic barriers, or habitat fragmentation caused by climate change during the Pleistocene [76]. Continental drift of South America and the uplift of the Andes resulted in the evolutionary dynamics that underpin the foundation of diversity in numerous ecoregions of the Neotropic [77–79]. These orogenic phenomena have raised questions about how many taxonomic groups were colonized and differentiated, and which ones would later inhabit the lowlands and slopes once the Andes reached their current elevations [80]. In this way, pitviper diversity in South America is connected to biotic exchange between North and Central America throughout the Tertiary period, especially after the emergence of the Isthmus of Panama [81].

Most speciation events in tropical regions are primarily influenced by the isolation induced by landscape alteration (e.g., vicariance) that is intrinsically linked to a broader range of biological and geological processes [60]. Despite this, the current phylogeographic hypotheses proposed for South American pitviper species have not been evaluated using a consideration of the influence of demographic processes [55,57,60,82]. In fact, examining the phylogeographic patterns of distinct species with overlapping or partially overlapping ranges can reveal common events that may have a similar impact on the evolutionary patterns of numerous taxa [83]. However, inconsistencies among sympatric taxa may arise because of lineage sorting, variation in effective population size, extinction, dispersal, sympatric speciation, or non-response to vicariance events [84–86].

In contrast, the explanation for parapatric speciation along the mountainous vertical axis could be attributed to specific adaptations associated with ecological gradients [87]. Consequently, the capacity of certain species to expand into novel niches that emerged during a specific time must have been pivotal in the diversification of inter-Andean pitvipers belonging to the genera *Bothrops*, *Bothrocophias*, *Bothriechis*, *Lachesis*, and *Porthidium*. These events in northwestern South America during the early Pliocene suggest that the final uplift of the Andes could have played a significant role in the species diversification (=cladogenesis) of pitviper groups [53].

Therefore, it has been posited that the rapid increase of viperids species from a common ancestor (with heat-sensitive pits) represents a significant evolutionary milestone, enabling the successful diversification of this family of snakes [3]. From an ecological standpoint, these lineages inhabit diverse environments and habitats, ranging from tropical lowland forests in Central America and the Amazon to open areas in the Andes and in Patagonia [2,55]. However, numerous species are still poorly known with respect to their taxonomy, natural history, and phylogenetic relationships [2,28,57,88]. In fact, several groups of pitvipers show apparently endemic patterns, and because some taxa are considered species complexes, several species groups are in the process of redescription and resolution of their phylogenetic patterns (see Chapter 1).

One of the significant challenges faced by herpetology in Colombia is the limited number of molecular markers that have been examined in species of snakes. Furthermore, the limited knowledge regarding the rates of molecular evolution of various groups of snake genes precludes a comprehensive phylogeographic and historical examination of the pitvipers found in northern South America.

2.2 A family with distinct species complexes

In Colombia, as well as throughout the Andean ecosystems, the most significant environmental factors affecting the distribution of the seven known pitviper genera are elevation and orographic complexity. These factors are proposed as some of the main drivers of the phylogenetic relationships and venomic diversity in several pitviper species. For instance, a select group of Colombian pitviper species attain elevations ranging from 1,500 to 3,200 meters above sea level (hereafter asl), with 1,500 m asl being the upper limit for most pitviper species inhabiting the lowlands; 3,600 m asl is the upper limit for high Andean species (such as *Bothrocophias tulitoi*, *B. myrringae*, *Bothriechis schlegelii,* and certain populations of the *B. atrox* complex) [2,75].

Within the diverse biogeographic regions of northern South American, the proportion of venomous species can range from 9% to 14% [89]. Therefore, the distributional zones of Colombian pitvipers have been primarily concentrated in areas inhabited by humans, in contrast to the notable presence of several snake populations in other forested areas [90]. As a result, the dispersion of the Colombian pitvipers is associated with primary forested areas, secondary forested areas, areas intended for agriculture at different elevations, and increasingly with urban areas (usually in expansion). Consequently, and due to their wide range of geographical distribution in Colombia, *B. asper* and *B. atrox* are the most medically important venomous snake species [64,91].

Historically, the genera *Bothrops* and *Porthidium* have been included in a substantial number of reviews and taxonomic approaches that have revealed a diversity previously unrecognized at the species level [63,92–97]. Over the past two decades, some species belonging to the

genus *Bothrops* have been the subject of numerous phylogenetic studies and biogeographic approaches pertaining to their diversification on the southern side of the western hemisphere of the earth [3,38,55,98,99]. These approaches have revealed a taxonomic complexity mainly within the *Bothrops*, although currently work is also being conducted with the *Porthidium*.

Research on the presence of cryptic diversity for several species of pitvipers found in Colombia reveals the presence of some species complexes identified through a series of common characteristics shared between several species and lineages (see Chapter 1) [53,63,75,92–95]. This difficulty in distinguishing morphologically distinct species (cryptic species) that are genetically divergent has become one of the most discussed points in work published in molecular systematics and in the process of species delimitation.

One of the objectives of this section is to document some of the pioneering research on the systematics, venoms, and evolution of Colombian pitvipers. Based on published data over the past three decades, we believe it is necessary to increase phylogeographic studies in order to analyze the cryptic diversity of recognized taxa [48,83,94,96]. This information could serve as an important tool for determining conservation efforts aimed at preserving viable populations of lineages that are yet to be discovered [83].

Below we present a description of the main groups and species complexes of the medically important pitvipers in Colombia, and we review the principal works that have laid the foundations of our knowledge. We also discuss pitviper systematics. Finally, we compile other published information about the different groups to show the association between the studies carried out on Colombian pitviper venoms and their phylogenetic correlation. Thus, we intend to show not only the phylogenetic complexity of the two main groups (*Bothrops* and *Porthidium*), but we also want to show the lineages and populations by species where there is no genetic or proteomic information.

The most medically important Colombian lancehead pitvipers: the *Bothrops asper-atrox* complex

The genus *Bothrops* exhibits more genetic divergence in comparison to the other South American pitvipers. In fact, the South American *Bothrops* exhibit paraphyletic characteristics in relation to the species found in Central America. The diversification of the genus has probably taken place in South America [60]. This hypothesis suggests that the common ancestor of all *Bothrops* was the first viperid to colonize South America sometime during the Miocene, about 10 million years ago [63]. As a result, only one lineage of *Bothrops* species (*B. asper*) spread throughout Central America, from Panama to Mexico. However, there are geographical distribution records for a second species of *Bothrops* (*B. punctatus*) in the eastern region of Panama.

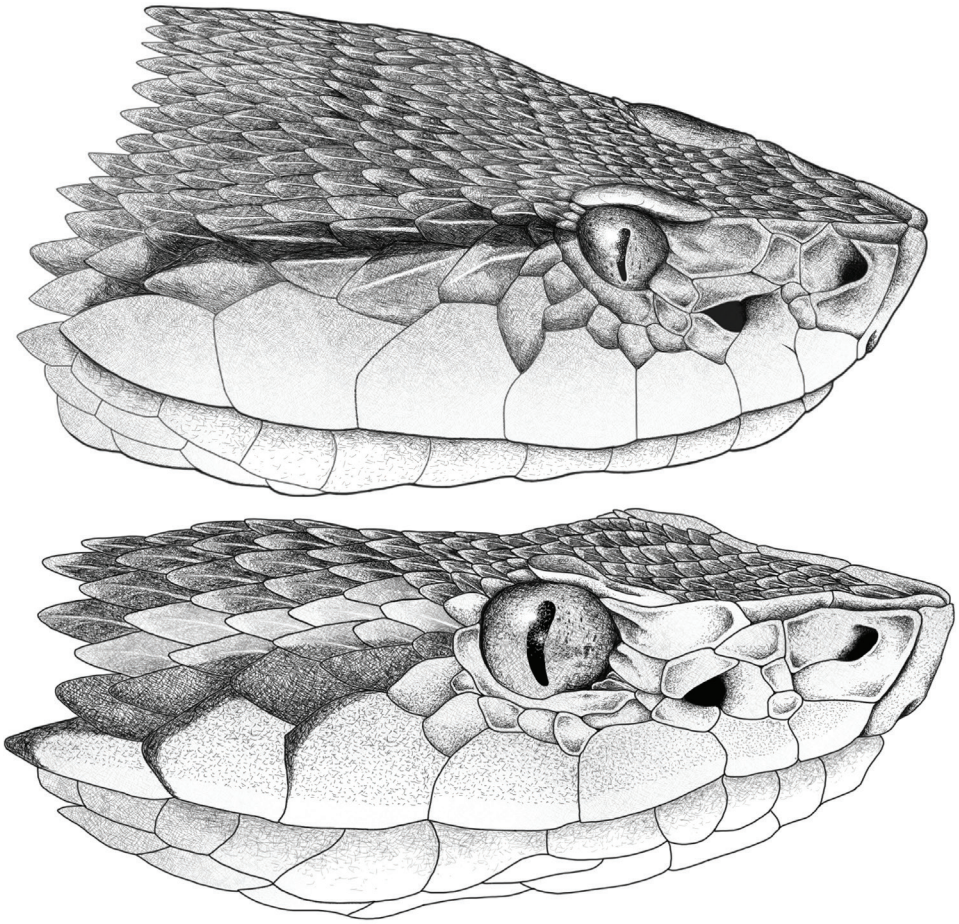


Figure 1. Head illustrations of *Bothrops asper-atrox* complex. (Top): head in lateral view of *Bothrops asper* from Melgar, Tolima, Colombia (INSZ 138). (Bottom): Head in lateral view of *Bothrops atrox* from Puerto Carreño; Vichada (BOTATR00061). Illustrations by Oscar A. Ramirez Ruiz.

Campbell and Lamar [2] suggested that Colombian populations of *B. asper* and *B. atrox* species have allopatric distributions. In fact, *B. asper* (Figure 1 Top) is distributed throughout the biogeographic regions of Choco and Magdalena Valley, with a focus on the Caribbean and inter-Andean basins [64,91], whereas *B. atrox* (Figure 1 Bottom) is found in the Orinoquia and Amazon regions. This distribution aligns with the dispersal hypothesis for the *B. atrox* species, positing that a preexisting population of forests located on the northern side of the Amazon gradually colonized the regions bordering the Amazonian River [97].

Nonetheless, there could be lineages of *B. asper* and *B. atrox* in sympatric geographical regions of Colombia's eastern and western Andes, like Boyaca, Cundinamarca, and Norte de Santander. Several studies have demonstrated that both species show recent lineage differentiation in relation to habitat diversity as well as numerous unrecognized evolutionary lineages associated with elevational gradients [53,60,92,97].

The taxonomic status of the *B. asper-atrox* complex within Colombian populations has been little explored from a genetic perspective, and there is no clear delimitation between the lineages of these species to

help recognize some of the previously described taxonomic hypotheses based on morphology. Particularly, the populations on the eastern slope of the Cordillera Oriental (*B. asper-atrox* complex) represent a challenging problem still unsolved: Some *B. atrox* mountain populations have been historically reported as *B. isabelae* [98], and *Bothrops colombiensis* [99]. *Bothrops isabelae* was initially synonymized with *B. atrox* in the 90s [33,100], but later it was recognized as a species in some of the pioneering work for South American *Bothrops* [92]. Recent analysis [101] shows *B. isabelae* together with *B. atrox* with an apparently low genetic distance. Even so, the authors recognize *B. isabelae* as a valid taxon, although a few years later it was again considered a synonym of *B. atrox* [55]. Campbell and Lamar [2] consider *B. colombiensis* as a species synonymous with the species complex formed by *B. asper* and *B. atrox* from Venezuelan populations.

Therefore, clarifying the phylogenetic connections between the Colombian *Bothrops* species and the populations of Venezuelan species/lineages would enable us to comprehend the dispersal dynamics of numerous of these widely distributed taxa, providing an alternative to the utilization of the unified species concept widely applied in analyses of geographic lineages [102]. The complexity of understanding the systematics of the *B. atrox* group is challenging [63,88,103]. Some studies suggest that the clade composed of *B. leucurus* and *B. moojeni* is the sister group of *B. atrox*; other studies indicate that *B. atrox* has closer evolutionary relationships with *B. asper* and *B. isabelae* [6,94,101,104]. Nonetheless, the *B. atrox* group originating from Colombia and Brazil requires a detailed taxonomic review, as the taxonomic hypotheses based on morphology do not align with the described mitochondrial lineages. Furthermore, these mitochondrial lineages also do not correspond with the taxonomically recognized species [103].

An example of this marked taxonomic complexity for *Bothrops* in Colombia was the naming of new species derived from *B. asper* [105]. That research provided a study that reevaluated the taxonomical boundaries within *B. asper* populations in Colombia and proposed *B. ayerbei* and *B. rhombeatus* as new species within the *B. asper* complex. Nonetheless, the unsuitable quantity and quality of characteristics employed in the species delimitation, the ambiguous descriptions of morphologic traits and diagnostic characters [106], and the lack of a phylogenetic analysis, impedes a clear and objective distinction between the species proposed (see Chapter 1). In fact, in the initial description made by Garcia [107] no type specimen was assigned nor was the description of a new taxon made, turning *B. rhombeatus* into a *nomen dubium* (see Chapter 1).

Another crucial point mentioned by Ramirez-Chaves and Solari [106] refers that Folleco-Fernandez [105] did not describe a neotype for *B. ayerbei* and *B. rhombeatus* nor were data on the geographical distributions provided for possible hybridization between the species nor was the relevance of distinguishing both taxa as valid species discussed. Although other authors have incorporated molecular markers to assess the phylo-

genetic relationships within the *B. asper-atrox* species complex [53]. Currently, the sequences from *B. ayerbei* and *B. rhombeatus* populations distributed in the southwest of Colombia (Cauca and Nariño departments), proposed by Folleco-Fernandez [105], are not available in official gene repositories, being lineages synonymous with *Bothrops asper* and *Bothrocophias colombianus*, respectively. So, further studies should include a larger or sufficient series of individuals collected in different habitats and analyzed with an integrative taxonomic approach in their sampling methodology [60,97].

In order to highlight the absence of genetic and venomic information of some representative clades and lineages within the Colombian pitviper species, we present as a novelty a heat map that shows the phylogenetic relationships of the *B. asper-atrox* complex and some proteome data published for some species in the last two decades. Thus, Figure 2 shows the result of a molecular phylogeny constructed from fragments of two mitochondrial genes, and supported by some genera of pitvipers as out groups (Appendix). We have recovered a topology that includes terminals for different populations and lineages, as well as the recently described Colombian *Bothrocophias* species [75]. We used three terminals from three populations in Brazil, including two terminals reported as species but recently synonymized for the species *B. atrox*. We used data from eight groups of protein/toxin families that have been frequently reported in venomic studies, and we performed the association of the percentages of these main components to present hypotheses related to the conformation of the clades and their geographical distribution (Figure 2).

We suggest that *B. asper* from Colombia presents three large and well-supported phylogeographic lineages that are morphologically and ecologically diverse with genetic divergences between 2.5% and 4%. On the other hand, *B. atrox* presents undefined population groups whose phylogenetic relationships still need to be well established (populations west of the Cordillera Oriental-Cundinamarca vs. populations east of the Cordillera Oriental-Meta, Vaupes and Caqueta). Furthermore, the geographical and taxonomic limits with its synonymous species/lineage *B. isabelae* (also distributed in the eastern versant of the Cordillera Oriental in some parts of Venezuela) continue to be a taxonomic problem to be resolved. Here we use information from ongoing work to indicate that revision of the taxonomic status of the species/lineage *B. isabelae* could be reported later.

It is important to note that the sister genus of *Bothrops*, *Bothrocophias*, has only three species with genetic data and a single published proteome (see section 3 in this chapter). Likewise, we were not able to calculate this relationship analysis for the proteomes of the *B. ayerbei* and *B. rhombeatus* lineages due to the absence of genetic data available. Finally, the absence of venomic data for two important lineages within *B. asper* (populations from the Caribbean region and Middle Magdalena) is noted here.

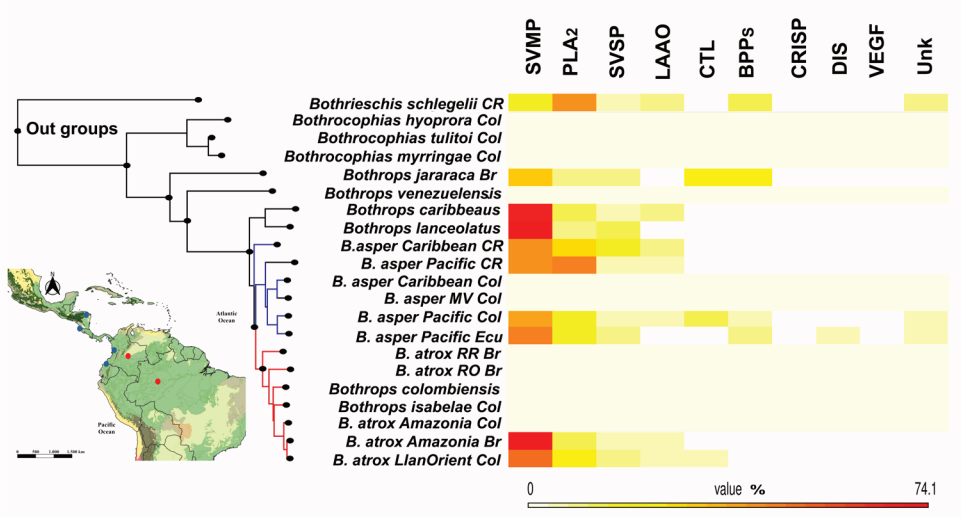


Figure 2. Molecular phylogeny of concatenated genes (MT-ND4 and MT-CYB sequences, produced by [3,50,53,63,75,94,96], and Cubides-Cubillos et al. (unpublished data) using maximum likelihood (ML) and a proteome heatmap of the Colombian pitvipers (*Bothrops asper-atrox* complex). Phylogenetic topology retrieves the species and their most important lineages and those for which genetic and proteomic information is available (see bibliographic information in Section 3). Besides, the nodes that support branches with bs > 90% are shown. Abbreviations: lineages/populations (Caribbean and Pacific CR: Costa Rica; Caribbean and Pacific Col: Colombia; Pacific Ecu: Ecuador; MV Col: Magdalena Valley, Colombia; RR Br: Roraima region from Brazil; RO Br: Rondonia region from Brazil; Amazonia Br: Amazonian region from Brazil; Amazonia Col: Amazonian region from Colombia; LlanOrient Col: Orinoquia region from Colombia; and protein family names: SVMPs: metalloproteinase; PLA_2s: phospholipase A_2; SVSPs: serine proteinase; LAAO: L-amino acid oxidase; CTL: C-type lectin/lectin-like; BPP: bradykinin-potentiating peptide; CRISP: cysteine-rich secretory protein; Unk: nucleotidase; phosphodiesterase; hyaluronidase; nerve growth factor; peptides and/or non-protein compounds.

With relation to the species *B. atrox* and the various lineages found in previous phylogenetic analyses, only records of proteomic data were found for a population from Colombia (Llanos Orientales region, Meta department) and the northern region of Brazil (Amazonas State). It remains under consideration for subsequent analysis, whether the populations of *B. atrox* distributed along the eastern versant of the Cordillera Oriental (encompassing Boyaca department), populations of the Colombian Amazon in the foothill region (Caqueta and Putumayo departments) of the Eastern Andes and those from the Amazon lowlands (departments of Guainia, Guaviare, Vaupes, and Amazonas), would require a review of their venoms, as well as other molecular data. Likewise, data from highland populations on the eastern versant of the Cordillera Oriental identified as the *B. isabelae* lineage, could show certain types of venomic adaptations such as biochemical diversity, functional specialization, and evolutionary dynamics as gene duplications, diversification and rapid evolution of toxin genes, which represents a challenge in future research for mountain populations pitvipers genus *Bothrops*.

The hemorrhagic potency of several types of Snake Venom Metalloproteinases (SVMP) is one of the important factors in the biological action of *Bothrops* venoms. Its relative abundance in venoms is related to local and systemic hemorrhage [43]. Our relationship analysis showed that the species and populations with the highest content of SVMPs were geographically distributed in both dry and humid tropical regions. Furthermore, venom composition exhibited high variability within closely related lineages, indicating a possible ecological response. Probably, the heterogeneous distribution and dispersal of species/lineages is a possible cause of the synergistic effects between several types of SVMPs. In fact, hemorrhage, which also contributes to myonecrosis [108], is one of the most documented effects in snakebite accidents of all members of the *B. asper-atrox* species group.

The PLA_2, the second most abundant toxin in the *B. asper-atrox* species venom is responsible for myonecrosis and is closely related to the

digestive functions of the venom [109-110] (see Chapter 5). This toxin usually represents about 20-40% of the proteome venom composition of the *B. asper-atrox* species group but shows moderate variability. In fact, the variability in PLA_2 percentage seems not to be related to a specific clade, region, or climate. However, due to large information gaps, this pattern could change in future studies or could be associated with ecological variables such as prey availability, habitat loss, or anthropogenic pressure.

Enzymes incompletely mimic the action of thrombin, and Snake Venom Serine Protease (SVSPs) exert a variety of actions on hemostasis and the kinin system or kinin-kallikrein system [111,112]. They constitute the third most important group of toxins and, in fact, show minor variation within the species/lineages of the *B. asper-atrox* group. Reports of snakebite accidents do not place much emphasis on variations in coagulopathies or inflammation, as these are typical symptoms of bothropic envenomation (see Chapter 9). Evolutionarily, this appears to be a characteristic intrinsically linked to constructive interactions with other components of the venom (see Chapter 5 and 9).

The Colombian hognose pitviper: *Porthidium* species group

Hognose pitvipers inhabit Central and South America, and it is hypothesized that their dispersal occurred along the Pacific coast during the final phase of the emergence of the Panamanian isthmus (3.5 mya), even after its formation [63]. This dispersion might have resulted from interspecific competition with other viper species such as *B. asper*, a species that has been identified as another pitviper that colonized South America before the Panamanian Isthmus [2,53,63]. The species of *Porthidium* are short-bodied terrestrial snakes that do not exceed 100 cm in length and whose hypothetical evolutionary origin is related to Central America [2,3,13,14,113].

This genus shares cryptic dorsal color patterns and a clearly defined canthus rostralis (rostral upward scale; see Figure 3) that presumably denotes important adaptations for the hunting forms typical of ambush predators [2]. In the 1990s, phylogenetic relationships between different species were initially reviewed using morphological characters and molecular data [6,50,104].

Currently, various taxonomic studies have revealed the evolutionary and systematic relationships between the different species that support the taxonomic validity of nine species of the genus *Porthidium*: *P. dunni*, *P. hespere*, *P. ophryomegas*, *P. volnicacum,* and *P. yucatanicum* with a strong affinity towards arid zones; and *P. nasutum*, *P. porrasi*, *P. lansbergii,* and *P. arcosae* that inhabit lowland tropical rainforests [95]. It is important to highlight that the description of the latest species for Central America, *P. porrasi* [114], was based on an analysis of adjacent populations of *P. nasutum* from Costa Rica. This implied the initiation of a discussion on phylogenetic diversity within the genus and marked the first strong revision suggested for northern South American populations [95].

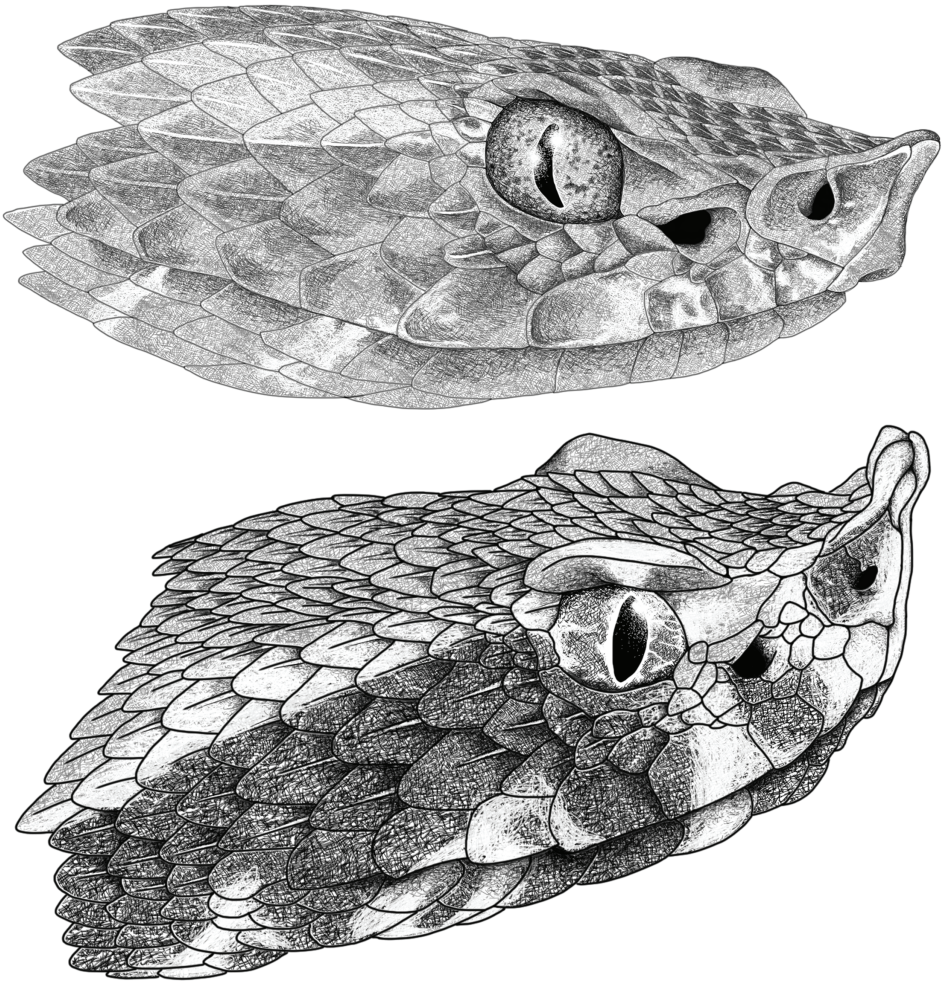


Figure 3. Head illustrations of *Porthidium lansbergii-nasutum* species complex. (Top): Head in lateral view of *Porthidium lansbergii* (INSV-SR-89) from Yondó, Antioquia, Colombia. (Bottom): Head in lateral view of *Porthidium nasutum* (QCAZR 15395) from Tundaloma Lodge, Durango, Ecuador. Illustrations by Oscar A. Ramirez Ruiz.

In Colombia, *P. nasutum* [115], and *P. lansbergii* [116] are closely related species, characterized by cryptic morphological traits such as the 'hog-nose snout' character. *Porthidium nasutum* is distributed from Mexico through the Isthmian-Atlantic moist forests, transitioning into the Choco-Darien ecoregion, as well as the biogeographic Chocoan ecoregion, including Ecuador [2]. Meanwhile, *P. lansbergii*, is distributed in the Isthmian-Pacific dry forests of Panama and the Colombian Caribbean, before reaching the tropical dry forest in northern Venezuela and the Choco-Darien region, where it transitions into the Uraba humid forests and the evergreen and dry forest in the Magdalena Valley [2,117].

Most of the previous scientific contributions for *P. nasutum* and *P. lansbergii*, performed phylogenetic approaches based on geographically restricted and small individual sampling [117,118]. The taxonomic complexity observed in Panama and in the diverse Colombian populations warrants large-scale research. For example, Castoe et al. [95] demonstrated that *P. nasutum* is a paraphyletic clade that shows two genetically well-differentiated lineages in Costa Rica and Ecuador.

Similarly, in our phylogeny of *P. nasutum* we recovered three principal lineages: Clade 1 and Clade 2 from Costa Rica and Ecuador, matching with previous results reported Castoe et al. [95], while Clade 3 clustering specimens distributed from the northeast of the Colombian Andes, and those from the central-eastern region of Antioquia department. Yet, in our phylogenetic analysis, the Colombian population (central-eastern Antioquia) was recovered as a clade related with the different lineages of *P. lansbergii* group. Previously, Cisneros-Heredia and Yanez-Muñoz [119] suggested that the Ecuadorian population ought to be regarded as a novel species, distinct from *P. nasutum* in Central America. However, our phylogeny does not include samples from the Chocoan populations of *P. nasutum* (Colombian Pacific Coast) or from the upper Magdalena River basin (Huila department), which could limit the resolution of the phylogenetic relationships between the *P. lansbergii* and *P. nasutum* Colombian lineages, as well as the inferences derived from this.

Our molecular phylogeny highlights that *Porthidium lansbergii* exhibits a wide range of diversification across diverse ecoregions encompassing elevation ranges from sea level to 1,200 meters. The Colombian lineages exhibit paraphyletic groups: Populations from the Darien-Panama are distinguished from those located in the Colombian Caribbean ecoregion, the Magdalena River basin, insular populations, and those to the east in Venezuela. Furthermore, certain genetic sequences associated with the two subspecies of *P. lansbergii* (*P. l. hutmanni* and *P. l. rozei*) were grouped in a polytomic clade, indicating a close association between the lineages of the Caribbean coast and Magdalena Valley with individuals analyzed from Venezuela, as also reported by De Arco-Rodriguez et al. [118].

Only a few species of *Porthidium* have published proteome composition. Despite of *Porthidium* second genus of viperid with the greatest medical importance in Colombia (see Chapter 9), only one population *P. lansbergii* has a proteome description

Nevertheless, the relationship analysis of molecular phylogeny and proteomic data of *Porthidium* species shows that there are significant differences between the proteomes of sister species. However, due to the fragmentary state of knowledge about their venom compositions, it is not possible to propose an explanation for the apparent variability observed between the lineages, nor about the concordance between phylogeographic lineages and ecoregions, or ecological characteristics.

The main toxins that vary between species correspond to the three main toxin groups: SVMP, PLA_2, and SVSP. Interestingly, these toxins vary in clades distributed in divergent climatic regions. However, missing data for several populations/lineages within the species *P. nasutum* and *P. lansbergii* could indicate different adaptive processes between populations of the same species. Therefore, we encourage both Colombian and foreign researchers to tackle biogeographic hypotheses that attempt to explain how the Andes promotes the random explosion of phenotypes (morphology, venom compositions, color patterns, etc.), as well as how selective

pressure on venoms correlates or does not correlate with lineages and proteome variability [188]. These hypotheses and speculations require future studies that significantly reduce the uncertainty surrounding the evolution of lineages within the *Porthidium* genus and their relationship with the functional response of their venoms.

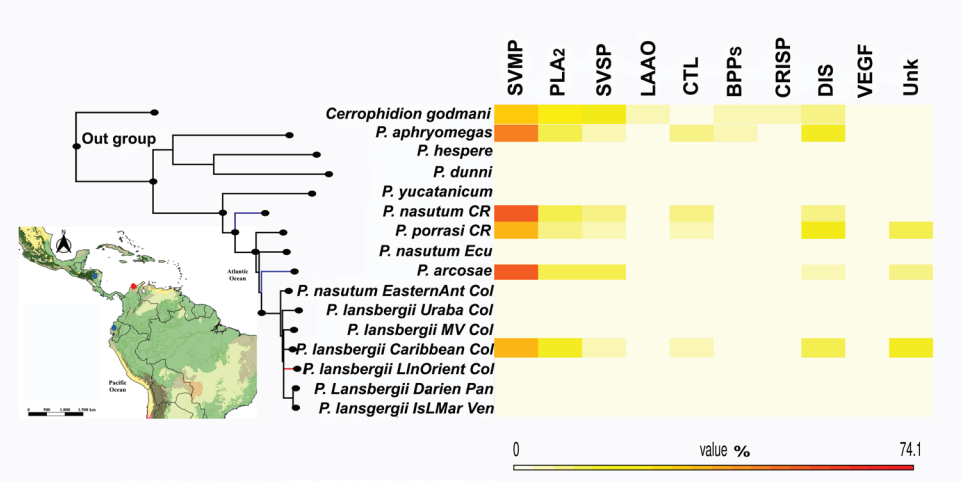
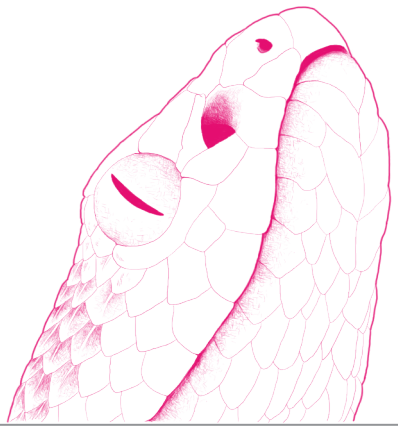


Figure 4. Molecular phylogeny of concatenated genes (MT-ND4 and MT-CYB sequences, produced by [95, 114], and Cubides-Cubillos et al.(unpublished data) using maximum likelihood (ML), and proteome heatmap of the Colombian pitvipers (*Porthidium* genus). Phylogenetic topology retrieves the species and their most important lineages, and those for which genetic and proteomic information is available (see bibliographic information in Section 3). Additionally, the nodes that support branches with BS > 90% are shown. Abbreviations: lineages/populations (CR: Costa Rica; Ecu: Ecuador; EasternAntCol: Eastern of Antioquia department, Colombia; Uraba Col: Uraba region from Colombia; MV Col: Magdalena Valey, Colombia; Caribbean Col: Caribbean region from Colombia; LlanOrient Col: Orinoquia region from Colombia; Darien Pan: Darien region from Panama; IslMarg Ven: Margarita Island from Venezuela); and protein family names: (SVMPs: metalloproteinase; PLA_2s: phospholipase A2; SVSPs: serine proteinase; LAAO: L-amino acid oxidase; CTL: C-type lectin/lectin-like; BPP: bradykinin-potentiating peptide; CRISP: cysteine-rich secretory protein; Unk: nucleotidase; phosphodiesterase; hyaluronidase; nerve growth factor; peptides and/or non-protein compounds).

Giant Colombian pitvipers: The South American rattlesnakes and bushmasters

The South American rattlesnake *Crotalus durissus* (*sensu lato*) is a remarkable species of *Crotalus*; its venom is of significant medical concern due to its characteristic actions of neuromuscular paralysis, rhabdomyolysis, acute kidney injury, and coagulopathy [120] (see Chapter 5 and 9). Recent research documents the diversity of subspecies for the species *C. durissus* [121], reported as follow: *C. d. durissus* [11]; *C. d. cascavella* [9]; *C. d. collilineatus* [52]; *C. d. cumanensis* [122]; *C. d. marojoensis* [123]; *C. d. maricelae* [124]; *C. d. ruruima* [123]; *C. d. terrificuss* [37]; and *C .d. trigonicus* [125]. These authors include two additional species from northern South America, *C. unicolor* [126] with a geographic distribution restricted to Aruba and *C. vegrandis* [127] with a geographic distribution restricted to Venezuela and previously regarded as a subspecies of *C. durissus* (see Figure 5, Top).



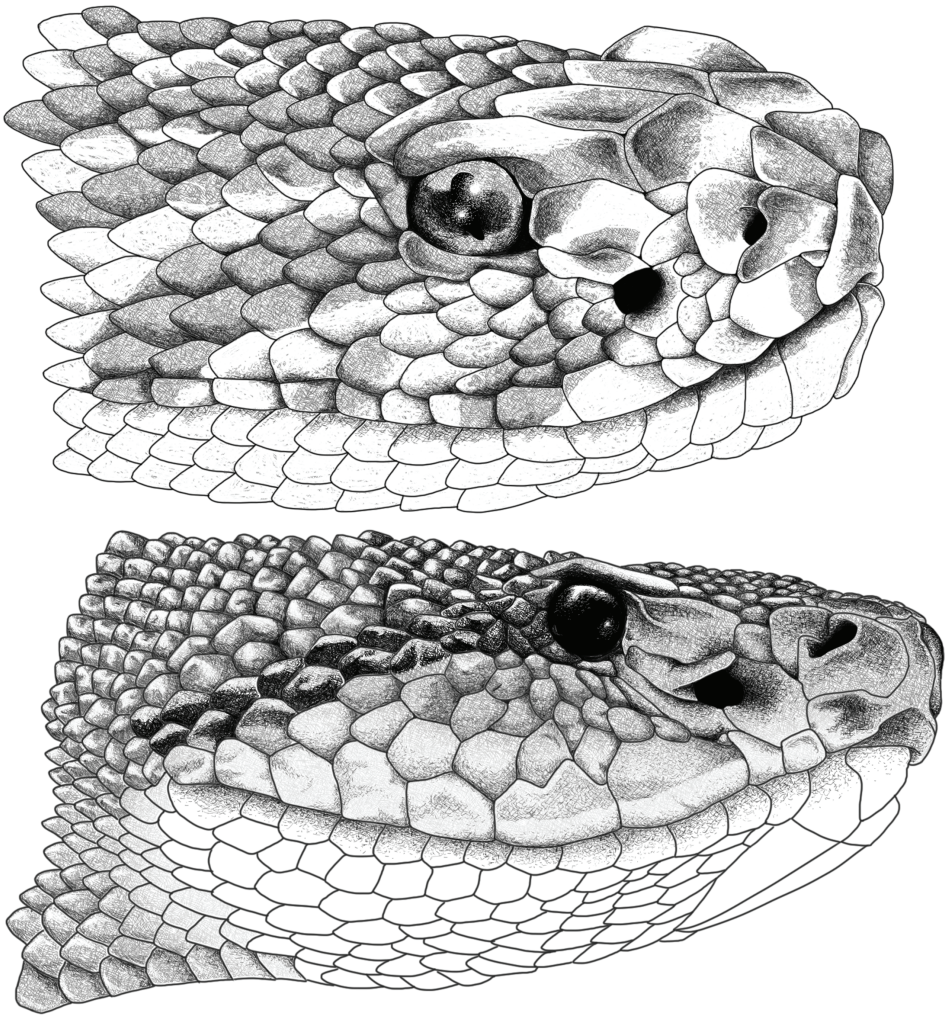


Figure 5. Head illustrations of giant Colombian pitvipers. (Top) Head in lateral view of *Crotalus durissus* from the municipality of El Paso, Cesar Colombia (INSZ 115). (Bottom) Head in lateral view of *Lachesis muta* from Miritíi-Paraná, municipality of La Pedrera, Amazonas, Colombia (IAvH-R-8721). Illustrations by Oscar A. Ramirez Ruiz.

Nonetheless, certain taxonomists have begun to recognize the subspecies as distinct species [128,129]. In recent work, the *C. durissus* group (including *C. vegrandis*) has been phylogenetically recovered as a sister group to *C. simus* (Central American rattlesnake) [121]. However, it appears that the cryptic diversity in this group is the result of processes of isolation. Furthermore, the molecular phylogeny published for the *C. durissus* group does not consider individuals from the trans-Andean and cis-Andean regions of Colombia.

In Colombia, *Crotalus durissus* is distributed in the trans-Andean region of the country over the lowlands of the Caribbean region, extending into the Magdalena Valley, with disjunct populations in the middle and upper Magdalena River basin. In the cis-Andean region, it is found in the well-drained high plains of the Orinoquia [2]. It is regarded as a species of terrestrial habits and is a prevalent resident of open areas that are relatively dry [130]. his rattlesnake exhibits ecological flexibility, allowing it to be active both within forests and outside their edges, and it is commonly found in heavily human-altered open areas [130,131]. Though it is one of the most venomous species in Colombia and one of the most

illegally traded [89], information on its natural history, registers of its occurrence, and evaluation of its state of conservation are still missing [64] (see Chapter 1). The disjunct distribution pattern of its populations within the trans-Andean region, as well as between the cis-Andean and trans-Andean populations, suggests that it is very likely that *Crotalus durissus* is composed of more than one evolutionary lineage that has diverged due to geographic and ecological isolation. Recently, significant intraspecific differences were found in the composition and biological activities of *C. durissus* venom among populations distributed in the middle Magdalena River basin, the Llanos Orientales foothills, and the Colombian Caribbean coast (see Chapter 5). These results suggest that the hypothesis that *Crotalus durissus* is a species complex with multiple evolutionary lineages that have diverged due to geographic and ecological isolation is plausible. However, future phylogenetic studies, including representative samples from all Colombian populations, as well as populations distributed in Brazilian ecosystems, are expected to help resolve these uncertainties.

The snakes of the genus *Lachesis* [12], typically called "bushmaster", are the largest pitviper snakes so far known, reaching more than 3.5 meters in length [2,89]. The snakes of this genus are the only oviparous species among all American pitvipers [132]. The species of the *Lachesis* genus can be divided into Central American and South American lineages, with an estimated divergence between 6 and 18 million years ago. The divergence within the Central American species seems to have occurred between 4 and 11 million years ago, likely due to the uplift of the Talamanca Range. However, the differentiation within South American lineages occurred only about 800,000 to 300,000 years ago [48,133]. Specifically, the emergence and structuring of the three Andean mountain ranges in Colombia has been proposed as the mechanism of isolation between *L. muta* (restricted to the Amazon) and the Central American bushmaster species (*L. melanocephala* and *L. stenophrys*), and *L. acrochorda*, the only *Lachesis* species inhabiting the trans-Andean region, distributed in the humid forests of the South American Pacific region, the Darien region in Panama, and the middle Magdalena River basin.

Recent literature reviews for *Lachesis* document the rarity of Colombian bushmasters, the number of individuals (specimens housed in biological museums; see Chapter 1), a few facts about the nature of the genus, and the small number of snakebite accidents annually [134]. In fact, it is surprising, given that *Lachesis* is considered the largest venomous snake species in South America, reaching up to 350 cm in length [89], and with a distribution encompassing four of the five Colombian ecoregions.

The systematics and taxonomy of the *Lachesis* species are the best studied aspects of the genus. In fact, this research has explored hemipenial morphology, color pattern and scale counts. Despite of these Colombian populations of the two species distributed in the country are poorly represented in these studies or have not been included [134].

It is important to highlight that in Colombia, the bushmaster species *L. acrochorda* (see Figure 5, Bottom) and *L. muta* have allopatric distribu-

tions: Populations of *L. acrochorda* are distributed in the Choco-Magdalena biogeographical province in western Colombia, and populations of *L. muta* are distributed on the eastern slopes of the Cordillera Oriental and in the Amazon ecoregion [135]. Colombian populations of bushmaster species have been poorly represented in phylogenetic assessments. For example, evolutionary relationships between the Colombian populations of *L. muta* in the Amazon and the populations of *L. muta* in the Atlantic Forest of Brazil have not been discussed.

Knowledge of the venomic and toxicological characteristics of Colombian bushmasters is still fragmentary. The protein composition of *L. acrochorda* venom is known from populations from the Pacific Coast and middle Magdalena River basin in Colombia, allowing the appreciation that the conformation of *Lachesis* venom is apparently very conservative in all species of the genus [136,137]. However, in the future some studies of the molecular systematics and new venomic analyzes will be published among populations of *L. acrochorda* from the southwest, northeast, and Pacific coast of Colombia (A.M. Franco-Vasquez personal communication 2024). For Amazonian *L. muta* from Colombia few records are available; and therefore, no biological and natural history information has been documented [138]. Thus, given medical importance of bushmaster populations from the Colombian Amazon, It is a priority to conduct venom proteomic characterization that allows to compare the venom compositions between populations from Colombia and Brazil.

Perspectives on poorly known species of Colombian pitvipers

The last 20 years of herpetological research have allowed us to clarify several of the different biological processes for the main species of pitvipers in Colombia. *Bothrocophias* and *Bothriechis* represent one of the challenges for researchers working with genetic and molecular data. The recent description of two new species of *Bothrocophias* reveals the importance of evaluating the cryptic populations of some species with a wide distribution (like *B. microphthalmus*) and of a genetic approach to some groups of species present in several South American countries [75].

Similarly, the proposal of new species *Bothriechis* for Colombia opens a space for deeper research on the venoms of the only group of arboreal pitvipers in Colombia [139]. Nevertheless, these efforts were hindered due to deep inconsistencies in species delimitation within Colombian populations, as well as the feeble evidence and unsupported new lineages proposed by Arteaga et al. [139] (see Chapter 1). Recently, Reyes-Velasco [222] re-evaluated the taxonomic proposal by Arteaga et al. [139], demonstrating that these authors misinterpreted their results. The genetic differences they interpreted as species boundaries may instead reflect clinal variation, not independent lineages. The rest of the taxa proposed by Arteaga et al. [139], including *B. khwargi*, *B. klebbai*, *B. rahimi*, *B. rasikusumorum*, and *B. torvus*, do not hold up under more rigorous analysis and should be synonymized with *B. schlegelii*. Therefore, we agree with Reyes-Velasco's [222] conclusions and recommend that future publica-

tions dealing with Colombian populations of *Bothriechis schlegelii* refer to it as the *B. schlegelii* species complex (see Chapter 1).

Published and available molecular data for different pitviper species inhabiting in Colombia were summarized in Table 1. However, despite several main phylogenies published on Neotropical pitvipers that provide our current understanding of the evolutionary paths of Colombian pitvipers, several taxa and populations of pitvipers in Colombian ecoregions are poorly represented or were not included in these phylogenies [3,55,63,92,93,101,55]. Therefore, evolutionary paths of Colombian pitvipers still fragmentary.

Bothrops asper has been the only Colombian species extensively genetically tested from molecular supports using partial sequences of the cytochrome b and NDH4 genes [53,60]. Also, parallel studies at the proteomic level could reveal the presence of geographical lineages for the main groups of species that are distributed North of South America, given that new research is being developed in the field of molecular systematics. We expect that the results of ongoing projects investigating the evolutionary trajectories of *B. atrox*, *P. nasutum*, *P. lansbergii*, and *Crotalus durissus* will arise in the coming years (Cubides-Cubillos et al.; and Arias-Sosa et al. in press), helping to unveil the complexity of the phylogenetic history of these Colombian pitvipers.

Table 1. Molecular marker data published for pitvipers from Colombia and other countries in the neotropical region.

Species	Cytochrome b	NDH4	Nuclear genes	Microsatellites
Bothriechis schlegelii	+*	*	-	-
Bothrocophias hyoprora	+*	+*	-	-
Bothrocophias tulitoi	+	+	-	-
Bothrocophias myrringae	+	+	-	-
Bothrocophias myersi	-	-	-	-
Bothrocophias colombianus	-	-	-	-
Bothrops asper	+*	+*	*	-
Lineage B. rhombeatus	-	-	-	-
Lineage B. ayerbei	-	-	-	-
Bothrops atrox	+*	+*	+*	*
Bothrops bilineatus	+*	+*	*	-
Bothrops punctatus	-	-	-	-
Bothrops pulcher	-	-	-	-

Crotalus durissus	+*	+*	+*	-
Lachesis acrochorda	+*	+*	-	-
Lachesis muta	+*	+*	-	-
Porthidium lansbergii	+*	+*	*	-
Porthidium nasutum	+*	+*	*	-

(+) Data for Colombian species. (*) Data for species in other countries. (-) unpublished data. For the species *P. lansbergii* and *P. nasutum*, the molecular data correspond to research in progress developed by Cubides-Cubillos et al. as well as the data for *C. durissus* corresponding to a review developed by Mario Vargas-Ramirez et al.; the other sequences were developed by [3,50,53,63,75,95,96,104], and [114].

3. Biological activities and proteome of the Colombian pitviper venoms

The evolutionary selection of specific types of toxic components appears to have been somewhat limited. Therefore, it is posited that the venomic proteins belong to a limited number of protein families [140]. Indeed, the restricted variety of proteins has undergone rapid evolution in its initial location, resulting in the production of numerous biological functions [141]. Hence, as venoms are trophic adaptations that facilitate subduing prey and their effects are highly variable according to the type of prey and its availability, the diverse toxic secretions of snakes exhibit significant variation in their composition [142].

Venom composition in snakes exhibits variation across all taxonomic levels (between families, genera, and species), yet understanding its mechanisms remains a subject of research [143,144]. A plausible explanation for this variation may require the conduct of adaptation evolutionary studies, coevolution, and the establishment of a phylogenetic and ecological framework [110,145,146,144]. The coarse description of the variability in venom composition has an impact on applied and biomedical sciences, influencing the efficacy of antivenom therapies and the treatment of snakebites, as well as the development of new drugs [111,147] (see Chapter 6 and 10).

Several countries in South America exhibit a high prevalence of bothropic snakebites associated with serious and life-threatening envenoming (see Chapter 9). This has led to the stigmatization of the remaining pitviper species, as well as similar non-venomous snakes, resulting in their deliberate killing by diverse human communities across their distribution ranges. Nonetheless, apprehension regarding pitviper species belonging to the genus *Bothrops* is not unfounded. Indeed, due to their capacity for inhabiting diverse ecological zones, *Bothrops* species are among the most dangerous venomous snakes in tropical regions of Central and South America. Particularly in Colombia, bothropic envenoming accounts for 62% of annual cases [111,148] (see Chapter 9).

Current research, focused on the study of venoms employing a multidisciplinary approach, using “omics” technologies such as genomics, transcriptomics, proteomics, and, more recently, metabolomics is used to investigate

low molecular weight components [149] of venoms. These research fields have had a significant impact on current biomedical science [150]. Hence, by incorporating advances in high-resolution mass spectrometry (MS) [151], and de novo and database-dependent annotation methods that need minimal amounts of venom [152], enables identification of toxin families, individual toxins, unique isoforms, splice variants, amines, amino acids, and alkaloids.

More than a hundred Neotropical pitvipers are recognized worldwide [4]; and given their extensive distribution, they constitute a medically important event with a significant impact on health, since they cause more than 2.7 million snakebites per year [108]. However, snake venoms, at the same time, are a source of raw materials for the acquisition of new molecular compounds [153], which facilitate various biotechnological advancements in molecular biology and biomedicine (see Chapter 10).

Chippaux et al. [154] paved the way for the study of snake venom, showing that these multifaceted secretions can differ at different levels and are affected by various intrinsic and extreme factors [144]. Currently, studies in venomic biology serve as models for studies in evolutionary genetics [155,156]. In the following sections, we will provide a summary of the knowledge about venomics of Colombian pitvipers. We will also discuss the gaps and research perspectives achievable in the near future.

3.1 The complexity of venom: more than a hunting weapon

Pitviper venom contains a complex mixture of proteins and enzymes with different biological activities [157]. Their primary function is to immobilize, pre-digest, and kill the prey (see Chapter 5). This enabled the transition from a mechanical subduction mechanism (body pinning and constriction) to a chemical subduction mechanism (the use of venom) [158]. Thus, the venom system became a main feature of advanced snakes [159], and it is an important trait for understanding the evolution and ecology of various snake species [141].

These complex mixtures of proteins are produced by specialized buccal glands that are homologous to the Duvernoy and salivary glands and are also present in other members of the Colubroidea superfamily [157,160]. These toxins originated from duplication events of genes encoding proteins with normal physiological functions (whose resulting proteins are recruited by the venom gland) and that show a selective expression on the structure [141,161].

Over time, venom gland-encoded proteins were subjected to selection pressures that resulted in their structural and functional modification and even suppression of their expression in the gland [161]. However, information about changes in the structural components and expression, as well as gene duplication related to the origin of the actual functions of various proteins and toxins in general, is still not well understood. Thus, understanding the order of such events that explain the origin of any protein is a challenge yet to be resolved [162].

Snake venoms present great intraspecific and interspecific variability, largely because these are ecological traits that evolve dynamically. There are several factors that influence their synthesis such as seasonality, geographic distribution, ecological variation between populations, and the variety in the diet that intrinsically diverges with the age/sex of the individual [154,163,164]. Since the properties of these venoms are useful when defending against predators, human beings have become part of this interaction.

For example, venoms from three clades of spitting cobras have the ability to activate mammalian sensory neurons, inducing pain [165]. In fact, the divergence of the spitting cobra clades in Africa (6.7 Mya) could be associated with the evolution of bipedalism and the development of larger brains in hominids [165], indicating that "spit venom" could be a mechanism that could have evolved in response to interactions with ancient hominins. In this sense, snakes will need to produce copious amounts of venom, which would implicitly make it difficult to infer whether the venom has a high-energy cost when compared to other ecological traits. In fact, it is difficult to determine experimentally this earlier premise due to the difficulty of experiments that would require specific prey management, taxon-specific toxicity testing, and, furthermore, an autonomous way in which snakes can modulate venom production and injection [166].

In fact, today those circumstantial or provoked events in which a snake bites a human has been continued, technically called "snakebite accidents" [167]. Hence, this incident leads to an intoxication resulting from venom inoculation from snakebite, resulting in a clinical case that is regarded by the World Health Organization (WHO) as one of the numerous neglected tropical diseases (NTDs; see Chapter 9). Unfortunately, these snakebite cases occur more often in developing countries, where almost 95% of the total number of snakebite cases worldwide are reported [168]. Moreover, since these accidents are frequent in low-income rural communities with limited access to healthcare services, addressing these envenomations is challenging due to the limited availability of antivenoms and the high costs of transporting the patients [168,169] (see Chapter 6).

In perspective, venoms are a rapidly evolving trait; it has been inferred that environmental factors can generate adaptive pressures that modulate their functional variation across species. As an adaptive characteristic used both for feeding and for deterring or defending against potential predators, developing effective antivenoms to neutralize their envenomations is currently a significant challenge, since these therapies must account for the wide intraspecific divergence in venom composition. In fact, recent research that reports on divergence of crotamine content in populations of the genus *Crotalus durissus* should be a warning about the need to develop an antidote capable of neutralizing this toxin [170,171].

One of the main "targets" of the venom of some snakes is the nervous system of the potential prey, even of one possible predator [172]. The presence of neurotoxins in these venoms is attributed to their capacity to disrupt the normal functioning of the central nervous and/or peripheral

nervous system [173,174]. The high specificity and selectivity of neurotoxins found in rattlesnake venoms have been widely used to study the structure-function of receptors and other elements involved in neuromuscular transmission, neurological mutations, and the causes of various neurological conditions [175] (e.g., Parkinson and Alzheimer, see Chapter 10). However, today, little research is available on the effect of toxins on the peripheral nervous system and some specific organs [172]. Researchers will continue to gain understanding about the targeted administration of drugs for the treatment of various nervous conditions, tumors, and neurodegenerative diseases in the future. In the interim, the persistent pursuit of research aimed at identifying novel ophidian peptides and comprehending their structure-function correlation with cellular receptors, will ultimately lead to the discovery of distinct molecules that have the potential to be utilized in diverse biomedical therapies (see Chapter 10).

In Colombia, the colonization of new niches in different mountainous, dry lowland, or humid tropical ecosystems must have played an important role in the phenotypic diversity of pitviper venoms that could be shaped by local adaptation to different habitats within ecoregions (e.g., the intraspecific differences in the venom of *Crotalus durissus* [171], see below). Nevertheless, further research efforts are still needed to understand the relationships between the composition of venoms, their ecological context, and their link to habitat transformations caused by human activities. For this, it is necessary to develop a more robust conceptual framework that allows for the proposal of hypotheses to explain the differentiation or specificity of venoms in an ecological and evolutionary context.

Understanding how these venoms have evolved in relation to interspecific interactions with humans would help to investigate the anthropogenic effect on natural populations of pitvipers, as well as to resolve some questions related to the effectiveness of antivenoms. Our goal in this section was to explain that snake venoms, before being a biological component that triggers a complex clinical picture (see Chapter 9), are a lethal hunting weapon modified and selected through the course of evolution of many species that grants the individual one of the most sophisticated natural resources for food and defense.

3.2. Studies in proteomics in Colombia

Despite the great diversity of pitviper snakes in Colombia, only nine species (40%) have been the object of proteomics studies of at least one population across their distribution range in the country (Table 2). The proteins present in these venoms are categorized into seven to twelve protein families. Generally, PLA_2s are the most abundant components identified, followed by SVMPs, SVSPs, and LAAOs. However, there are some exceptions. For instance, the venom of *C. durissus* exhibits a significant concentration of crotoxin (64.71%), which is a characteristic of South American rattlesnakes [170,176], but between Colombian populations there are intraspecific differences in which crotamine is only present in the Caribbean ecoregion [171].

Table 2. Percentage of toxins in the venom composition of the pitviper species distributed in Colombia with proteome characterization.

Toxins	*B. atrox*	*B. asper*	*B. ayerbei***	*B. rhombeatus***	*B. punctatus*	*P. lansbergii*	*B. myersi*	*L. acrochorda*	*C. durissus*
SVMPs	48.5	33.17	53.7	39.7	41.4	35.5	21.50	23.2	3.3
PLA_2s	24.1	31.29	0.7	23.0	9.3	16.2	54.04	2.3	0.58
SVSPs	10.9	3.89	9.3	4.9	5.4	4.5	3.43	35.1	6.33
LAAO	4.7	3	3.3	2.1	3.1	3.6	1.10	9.6	3.16
Hya	*	*	*	*	*	*	0.01	*	*
Crotoxin	*	*	*	*	*	*	*	*	64.71
CRISP	2.6	1.45	1.1	0.7	1.2	1.4	*	0.9	1.27
Dis	1.7	3.27	2.3	4	3.8	12.9	0.62	*	13.7
PLB	*	*	*	*	*	0.7	0.37	*	*
PDE	*	*	0.7	0.2	*	0.3	0.08	*	*
CTL	7.1	8.54	10.1	3.1	16.7	6.7	0.56	6.9	1.18
VEGF	*	*	*	*	1.7	2.2	*	0.6	*
NGF	*	*	0.1	*	*	*	0.07	*	*
PNP	*	*	*	*	*	*	*	*	*
BPP	0.3	*	8,3	7	*	*	*	21.5	*
PEP	*	*	8.7	*	10.7	*	9.07	*	*
Nuc	*	*	*	*	*	0.4	0.02	*	*
SVMPI	*	*	*	10	*	*	*	*	*
Crotamine	*	*	*	*	*	*	*	*	5.77

Abbreviations for protein family names: PLA_2s: phospholipase A_2; SVMPs: metalloproteinase; LAAO: L-amino acid oxidase; CTL: C-type lectin/lectin-like; CRISP: cysteine-rich secretory protein; SVSPs: serine proteinase; Nuc: nucleotidase; PDE: phosphodiesterase; Hya: hyaluronidase; NGF: nerve growth factor; PLB: phospholipase B; PNP: peptides and/or non-protein compounds; BPP: bradykinin-potentiating peptide. * Toxins no detected in the proteome. ** Dubious species of the *Bothrops asper* species complex (see Chapter 1) [118,136,171,181,184,187,192,195].

Until now, the venoms of *Bothrops* species had higher proportions of SVMPs than PLA_2. This same pattern was observed in the venoms of *P. lansbergii* and *L. acrochorda.* In contrast, the venom of *Bothrocophias myersi* has a superior percentage of PLA_2s. In addition, the venom of this species is the most complex, with twelve families of proteins. Special characteristics of *L. acrochorda* venom are their elevated amounts of SVSPs and BBPs (35.1% and 25.5%), even more than PLA_2s, which is a unique pattern. While the venom of the *L. muta* (Amazon bushmaster) has the highest quantity of LAAOs with 9.6%.

Interestingly, the venom of *B. punctatus* exhibits a higher concentration of C-type lectins (16.7%); however, these toxins were identified in all venoms through proteomic studies, ranging from 0,56% to 10,1% in the remaining species with proteomes available. It should be noted that the venom of *P. lansbergii* possesses the highest concentrations of desintegrins, whereas these toxins were not detected in the venom of *L. acrochorda*. One noteworthy discovery was the presence of hyaluronidases, the spreading factor of venoms (as discussed in Chapter 5) that were only detected in the venom of *B. myersi,* but in a minimal amount (0.01%). This result may be attributed to the fact that in certain instances not all proteins were classified. On average eight groups of toxins were described in most of the research, where percentages were usually informed for the following toxins: SVMPs, PLA_2s, SVSPs, LAAO, CRISP, Dis, CTL and BPP. Crotoxin and crotamine are exclusive components for the *Crotalus* genus.

The venom of *Crotalus durissus* requires special attention because it is the only one with neurotoxic activity and low molecular weight myotoxins among Colombian viperids. The venom of Colombian rattlesnakes is composed of a considerable proportion of crotoxin (64.71%), the toxin responsible for inducing diaphragm-flaccid paralysis. However, another exceptional toxin present in this venom is crotamine, a small basic polypeptide with myotoxic and cell-penetrating activities [177,178]. Interestingly, the expression of crotamine can be different in venom from different geographical regions, showing an intraspecific variation in the composition of the *C. durissus* venom [171,179].

3.3 The biological activities of Colombian pitvipers

All venom activities evaluated for species from Colombia are correlated with the signs and symptoms observed in snakebite cases reported (see Chapter 9). Venoms from *Bothrops* species (*B. atrox*, *B. asper*, *B. ayerbei*, *B. rhombeatus* and *B. punctatus*; see Figure 6) have different lethal activity values (LD_{50} µg/mice), such as 81.4 (80.2–83.6) [42], 63 (50–81 ranges for several ecoregions from Colombia) [180], 50.1 (37.5–58.3) [181], 54.9 (36.0–83.8) [181], and 47 (36–61) [180]. Hence, the most lethal venoms among the *Bothrops* species tested so far are *B. asper* and *B. punctatus*. It is important to mention that not all Colombian *Bothrops* species have been evaluated. However, the venom of *C. durissus* is the most lethal venom within pitvipers in Colombia, with a lethal dose average of 1 µg/mice (0.02–2.5) [171,180]. This lethal activity value is considered a consequence

of the venom's neurotoxicity, a widely described functional feature that is biologically absent in the venoms of other pitviper species [182,183].

Saldarriaga et al. [42] carried out one of the pioneering studies on the ontogeny of *Bothrops* venoms that allowed us to understand how the species in its first stages of life develops venoms with fractions endowed with high hemorrhagic activity, while in the adult stage mostly fractions are found with indirect hemolytic activity. Furthermore, when *B. asper* venoms from Colombia and Costa Rica are compared, there are specific divergences associated with variations in lethal, hemorrhagic, edematogenic, myotoxic and indirect hemolytic activities [42].

In this study, we compared the biological activities of the principal biological important pitviper species from Colombia. We found that within the *B. asper* lineages (*B. asper*, *B. ayerbei,* and *B. rhombeatus* [184]) the venom of main lineage of *B. asper* (distributed throughout the Caribbean region and the middle Magdalena Valley) is significantly more lethal than *B. ayerbei,* and *B. rhombeatus* lineages. *Bothrops asper* venom turned out to be more hemorrhagic than venom of the *B. rhombeatus* lineage, but the minimum hemorrhagic dose of *B. ayerbei* lineage was significantly lower, showing its greater capability to cause hemorrhage.

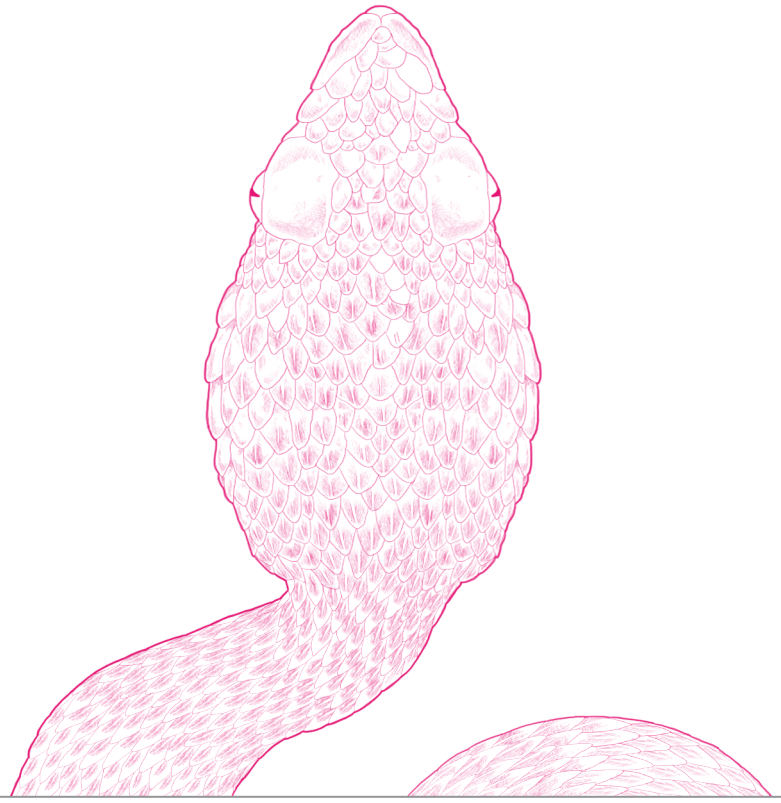
On the other hand, the venom of *B. rhombeatus* lineage showed a greater coagulating capacity while *B. ayerbei* lineage exhibited the lowest coagulating capacity. Moreover, despite the geographic isolation between the populations of *B. asper* from Gorgona Island (Pacific Ocean) and the continental populations from the Valle del Cauca, the biological activities of their venoms showed very similar activities, with the exception of coagulating activity, which is much lower in the populations from Gorgona Island. However, the biological activities of the venom of these two populations are significantly different when compared to the venoms from the Caribbean cost and Magdalena River Basin [185]. It has been inferred that the divergence of populations within some lineages of *B. asper* is strongly influenced by the orography of the Andes mountain range, and the dynamically changing nature of their ecosystems that could drive specificity in different ecological niches [184]. In fact, a proteomic comparison of *B. asper* venoms based on the genetic diversity documented for lineages of the *Bothrops asper-atrox* complex found intraspecific variation in four of the main components of the venom [53,60]: SVMP, SVSP, PLA_2 and C-type lectin-like proteins (CTL) [184].

There is controversy regarding the biological activities of the venom of various populations of the *Bothriechis schlegelii* species complex. As we explained earlier, this species exhibits clinal variation among its populations, which is possibly related to the variability in the biological activities of their venoms (Figure 6A, C-E). For example, the Colombian populations from the central-eastern Andes (Antioquia department) [180,186], and those from the southwestern region of Colombia (Valle del Cauca department) [187] do not exhibit hemorrhagic activity. However, the venom of these populations shows coagulating activity and a high capacity for ede-

ma formation, similar to what is observed in the populations from Costa Rica (*Bothriechis nigroadspersus*) [188]. Given the medical importance of this species and its wide distribution along the agricultural production zones of the Colombian Andes, it is a priority to conduct rigorous studies exploring the determinants of the variability in the biological activities of the *Bothriechis schlegelii* species complex. This will enable physicians treating envenomations to generate more accurate diagnoses, establish timely and appropriate therapy, and anticipate possible clinical complications.

Hemorrhagic activity of snake venoms is attributed to the activity of SVMPs [189]. All pitviper venoms tested up till now have hemorrhagic activity; however, their potency is variable. Generally, *Bothrops* spp., *Porthidium* spp., *L. acrochorda* and *Bothrocophias* spp. are more hemorrhagic than the *B. schlegelii* species complex, whereas *C. durissus* rarely induces local hemorrhage [190–193]. Nevertheless, *Bothrocophias campbelli* venom induces mild hemorrhage and coagulation disorders [194].

Both *Porthidium lansbergii* and *P. nasutum* show medium cytotoxic activity and low lethality when assessed on mice, but both species show high hemorrhagic activity [195]. In addition, pitviper venoms from Colombian populations induce hemostatic disorders that are related to their pro-coagulant activity shown in vitro, their fibrinogenolytic displayed in vivo, and the alteration of coagulation times. These venoms are characterized by a massive consumption of fibrinogen that can only be recovered by antivenom therapy [90,193,196].



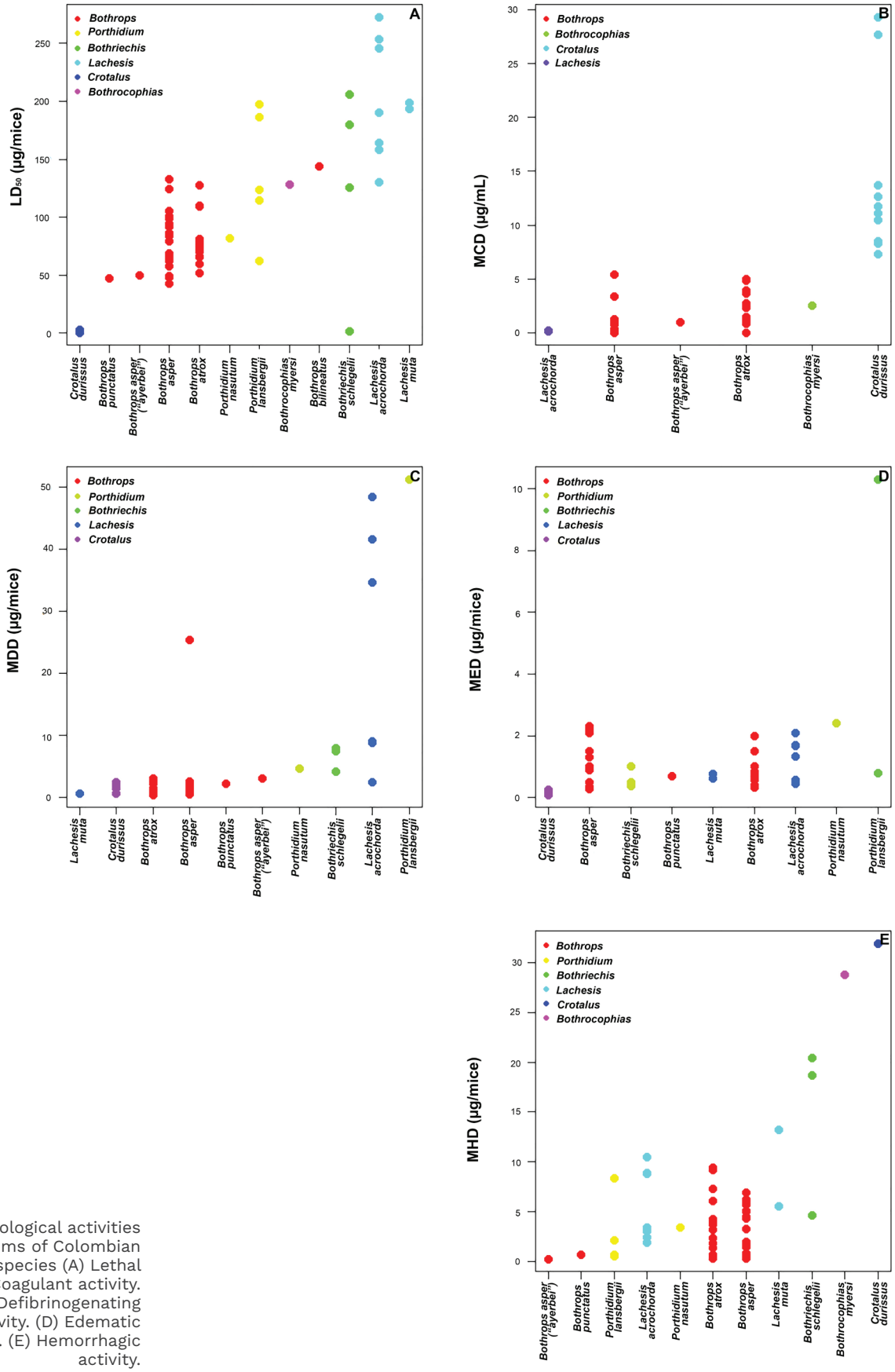


Figure 6. Biological activities of venoms of Colombian pitviper species (A) Lethal dose. (B) Coagulant activity. (C) Defibrinogenating activity. (D) Edematic activity. (E) Hemorrhagic activity.

Table 3. Biological activities tested for Colombian Viperidae

Species	Lethality	Hemorrhagic	Myotoxicity	Edematic	Coagulant	Hemolytic (PLA_2 activity)	Defibrinogenating	Neurotoxic
Bothriechis schlegelii	Evaluated	Present	Present	Present	Present	Present	Present	Absent
Bothrocophias myersi	Evaluated	Present	Present	Present	Present	Unevaluated	Unevaluated	Absent
Bothrops asper	Evaluated	Present	Present	Present	Present	Present	Present	Absent
Lineage *B. ayerbei**	Evaluated	Present	Present	Present	Present	Present	Present	Absent
Lineage *B. rhombeatus**	Evaluated	Present	Present	Present	Present	Present	Present	Absent
Bothrops atrox	Evaluated	Present	Present	Present	Present	Present	Present	Absent
Bothrops punctatus	Evaluated	Present	Present	Present	Present	Present	Present	Absent
Crotalus durissus	Evaluated	Present	Present	Present	Present	Present	Present	Present
Lachesis acrochorda	Evaluated	Present	Present	Present	Present	Present	Present	Absent
Lachesis muta	Evaluated	Present	Present	Present	Present	Present	Present	Absent
Porthidium lansbergii	Evaluated	Present	Present	Present	Present	Present	Present	Absent
Porthidium nasutum	Evaluated	Present	Present	Present	Present	Present	Present	Absent
Bothrocophias campbelli	Unevaluated							
Bothrocophias colombianus								
Bothrocophias hyoprora								
Bothrocophias myrringae								
Bothrocophias tulitoi								
Bothrops bilineatus								
Bothrops oligobalius								
Bothrops pulcher								
Bothrops taeniatus								
Bothrops venezuelensis								

* Dubious species of the *Bothrops asper* species complex (see Chapter 1). [42,118,136,171,180-190,194]

3.4 Outlook for antivenom research

Antivenom therapy, (based on serum therapy; see Chapter 6), was developed in the 19th century; and although it has had huge improvements throughout its existence, this essentially uses immunoglobulins as therapy to this day [197]. In fact, since their production, antivenoms have been used successfully for the treatment of snakebite envenomation in various regions of the world [108]. In context, the antivenom "works" from purified immunoglobulins, which act initially by activating the immune response to the most pathogenic venom proteins, and then, trying to reduce and stop the severity of the envenomation. However, in some cases this therapy can cause adverse reactions (e.g., anaphylactic shock) [111].

The administration of antivenoms is done intravenously. Once in the bloodstream, the antibodies or antibody fragments bind to the venom toxins, preventing them from damaging tissues or interfere with critical bodily functions. These immunoglobulins are obtained and purified from the plasma of large mammals (usually horses), which have been previously immunized with sub-lethal doses of snake venom [198,199] (see Chapter 6). Antivenoms can be either monovalent or polyvalent, depending on whether the horses are immunized with venom from a single species or with a mixture of venoms from multiple species, respectively [200].

The incidence and severity of adverse reactions associated with the administration of antivenom have been reduced by the purification of immunoglobulins [201], thus demonstrating the therapeutic efficacy of antivenom to control the systemic manifestations of snake envenoming [202]. However, the venom heterogeneity between species could explain the differences in the clinical symptoms recorded from different geographic regions during snakebite cases [154]. This phenomenon reflects a series of local adaptations that confer a series of biological advantages on a certain population of snakes. Currently, snake envenoming represents a huge challenge for the design of new approaches to immunotherapy [111].

The description of venoms from various species and the knowledge of implicit inter- and intraspecific variability raises a scenario of new and necessary research approaches for the selection of phylogeographic regions of interest to help capture and describe venom variability. For example, neutralization measurements of the *C. durissus* venom using National Institute of Health of Colombia (INS) antivenom to calculate the median effective dose (ED_{50}) have shown that more antivenom is required to neutralize venom from Caribbean populations (2.3 mg venom/mL) than those from middle Magdalena basin (1.4 mg venom/mL), or the Orinoquia ecoregion (0.6 mg venom/mL) [171]. Therefore, there is a need to use venoms combined as a substrate to produce antivenom and, thus, prioritize the investigation of the snake venom using a combination of proteomic tools, functional toxicological and biochemical [199]. In fact, Juan Calvete et al. [111] presents a useful protocol to investigate antigen-antibody immunoreactivity, opening the door to the possible therapeutic utility of antivenoms against homologous and heterologous venoms in pitviper species.

Thus, based on the immuno-reactivity of the various toxins against antivenoms, these toxic components could be grouped into three types of toxins: completely immunodepletable, partially immunodepleted, and non-immunodepleted. Thus, immunization protocols must be improved by using immunogen mixtures [111]. This approach has recently established the basis for the development of antivenoms on an immunologically sound foundation and has proposed the use of immunochemical analysis based on proteomics for the identification of the most medically relevant venoms from snakes present in a specific region [111].

A first example of this type of new therapy, which has impacted and driven research in the field of antivenoms, is the development of an antivenom designed for the treatment of snakebites caused by *Bothrops lanceolatus* (endemic to the island of Martinique in the Lesser Antilles). This Sanofi-Pasteur Bothrofav®, has demonstrated good preclinical efficacy in neutralization, timely preventing the development of the most severe systemic events [203,204].

The limited availability of antivenoms in several Central and South American countries, as a result of various anthropogenic processes (social and environmental; see Chapter 6), has created the need and challenge to improve the neutralizing capacity of the available antivenoms. As an alternative, the use of small molecular inhibitors has been proposed to counteract the effect of the main snake venom proteins that cause clinical symptoms. Research in this field has recently gained relevance [144]. The effect of these inhibitors has been studied both in vitro and in vivo, yielding promising results for the inhibition of PLA_2, SVMP, and SVSP [205,206].

Researchers from the Clodomiro Picado Institute, led by Dr. José María Gutiérrez, conducted detailed proteomic studies of the venoms of *Bothrops caribbaeus* and *B. lanceolatus* (species included within the *B. atrox* complex), finding immunoreactivity to the polyvalent antivenom produced in Costa Rica to neutralize the venom of Central American rattlesnakes. This antivenom was able to immunodegrade 80% of the proteins and neutralize the lethal activities of the venoms in these two species [205]. Other studies highlight the importance of comparing proteomes between species with a similar natural history but with taxonomic uncertainties or undefined lineages within the *Bothrops asper-atrox* complex [111,207,208]. The findings of the Costa Rican researchers represent a significant advancement in understanding the cross-reactivity of antivenoms that counteract the symptoms of envenomation caused by American pit vipers.

In Colombia, experiments have been conducted to explore complementary options to antivenom treatment. Recently, recombinant proteins have been produced to improve the immunogenicity of proteins such as PLA_2 and three-finger toxins, representing a novel advancement for the future development of antivenoms that neutralize the toxins of snakes from the *Micrurus* genus [209]. Additionally, experiments using purified molecules from plant extracts to inhibit certain biological activities of snake venoms [192,210,211], as well as synthetic inhibitors that

have shown inhibitory activity against PLA_2 and SVMP [212] have all been carried out in preclinical trials.

The gradual incorporation of chromatographic and mass spectrometry techniques to complement the current platforms recommended by the WHO in the evaluation of antivenoms are ideal for establishing a geographical range of their clinical validity [213]. Besides, when combined with *in vivo* neutralization assays, antivenoms represent a good complement for evaluating the therapeutic efficacy of antivenoms against the same venoms used in its production [214].

Polyvalent antivenoms such as those from ICP (Costa Rica), INS (Colombia), PROBIOL (Colombia), UCV (Venezuela), BIOL (Argentina), and INS-PERU (Peru) can recognize the main toxin families present in *Bothrops asper* venoms (SVMP, PLA_2s, CRISP, SVSP, CTL); among these, the INS antivenom is the most effective and safest therapy [181]. In comparative studies of the antivenoms available in Colombia, the INS antivenom showed the most effective immunorecognition capacity, as well as the best neutralizing capacity against the biological activities of venoms from *Bothrops* spp., *Bothrocophias* spp., *Bothriechis* spp., *Crotalus durissus*, *Lachesis* spp., and *Porthidium* spp., followed by the Silanes (Bioclon) antivenom from Mexico [190]. In a more recent study [185], *B. asper* venoms from Gorgona Island and the Pacific and Western ecoregions of Colombia were evaluated from immunoreactivity assays using INS and Probiol antivenoms. The results obtained indicate again that the INS antivenom had higher antibody titers and neutralization compared to Probiol antivenom, showing the greater neutralizing capacity for the venoms of *B. asper* from Gorgona island population.

It is important to study the variability in the protein composition of different pitviper venoms and to develop studies that describe the structure-function correlations of individual toxins that will help to improve an understanding of venoms as trophic and defensive adaptations in different species [213]. Likewise, it is important to continue exploring alternatives for the potential use of venom toxins as active compounds in the development of new medications. However, this should be preceded by a deeper study of the natural history of viperid genera present in Colombia (e.g., distribution), to better understand how the destruction of their natural habitats and climate change affect the variability of their venoms and their survival in both natural and altered environments.

Appendix: Material and Methods

Phylogenetic analyses
Phylogenetic analyses were performed using maximum-likelihood estimation in the IQ tree software [215]. The evolutionary models were performed using the model finder [216]: TN+F+I: CYTB1_ND41, TIM+F+R2: CYTB2, GTR+F+I+G4: CYTB3_ND43, TN+F+R2: ND42. The analyses were performed using an array of 102 concatenated sequences for 51 terminals for the *Bothrops atrox* complex and the *Porthidium* genus from Colombia, Brazil, Ecuador, Venezuela, and Costa Rica. We used 20 species as out groups: *Gloydius halys*, *Sistrurus miliarius* and *S. catenatus*; *Crotalus horridus*, *C. ruber*, *C. atrox*, and *C. durissus*; *Agkistrodon piscivorus* and *A. contortrix*; *Mixcoatlus melanurus*; *Ophryacus ondulatus*; *Cerrophidion godmani*; *Metlapilcoatlus* (*Atropoides*) *nummifer* and *M. mexicanus*; *Atropoides picadoi* and *Bothrocophias hyoprora*, *B. tulitoi* and *B. myrringae*; *Bothriechis schlegelii* (currently redescribed as *Bothriechis nigroadspersus*) and *Bothrops jararaca.* Besides, phytools were used to perform a relationship analysis (heatmap) at the phylogenetic level [217], using two published proteomes for Colombian pitviper species and some related lineages/clades.

Biological activity assays
The biological assays for lethality, coagulant, defibrinogenating, hemorrhagic, and edematogenic activity were conducted by the INS (National Institute of Health) as part of its antivenom production activities, in accordance with venom characterization guidelines established by the WHO in "Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins; 2017". All tests were performed on CD1 strain mice weighing 18-22 g, following INS protocols for the use of laboratory animals.

Median Lethal Dose ($LD50_{50}$).— We assessed venom lethality following the procedures and protocols described by [200,218], as well as the INS production protocol MEN-R04.6022-011. The median lethal dose (LD_{50}) of a venom is defined as the amount of venom that causes death in 50% of inoculated mice. To determine the LD_{50}, different doses of venom were prepared in saline solution and administered intraperitoneally to groups of five mice. Mortality was recorded after 48 hours. A control group of five mice received saline solution. Finally, the LD_{50} was estimated using statistical methods such as the Spearman-Karber, Probit function, or other non-parametric methods [219].

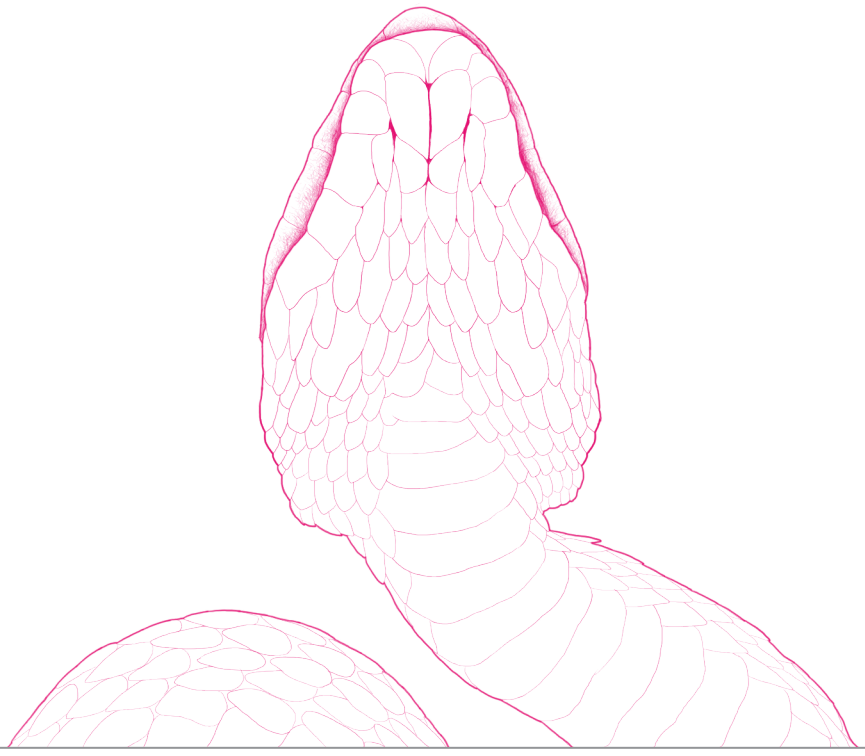
Minimum Coagulant Dose (MCD).— In vitro coagulation assays were performed following the procedures and protocols described by [200,220], as well as the INS production protocol MEN-R04.6031-001. The minimum coagulant dose (MCD) was defined as the lowest venom dose that induced coagulation in citrated human plasma.

Minimum Defibrinogenating Dose (MDD).— In vivo defibrinogenation assays were conducted following the INS production protocol MEN-R04.6022-046, as well as the procedures and protocols described by [200,220]. The MDD corresponds to the venom dose that, when adminis-

tered intravenously to five mice injected with a venom dilution, induces incoagulability after 2 hours of sample collection.

Minimum Edematogenic Dose (MED).— In vivo edema assays were performed following the INS production protocol MEN-R04.6022-043, as well as the procedures and protocols described by [200]. The MED is defined as the venom dose that induces a 30% increase in mouse paw edema following the injection of a venom dilution. For this purpose, venom solutions at different concentrations were prepared. Each dilution was injected into the right hind paw plantar pad of five mice, while the left paw received sterile saline (0.85%). One hour after inoculation, the animals were ethically euthanized [221]. Both paws (at the tibiotarsal joint) were excised and weighed.

Minimum Hemorrhagic Dose (MHD).— In vivo hemorrhagic assays were conducted following the INS production protocol MEN-R04.6022-049, as well as the procedures and protocols described by [200,220]. The minimum hemorrhagic dose (MHD) was defined as the smallest amount of venom capable of inducing a hemorrhagic lesion with a 10 mm diameter. To determine this, different venom dilutions in saline solution were prepared, with a final injection volume of 50 µL. Each dilution was then injected intradermally into the shaved ventral skin of five mice. After 2-3 hours, the animals were ethically euthanized [221]. The skin was dissected, and the diameter of the resulting hemorrhagic lesion was measured.



References

1. Cubides-Cubillos, S.D. Actitudes, Creencias y Prácticas Desarrolladas En Comunidades Rurales Del Urabá Antioqueño Con Relación a Las Serpientes y Su Mordedura. Trabajo presentado para optar al título de Magister en Educación – Línea de formación: pedagogía y diversidad cultural, Universidad de Antioquia: Medellín, Colombia, **2016**.
2. Campbell, J.A.; Lamar, W. *The Venomous Reptiles of the Western Hemisphere*; Cornell University Press, Ed; Comstock Publishing: Ithaca, New York, **2004**.
3. Alencar, L.R.V.; Quental, T.B.; Grazziotin, F.G.; Alfaro, M.L.; Martins, M.; Venzon, M.; Zaher, H. Diversification in Vipers: Phylogenetic Relationships, Time of Divergence and Shifts in Speciation Rates. *Molecular Phylogenetics and Evolution* **2016**, *105*, 50–62, doi:10.1016/j.ympev.2016.07.029.
4. Uetz, P.; Freed, P.; Aguilar, R.; Hošek, J. The Reptile Database. **2023**.
5. Palisot de Beauvois Memoir on Amphibia. Serpents. *Transactions of the American Philosophical Society* **1799**, *4*, 362–381.
6. Werman, S.D. Phylogenetic Relationships of Central and South American Pitvipers of the Genus *Bothrops* (Sensu Lato): Cladistic Analyses of Biochemical and Anatomical Characters. In *Biology of the Pitvipers*; J.A. Campbell, E.D. Brodie, Eds.; Selva: Texas, **1992**; pp. 21–40.
7. Peters, W. Über Die von Hrn. Dr. Hoffmann in Costa Rica Gesammelten Und an Das Königl. *Zoologische Museum gesandten Schlangen* **1859**, pp. 275-278.
8. Gutberlet, R.L.; Campbell, J.A. Generic Recognition for a Neglected Lineage of South American Pitvipers (Squamata: Viperidae: Crotalinae), with the Description of a New Species from the Colombian Choco. *American Museum Novitates* **2001**, 1–15.
9. Wagler, J. Serpentum Brasiliensium Species Novae, Ou Histoire Naturelle Des Espèces Nouvelles de Serpens. *Jean de Spix, Animalia nova sive species novae* **1824**, *VII*, 1–75.
10. Campbell, J.A.; Lamar, W.W. Taxonomic Status of Miscellaneous Neotropical Viperids, with the Description of a New Genus. *Occasional Papers, Museum of Texas Tech University*. **1992**, *153*, 1–31.
11. Linnaeus, C. Systema Naturæ per Regna Tria Naturæ, Secundum Classes, Ordines, Genera, Species. *Cum Characteribus, Differentiis, Synonymis, Locis*; 10th ed.; **1758**; Vol. 1.
12. Daudin, F.M. Histoire Naturelle, Générale et Particulière*, Des Reptiles*; F. Dufart.; Paris, **1802**; Vol. 4.
13. Campbell, J.D.; Frost, R.; and Castoe, T.A. A new generic name for jumping pitvipers (Serpentes: Viperidae). *Revista Latinoamericana de Herpetología* **2019**, *2*, 52–53.
14. Jadin, R.C.; Gutberlet Jr, R.L.; Smith, E.N. Phylogeny, Evolutionary Morphology, and Hemipenis Descriptions of the Middle American Jumping Pitvipers (Serpentes: Crotalinae: *Atropoides*). *Journal of Zoological Systematics and Evolutionary Research* **2010**, *48*, 360–365, doi:10.1111/j.1439-0469.2009.00559.x.
15. Cope, E.D. Catalogue of Batrachians and Reptiles [Batrachia and Reptilia] of Central America and Mexico. **1887**, *32*, 1–98.

16. Cope, E.D. “Ninth Contribution to the Herpetology of Tropical America.” *Proceedings of the Academy of Natural Sciences of Philadelphia* **1871**, *23*, 200–224.
17. Garman, S. The Reptiles and Batrachians of North America. *Memoirs of the Museum of Comparative Zoölogy,* Cambridge (Massachusetts) **1884**, *8*, 34–185.
18. Cope, E.D. «Notes upon Some REPTILES of the Old World». *Proceedings of the Academy of Natural Science of Philadelphia.* **1862**, *14*, 337–344.
19. Boulenger, G.A. “An Account of the Reptilia Obtained in Burma, North of Tenasserim, by Monsieur. L. Fea, of the Genoa Civic Museum.” *Annali del Museo Civico di Storia Naturale di Genova* **1888**, *6*, 593–604.
20. Gray, J.E. Monographic Synopsis of the Vipers, or the Family Viperidæ. *Zoological Miscellany, London* **1842**, *2*, 68–71.
21. Cope, E.D. Catalogue of the Venomous Serpents in the Museum of the Academy of Natural Sciences of Philadelphia, with Notes on the Families, Genera and Species. *Proceedings of the Academy of Natural Sciences of Philadelphia* **1860**, *11*, 332–347.
22. Wagler, J.G. Natürliches System Der Amphibien, Mit Vorangehender Classification Der Säugetiere Und Vögel. *Ein Beitrag zur vergleichenden Zoologie* **1830**, 1–352.
23. Kuhl, K.; Van Hasselt, J.C. Uittreksel Uit de Brieven van Heeren Kuhl En van Hasselt, Aan de Heeren C.J. Temminck, Th. van Swinderen En W. de Haan. Algemeene Konst. *Letterbode* **1822**, *7*, 99–104.
24. Gloyd, H.K. “A New Generic Name for the Hundred-Pace Viper.” *Proceedings of the Biological Society of Washington* **1979**, *91*, 963–964.
25. Merrem, B. Versuch Eines Systems Der Amphibien. *Tentamen Systematis Amphibiorum. Marburg: J.C. Krieger* **1820**, 191.
26. Alcoock, A., F.F. “An Account of the Reptilia Collected by Dr. F. P. Maynard, Captain A. H. McMahon, C.I.E., and the Members of the Afghan-Baluch Boundary Commission of 1896.” *Journal of the Asiatic Society of Bengal* **1897**, *65*, 550–556.
27. Malhotra, A.; Thorpe, R.S. A Phylogeny of Four Mitochondrial Gene Regions Suggests a Revised Taxonomy for Asian Pitvipers (*Trimeresurus* and *Ovophis*). *Molecular Phylogenetics and Evolution* **2004**, *32*, 83–100, doi:10.1016/j.ympev.2004.02.008.
28. Hoge, A.R.; Romano-Hoge, S.A.R. Poisonous Snakes of the World. Part 1: Check List of the Pitvipers, Viperoidea, Viperidae, Crotalinae. *Memorias do Instituto Butantan* **1981**, *42*, 179–309.
29. Fitzinger, L. Systema Reptilium. Fasciculus Primus: Amblyglossae. *Vindobonae: Braumüller und Seidel* **1843**, 1–106.
30. Reuss, A.F. Sechs Europaïsche Giftschlangengattungen. *Zoologischer Anzeiger* **1927**, *75*, 124–129.
31. Broadley, D.G. A Review of the Tribe Atherini (Serpentes: Viperidae), with Descriptions of Two New Genera. *African Journal of Herpetology* **1996**, *45*, 40–48.
32. Nilson, G., T.B., A.C., O.N., J.U.& H.H.W. Taxonomic Position of the *Vipera xanthina* Complex. *Kaupia* **1999**, *8*, 99–102.
33. McDiarmid, R.W., C.J.A., T.T. Snake Species of the World: A Taxonomic and Geographic Reference. *Herpetologists’ League* **1999**, *1*, 511.

34. Hoge A.R.; Romano-Hoge S.A.L. «Notes on Micro and Ultrastructure of 'Oberhäutschen' in Viperoidea. Viperoidea, Viperidae, Crotalinae». *Memorias do Instituto Butantan* **1983**, *44*, 81–118.
35. Boulenger, G.A. Catalogue of the Snakes in the British Museum (Natural History. Volume III., Containing the ... Viperidæ. *London: Trustees of the British Museum (Natural History). (Taylor and Francis, printers)* **1896**, *3*, 772.
36. Lacepede, B.G.E.L. Memoire Sur Plusieurs Animaux de La Nouvelle Hollande Dont La Description n'a Pas Encore Ete Publiee. *Annales du Museum d'Histoire naturelle* **1804**, *4*, 184–211.
37. Laurenti, J. Specimen Medicus Exhibens Synopsin Reptilium. *J. Thoma* (Vienna) **1768**, 1–2.
38. Wüster, W.; Peppin, L.; Pook, C.E.; Walker, D.E. A Nesting of Vipers: Phylogeny and Historical Biogeography of the Viperidae (Squamata: Serpentes). *Molecular Phylogenetics and Evolution* **2008**, *49*, 445–459, doi:10.1016/j.ympev.2008.08.019.
39. Greene, H. The Ecological and Behavioral Context for Pitviper Evolution. In *Biology of the Pitvipers*; **1992**; pp. 107–117.
40. Mackessy, S. Venom Ontogeny in the Pacific Rattlesnakes *Crotalus viridis helleri* and *C. d. oreganus*. *Copeia* **1988**, *2*, 92–101.
41. Guércio, R.A.P.; Shevchenko, A.; Shevchenko, A.; López-Lozano, J.L.; Paba, J.; Sousa, M. V.; Ricart, C.A.O. Ontogenetic Variations in the Venom Proteome of the Amazonian Snake *Bothrops atrox*. *Proteome Science* **2006**, *4*, doi:10.1186/1477-5956-4-11.
42. Saldarriaga, M.M.; Otero, R.; Núñez, V.; Toro, M.F.; Díaz, A.; Gutiérrez, J.M. Ontogenetic Variability of *Bothrops atrox* and *Bothrops asper* Snake Venoms from Colombia. *Toxicon* **2003**, *42*, 405–411, doi:10.1016/S0041-0101(03)00171-5.
43. Alape-Girón, A.; Sanz, L.; Escolano, J.; Flores-Díaz, M.; Madrigal, M.; Sasa, M.; Calvete, J.J. Snake Venomics of the Lancehead Pitviper Bothrops Asper. Geographic, Individual, and Ontogenetic Variations. *Journal of Proteome Research* **2008**, *7*, 3556–3571, doi:10.1021/pr800332p.
44. Kardong, K.; Kiene, T.; Bels, V. Evolution of Trophic Systems in Squamates. *Neth Journal Zoology* **1997**, *47*, 1–17.
45. Burger, W.L. Genera of Pitvipers (Serpentes: Crotalidae), University of Kansas: Lawrence, **1971**.
46. Avise, J.C. *Molecular Markers, Natural History and Evolution*; Springer US, **1994**.
47. Avise, J.C. Phylogeography; Harvard University Press, **2000**; ISBN 9780674268708.
48. Zamudio, K.R.; Greene, H.W.; Zamudio, K.R.; Greene, H.W. Phylogeography of the Bushmaster (Lachesis Muta: Viperidae): Implications for Neotropical Biogeography. *Systematics and Conservation*; **1997**; Vol. 62.
49. Thorpe, R.S.; Mcgregor, D.P.; Cumming, A.M.; Jordan, W.C. DNA Evolution and Colonization Sequence of Island Lizards in Relation to Geological History: MtDNA RFLP, Cytochrome b, Cytochrome Oxidase, 12s RRNA Sequence, and RAPD Nuclear Analysis. *Evolution* (NY) **1994**, *48*, 230–240.
50. Parkinson, C.L. Molecular Systematics and Biogeographical History of Pitvipers as Determined by Mitochondrial Ribosomal DNA Sequences. *Copeia* **1999**, 576–586.

51. Gutberlet Jr, R.L.; Harvey, M.B. Phylogenetic Relationships of New World Pit Vipers as Inferred from Anatomical Evidence. In *Biology of the Vipers*; G.W. Schuett, M. Höggren, M.E. Douglas, H.W. Greene, Eds.; Eagle Mountain Publishing: Salta Lake City, **2002**; Vol. 1, pp. 51–68.
52. Amaral, A. Contribuicao ao conhecimento dos ofidios neotropicos XXXVI. Redescricao da espécie *Bothrops hyoprora* Amaral, **1935**. Mem. Inst. Butantan 26: 221-225.
53. Saldarriaga-Cordoba, M.; Parkinson, C.L.; Daza, J.M.; Wüster, W.; Sasa, M. Phylogeography of the Central American Lancehead *Bothrops asper* (Serpentes: Viperidae). *PLoS One* **2017**, *12*, e0187969, doi:10.1371/journal.pone.0187969.
54. Gregory-Wodzicki, K.M. Uplift History of the Central and Northern Andes: A Review. *Bulletin of the Geological Society of America* **2000**, *112*, 1091–1105, doi:10.1130/0016-7606(2000)112<1091:UHOTCA>2.3.CO;2.
55. Carrasco, P.A.; Mattoni, C.I.; Leynaud, G.C.; Scrocchi, G.J. Morphology, Phylogeny and Taxonomy of South American Bothropoid Pitvipers (Serpentes, Viperidae). *Zoologica Scripta* **2012**, *41*, 109–124, doi:10.1111/j.1463-6409.2011.00511.x.
56. Bagley, J.C.; Johnson, J.B. Phylogeography and Biogeography of the Lower Central American Neotropics: Diversification between Two Continents and between Two Seas. *Biological Reviews* **2014**, *89*, 767–790, doi:10.1111/brv.12076.
57. Carrasco, P.A.; Koch, C.; Grazziotin, F.G.; Venegas, P.J.; Chaparro, J.C.; Scrocchi, G.J.; Salazar-Valenzuela, D.; Leynaud, G.C.; Mattoni, C.I. Total-evidence Phylogeny and Evolutionary Morphology of New World Pitvipers (Serpentes: Viperidae: Crotalinae). *Cladistics* **2023**, *39*, 71–100, doi:10.1111/cla.12522.
58. Antonelli, A.; Quijada, A.; Crawford, A.J.; Bates, J.M. Molecular Studies and Phylogeography of Amazonian Tetrapods and Their Relation to Geological and Climatic Models Origin, Evolution, Genetics and Selective Drivers of Snake Venom Evolution. *View Project Population Genetics and Molecular Ecology of Ectothermic Vertebrates* **2010**.
59. Lagomarsino, L.P.; Condamine, F.L.; Antonelli, A.; Mulch, A.; Davis, C.C. The Abiotic and Biotic Drivers of Rapid Diversification in Andean Bellflowers (Campanulaceae). *New Phytologist* **2016**, *210*, 1430–1442, doi:10.1111/nph.13920.
60. Salazar-Valenzuela, D.; Kuch, U.; Torres-Carvajal, O.; Valencia, J.H.; Gibbs, H.L. Divergence of Tropical Pitvipers Promoted by Independent Colonization Events of Dry Montane Andean Habitats. *Journal of Biogeography* **2019**, *46*, 1826–1840, doi:10.1111/jbi.13661.
61. Solís-Lemus, C.; Ané, C. Inferring Phylogenetic Networks with Maximum Pseudolikelihood under Incomplete Lineage Sorting. *PLoS Genetics* **2016**, *12*, doi:10.1371/journal.pgen.1005896.
62. Sousa, V.; Hey, J. Understanding the Origin of Species with Genome-Scale Data: Modelling Gene Flow. *Nature Reviews Genetics* **2013**, *14*, 404–414, doi:10.1038/nrg3446.
63. Wüster, W.; Salomão, M.; Quijada–Mascareñas, J.A.; Thorpe, R.; BBBSP Origins and Evolution of the South American Pitviper Fauna: Evidence from Mitochondrial DNA Sequence Analysis. In Proceedings

of the Biology of the Vipers; Campbell, J.A., Brodie, E.D., Schuett, G.W., Hoggren, M., Douglas, M.E., Greene, H.W., Eds.; Eagle Mountain Publishing: UT, USA, **2002**; pp. 111–128.
64. Díaz-Ricaurte, J.; Cubides-Cubillos, S.D.; Ferreto Fiorillo, B. *Bothrops asper* (Garman, 1884). *Catálogo de Anfibios y Reptiles de Colombia* **2018**, *4*, 4–22.
65. Cubides-Cubillos, S.D.; Loaiza-López, F.; Molina-Betancourt, J. Porthidium Nasutum (Bocourt, 1868). *Catálogo de Anfibios y Reptiles de Colombia* **2021**, *7*, 64–73.
66. Molina-Betancourth, J.; Loaiza-López, F.; Cubides-Cubillos, S.D. Porthidium Lansbergii (Schlegel, 1841). *Catálogo de Anfibios y Reptiles de Colombia* **2021**, *7*, 51–63.
67. Gutenkunst, R.N.; Hernandez, R.D.; Williamson, S.H.; Bustamante, C.D. Inferring the Joint Demographic History of Multiple Populations from Multidimensional SNP Frequency Data. *PLoS Genetics* **2009**, *5*, doi:10.1371/journal.pgen.1000695.
68. Pickrell, J.K.; Pritchard, J.K. Inference of Population Splits and Mixtures from Genome-Wide Allele Frequency Data. *PLoS Genetics* **2012**, *8*, doi:10.1371/journal.pgen.1002967.
69. Rittmeyer, E.N.; Austin, C.C. Combined Next-Generation Sequencing and Morphology Reveal Fine-Scale Speciation in Crocodile Skinks (Squamata: Scincidae: Tribolonotus). *Molecular Ecology* **2015**, *24*, 466–483, doi:10.1111/mec.13030.
70. Leaché, A.D.; Banbury, B.L.; Felsenstein, J.; De Oca, A.N.M.; Stamatakis, A. Short Tree, Long Tree, Right Tree, Wrong Tree: New Acquisition Bias Corrections for Inferring SNP Phylogenies. *Systematic Biology* **2015**, *64*, 1032–1047, doi:10.1093/sysbio/syv053.
71. McCormack, B.; Rycroft-Malone, J.; DeCorby, K.; Hutchinson, A.M.; Bucknall, T.; Kent, B.; Schultz, A.; Snelgrove-Clarke, E.; Stetler, C.; Titler, M.; et al. A Realist Review of Interventions and Strategies to Promote Evidence-Informed Healthcare: A Focus on Change Agency. *Implementation Science* **2013**, *8*, doi:10.1186/1748-5908-8-107.
72. Pyron, R.A.; Costa, G.C.; Patten, M.A.; Burbrink, F.T. Phylogenetic Niche Conservatism and the Evolutionary Basis of Ecological Speciation. *Biological Reviews* **2015**, *90*, 1248–1262, doi:10.1111/brv.12154.
73. Shanker, K.; Vijayakumar, S.P.; Ganeshaiah, K.N. Unpacking the Species Conundrum: Philosophy, Practice and a Way Forward. *Journal of Genetics* **2017**, *96*, 413–430, doi:10.1007/s12041-017-0800-0.
74. Mallik, A.K.; Srikanthan, A.N.; Ganesh, S.R.; Vijayakumar, S.P.; Campbell, P.D.; Malhotra, A.; Shanker, K. Resolving Pitfalls in Pit Viper Systematics - A Multi-Criteria Approach to Species Delimitation in Pitvipers (Reptilia, Viperidae, Craspedocephalus) of Peninsular India Reveals Cryptic Diversity. *Vertebrate Zoology* **2021**, *71*, 577–619, doi:10.3897/vz.71.e66239.
75. Angarita-Sierra, T.; Cubides-Cubillos, S.D.; Hurtado-Gómez, J.P. Hidden in the Highs: Two New Species of the Enigmatic Toadheaded Pitvipers of the Genus *Bothrocophias*. *Vertebrate Zoology* **2022**, *72*, 971–996, doi:10.3897/vz.72.e87313.
76. Haffer, J. Speciation in Amazonian Forest Birds. *Science* **1969**, *165*, 131–137.
77. Fjelds, J. Geographical Patterns for Relict and Young Species of Birds

in Africa and South America and Implications for Conservation Priorities; **1994**; Vol. 3.
78. Duellman, W.E. Global Distribution of Amphibians: Patterns, Conservation and Future Challenges. In *Patterns of distribution of amphibians: A global perspective*; Duellman, W.E., Ed.; John Hopkins University Press, **1999**; pp. 1–30.
79. Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; Da Fonseca, G.A.B.; Kent, J. *Biodiversity Hotspots for Conservation Priorities*; **2000**; Vol. 403.
80. Brumfield, R.T.; Edwards, S. V. Evolution into and out of the Andes: a bayesian analysis of historical diversification in *Thamnophilus* antshrikes. *Evolution* (NY) **2007**, *61*, 346–367, doi:10.1111/j.1558-5646.2007.00039.x.
81. Coates, A.; Obando, J. "The Geologic Evolution of the Central American Isthmus." In *Evolution and Environment in Tropical America*; Jackson, J., Budd, A., Coates, A., Eds.; The University of Chicago Press: Chicago, **1996**; pp. 21–56.
82. Harvey, M.G.; Brumfield, R.T. Genomic Variation in a Widespread Neotropical Bird (*Xenops minutus*) Reveals Divergence, Population Expansion, and Gene Flow. *Molecular Phylogenetics and Evolution* **2015**, *83*, 305–316.
83. Burbrink, F.T.; Castoe, T. Molecular Phylogeography of Snakes Rattlesnake Phylogeonomics and Morphology in MX: *Crotalus atrox* and *Crotalus scutulatus*. *View Project*; **2009**.
84. Mason-Gamer, R.J.; Kellogg, E.A. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae); **1996**; Vol. 45.
85. van Veller, M.G.P.; Zandee, M.; Kornet, D.J. Two Requirements for Obtaining Valid Common Patterns under Assumptions Zero, 1 and 2 in Vicariance Biogeography. *Cladistics* **1999**, *15*, 393–406.
86. Crisci, J. V.; Katinas, L.; Posadas, P. Historical Bioegeography: An Introduction; Harvard University Press: Cambridge, **2003**.
87. Guarnizo, C.E.; Amézquita, A.; Bermingham, E. The Relative Roles of Vicariance versus Elevational Gradients in the Genetic Differentiation of the High Andean Tree Frog, *Dendropsophus labialis*. *Molecular Phylogenetics and Evolution* **2009**, *50*, 84–92, doi:10.1016/j.ympev.2008.10.005.
88. Martins, M.; Marques, O.; Sazima, I. Ecological and Phylogenetic Correlates of Feeding Habits in Neotropical Pitvipers of the Genus *Bothrops*. In *Biology of the Vipers*; G.W. Schuett, M. Hoggren, M.E. Douglas, H.W. Greene, Eds.; Eagle Mountain ¨ Publishing, **2002**; pp. 307–328.
89. Lynch, J.D. El Contexto de Las Serpientes de Colombia Con Un Análisis de Las Amenazas En Contra de Su Conservación. *Revista de la academia colombiana de Ciencias Exactas, Físicas y Naturales* **2012**, *36*, 435–449.
90. Cañas-Dávila, C.A.; Castro-Herrera, F.; Castaño-Valencia, R.S. Serpientes Venenosas: Lecciones Aprendidas Desde Colombia; Tobón García, G.J., Osorno Reyes, J., Cañas Dávila, C.A., Castro Herrera, F., Castaño Valencia, R.S., Eugenia Rebolled, V., Eds.; Fundación Valle del Lili: Cali, Colombia., **2016**; Vol. 1.

91. Quiñones-Betancourt, E.; Díaz-Ricaurte, Juan.; Angarita-Sierra, T.; Guevara- Molina, C.; Díaz-Morales, R. *Bothrops Atrox* (Linnaeus, 1758). *Catálogo de Anfibios y Reptiles de Colombia* **2018**, *4*, 7–23.
92. Salomão, M.G.; Wüster, W.; Thorpe, R.S. MtDNA Phylogeny of Neotropical Pitvipers of the Genus *Bothrops* (Squamata: Serpentes: Viperidae). *Kaupia (Darmstadt)* **1999**, *8*, 127–134.
93. Wuster, W.; Salomao, M.G.; Duckett, D.; Thorpe, R.S. Systematics of the *Bothrops atrox* Complex: New Insights from Multivariate Analysis and Mitochondrial DNA Sequence Information. *Symposia of the Zoological Society of London* **1997**, *70*, 99–113.
94. Castoe, T.A.; Parkinson, C.L. Bayesian Mixed Models and the Phylogeny of Pitvipers (Viperidae: Serpentes). *Molecular Phylogenetics and Evolution* **2006**, *39*, 91–110, doi:10.1016/j.ympev.2005.12.014.
95. Castoe, T.A.; Sasa, M.M.; Parkinson, C.L. Modeling Nucleotide Evolution at the Mesoscale: The Phylogeny of the Neotropical Pitvipers of the Porthidium Group (Viperidae: Crotalinae). *Molecular Phylogenetics and Evolution* **2005**, *37*, 881–898, doi:10.1016/j.ympev.2005.05.013.
96. Parkinson, C.L.; Zamudio, K.R.; Greene, H.W. Phylogeography of the Pitviper Clade *Agkistrodon*: Historical Ecology, Species Status, and Conservation of Cantils. *Molecular Ecology* **2000**, *9*, 411–420.
97. Gibbs, H.L.; Sovic, M.; Amazonas, D.; Chalkidis, H.; Salazar-Valenzuela, D.; Moura-Da-Silva, A.M. Recent Lineage Diversification in a Venomous Snake through Dispersal across the Amazon River. *Biological Journal of the Linnean Society* **2018**, *123*, 651–665, doi:10.1093/biolinnean/blx158.
98. Sandner-Montilla, F. Una Nueva Especie de Genero *Bothrops* (Serpentes, Crotalidae, Bothropinae) de La Region de Guanare, Estado Portuguesa, Venezuela. *Memorias cientifica de ofidiologia* **1979**, *4*, 1–19.
99. Hallowell, E. Descriptions of Reptiles from South America, Supposed to Be New. *Proceedings of the Academy of Natural Sciences of Philadelphia* **1845**, *2*, 241–247.
100. Markezich, A.L.; Taphorn, D.C. A Variational Analysis of Populations of *Bothrops* (Serpentes: Viperidae) from Western Venezuela. *Journal of Herpetology* **1993**, *27*, 248–254.
101. Fenwick, A.M.; Gutberlet Jr, R.L.; Evans, J.A.; Parkinson, C.L. Morphological and Molecular Evidence for Phylogeny and Classification of South American Pitvipers, Genera *Bothrops, Bothriopsis, and Bothrocophias* (Serpentes: Viperidae). *Zoological Journal of the Linnean Society* **2009;** 156(3):617 – 640. DOI:10.1111/j.1096-3642.2008.00495.x
102. de Queiroz, K. "The General Lineage Concept of Species, Species Criteria, and the Process of Speciation." In book: *Endless Forms: Species and Speciation* **1998**, 57–75.
103. Nascimento, D. dos S. Filogenia Molecular de Serpentes Neotropicais Do Grupo *Bothrops atrox* (Linnaeus, 1758) (Viperidae: Crotalinae). Tese de doutorado; **2014**.
104. Parkinson, C.L.; Campbell, J.A.; Chippindale, P. Multigene Phylogenetic Analyses of Pitviper Relationships, with Comments on Their Biogeography. In *Biology of the Vipers*; G.W. Schuett, M. Höggren, H.W. Greene, Eds.; *Biological Sciences Press*, Traverse City, **2002**; pp. 3–110.

105. Folleco-Fernández, A.J. Taxonomía del complejo *Bothrops asper* (serpentes: viperidæ) en el sudoeste de Colombia. Revalidación de la especie *Bothrops rhombeatus* (García 1896) y descripción de una nueva especie. *Revista Novedades Colombianas* **2010**, *10*, 33–70.
106. Ramírez-Chaves, H.E.; Solari, S. *Bothrops ayerbei* Folleco-Fernández, 2010 y *Bothrops rhomboatus* García, 1896 (Serpentes: Viperidae) Son Un Nombre No Disponible y Un Nomen Dubium, Respectivamente. *Boletín Científico Centro de Museos Museo de Historia Natural*, Universidad del Caldas **2014**, *18*, 138–141.
107. Garcia, E. Los Ofidios Venenosos Del Cauca. Métodos Empíricos y Racionales Empleados Contra Los Accidentes Producidos Por La Mordedura de Esos Reptiles. *Cali: Librería Colombiana* **1896**, 102.
108. Gutiérrez, J.M.; Calvete, J.J.; Habib, A.G.; Harrison, R.A.; Williams, D.J.; Warrell, D.A. Snakebite Envenoming. *Nature Reviews Disease Primers* **2017**, *3*, doi:10.1038/NRDP.2017.63.
109. Gutiérrez, J.M. Comprendiendo Los Venenos de Serpientes: 50 Años de Investigaciones En América Latina; *Revista de Biología Tropical* **2002**; Vol. 50.
110. Daltry, J.C.; Wüster, W.; Thorpe, R.S. Diet and Snake Venom Evolution. *Nature* **1996**, *379*, doi:10.1038/379537a0.
111. Calvete, J.J.; Sanz, L.; Angulo, Y.; Lomonte, B.; Gutiérrez, J.M. Venoms, Venomics, Antivenomics. *FEBS Letters* **2009**, *583*, 1736–1743, doi:10.1016/j.febslet.2009.03.029.
112. Gutiérrez, J.M.; Sanz, L.; Escolano, J.; Fernández, J.; Lomonte, B.; Angulo, Y.; Rucavado, A.; Warrell, D.A.; Calvete, J.J. Snake Venomics of the Lesser Antillean Pit Vipers *Bothrops caribbaeus* and *Bothrops lanceolatus*: Correlation with Toxicological Activities and Immunoreactivity of a Heterologous Antivenom. *Journal of Proteome Research* **2008**, *7*, 4396–4408, doi:10.1021/pr8003826.
113. Savage, J.M. *The Amphibians and Reptiles of Costa Rica. A Herpetofauna between Two Continents, between Two Seas*; The University of Chicago: Chicago and London, **2002**.
114. Lamar, W.; Sasa, Mahmood. A New Species of Hognose Pitviper, Genus *Porthidium*, from the Southwestern Pacific of Costa Rica (Serpentes: Viperidae). *Revista de Biología Tropical.* **2003**, *51*, 797–804.
115. Bocourt, M.F. Descriptions de Quelques Crotaliens Nouveaux Appartenant au Genre *Bothrops*, Recueillis Dans Le Guatémala. *Annales des sciences naturelles., Paris* **1868**, 201–202.
116. Schlegel, H. Description d'une Nouvelle Espèce Du Genre *Trigonocéphale* (*Trigonocephalus lansbergii*). *Mag. Zool. Rept* **1841**, 1–3.
117. Monteza-Moreno, C.M.; Ramos, C.; Martínez, V.; Sasa, M. On the identity of hog-nosed pit-vipers from western panama: a review of specimens of *Porthidium lansbergii* (Schlegel, 1841) in lower Central America. *Tecnociencia (Panama)* **2020**, *22*, 27–44, doi:10.48204/j.tecno.v22n2a2.
118. De arco-Rodríguez, B.; Montealegre-Sánchez, L.; Solano-Redondo, L.; Castro-Herrera, F.; Ortega, J.G.; Castillo, A.; Vargas-Zapata, C.; Jiménez-Charris, E. Phylogeny and Toxicological Assessments of Two *Porthidium lansbergii lansbergii* Morphotypes from the Caribbean Region of Colombia. *Toxicon* **2019**, *166*, 56–65, doi:10.1016/j.toxicon.2019.05.010.

119. Cisneros-Heredia, D.F.; Yánez-Muñoz, M. Reptilia, Viperidae, Crotalinae, *Porthidium nasutum*: Distribution Extension and Remarks on Its Range and Records. *Check List* **2005**, *1*, 16–17, doi:10.15560/1.1.16.
120. Warrell, D.A. Snakebites in Central and South America: Epidemiology, Clinical Features, and Clinical Management. In *Venomous Reptiles of the Western Hemisphere*; Campbell, J.A., Lamar, W.W., Comstock, Eds.; Cornell University: Ithaca, NY, **2004**; Vol. 2, pp. 709–761.
121. Reyes-Velasco, J.; Cox, C.L.; Jones, J.M.; Borja, M.; Campbell, J.A. how many species of rattlesnakes are there in the *Crotalus durissus* species group (Serpentes: Crotalidae); *Revista Latinoamericana de Herpetologia* **2022**, *5*, 43–55, doi:10.22201/fc.25942158e.2022.1.330.
122. Humboldt, A. Sur Deux Nouvelles Espèces de Crotles. In: Humboldt and Bonpland, 1813. *Recueil d'observations de zoologie.* **1811**, *2*, 1–8.
123. Hoge, A.R. Preliminary Account on Neotropical Crotalinae (Serpentes Viperidae). *Mem Inst Butantan* **1966**, *32*, 137–184.
124. García-Perez, J. Una Nueva Especie de Cascabel (Serpentes: Crotalidae) Para El Bolsón Árido de Lagunillas, Cordillera de Mérida, Venezuela. *Latin American scientific contribution to ecology* **1995**, *3*, 7–12.
125. Harris, H.S.JR.; Simmons, R.S. A New Subspecies of *Crotalus durissus* (Serpentes: Crotalidae) from the Rupununi Savanna of Southwestern Guyana. *Instituto Butantan* **1978**, 305–311.
126. van Lidth de Jeude, T.W. On a Collection of Reptiles and Fishes from the West-Indies. *Notes from the Leyden Museum* **1887**, *9*, 129–139.
127. Klauber, Laurence. A New Species of Rattlesnake from Venezuela. *Transactions of the San Diego Society of Natural History* **1941**, *9*, 333–336.
128. Wallach, Van.; Williams, K.; Boundy, J. Snakes of the World: A Catalogue of Living and Extinct Species. *Taylor and Francis, CRC Press* **2014**, 1–1237.
129. Zaher, H.; Murphy, R.W.; Arredondo, J.C.; Graboski, R.; Machado-Filho, P.R.; Mahlow, K.; Montingelli, G.G.; Quadros, A.B.; Orlov, N.L.; Wilkinson, M.; et al. Large-Scale Molecular Phylogeny, Morphology, Divergence-Time Estimation, and the Fossil Record of Advanced Caenophidian Snakes (Squamata: Serpentes). *PLoS One* **2019**, *14*, e0216148, doi:10.1371/journal.pone.0216148.
130. Sawaya, R.J.; M.O.A.V.& M.M. Composition and Natural History of a Cerrado Snake Assemblage at Itirapina, São Paulo State, Southeastern Brazil. *Biota Neotropica* **2008**, *8*, 127–149.
131. Hartmann, P.A. Ecology of a Snake Assemblage in the Atlantic Forest of Southeastern Brazil. *Papéis Avulsos de Zoologia* **2009**, *49*, 343–360.
132. McDiarmid, R.W.; Campbell, J.A.; Touré, T.A. *Snake Species of the World: A Taxonomic and Geographic Reference*; 1st ed.; *Herpetologists' League*, **1999**; Vol. 1.
133. Fernandes, D.S.; Franco, F.L.; Fernandes, R. Systematic revision of the genus *Lachesis* Daudin, 1803 (Serpentes, Viperidae). *Herpetologica* **2004**, *60*, 245–260, doi:10.1655/02-85.
134. Cubides-Cubillos, Sergio.; Loaiza-López, F.; Molina-Betancourth, J.; Agudelo-Sánchez, W. *Lachesis acrochorda* (García, 1896). *Catálogo de Anfibios y Reptiles de Colombia* **2021**, *7*, 39–50.

135. Barrio-Amorós, C.L.; Corrales, G.; Rodríguez, S.; Culebras, J.; Dwyer, Q.; Flores, D.A. The Bushmasters (*Lachesis* Spp.): Queens of the Rainforest. *Reptiles & Amphibians* **2020**, *27*, 358–381, doi:10.17161/randa.v27i3.14978.
136. Madrigal, M.; Sanz, L.; Flores-Díaz, M.; Sasa, M.; Núñez, V.; Alape-Girón, A.; Calvete, J.J. Snake Venomics across Genus *Lachesis*. Ontogenetic Changes in the Venom Composition of *Lachesis stenophrys* and Comparative Proteomics of the Venoms of Adult *Lachesis melanocephala* and *Lachesis acrochorda*. *Journal in Proteomics* **2012**, *77*, 280–297, doi:10.1016/j.jprot.2012.09.003.
137. Diniz-Sousa, R.; Moraes, J. do N.; Rodrigues-da-Silva, T.M.; Oliveira, C.S.; Caldeira, C.A. A Brief Review on the Natural History, Venomics and the Medical Importance of Bushmaster (*Lachesis*) Pit Viper Snakes. *Toxicon X* **2020**, *7*, 100053, doi:10.1016/j.toxcx.2020.100053.
138. Díaz-Ricaurte, Juan.; Guevara-Molina, C.; Cubides-Cubillos, Sergio. *Lachesis muta* (Linnaeus 1766). *Catálogo de Anfibios y Reptiles de Colombia* **2017**, *3*, 20–24.
139. Arteaga, A.; Pyron, R.A.; Batista, A.; Vieira, J.; Meneses Pelayo, E.; Smith, E.N.; Barrio Amorós, C.L.; Koch, C.; Agne, S.; Valencia, J.H.; et al. Systematic Revision of the Eyelash Palm-Pitviper *Bothriechis schlegelii* (Serpentes, Viperidae), with the Description of Five New Species and Revalidation of Three. *Journal of Systematics and Evolution* **2024**, *8*, 15–64, doi:10.3897/evolsyst.8.114527.
140. Calvete, J.J.; Escolano, J.; Sanz, L. Snake Venomics of *Bitis* Species Reveals Large Intragenus Venom Toxin Composition Variation: Application to Taxonomy of Congeneric Taxa. *Journal of Proteome Research* **2007**, *6*, 2732–2745, doi:10.1021/pr0701714.
141. Fry, B.G. From Genome to "Venome": Molecular Origin and Evolution of the Snake Venom Proteome Inferred from Phylogenetic Analysis of Toxin Sequences and Related Body Proteins. *Genome Research* **2005**, *15*, 403–420, doi:10.1101/gr.3228405.
142. Earl, S.T.H.; Birrell, G.W.; Wallis, T.P.; St Pierre, L.D.; Masci, P.P.; de Jersey, J.; Gorman, J.J.; Lavin, M.F. Post-Translational Modification Accounts for the Presence of Varied Forms of Nerve Growth Factor in Australian Elapid Snake Venoms. *Proteomics* **2006**, *6*, 6554–6565, doi:10.1002/pmic.200600263.
143. Chippaux, J. P.; Williams2, V.; White', J.; Chippaux, J. P.; Williabis, V. State Toxinology Service, Adelaide Children's Hospital; **1991**; Vol. 29.
144. Casewell, N.R.; Jackson, T.N.W.; Laustsen, A.H.; Sunagar, K. Causes and Consequences of Snake Venom Variation. *Trends in Pharmacological Sciences* **2020**, *41*, doi:10.1016/j.tips.2020.05.006.
145. Fry, B.G.; Wüster, W. Assembling an Arsenal: Origin and Evolution of the Snake Venom Proteome Inferred from Phylogenetic Analysis of Toxin Sequences. *Molecular Biology and Evolution* **2004**, *21*, 870–883, doi:10.1093/molbev/msh091.
146. Wüster, W.; Peppin, L.; Pook, C.E.; Walker, D.E. A Nesting of Vipers: Phylogeny and Historical Biogeography of the Viperidae (Squamata: Serpentes). *Molecular Phylogenetics and Evolution* **2008**, *49*, doi:10.1016/j.ympev.2008.08.019.
147. Fry, B.G.; Winkel, K.D.; Wickramaratna, J.C.; Hodgson, W.C.; Wüster, W. Effectiveness of Snake Antivenom: Species and Regional Venom

Variation and Its Clinical Impact. *Journal of Toxicology: Toxin Reviews* **2003**, *22*, 23–34, doi:10.1081/TXR-120019018.
148. Calvete, J.J.; Borges, A.; Segura, Á.; Flores-Díaz, M.; Alape-Girón, A.; Gutiérrez, J.M.; Diez, N.; De Sousa, L.; Kiriakos, D.; Sánchez, E.; et al. Snake Venomics and Antivenomics of *Bothrops colombiensi*s, a Medically Important Pitviper of the *Bothrops atrox-aspe*r Complex Endemic to Venezuela: Contributing to Its Taxonomy and Snakebite Management. *Journal in Proteomics* **2009**, *72*, 227–240, doi:10.1016/j.jprot.2009.01.005.
149. Klupczynska, A.; Pawlak, M.; Kokot, Z.J.; Matysiak, J. Application of Metabolomic Tools for Studying Low Molecular-Weight Fraction of Animal Venoms and Poisons. *Toxins (Basel)* **2018**, *10*, 306, https://doi.org/10.3390/toxins10080306
150. Modahl, C.M.; Brahma, R.K.; Koh, C.Y.; Shioi, N.; Kini, R.M. Omics Technologies for Profiling Toxin Diversity and Evolution in Snake Venom: Impacts on the Discovery of Therapeutic and Diagnostic Agents. *Annual Review of Animal Bioscience* **2020**, *8*, 91–116.
151. Abd El-Aziz, T.M.; Soares, A.G.; Stockand, J.D. Advances in Venomics: Modern Separation Techniques and Mass Spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* **2020**, *1160*.
152. Walker, A.A.; Robinson, S.D.; Hamilton, B.F.; Undheim, E.A.B.; King, G.F. Deadly Proteomes: A Practical Guide to Proteotranscriptomics of Animal Venoms. *Proteomics* **2020**, *20*, doi:10.1002/pmic.201900324.
153. Damm, M.; Hempel, B.F.; Süssmuth, R.D. Old World Vipers-a Review about Snake Venom Proteomics of Viperinae and Their Variations. *Toxins (Basel)* **2021**, *13*.
154. Chippaux, J.-P.; Williams, V.; White, J. Snake Venom Variability: Methods of Study, Results and Interpretation. *Toxicon* **1991**, *29*, doi:10.1016/0041-0101(91)90116-9.
155. Arbuckle, K. From Molecules to Macroevolution: Venom as a Model System for Evolutionary Biology across Levels of Life. *Toxicon X* **2020**, *6*, doi:10.1016/j.toxcx.2020.100034.
156. Zancolli, G.; Casewell, N.R. Venom Systems as Models for Studying the Origin and Regulation of Evolutionary Novelties. *Molecular Biology and Evolution* **2020**, *37*, doi:10.1093/molbev/msaa133.
157. Fry, B.G. *Venomous Reptiles and Their Toxins. Evolution, Pathophysiology, and Biodiscovery*; Oxford University Press, USA., Ed.; Oxford University Press: New York, NY., **2015**; Vol. 1.
158. Mackessy, S.P.; Saviola, A.J. Understanding Biological Roles of Venoms Among the Caenophidia: The Importance of Rear-Fanged Snakes. *Integrative and Comparative Biology* **2016**, *56*, 1004–1021, doi:10.1093/icb/icw110.
159. Fry, B.G.; Scheib, H.; van der Weerd, L.; Young, B.; McNaughtan, J.; Ryan Ramjan, S.F.; Vidal, N.; Poelmann, R.E.; Norman, J.A. Evolution of an Arsenal: Structural and Functional Diversification of the Venom System in the Advanced Snakes (Caenophidia). *Molecular and Cellular Proteomics* **2008**, *7*, 215–246, doi:10.1074/mcp.M700094-MCP200.
160. Jackson, T.; Koludarov, I.; Ali, S.; Dobson, J.; Zdenek, C.; Dashevsky, D.; op den Brouw, B.; Masci, P.; Nouwens, A.; Josh, P.; et al. Rapid Radiations and the Race to Redundancy: An Investigation of the Evo-

lution of Australian Elapid Snake Venoms. *Toxins (Basel)* **2016**, *8*, 309, doi:10.3390/toxins8110309.
161. Casewell, N.R.; Wüster, W.; Vonk, F.J.; Harrison, R.A.; Fry, B.G. Complex Cocktails: The Evolutionary Novelty of Venoms. *Trends in Ecology & Evolution* **2013**, *28*, 219–229, doi:10.1016/j.tree.2012.10.020.
162. Giorgianni, M.W.; Dowell, N.L.; Griffin, S.; Kassner, V.A.; Selegue, J.E.; Carroll, S.B. The Origin and Diversification of a Novel Protein Family in Venomous Snakes. *Proceedings of the National Academy of Sciences* **2020**, *117*, 10911–10920, doi:10.1073/pnas.1920011117.
163. Amorim, F.; Costa, T.; Baiwir, D.; De Pauw, E.; Quinton, L.; Sampaio, S. Proteopeptidomic, Functional and Immunoreactivity Characterization of *Bothrops moojeni* Snake Venom: Influence of Snake Gender on Venom Composition. *Toxins (Basel)* **2018**, *10*, 177, doi:10.3390/toxins10050177.
164. Queiroz, G.P.; Pessoa, L.A.; Portaro, F.C.V.; Furtado, M. de F.D.; Tambourgi, D. V. Interspecific Variation in Venom Composition and Toxicity of Brazilian Snakes from *Bothrops* Genus. *Toxicon* **2008**, *52*, 842–851, doi:10.1016/j.toxicon.2008.10.002.
165. Kazandjian, T.D.; Petras, D.; Robinson, S.D.; van Thiel, J.; Greene, H.W.; Arbuckle, K.; Barlow, A.; Carter, D.A.; Wouters, R.M.; Whiteley, G.; et al. Convergent Evolution of Pain-Inducing Defensive Venom Components in Spitting Cobras. *Science* **2021**, *371*, 386–390, doi:10.1126/science.abb9303.
166. Barua, A.; Mikheyev, A.S. Toxin Expression in Snake Venom Evolves Rapidly with Constant Shifts in Evolutionary Rates. *Proceedings of the Royal Society: Biological Sciences* **2020**, *287*, 20200613, doi:10.1098/rspb.2020.0613.
167. Cubides-Cubillos, S.D.; Alarcón–Pérez, J.C. Accidente Ofídico En Antioquia, Colombia: Análisis Etnobiológico de Las Construcciones Culturales. *Revista Etnobiología* **2018**, *16*, 18–29.
168. Chippaux, J.-P. Snakebite Envenomation Turns Again into a Neglected Tropical Disease! *Journal of Venomous Animals and Toxins including Tropical Diseases* **2017**, *23*, 38, doi:10.1186/s40409-017-0127-6.
169. Chippaux, J.-P. Epidemiology of Envenomations by Terrestrial Venomous Animals in Brazil Based on Case Reporting: From Obvious Facts to Contingencies. *Journal of Venomous Animals and Toxins including Tropical Diseases* **2015**, *21*, 13, doi:10.1186/s40409-015-0011-1.
170. Boldrini-França, J.; Corrêa-Netto, C.; Silva, M.M.S.; Rodrigues, R.S.; De La Torre, P.; Pérez, A.; Soares, A.M.; Zingali, R.B.; Nogueira, R.A.; Rodrigues, V.M.; et al. Snake Venomics and Antivenomics of *Crotalus durissus* Subspecies from Brazil: Assessment of Geographic Variation and Its Implication on Snakebite Management. *Journal in Proteomics* **2010**, *73*, 1758–1776, doi:10.1016/j.jprot.2010.06.001.
171. Rodríguez-Vargas, A.; Vega, N.; Reyes-Montaño, E.; Corzo, G.; Neri-Castro, E.; Clement, H.; Ruiz-Gómez, F. Intraspecific Differences in the Venom of *Crotalus durissus cumanensis* from Colombia. *Toxins (Basel)* **2022**, *14*, doi:10.3390/toxins14080532.
172. Talukdar, A.; Maddhesiya, P.; Namsa, N.D.; Doley, R. Snake Venom Toxins Targeting the Central Nervous System. *Toxin Reviews* **2023**, *42*, 382–406, doi:10.1080/15569543.2022.2084418.

173. Lewis, R.; Gutmann, L. Snake Venoms and the Neuromuscular Junction. *Semin Neurol* **2004**, *24*, 175–179, doi:10.1055/s-2004-830904.
174. Rossetto, O.; Morbiato, L.; Caccin, P.; Rigoni, M.; Montecucco, C. Presynaptic Enzymatic Neurotoxins. *Journal Neurochem* **2006**, *97*, 1534–1545, doi:10.1111/j.1471-4159.2006.03965.x.
175. Gawade, S.P. Snake Venom Neurotoxins: Pharmacological Classification. *Journal of Toxicology: Toxin Reviews* **2004**, *23*, 37–96, doi:10.1081/TXR-120030647.
176. Calvete, J.J.; Sanz, L.; Cid, P.; De La Torre, P.; Flores-Díaz, M.; Dos Santos, M.C.; Borges, A.; Bremo, A.; Angulo, Y.; Lomonte, B.; et al. Snake Venomics of the Central American Rattlesnake *Crotalus simus* and the South American Crotalus Durissus Complex Points to Neurotoxicity as an Adaptive Paedomorphic Trend along Crotalus Dispersal in South America. *Journal of Proteome Research* **2010**, *9*, 528–544, doi:10.1021/pr9008749.
177. Batista da Cunha, D.; Pupo Silvestrini, A.V.; Gomes da Silva, A.C.; Maria de Paula Estevam, D.; Pollettini, F.L.; de Oliveira Navarro, J.; Alves, A.A.; Remédio Zeni Beretta, A.L.; Annichino Bizzacchi, J.M.; Pereira, L.C.; et al. Mechanistic Insights into Functional Characteristics of Native Crotamine. *Toxicon* **2018**, *146*, 1–12, doi:10.1016/j.toxicon.2018.03.007.
178. Rádis-Baptista, G.; Kerkis, I. Crotamine, a Small Basic Polypeptide Myo-toxin from Rattlesnake Venom with Cell-Penetrating Properties. Curr Pharm Des **2011**, 17 (38), 4351–4361, doi:10.2174/138161211798999429
179. Schenberg, S. Geographical Pattern of Crotamine Distribution in the Same Rattlesnake Subspecies. *Science* (1979) **1959**, *129*, 1361–1363.
180. Otero, R.; Osorio, R.G.; Valderrama, R.; Giraldo, C.A. Efectos farmacoló-gicos y enzimaticos de los venenos de serpientes de Antioquia y Chocó (Colombia); *Toxicon* **1992**, 30 (5-6): 611-620, doi: https://doi.org/10.1016/0041-0101(92)90855-Y
181. Mora-Obando, D.; Plaid, D.; Lomonte, B.; Guerrero-Vargas, J.A.; Ayerbe, S.; Calvete, J.J. Antivenomics and in Vivo Preclinical Efficacy of Six Latin American Antivenoms towards Southwestern Colombian *Bothrops asper* Lineage Venoms. *PLOS Neglected Tropical Diseases* **2021**, *15*, 1–36, doi:10.1371/journal.pntd.0009073.
182. Arévalo-Páez, M.; Rada-Vargas, E.; Betancur-Hurtado, C.; Renjifo, J.M.; Renjifo-Ibáñez, C. Neuromuscular Effect of Venoms from Adults and Juveniles of *Crotalus durissus cumanensis* (Humboldt, 1811) from Guajira, Colombia. *Toxicon* **2017**, *139*, 41–44, doi:10.1016/j.toxicon.2017.09.016.
183. Pereañez, J.A.; Núñez, V.; Huancahuire-Vega, S.; Marangoni, S.; Ponce-Soto, L.A. Biochemical and Biological Characterization of a PLA_2 from Crotoxin Complex of *Crotalus durissus cumanensis*. *Toxicon* **2009**, *53*, 534–542, doi:10.1016/j.toxicon.2009.01.021.
184. Mora-Obando, D.; Salazar-Valenzuela, D.; Pla, D.; Lomonte, B.; Guerrero-Vargas, J.A.; Ayerbe, S.; Gibbs, H.L.; Calvete, J.J. Venom Variation in *Bothrops asper* Lineages from North-Western South America. *Journal in Proteomics* **2020**, *229*, 103945, doi:10.1016/j.jprot.2020.103945.
185. Jiménez-Charris, E.; Montoya-Gómez, A.; Torres, J.K.; Gómez-Díaz, M.; Bolivar-García, W. First Functional and Proteomic Analysis of *Bothrops asper* Snake Venom from Gorgona Island - Colombia, and Its Compa-

rative Characterization with Two Colombian Southwest Ecoregions. *Biochimie* **2022**, *194*, 19–27, doi:10.1016/j.biochi.2021.12.005.

186. Quintana, J.; Otero, R.; Nuñez, V.; Toro, F. Estudio de La Variabilidad En El Veneno de 2 Poblaciones de *Bothriechis schlegelii* Del Suroeste y Norte de Antioquia y Correlación Morfométrica. *Iatreia* **2000**, 1–107.
187. Montealegre-Sánchez, L.; Montoya-Gómez, A.; Jiménez-Charris, E. Individual Variations in the Protein Profiles and Functional Activities of the Eyelash Palm Pit-Viper (*Bothriechis schlegelii*) Venom from the Colombian Southwest Region. *Acta Tropica* **2021**, *223*, 106113, doi:10.1016/j.actatropica.2021.106113.
188. Lomonte, B.; Escolano, J.; Fernández, J.; Sanz, L.; Angulo, Y.; Gutiérrez, J.M.; Calvete, J.J. Snake Venomics and Antivenomics of the Arboreal Neotropical Pitvipers *Bothriechis lateralis* and *Bothriechis schlegelii*. *Journal of Proteome Research* **2008**, *7*, 2445–2457, doi:10.1021/pr8000139.
189. Gutiérrez, J.; Escalante, T.; Rucavado, A.; Herrera, C. Hemorrhage Caused by Snake Venom Metalloproteinases: A Journey of Discovery and Understanding. *Toxins (Basel)* **2016**, *8*, 93, doi:10.3390/toxins8040093.
190. Otero, R.; Gutiérrez, J.; Beatriz Mesa, M.; Duque, E.; Rodríguez, O.; Luis Arango, J.; Gómez, F.; Toro, A.; Cano, F.; María Rodríguez, L.; et al. Complications of *Bothrops*, *Porthidium*, and *Bothriechis* Snakebites in Colombia. A Clinical and Epidemiological Study of 39 Cases Attended in a University Hospital. *Toxicon* **2002**, *40*, 1107–1114, doi:10.1016/S0041-0101(02)00104-6.
191. Otero-Patiño, R. Snake Bites in Colombia. In: Vogel, CW., Seifert, S., Tambourgi, D. (eds) Clinical Toxinology in Australia, Europe, and Americas. *Toxinology. Springer*, Dordrecht; **2018**; pp. 3–50. https://doi.org/10.1007/978-94-017-7438-3_41
192. Preciado, L.M.; Pereañez, J.A.; Comer, J. Potential of Matrix Metalloproteinase Inhibitors for the Treatment of Local Tissue Damage Induced by a Type P-I Snake Venom Metalloproteinase. *Toxins (Basel)* **2019**, *12*, 8, doi:10.3390/toxins12010008.
193. Pereañez, J.A.; Preciado, L.M.; Fernández, J.; Camacho, E.; Lomonte, B.; Castro, F.; Cañas, C.A.; Galvis, C.; Castaño, S. Snake Venomics, Experimental Toxic Activities and Clinical Characteristics of Human Envenomation by *Bothrocophias myersi* (Serpentes: Viperidae) from Colombia. *Journal in Proteomics* **2020**, *220*, doi:10.1016/j.jprot.2020.103758.
194. Sevilla-Sánchez, M.-J.; Guerrero-Vargas, J.A.; Ayerbe-González, S.; Calderón-Leytón, J.J.; Lomonte, B.; Mora-Obando, D. Toxinological Profile and Histopathological Alterations Induced by *Bothrocophias campbelli* Venom from Colombia. *Acta Tropica* **2024**, *250*, 107094, doi:10.1016/j.actatropica.2023.107094.
195. Jiménez-Charris, E.; Montealegre-Sanchez, L.; Solano-Redondo, L.; Mora-Obando, D.; Camacho, E.; Castro-Herrera, F.; Fierro-Pérez, L.; Lomonte, B. Proteomic and Functional Analyses of the Venom *of Porthidium lansbergii lansbergii* (Lansberg's Hognose Viper) from the Atlantic Department of Colombia. *J Proteomics* **2015**, *114*, 287–299, doi:10.1016/j.jprot.2014.11.016.

196. Otero-Patiño, R.; Segura, Á.; Herrera, M.; Angulo, Y.; León, G.; Gutiérrez, J.M.; Barona, J.; Estrada, S.; Pereañez, A.; Quintana, J.C.; et al. Comparative Study of the Efficacy and Safety of Two Polyvalent, Caprylic Acid Fractionated [IgG and F(Ab')2] Antivenoms, in *Bothrops asper* Bites in Colombia. *Toxicon* **2012**, *59*, 344–355, doi:10.1016/j.toxicon.2011.11.017.
197. Kalil, J.; Fan, H.W. Production and Utilization of Snake Antivenoms in South America. In; **2017**; pp. 81–101.
198. Bermúdez-Méndez, E.; Fuglsang-Madsen, A.; Føns, S.; Lomonte, B.; Gutiérrez, J.; Laustsen, A. Innovative Immunization Strategies for Antivenom Development. *Toxins (Basel)* **2018**, *10*, 452, doi:10.3390/toxins10110452.
199. Gutiérrez, J.M.; León, G.; Burnouf, T. Antivenoms for the Treatment of Snakebite Envenomings: The Road Ahead. *Biologicals* **2011**, *39*, 129–142.
200. Theakston, R.D.G.; Reid, H.A. Development of Simple Standard Assay Procedures for the Characterization of Snake Venoms. *Bulletin of the World Health Organization* **1983**, *61*, 949–956.
201. Rojas, G.; Jiménez, J.M.; Gutiérrez, J.M. Caprylic Acid Fractionation of Hyperimmune Horse Plasma: Description of a Simple Procedure for Antivenom Production; *Toxicon* **1994**, *32*, 351-363, https://doi.org/10.1016/0041-0101(94)90087-6.
202. Lalloo, D.G.; Theakston, R.D.G. Snake Antivenoms. *Journal of Toxicology: Clinical Toxicology* **2003**, *41*, 277–290, doi:10.1081/CLT-120021113.
203. Bogarín, G.; Romero, M.; Rojas, G.; Lutsch, C.; Casadamont, M.; Lang, J.; Otero, R.; Gutiérrez, J.M. Neutralization, by a Monospecific *Bothrops lanceolatus* Antivenom, of Toxic Activities Induced by Homologous and Heterologous *Bothrops* Snake Venoms. *Toxicon* **1999**, *37*, 551–557, doi:10.1016/S0041-0101(98)00193-7.
204. Bucher, B.; Canongel, D.; Thomas, L.; Tyburn, B. Clinical Indicators of Envenoming and Serum Levels of Venom Antigens Bitten by *Bothrops lanceolatus* in Martinique. *Transactions of The Royal Society of Tropical Medicine*. **1997** (2):186-90. doi: 10.1016/s0035-9203(97)90219-4..
205. Gutiérrez, J.M.; Theakston, R.D.G.; Warrell, D.A. Confronting the Neglected Problem of Snake Bite Envenoming: The Need for a Global Partnership. *PLoS Medicine* **2006**, *3*, 0727–0731.
206. Layfield, H.J.; Williams, H.F.; Ravishankar, D.; Mehmi, A.; Sonavane, M.; Salim, A.; Vaiyapuri, R.; Lakshminarayanan, K.; Vallance, T.M.; Bicknell, A.B.; et al. Repurposing Cancer Drugs Batimastat and Marimastat to Inhibit the Activity of a Group I Metalloprotease from the Venom of the Western Diamondback Rattlesnake, *Crotalus atrox*. *Toxins (Basel)* **2020**, *12*, 309, doi:10.3390/toxins12050309.
207. Calvete, J.J.; Pérez, A.; Lomonte, B.; Sánchez, E.E.; Sanz, L. Snake Venomics of *Crotalus tigris*: The Minimalist Toxin Arsenal of the Deadliest Neartic Rattlesnake Venom. Evolutionary Clues for Generating a Pan-Specific Antivenom against Crotalid Type II Venoms. *Journal of Proteome Research* **2012**, *11*, 1382–1390, doi:10.1021/pr201021d.
208. Calvete, J.J.; Fasoli, E.; Sanz, L.; Boschetti, E.; Righetti, P.G. Exploring the Venom Proteome of the Western Diamondback Rattlesnake, *Crotalus atrox*, via Snake Venomics and Combinatorial Peptide Ligand Library Approaches. *Journal of Proteome Research* **2009**, *8*, 3055–3067, doi:10.1021/pr900249q.

209. Romero-Giraldo, L.E.; Pulido, S.; Berrío, M.A.; Flórez, M.F.; Rey-Suárez, P.; Nuñez, V.; Pereañez, J.A. Heterologous Expression and Immunogenic Potential of the Most Abundant Phospholipase A2 from Coral Snake *Micrurus dumerilii* to Develop Antivenoms. *Toxins (Basel)* **2022**, *14*, 825, doi:10.3390/toxins14120825.
210. Carvalho, B.M.A.; Santos, J.D.L.; Xavier, B.M.; Almeida, J.R.; Resende, L.M.; Martins, W.; Marcussi, S.; Marangoni, S.; Stábeli, R.G.; Calderon, L.A.; et al. Snake Venom PLA_2s Inhibitors Isolated from Brazilian Plants: Synthetic and Natural Molecules. *BioMed Research International* **2013**, *2013*, 1–8, doi:10.1155/2013/153045.
211. Gómez-Betancur, I.; Gogineni, V.; Salazar-Ospina, A.; León, F. Perspective on the Therapeutics of Anti-Snake Venom. *Molecules* **2019**, *24*, 3276, doi:10.3390/molecules24183276.
212. Quiroz, S.; Henao Castañeda, I.C.; Granados, J.; Patiño, A.C.; Preciado, L.M.; Pereañez, J.A. Inhibitory Effects of Varespladib, CP471474, and Their Potential Synergistic Activity on *Bothrops asper* and *Crotalus durissus cumanensis* Venoms. *Molecules* **2022**, *27*, 8588, doi:10.3390/molecules27238588.
213. Mora-Obando, D.; Lomonte, B.; Pla, D.; Guerrero-Vargas, J.A.; Ayerbe-González, S.; Gutiérrez, J.M.; Sasa, M.; Calvete, J.J. Half a Century of Research on *Bothrops asper* Venom Variation: Biological and Biomedical Implications. *Toxicon* **2023**, *221*, 106983, doi:10.1016/j.toxicon.2022.106983.
214. Calvete, J.J.; Rodríguez, Y.; Quesada-Bernat, S.; Pla, D. Toxin-Resolved Antivenomics-Guided Assessment of the Immunorecognition Landscape of Antivenoms. *Toxicon* **2018**, *148*, 107–122, doi:10.1016/j.toxicon.2018.04.015.
215. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and Evolution* **2015**, *32*, 268–274, doi:10.1093/molbev/msu300.
216. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; von Haeseler, A.; Jermiin, L.S. ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. *Nature Methods* **2017**, *14*, 587–589, doi:10.1038/nmeth.4285.
217. Revell, L.J. Phytools: An R Package for Phylogenetic Comparative Biology (and Other Things). *Methods in Ecology and Evolution* **2012**, *3*, 217–223, doi:10.1111/j.2041-210X.2011.00169.x.
218. Warrell, D.A. Snake Bite. *The Lancet* **2010**, *375*, 77–88.
219. Ramakrishnan, M.A. Determination of 50% Endpoint Titer Using a Simple Formula. *World Journal of Virology* **2016**, *5*, 85, doi:10.5501/wjv.v5.i2.85.
220. Sells, P.G. Animal Experimentation in Snake Venom Research and in Vitro Alternatives. *Toxicon* **2003**, *42*, 115–133.
221. Boivin, G.P.; Hickman, D.L.; Creamer-Hente, M.A.; Pritchett-Corning, K.R.; Bratcher, N.A. Review of CO_2 as a Euthanasia Agent for Laboratory Rats and Mice. *Journal of the American Association for Laboratory Animal Science* **2017**, *56*, 491–499.
222. Reyes-Velasco, J. A revision of recent taxonomic changes to the eyelash palm pitviper (Serpentes, Viperidae, *Bothriechis schlegelii*). *Herpetozooa* **2024**, *37*, 1–14, doi:10.3897/herpetozoa.

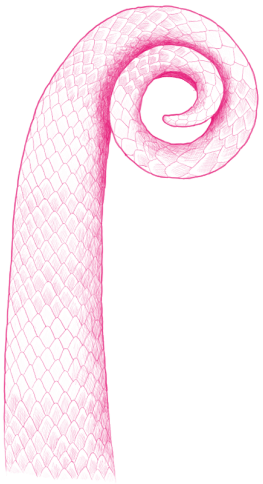
Chapter 4

An unexplored universe: Venoms and toxins from colubrid snakes

Juan David Bayona-Serrano, Kristian Alberto Torres-Bonilla, Teddy Angarita-Sierra

Abstract: Among toxinological studies of snake venoms there has been a historical bias towards front-fanged snakes (viperids and elapids), based on their higher medical relevance. This caused rear-fanged and aglyphous colubrid species to be left neglected in terms of the availability and generation of venom-related data. However, several studies in the last two decades have partially unveiled the diverse array of toxins that lies hidden in colubrid venoms. This research has turned colubrid venoms into a new and interesting group to search for novel protein scaffolds and functions. Most colubrid species lack information about their venom composition or function. In this chapter, we summarize the available information about the venom of Colombian colubrids integrating it with the available information about their epidemiology and their biological significance. Also, we contrast that information with its possible applications, evolutionary data, and future perspectives, hoping to enhance the interest of the scientific community for this amazing group of venomous but generally harmless snakes.

Keywords: Colubridae, rear-fanged snakes, venom, proteolytic enzymes, envenomation.



Citation: Bayona-Serrano JD.; Torres-Bonilla KA.; Angarita-Sierra T. Chapter 4. An unexplored universe: Venoms and toxins from colubrid snakes. In Book: *Bites, venoms, and venomous snakes of Colombia*; Angarita-Sierra, T., Ruiz-Gomez, FJ, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 165–200. doi:10.33610/769527ncjuqd

Copyright: © 2024 by the authors. Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0) license (http://creativecommons.org/licenses/by/4.0/).

Illustrations by:
Oscar A. Ramirez Ruiz

1. Introduction

During the last four decades, toxins found in the venoms of the families Viperidae and Elapidae (see Chapters 2 and 3) have been thoroughly studied using compositional, functional, and evolutionary approaches because of their paramount medical relevance (see Chapter 8, 9 and 10) [1–3]. Viperid and elapid venoms have received enormous attention since they possibly might cause serious and life-threatening envenomation when human populations expand into their natural habitat and increased contacts take place between humans and snakes (see Chapter 9) [1,4–7]. These studies revealed an incredible diversity of functions and protein scaffolds that were hidden within those venoms; unveiling a huge amount of intraspecific variation with ontogenetic and geographical bases [1]. Nevertheless, most of snake diversity lies within a group commonly known as colubrids (Superfamily Colubroidea sensu Zaher et al. [9]), that have been out of the spotlight of toxinological studies for most of history [8–11].

In Colombia, colubrids account for ~70% of the ~340 currently recognized snake species; and they are distributed in 60 genera [8]. These snakes possess a wide spectrum of morphological and physiological traits that separates them from front-fanged snakes (i.e., vipers and coralsnakes) [12–14]. Colubrids are endowed with a secretory maxillary gland, called Duvernoy's gland, that is homologous (similar in position, structure, and evolutionary origin) to the venom gland of vipers and elapids [13–15]. This gland produces toxins, and it is connected to one of the two different types of maxillary dentition present in colubrids. The first type of dentition consists of rows of undifferentiated teeth with an absence of grooved fangs, known as aglyphous. The second is characterized by the presence of posteriorly enlarged grooved fangs, known as opisthoglyphous [13–17].

These characteristics granted them different mechanisms of subduing prey including constriction, venom inoculation, or a mixture of both methods. In addition, colubrids exhibit a wide spectrum of ecological specializations that allow them to adapt to a wide variety of habitats, diets, and behaviors [12,18–24]. While most colubrids are terrestrial or arboreal, feeding mostly on birds, mammals, amphibians, or other reptiles, some species are specialized to thrive in acuatic or semifossorial environments, changing their preferred prey to fish and invertebrate animals [16,19,22,24–27].

Colubrids exhibit a myriad of ecological specializations that are accompanied by similarly diverse changes in the function of their venoms. Some species possess venoms dominated by proteolytic enzymes (enzymes that break down proteins) similar to those found in vipers; others emulate the neurotoxic profile common in elapids; and some groups even have lost their Duvernoy's gland altogether [1,11,15,16,28–33].

Notably, the venom inoculation system of colubrids tends to be less efficient than that of viperids and elapids, mainly because their Duvernoy's gland does not possess the insertion of a compressor muscle that can

generate the pressure needed to efficiently penetrate the tissue of their prey [1,15,16]. The lack of an efficient venom delivery system is one of the main reasons that cause them to be considered less medically relevant. Thus, their venoms have been poorly studied in comparison to their viperid and elapid counterparts [10,11,34]. This medical bias transformed these species into a neglected group within toxinological sciences worldwide, and Colombian species are no exception.

Although several colubrids possess venom, only a small number of genera distributed in Colombia have been reported to cause severe or mild envenomation in humans [35]. However, accidents with some species might be far from harmless and may be underestimated in epidemiological studies done in the country [17,36] (see Chapter 9). Moreover, the dearth of information about the real number of yearly accidents involving colubrids increases the already existing bias against understanding the biological activities and composition of their venoms [10].

This bias is not only caused by the relatively low medical significance they have, but also by several factors that make the research of colubrid venoms an arduous task. For example, the difficulty of efficiently maintaining these snakes in captivity and extracting venom for research purposes, the low venom yields, the scarce chance of encountering venom-producing rear-fanged species, the low toxicity for humans and in murine models, the high prey specificity of their toxins, and the slow spread of their venom when injected into tissues are some of the main reasons that slow venom studies of colubrid species [37–41].

Most Colombian colubrids lack data about the composition, pharmacological effects, or biological function of their venom or oral secretions. Fortunately, recent studies performed with Colombian and non-Colombian species have partially unveiled the enormous diversity of toxins that are present in the venoms of this group of snakes [11,32–34,42]. Complex mixtures of proteins, like those found in vipers and elapids with myriad enzymatic and non-enzymatic activities have been reported in various venoms of colubrid species, turning them into an interesting prospect for searching for new, previously undescribed toxins.

Functional studies have revealed that some venoms from colubrids show similar potencies to those from vipers. Bites from these colubrid species can even induce serious clinical manifestations that might incapacitate a patient [17,36,43–51]. In this chapter, we will review the available information about the composition, function, and clinical manifestations of the venom of colubrids distributed in Colombia, focusing on the untapped potential that lies in this group and the future of toxinological studies in the Colombian panorama.

2. The venom of “colubrid” snakes

Most snake diversity in Colombia is contained within a single group of opisthoglyphous and aglyphous species (see Chapter 1) known as the su-

perfamily Colubroidea [8]. Currently, there are three recognized subfamilies inhabiting Colombia: Colubrinae (~50 species in ~15 genera), Dipsadinae (~187 species in ~44 genera) and Sybinophiinae (one species in one genus). Venom-related information is available only for some genera of Colubrinae and Dipsadinae, focusing on compositional and functional data [11,28,29,32–34,42,52–57]. In this section we shall discuss the trends observed in the venoms of both subfamilies of Colombian colubrids.

2.1. Subfamily Colubrinae

Historically, most genera and species from this subfamily lack any type of venom-related information [10,11]. Recently there has been a continuous increase in the amount of published data tackling colubrine venoms. Several works including Asian, African and American species have unveiled a large repertoire of functionally diverse toxins [11,28,29,52,55,58–61].

Some species from this group resembling viper venoms (see Chapter 3) possess a venom profile dominated by snake venom metalloproteinases (SVMPs), a group of enzymes that break down tissue and cause hemorrhage and other local manifestations [58,61]. However, most of the toxin diversity found in this subfamily is composed of non-enzymatic post-synaptic neurotoxins known as three-finger toxins (3FTxs) [28,52,55,59,60]. These proteins are common components of elapid venoms (see Chapter 5), causing a potent neuromuscular blockade that leads to asphyxiation by respiratory arrest and a rapid death [3,62]. Interestingly, 3FTxs of Colubrinae appear to have evolved to become prey-specific, being more toxic towards certain types of prey [63]. Due to the broad spectrum of prey items that Colubrinae species with 3FTxs feed upon, we can only hypothesize about the different amount of specific functional motifs that are hidden in their neurotoxins.

In Colombia, all species with venom-related data have a noticeable neurotoxic profile with a rather simple compositional pattern in terms of the number of toxin classes. Genera *Spilotes*, *Leptophis*, *Tantilla*, *Rhinobothryum* and *Oxybelis* contain the species with available compositional information [29,30,52,55,60]. Their venoms are dominated by neurotoxic 3FTxs and cysteine-rich secretory proteins (CRISPs, see Chapter 5). The latter are a widespread class of toxins commonly seen in most member of the Colubridae that are known to interfere with smooth muscle contraction by blocking ion channels [1,64,65].

In addition, other minor components reported in most studied genera are SVMPs and C-type lectins (CTLs) that are associated with local effects such as hemorrhage, edema, necrosis, and platelet-related hemostatic disorders [66]. This compositional information corresponds with the low hemorrhagic and myonecrotic activities observed in most Colombian Colubrinae [30], although those studies were carried out with venom samples from foreign populations. Intraspecific geographic variation of colubrid venoms is still poorly explored and an unresolved research area for Colombian populations (see Chapter 5).

Interestingly, the Duvernoy's gland is not the only toxin-producing cephalic gland found in Colubrinae species. A recent study with a species of *Tretanorhinus*, revealed that the Harderian gland is also capable of producing highly expressed venom-like transcripts [57]. This gland is located behind the eye of the snake and is proximal to the Duvernoy's gland. It is thought to be closely related to the lacrimal gland aiding in the lubrication of the eye, but it has been proposed to fulfill several other roles such as acting as an accessory salivary gland that may secrete digestive enzymes or provide a source of lubrication [67,68].

The expression profile of the Harderian gland of *Tretanorhinus variabilis* revealed a series of putative toxins including P-III SVMPs, CTLs, and CRISPs that are three of the main toxins reported in other Colubrinae [57]. These findings indicate that the production of toxin-like proteins is not limited to the Duvernoy's gland in colubrids, but it is not clear if these other oral glands that are producing putative toxins represent a trophic advantage (ecological function).

Overall, this subfamily contains species with prey-specific highly neurotoxic venoms dominated by 3FTxs and CRISPs. The molecular targets, biochemical characterization, and function of these colubrid toxins remain poorly studied. Therefore, they represent an interesting model to explore novel molecular mechanisms that might be pharmacologically exploitable since they interact with several ion channels that modulate nerve responses [62,65]. Nevertheless, most species and genera distributed within Colombian territory lack compositional data that is needed to gain a better understanding of the evolutionary processes that might be shaping venom composition and function.

2.2. Subfamily Dipsadinae

Contrasting with the neurotoxin dominated venoms that are widespread across the Colubrinae, this subfamily has a more proteolytic venom profile with some exceptions (e.g. genus *Helicops*) [11,42]. The subfamily Dipsadinae contains some genera that are known to be medically important and cause serious cases of envenomation with a predominance of local symptoms (i.e., hemorrhage, edema, necrosis) [31–33,56,69–71]. Compositional studies performed on Colombian genera revealed that Dipsadinae venoms are extensively dominated by proteolytic P-III SVMPs and CTLs that directly correlate with most envenomation clinical symptoms informed in the case reports [11,32–34,42].

However, a recent study found that three tribes of this subfamily (Tachymenini, Xenodontini and Conophiini) had a different type of metalloproteinase as the main enzymatic component of their venoms [42,53]. These enzymes were identified as snake venom matrix-metalloproteinases (svMMPs) and represent three independent events of gene recruitment in the evolutionary history of this group of snakes (see section 4 on this chapter).

Moreover, the tribe Hydropsini possesses a compositional pattern that is neither SVMP nor svMMP dominated, agreeing with the neurotoxic venom profile reported in *Helicops angulatus* an the CTL-dominated profiles observed in other species of the genus [42,72,73]. So, we can separate three big compositionally different groups of Dipsadinae venoms: (1) Genera with venoms dominated by P-III SVMPs (e.g. *Leptodeira*, *Pseudoboa*, *Philodryas* and *Oxyrhopus*) [11,32,34,54]; (2) genera with venoms dominated by svMMPs (e.g. *Erythrolamprus*, *Lygophis* and *Thamnodynastes*) [11,42,53]; and (3) genera with a unique profile with an abundance of CRISPs and CTLs, similar to what is found in some Colubrinae species (e.g. *Helicops*) [42,72,73].

Functional analyses performed in species of *Philodryas*, *Leptodeira*, *Thamnodynastes*, *Erythrolamprus,* and *Pseudoboa* verified the proteolytic effects predicted by the analyses of venom composition. Most species of dipsadids show strong proteolytic capabilities *in vitro*, even comparable to those of some *Bothrops* species [32–34,42,56].

Interestingly, in some species of the Pseudoboini tribe (Genera *Clelia* and *Boiruna)*, a new type of phospholipase A_2 (PLA_2) has been detected as a dominant toxin [11,61,73]. These PLA_2s are different from those found in vipers and elapids (see Chapter 5) as they belong to the IIE group, typically found in brain, heart and uterine tissue of mammals [1,74]. Recently, a transcriptomic and proteomic analysis of the whole tribe revealed that these PLA_2-IIE that dominate the venom of these genera possess a shorter C-terminal tail when compared to the endogenous protein that fulfills physiological functions in other snakes [73]. This shortening could be a convergent process to what is observed in other enzymatic toxins that have evolved into more compact scaffolds from a larger endogenous gene [73].

Although there is still little information available about these toxins, they are likely secretory, calcium-dependent enzymes that primarily target extracellular phospholipids from their primary and tertiary structures, that resemble the ones observed in PLA_2s from vipers [73]. Moreover, enzymatic assays with venoms of the Pseudoboini tribe confirmed that they are catalytically active, with *in vitro* activity levels similar to those observed in viper venoms rich in PLA_2s [1,73,74]. This PLA_2 dominance is unique amongst colubrids so far, and represents the fifth recruitment of the PLA_2 scaffold to snake venoms, showing that venom tends to converge towards the same optimal toxin scaffolds across different snake families [73].

Additionally, an acidic lipase was reported as a new putative toxin in the venom of *Phalotris mertensi* (False coral) that might represent the recruitment of a new toxin scaffold to the venom repertoire of colubrids [74]. Overall, studies of colubrid venoms have resulted in the discovery of several novel scaffolds of toxins that in some cases lead to phenotypical convergence with front-fanged snakes and in others they result in new, unique venom components [11,42,61,75].

2.3. Compositional trends of colubrid venoms

Comparing the general trends observed in the venom compositions of colubrids of both transcriptome and proteome, some interesting patterns appear (Figures 1 and 2). The overall dominance of proteolytic venoms in the Dipsadinae subfamily is clear where two distinct types of zinc-dependent metalloproteinases appear to have been recruited into the venom arsenal of this subfamily (Figure 1) [42]. Snake venom metalloproteinases are present across several groups of advanced snakes (see Chapters 3 and 5) and are likely to have been ancestrally recruited before the separation of vipers and all other venomous snakes [11,76–79].

On the other hand, some tribes (Conophiini, Tachymenini, and Xenodontini) of the Dipsadinae subfamily possess another type of proteolytic enzyme (svMMPs) that dominates their venoms. These proteins are likely to have been recently recruited into the venom glands of species of Dipsadinae and are the dominant toxin class in some groups [42].

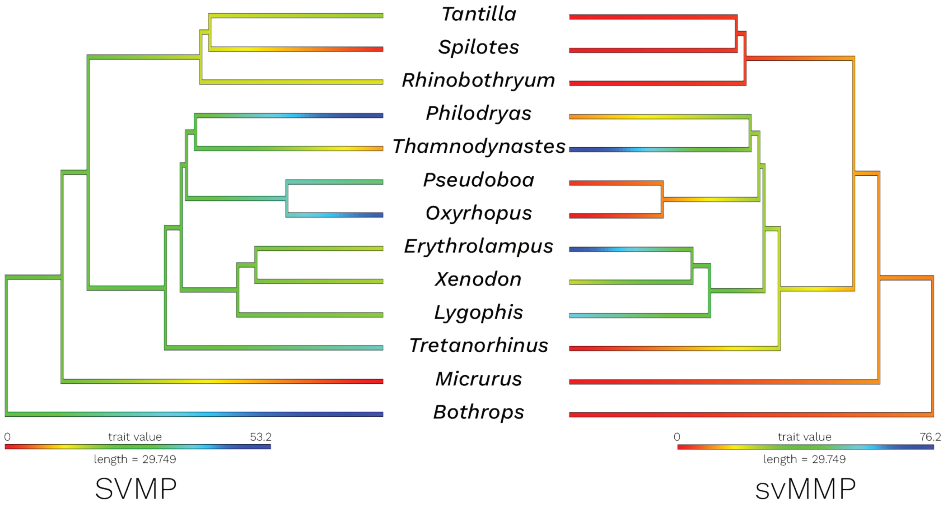


Figure 1. Ancestral state reconstruction of compositional data for SVMPs (left) and svMMPs (right). Red color indicates lower proportions of that toxin while blue colors indicate higher proportions of that toxin; yellow to green color scale depicts moderate proportions. Data for *Rhinobothryum* were obtained from proteomic analyses [55], and data for *Tretanorhinus* belong to a study that analyzed the Harderian gland [57]. *Micrurus* and *Bothrops* were used as outgroups [80,81]. Branch lengths were obtained from Zaher et al. [9].

Venoms of Colubrinae species show highly neurotoxic venoms dominated by 3FTxs that are post-synaptic toxins that cause neuromuscular blockades by acting at the motor endplate (Figure 2) [62]. This compositional pattern resembles the one observed in some coralsnakes and marks a conspicuous difference between Colubrinae and Dipsadinae venoms. Nevertheless, some species of Dipsadinae possess neurotoxic effects, but these are thought to be mediated by CRISPs or to be secondary effects of the proteolytic action of their toxins [56,72].

Overall, venoms from colubrid species possess a diversity of toxins similar to that of vipers and elapids (see Chapters 3 and 5) with some groups favoring 3FTx-dominated highly neurotoxic venoms and others SVMP/svMMP-dominated highly proteolytic venoms (Figure 3). Moreover, even though the vast majority of colubrid species lack any kind of venom-related information, an enormous diversity of toxin scaffolds and functions unique to this group of snakes has already been unveiled [11,37,42]. These convergent and divergent patterns indicate that changes in venom

composition could be linked to both phylogenetic inertia and ecological factors leading to convergence [42,82,83].

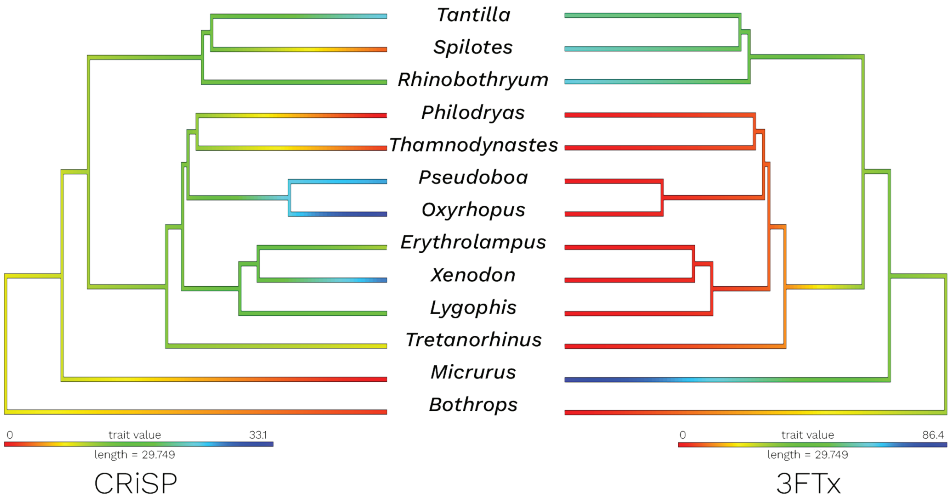


Figure 2. Ancestral state reconstruction of compositional data for CRISPs (left) and 3FTxs (right). The red color indicates lower proportions of that toxin while blue colors indicate higher proportions of that toxin. Yellow to green color scale depicts moderate proportions. Data for *Rhinobothryum* were obtained from proteomic analyses [55], and data for *Tretanorhinus* belong to a study that analyzed the Harderian gland [57]. *Micrurus* and *Bothrops* were used as outgroups[80,81]. Branch lengths were obtained from Zaher et al. [9].

This duality has already been noted in the past for the variation of the activity of two enzymatic toxins from Australian elapid species. Although enzymatic activity showed a stronger correlation to phylogeny in most genera, divergent activity levels were reported for two closely related species of *Denisonia* that are frog specialists [82]. Therefore, there might be unknown underlying factors shaping venom composition and function in front-fanged, rear-fanged, and even aglyphous snake species. Studies that link ecological variables to compositional and functional venom data, along with phylogenomic information, are needed in order to gain a better understanding of the evolutionary processes that mold the venom phenotype of extant snakes, especially in colubrid species.

NON-VENOMOUS SNAKE TOXINS

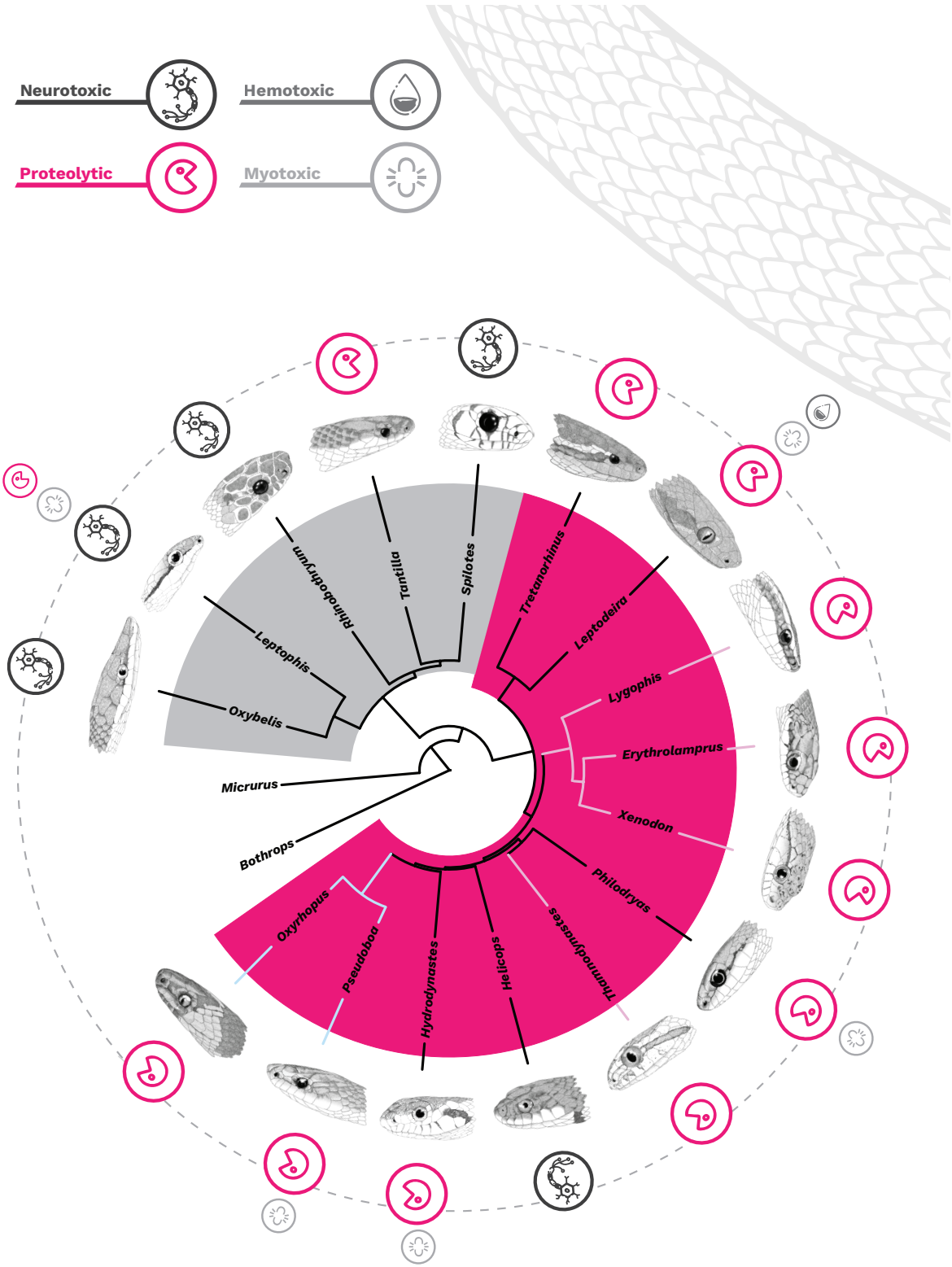


Figure 3. General overview of the known venom profiles of colubrids.

2.4 Venomic notes of Colombian colubrids

Envenomation by colubrid snakes in Colombia is a poorly known phenomenon, consequently, the clinical relevance of these species in snakebite accidents has been historically underestimated [106]. This does not mean that envenomation by colubrid snakes do not occur. In fact, during the last decade a total 347 cases were notified in Colombia (see Chapter 9), and those caused by *Leptodeira annulata* and *Erythrolamprus bizona* were documented with detail [106,107]. However, the true snakebite incidence caused by colubrid snakes is masked. Following, we present the available venomic information of the colubrid snakes (aglyphous and opisthoglyphous) with potential medical importance in Colombia.

Genus Erythrolampus.— Recently, it was found that venoms from this genus are mainly dominated by a novel proteolytic scaffold known as snake venom matrix metalloproteinases (svMMPs) and a few other minor toxins such as SVMPs, CTLs and CRISPs [42]. Functionally, the venom of Colombian populations of *Erythrolampus bizona* produces partial neuromuscular blockade causing notable morphological alterations with higher doses involving disorganization of muscle fascicles, interstitial edema, and expressive muscle fibers necrosis [56]. The neuromuscular effects observed seem to be the result of the high proteolytic activity rather than a neurotoxic mechanism which fits perfectly with the mainly proteolytic profile of the reported venom proteins [56].

Leptodeira annulata species complex.— This species is known for its harmless and inoffensive behavior [108] (Figure 4). Nevertheless, there are reported cases of moderate local envenoming when its bite is provoked due to improper handling [106,109]. Venom of Colombian populations from the Magdalena Valley has been explored [33,34], finding protein bands with molecular masses from ~15 kDa to ~140 kDa. The most conspicuous bands were seen at ~35 kDa, ~40 kDa and ~50 kDa, as well as a prominent band at the migratory front (~15 kDa). In addition, chromatographic analysis (RP-HPLC) showed a total of 38 separate peaks, indicating a highly complex venom composition, which can be equal or superior that observed the venom of some *Bothrops* species [34].

Recently, a complete toxinological characterization of venom from *L. annulata* was reported. The venom had no esterase, L-amino acid oxidase or thrombin-like activity, but was proteolytic active towards several substrates including fibrin, fibrinogen, gelatin and casein. The proteolytic activity is probably mediated by metalloproteinases as it was only inhibited by metal ion chelators (EDTA and phenanthroline) [34]. Besides, the venom did not aggregate rat platelets or inhibit collagen-induced aggregation, but partially inhibited thrombin-induced aggregation. The venom was also hemorrhagic (inhibited by EDTA) and increased the vascular permeability in rat dorsal skin. Additionally, the venom produced myonecrosis and increased serum creatine kinase concentrations in the gastrocnemius muscle [34].

Leptodeira annulata venom preparations applied to nerve-muscle, produce a moderate neuromuscular blockade without causing a complete

blockades. Moreover, it significantly inhibits the contracture response to exogenous potassium chloride. In contrast, in mice nerve-muscle preparations, the venom of *L. annulata* does not cause significant neuromuscular blockade. However, it induced minor muscle membrane depolarization without affecting the postsynaptic receptors [33]. These neuromuscular effects observed are the result of an unspecific proteolytic activity of the venom rather than a neurotoxic mechanism. On the other hand, *L. annulata* venom causes clear histological alterations in both chick and mice models, including swollen fibers and necrosis. These effects seem to be related to the high proteolytic and mild PLA_2 activity exerted by the venom [33].

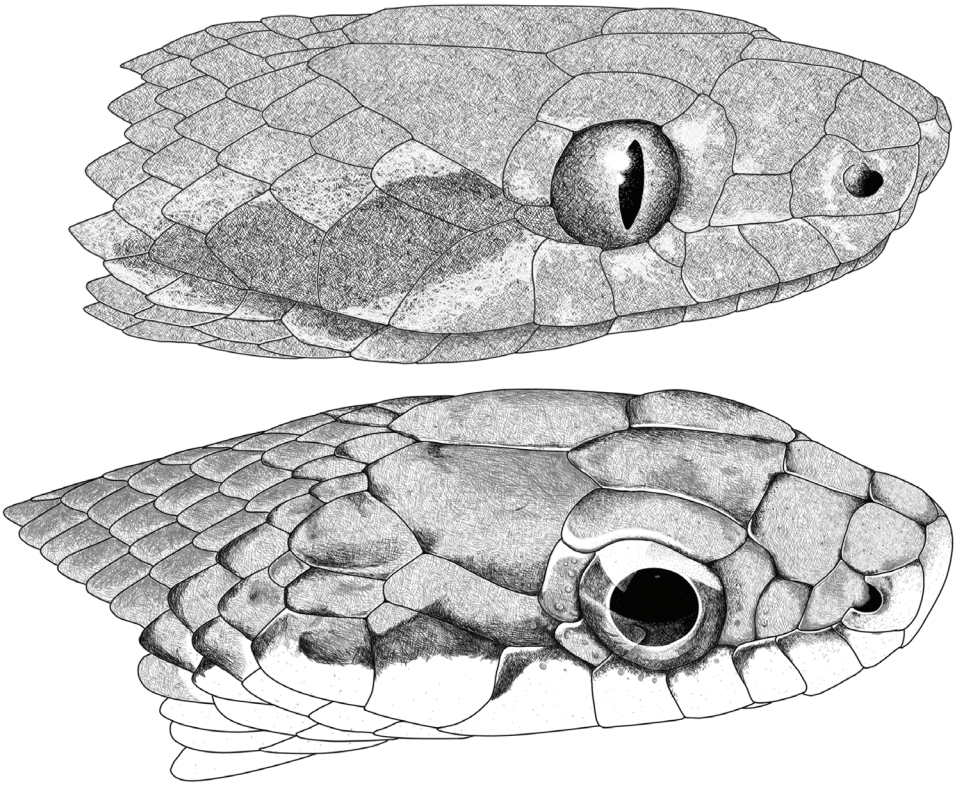


Figure 4. Head illustrations of *Leptodeira annulata* and *Leptophis ahaetulla* species complex. (Top) head in lateral view of *Leptodeira annulata* from Pacurita, Quibdó, Choco, Colombia (JDL 31754). (Bottom) Head in lateral view of *Leptophis ahaetulla* from Caño Cristales, Riosucio, Choco, Colombia (IAvH-R1458). Illustrations by Oscar A. Ramirez Ruiz.

Genus *Leptophis*.— Among the *Leptophis* species inhabiting Colombia, only *Leptophis ahaetulla* has venom studies. Although it is a commonly found genus across its distribution range, there are few studies regarding venom composition or function with Colombian populations (Figure 4). Rodriguez-Gonzalez and Portillo-Portillo [110] made the first work testing the toxic capacity of *L. ahaetulla* venom. They tested the neurotoxic effect of this species venom in the sciatic nerve of the frog *Rhinella horribilis*. However, the venom did not disturb the action potential of the nerve, and the author hypothesized that it had a minor biological function.

Recently, Sanchez et al. [30] performed a functional and compositional characterization of venom from an Argentinian population of *L. ahaetulla* revealing a compositional profile dominated by small proteins (<10.0-30.0

kDa) identified as CTLs, CRISPs and 3FTxs. Consequently, proteolytic and myotoxic activities of the venom from *L. ahaetulla* were practically null, as no enzymatic protein was identified as an important component of the venom. Moreover, lethality tests in mice showed no signs of lethal effects in doses as high as 20 µg/g, although this might be explained by prey-specific toxicity (frogs and lizards) as has been observed in other colubrid with neurotoxic venoms [60,63,111]. Nevertheless, studies about functional and compositional characterization of *L. ahaetulla* venom from Colombian populations have not been performed yet.

Genus *Lygophis*.— There is not much information regarding the venom composition and function of *Lygophis* snakes. A recent work analyzing the venom gland transcriptome of *L. flavifrenatus* from Brazil found that its venom shows a highly proteolytic profile following the trend observed in other closely related genera (e.g., *Erythrolamprus*), with svMMPs as the main type of proteolytic enzymes among the other minor venom components [42].

Genus *Oxybelis*.— A few human accidents involving *Oxybelis* species have been reported across its known distribution [112–114]. Generally, *Oxybelis* envenomation consists of local symptoms like pain, erythema, and hemorrhage, rarely accompanied by tachycardia and dizziness (Figure 5).

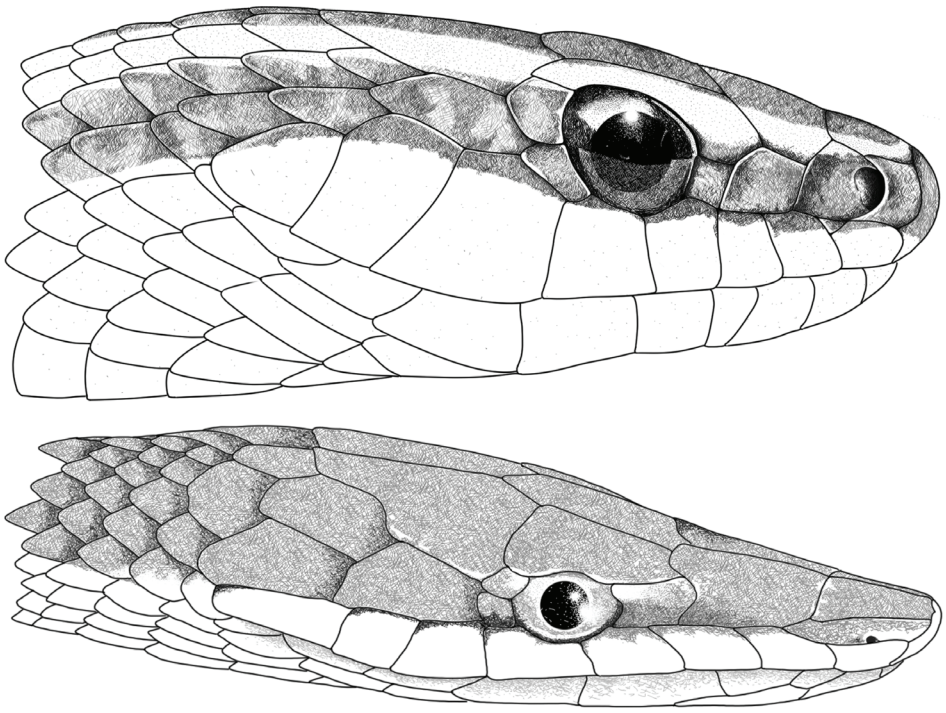


Figure 5. Head illustrations of *Lygophis lineatus* and *Oxybelis aeneus*. (Top) head in lateral view of *Lygophis lineatus* from Universidad Nacional de Colombia sede de La Paz, La Paz, Cesar, Colombia (TAS 2576). (Bottom) Head in lateral view of *Oxybelis aeneus* from Riosucio, Choco, Colombia (IAvH-R1458). Illustrations by Oscar A. Ramirez Ruiz.

Regarding its venom, in the last decades some interesting research has been published. The venom from the green vine snake (*O. fulgidus*) seems to be compositionally simple with venom proteins within the 6-9 kDa, 25 kDa and 50-66 kDa size ranges, highlighting the presence of 3FTx [111].

Moreover, a neurotoxin from this venom, which is the most abundant component, was purified and characterized. Fulgimotoxin is a 3FTx with very high lethal toxicity towards native lizards (*Anolis* species were more sensitive than *Hemidactylus* species) and little to no effects on mammals (mice) [111].

Recently, the venom proteome from two species was described. The venom proteomes were of low compositional complexity with four detected protein families (LAAO, P-III SVMPs, CRISP and 3FTx) [60]. In general terms, both venoms are highly neurotoxic with lethal activity in lizards, low proteolytic activity, and lacking fibrinogenolytic activity [60].

Genus *Oxyrhopus*.— Unfortunately, there is not much information available about the venom composition or function of this group of snakes. However, the little data that exists suggests that venoms of *Oxyrhopus* species are dominated by SVMPs, CRISPs and CTLs, following the same trend observed in most of Dipsadinae [11]. Nevertheless, other venom components include PLA_2s-IIE which are of special interest because it represents a class of PLA_2 different than that found in vipers and elapids, perhaps being a new event of toxin recruitment into the venom gland arsenal of this group of snakes as was recently observed in other species of the Peudoboini tribe [11,73]

Genus *Philodryas*.— Among non-front fanged colubrid envenoming in South America (Figure 6), *Philodryas* species cause the most cases reported [17,84,115–118]. Thus, they are the colubrid snakes with the most comprehensive venom-related data compared to other colubrid genera [54,119,120]. Particularly, due to the wide distribution of *P. olfersii*, its venom has been widely studied. This venom is highly proteolytic, produces edema, hemorrhage and myonecrosis in animal models [121–124]. Some components of the venom have been isolated: a myotoxin [125], four metalloproteinases and one serine protease [126]. The characterization and identification of these venom components agree with the proteomic and transcriptomic analyses performed on this species venom [54,127]. Functionally, the venom of *P. olfersii* degrades fibrinogen and fibrin, and it is devoid of thrombin-like activity, platelet-aggregating inhibitory effects [123]. The venom also does not produce hematological alterations having limited effects on circulating cytokine concentrations [128]. Although different effects are observed *in vivo* using murine models, primary manifestations in human envenoming are local effects such as pain, edema, erythema and ecchymosis [85]. Currently, no cases of envenoming by species of the *Philodryas* genus have been reported in Colombia.

Genus *Rhinobothryum*.— Field encounters with these species are usually scarce, thus, *Rhinobothryum* venoms are poorly known (Figure 6). Recently, a study with *R. bovallii* revealed that it possesses a neurotoxic venom as observed in other genera of the colubrinae subfamily (e.g., *Spilotes*, *Leptophis* and *Oxybelis*) [55]. Proteomic analyses revealed a rather simple compositional profile dominated by neurotoxic CRISPs (8.2%) and 3FTxs (86.5%) with a small proportion of proteolytic SVMPs (2.4%). Taxon-

specific neurotoxins have been repeatedly reported in venoms of colubrids with specialized diets mainly focusing on birds and/or lizards. However, *Rhinobothryum* species have a very diverse diet, so functional studies aiming to uncover the specificity of their neurotoxins are needed to gain a better understanding on toxin evolution and venom resistance in those prey models. Currently, cases of envenomation by *Rhinobothryum* species have not been reported in Colombia.

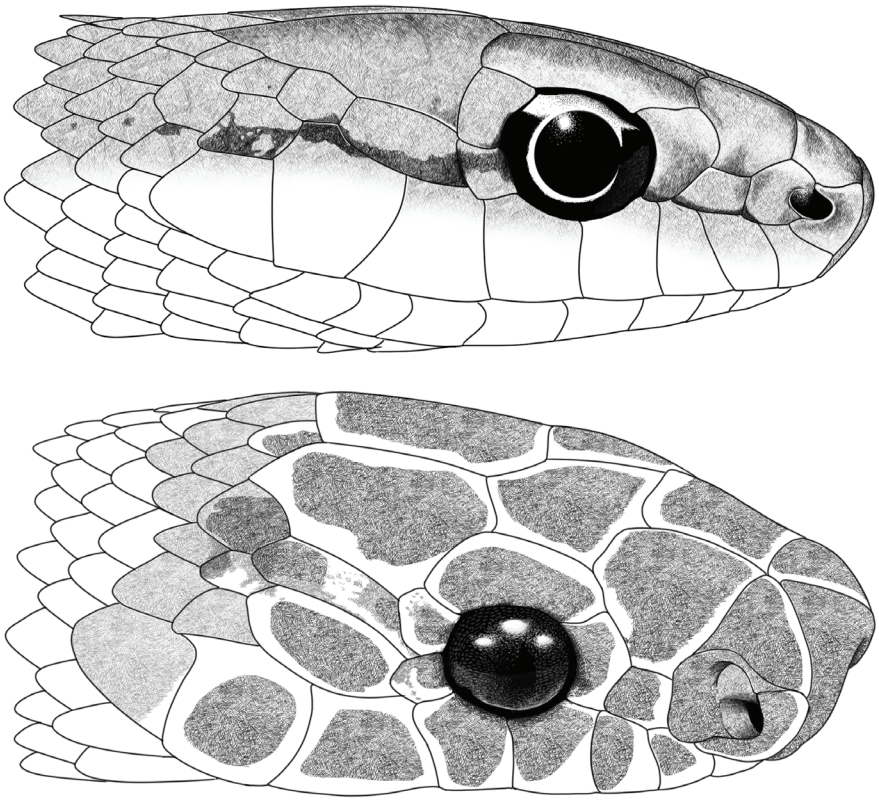


Figure 6. Head illustrations of *Philodryas olfersii* and *Rhinobothryum bovallii*. (Top) head in lateral view of *Philodryas olfersii* from Puerto Rondón, Arauca, Colombia (IAvH-R2878). (Bottom) Head in lateral view of *Rhinobothryum bovallii* from Pedernales, Manabí, Ecuador (QCAZR -R5757). Illustrations by Oscar A. Ramirez Ruiz.

Pseudoboa neuwiedii.— Venom of *P. neuwiedii* from Tolima was biochemically characterized by Torres-Bonilla et al. [129]. In their analyses they report its SDS-PAGE profile and the identification of its components by LC-MS/MS. Venom from this species shows protein bands ranging from ~15 kDa to ~200 kDa, with the most conspicuous bands between ~50-75 kDa and ~20-25 kDa. These bands were identified as SVMPs and CRISPs, resembling the reported compositional pattern observed in other closely related species at the transcriptomic level [11]. Among components identified, less intense bands were CTLs, PLA_2s and more notably, svMMPs, a scarcely studied family of proteinases recently reported as a major component of the venoms of some dipsadids [129].

Moreover, venom from *P. neuwiedii* produces moderate neuromuscular blockade (50% blockade after ~80 min) with significant reduction of the contracture responses to exogenous potassium chloride and acetylcholine after 120 min of incubation. The venom caused extensive histopatho-

logical alterations in muscle preparations. The muscle fibers lost their normal and typical polygonal profile with swollen or vacuolated fibers leading to failure of permeability regulation. The neuromuscular effects observed are the result of a general proteolytic activity rather than a neurotoxic mechanism [56]. *Pseudoboa neuwiedii* venom is highly proteolytic towards azocasein with optimal temperature of 37 °C, pH 8.0, extremely active in gelatin and displays moderate PLA_2 activity. The venom also degrades the Aα chain of fibrinogen in vitro, lacks coagulant activity and delays the coagulation time during plasma recalcification [129].

Genus *Spilotes*.— Currently, there are no reports of envenomation cases or characterized venom properties from this species. However, there is recent research addressing the venom evolution of another species of the genus (*Spilotes sulphureus*). The venom from *S. sulphureus* was analyzed combining specific prey assays with venom gene expression and venom phenotype [63]. Two novel neurotoxins (3FTx) were identified: sulditoxin and sulmotoxin 1, which are the most abundant venom proteins with opposite prey-specific toxicities. Sulditoxin is highly toxic to lizards and non-toxic to mammals, whereas sulmotoxin 1 exhibits the reverse trend [63]. This is also the first case in which a bimodal and opposite pattern of toxicity for toxins is shown for a venom in relation to diet [63].

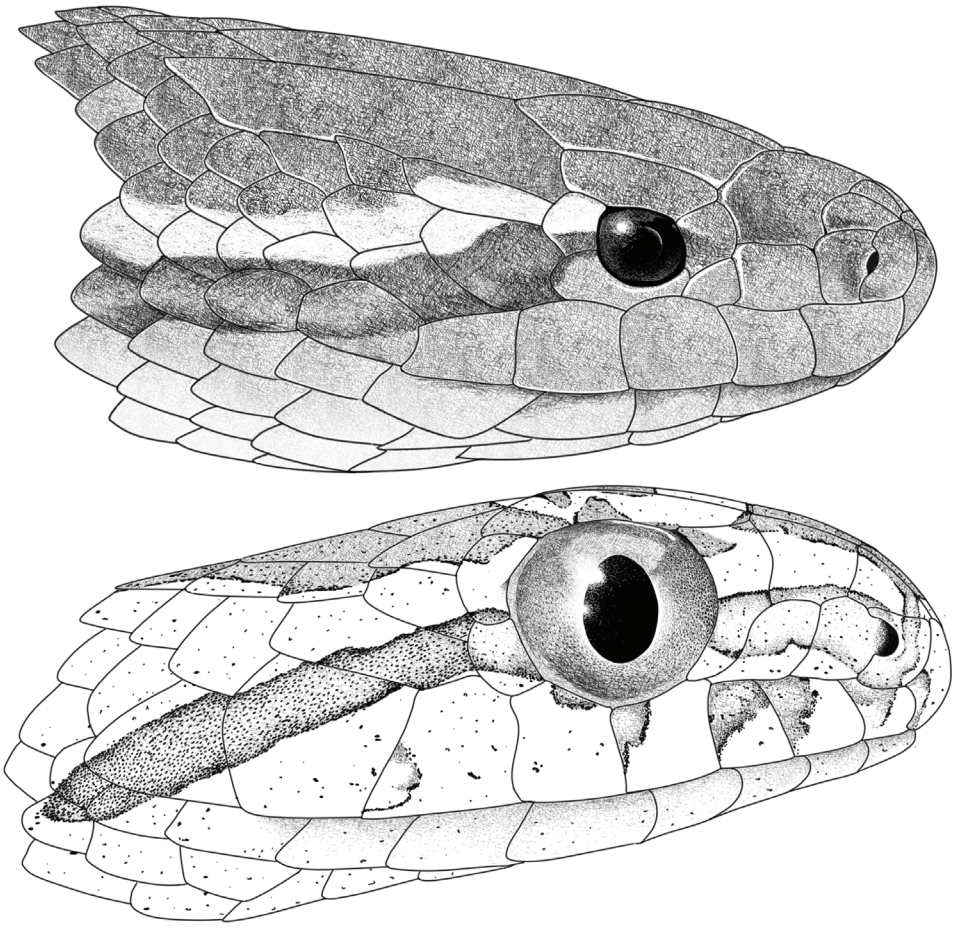


Figure 7. Head illustrations of *Tretanorhinus nigroluteus* and *Thamnodynastes dixoni*. (Top) Head in lateral view of *Tretanorhinus nigroluteus* from vereda Cacarica, Riosucio, Choco, Colombia (IAvH-R 2002). (Bottom) Head in lateral view of *Thamnodynastes dixoni* from Puerto Carreño, Vichada, Colombia (JDL 31383). Illustrations by Oscar A. Ramirez Ruiz.

Genus *Tantilla*.— Despite the enormous species richness within the *Tantilla* genus, only *T. nigriceps,* a species distributed in southern North America, has venom studies. These studies highlight that this genus possesses a venom highly neurotoxic. However, their small size and very low venom yields make them harmless to humans [37,38]. The venom gland transcriptome of *T. nigriceps* was dominated by neurotoxic 3FTxs (54%) and CRISPs (24%), with smaller proportions of SVMPs (18%) and CTLs (3%) [52]. The highly specialized diet of *Tantilla* species would make their neurotoxins an amazing model to study toxin specificity and evolution.

Genus *Thamnodynastes*.— Snake species of the genus *Thamnodynastes* are notorious for their reported envenoming cases which generally include symptoms like edema, radiating pain, ecchymosis and hemorrhage [46,130,131] (Figure 7). Nevertheless, no venom related information was available until 2012 when Ching et al. [132] published the proteomic and transcriptomic characterization of the venom of *T. strigatus*. Their findings revealed that a new type of metalloproteinases, called svMMPs, were the dominant toxin class in both the venom proteome and the venom gland transcriptome. svMMPs accounted for 46% of the whole transcriptome and 76% of all the annotated toxins which included SVMPs, CTLs, 3FTxs, CRISPs and other minor toxins. Recently, a transcriptomic screening including more species confirmed the dominance of svMMPs in the venom of this genus and showed that they maintain a highly proteolytic profile [42].

Genus *Tretanorhinus*.— Due to the scarce sampling and information readily available for this genus, there has not been a single study regarding venom composition for either of the two Colombian species (Figure 7). Nevertheless, the transcriptome of the harderian gland from a Cuban swamp snake *T. variabilis* has been analyzed. This gland is located behind the eye of the snake and is proximal to the duvernoy's gland. It is thought to be closely related to the lacrimal gland aiding in the lubrication of the eye, but it has been proposed to fulfill several other roles [67,68,133]. These hypothetical functions include acting as an accessory salivary gland which may secrete digestive enzymes or provide a source of lubrication. In *T. variabilis* the expression profile of the harderian gland revealed a series of putative toxins including P-III SVMPs, CTLs, and CRISPs, which are three of the main toxins reported in other colubrids [57]. These findings indicate that the production of toxin-like proteins is not limited to the duvernoy's gland of colubrids, but it is not clear if these other oral glands that are producing putative toxins represent a trophic advantage.

3. Epidemiology and clinical manifestations

Envenomation by colubrid species has been greatly overlooked by epidemiological studies worldwide [10,11,35,47,84]. This might be related to the fewer number of bites involving colubrids or underreporting, as well as the relatively minor clinical manifestations caused by their venoms [35,43,45,47,49,85,86]. However, these mild effects could result in partial

or full immobilization of affected limbs, causing a potential loss of productive work capacity for the patient (commonly a farmer or agricultural worker) [17,36,44,48,51,85].

In Colombia the incidence and epidemiological records of snakebite as a public health metric is provided by the Surveillance System of the National Health Institute (Sistema Nacional de Vigilancia en Salud Pública: SIVIGILA, Spanish acronym) (see Chapter 9). The SIVIGILA publishes annual reports with the total number of snakebite cases including numerous amounts of data including information about the identification of the snake involved in the accident. However, the process of data collection and analysis involves the arduous revision of handwritten forms filled by medical practitioners from all around the country that had varying degrees of available taxonomical information. This complication is generally bypassed for commonly identified groups (e.g. genera *Bothrops*, *Crotalus* and *Micrurus*), but it still is a difficult bias to overcome for most colubrid species that typically have various common names that change between different regions, and encompasses most of the snake diversity of the country [8,10].

Therefore, the number of ambiguously identified or undetermined snakes that were reported is generally high with ~11,280 out of the ~49,143 cases between 2010-2020 (~20% of total cases). This is likely to increase the already existing identification bias that colubrid envenomation suffers, widening the gap of medical relevance between front-fanged and rear-fanged snakes [10,11].

Fortunately, since 2012 the National Health Institute (Instituto Nacional de Salud: INS, Spanish acronym) has conducted several campaigns to educate about the prevention and handling of snakebite incidents, helping medical professionals to gain a better insight into snake identification. The positive impact of these initiatives becomes clear when analyzing the cumulative average of annual snakebites that were not identified in the country (Figure 8).

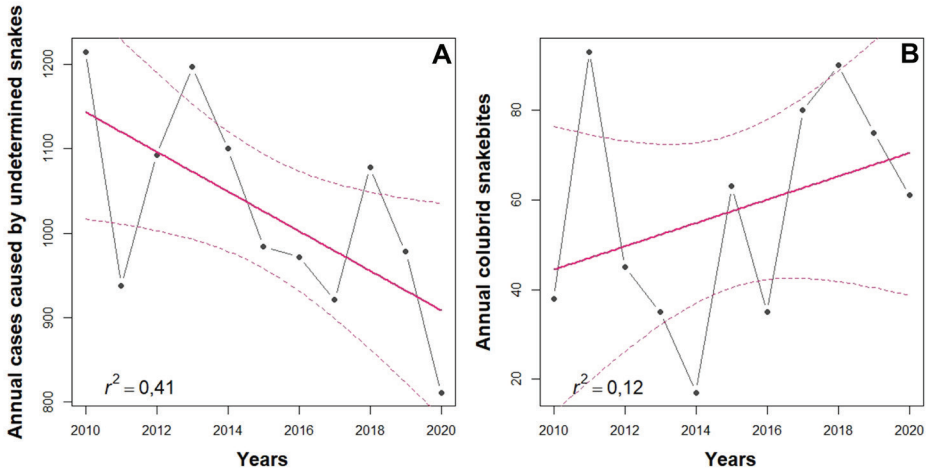


Figure 8. Linear regression model of the cumulative average of annual undetermined snakebites (A) and the annual colubrid snakebites (B) reported by the SIVIGILA (2010-2020). Grey lines and dots represent the yearly variation of snakebite cases. The solid pink line represents the best fit of the linear regression model, and the dashed pink lines shows the 95% confidence interval for the model. r^2 values are shown on the bottom left of each graph.

The number of undetermined cases shows a diminishing trend in the last ten years (F= 6.15, p= 0.035). Therefore, we could expect an increase in the number of identified colubrid snakebites that were previously hindered as undetermined cases. When analyzing the annual occurrences of colubrid snakebites we observe an increasing trend. However, this tendency is not significant due to the heterogeneity of the data (F = 1.19, p= 0.30). Nevertheless, as the number of undetermined cases decreases, we expect to see an increase in the number of reported snakebites involving colubrids.

Currently, there are ~30 confirmed annual snakebite cases caused by recognizable snake species belonging to the Colubridae or the Boidae families in the SIVIGILA database (~541 and ~91 respectively from 2010-2020). These cases have a rather consistent profile of clinical manifestations (Figure 9). Pain, local symptoms, edema, erythema, systemic symptoms, paresthesia, and general sickness are among the major reported symptoms. Moreover, when comparing these occurrences with the clinical profile of undetermined cases we see a similar profile that might relate to the likely high proportion of colubrid-related cases that are labeled as undetermined.

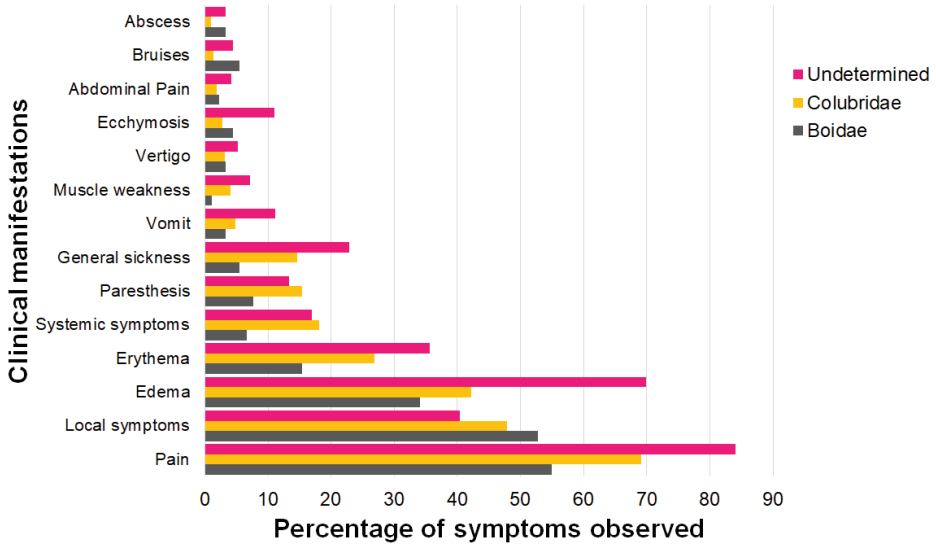


Figure 9. Clinical manifestations reported in the SIVIGILA for Boidae, Colubridae, and undetermined snakebite cases in Colombia during the period 2010-2020.

Interestingly, the SIVIGILA database reports the application of antivenom in some cases attributed to either Colubridae or Boidae species (~159 and ~19 respectively). This is problematic as allergic reactions to polyvalent antivenom are known to cause medical complications [87]. So, the correct and accurate identification of the species that caused the accident is a crucial stage to ensure the safety of the patient. Increasing the percentage of accurate identification of non-front-fanged snake species is key to obtain a complete panorama of the true epidemiological relevance of snakebites in the country.

Overall, the management of snakebite incidence in Colombia has improved in the last decade, although some challenges remain. There is an

urgent need to increase the percentage of identified snakes involved in snakebite cases and to implement a more efficient system to compile and organize all the clinical information of each snakebite case. Moreover, colubrids and other non-front-fanged snake groups should be given a higher relevance in clinical studies and courses in order to decrease the number of undetermined snakebite incidents and ensure that each snakebite case is treated correctly.

4. The untapped biological potential of "colubrid" snakes

Historically, snake venoms have been a source of novel protein scaffolds with evolutionarily intriguing and pharmacologically applicable functions [86–88] (see Chapter 10). However, as previously discussed, most research on venom diversity has focused on vipers and elapids, neglecting the majority of venom producing species [11,39]. Fortunately, in recent years, there have been transcendent advances in our knowledge of colubrid venom composition that reveals a toxin diversity of similar magnitude to that of front-fanged snakes [11,42,73]. In this section we will present the main direct and indirect applications of colubrid venoms and why they represent an interesting biological model for both evolutionary and pharmacological studies.

4.1 Colubrid venoms as a tool for understanding evolutionary processes

Snake venom is a trophic adaptation that is directly linked to the capacity for effective hunting and feeding, since it aids in paralyzing and killing prey [63]. Therefore, venom composition and function are under heavy selective pressure and can only vary between a few optimal thresholds [91]. Moreover, genes controlling toxins are generally recruited into the venom gland through a process known as gene duplication that allows for rapid diversification as new gene copies escape selective constraints, being free to mutate [42,79,83,92].

Hence, snake venom toxins display a broad spectrum of diverse functions and molecular targets contained within few conserved structural scaffolds [62,76,77,91,93]. These peculiarities have turned them into interesting models for studying phenotypical convergence, gene recruitment and co-option, predator-prey relationships, and several other evolutionary phenomena [29,42,76,83,93].

Snake venoms are dominated by a relatively small number of protein families, with SVMPs, PLA_2s, 3FTxs, and SVSPs as major components of a few optimal compositional profiles. However, most studies have few data from colubrid species; as they remain a sub-sampled group in venom-related research [11,91]. Nevertheless, the few colubrid venoms that have been thoroughly studied have already revealed the occurrence of new toxin scaffolds that are likely to represent unique gene recruitment events [42].

Recent studies of colubrid venoms have detected the occurrence of phenotypical convergence and recruitment of novel gene families in their venom repertoire. For example, snake venom matrix metalloproteinases (svMMPs) were recently found to be a major toxin class in three tribes of the dipsadinae subfamily, replacing SVMPs as the main proteolytic toxins in Xenodontini, Tachymenini and Conophiini [42]. This toxin class was recruited independently in each of those tribes and represents a curious event of convergence among them. Interestingly, these newly reported proteolytic toxins evolved into simplified structures resembling the SVMPs of vipers, and they are thought to possess similar proteolytic capabilities [42,53,76,77,94] (see Chapter 5). Examples of the maintenance of a phenotypic trait by recruiting new genes into a tissue are scarce [42,83], but snake venoms, as a multi-factorial trait determined by countless proteins with overlapping and synergistic functions, compose a prime model for studying these intricate evolutionary events.

As a trophic adaptation, snake venom is a useful tool for understanding predator-prey relationships and species adaptations to changing environments. This is especially true for evolutionary hypotheses like "The red queen hypothesis" proposed by Leigh Van Valen, in which predator and prey are in a constant arms race to surpass one another [95,96]. In this example, venomous snakes would evolve more specific and potent toxins towards their prey, while their prey would evolve resistance mechanisms or specific behaviors to avoid being preyed upon.

Several examples of toxin resistance/specificity have been reported for front-fanged snake venoms, involving both proteolytic and neurotoxic toxins. Venom resistance mechanisms are generally mediated by circulating serum factors or by adaptive changes in venom-targeted molecules [97–99]. A classic example of the former are α1B-glycoprotein homologs found in mongooses and opossums that inhibit either SVMPs or PLA_2 myotoxins [98].

Resistance to neurotoxic toxins generally involves residue modifications in muscular nicotinic cholinergic receptors (nAChRs) that are targeted by α-neurotoxins. These mechanisms have been primarily reported in front-fanged snakes, transforming colubrids into a novel and outstanding model for exploring toxin resistance and specificity [99].

Several colubrid species have already been shown to possess venoms with highly specific neurotoxins that exhibit varying potency towards different prey [29,55]. However, these studies have only been performed on a limited number of genera and the evolutionary consequences of colubrid venom specificity/resistance are yet to be unveiled. Overall, colubrid venoms represent an interesting source to look for novel protein scaffolds and to study evolutionary processes that are poorly understood. An increased amount of venom-related data is needed in order to truly understand the mechanisms that shape venom composition, function, and evolution in this extremely diverse group.

4.2 Colubrid venoms and pharmacological applications

Current research focusing on venoms from rear-fanged snakes is still neglected compared to the amount of work describing the composition and biological diversity of front-fanged snake venoms (see Chapter 5 and 10). Therefore, the amount of fully characterized toxins from colubrid venoms is contrastingly low [11]. The transition from toxin discovery/characterization to the development of a possible therapeutic agent is a very complex process [88,90]. Historically, venom research has been a great source of proteins and peptides with a vast potential application in numerous biomedical and pharmacological fields (see Chapter 10). Hence, there are several therapeutic compounds in circulation and/or in clinical trials used in humans that have been successfully designed and developed from animal poisons and venoms [88,90,100–102].

Considering that rear-fanged snake venoms contain a vast number of different proteins and peptides with complex and potent biological activities resembling those found in vipers and elapids, they represent a novel source of potentially bioactive molecules [28,37,39–41]. The implementation of new extraction methods, the improvement of methodological assays, and the increasing interest in these venoms have resulted in a better understanding of their complex, unique, and new biological activities and molecules [11,40,103]. The huge taxonomic diversity of colubrids has resulted in a great variety of compositional and functional venom profiles as previously described in this chapter. All of these factors set an enormous potential for the discovery of novel protein families or enhanced biological effects of some compounds in terms of potency or efficacy that may represent an excellent basis for drug design and development [10,40,103].

Research on rear-fanged snake venoms has revealed a complex variety of biological activities and compositional patterns. However, the most ubiquitous and commonly studied activities in colubrid venoms are proteolytic activity that leads to hemorrhage and coagulation disturbances, and myotoxic and edematogenic activities that resemble the effects observed in *Bothrops* venoms [32–34,42,56]. Moreover, some protein families like SVMPs and PLA_2 found in colubrid venoms show different toxic and enzymatic potencies compared to the ones found in species of viperids [32,34]. These functional differences, as well as prey specific toxicities, might be interesting approaches for future research looking for compounds with therapeutic potential [33,56].

The presence of conserved structures in common toxins (e.g. CRISPs) that are spread throughout many venomous reptiles, together with non-enzymatic proteins known to produce several pharmacological activities, also provides venoms with different and unknown biological properties to be examined [11,32,37,39]. A prime example of this is *patagonin*, a CRISP isolated from the venom of *Philodryas patagoniensis* that shows antibacterial activity at very low concentrations without signs of cytotoxic activity [104,105].

Another very interesting fact that underlines research on colubrid venoms as a great source of potential discoveries is the presence of unique and putative toxins in these venoms [11,32,33,42]. The presence of these unique protein families provides different research approaches for discovering the biological role and activities from these toxins such as possible new physiological mechanisms, pharmacological targets, and even therapeutic potential.

On the other hand, the diversity of habitats, diets and behaviors found in Colombian colubrids might translate into drastically different toxin scaffolds from that of elapid and viperid venoms. However, the lack of venom-related data has hindered the advance of studies that unveil the pharmacological and functional applications of colubrid venoms. The advance and development of new methods and tools for venom research represents a great opportunity to better understand the biological implications of these toxins and to provide valuable information for possible future novel drug discoveries.

5. The Colombian perspective

During the last few decades, an interest in research focused on colubrid venoms and envenomation cases has bloomed. The implementation of novel venom extraction methods, including manual and pharmacological protocols, has resulted in greater venom yields. This has eased the development of better transcriptomic and proteomic methodologies that have increased our understanding of the composition and biological properties of colubrid toxins using small quantities of venom [11,37–40].

Knowledge about these venoms obtained during recent years and the awareness of the potential hazards that some colubrid species represent around the world has led to the implementation of different strategies to record and control envenomation cases including these species [134,135]. These strategies focus on engaging with communities to increase their awareness, ensuring the availability of efficient and specific medical treatment, strengthening the health system, and establishing interdisciplinary partnerships between the public and private sectors. In many countries, institutions are encouraged to develop security measures to handle accidents involving these species and to achieve a faster and more accurate taxonomical identification in eventual envenomation cases [135]. In Brazil, for example, colubrid snakebites represent a collective health problem with many cases reported per year and with the awareness that some snakes previously considered harmless could be of medical interest [35,45,49].

In Colombia, there are around 340 species of snakes and 18% (~20 viperid and ~29 elapid) represent species of medical interest [8,136,137] (See Chapter 1). Colubrids contain the majority of snake species found in the country (~291 species) and many of these possess an opistoglyphous dentition (enlarged grooved posterior maxillary teeth) connected to the Duvernoy's venom gland that makes them a potential hazard for humans [17,36].

However, this group of snakes receives almost no medical or epidemiological interest in Colombia. The lack of ability to identify these species by the community in general and the medical staff, as well as the shortfalls in the surveillance system, are the main constraints facing snakebites caused by non-front-fanged snake species [10]. An improved surveillance and understanding of these cases would lead to better treatment for the patients and would avoid some practices such as the injection of bothropic or elapidic antivenom that might not be necessary.

Because of minimal medical interest for these species and venoms, there is also a large knowledge gap and a lack of information of the compositional and biological properties of venom samples from Colombian colubrid species [33,56]. The relevance of the toxinological research of this group lies in the vast diversity of new family proteins/toxins and biological functions that also represent a great fountain of resources for bioprospection and evolutionary/phylogeny approaches [32,34].

It is necessary to include these species in the snakebite programs in Colombia. They should also be included in epidemiological surveillance strategies. This will lead to the restoration of security measures and protocols for the maintenance of colubrid snakes in captivity and to a better system of notification and registration of accidents that include these species. Along with these data, regional epidemiological studies of these accidents would aid in the identification of the most affected communities and the implementation of educational programs that increase medical awareness of these species. Finally, these approaches will result in increased interest of research groups for investigating these venoms and for carrying out interdisciplinary projects to understand the composition, evolution, and mechanisms of action of these venoms.

Appendix: Material and Methods

The search methods for identifying studies were based on weekly searches from January to June 2024. We performed the searches as follow:

1. Weekly searches of google scholar
2. Weekly searches of Scielo
3. Weekly searches of Scopus

The search strategies contained the following keywords and were combined in different ways using the boolean connectors AND OR: snake venoms, colubrid, Colubridae, Dipsadidae, *Xenodon, Leptophis, Erythrolamprus, Oxybelis, Helicops, Thamnodynastes, Leptodeira, Philodryas, Pseudoboa, Tantilla, Spilotes, Rhinobothryum, Tretanorhinus, Oxyrhopus, Lygophis*, transcriptome, proteome, evolution, epidemiology, pharmacology, and drug development.

To compare the relationship between different venom toxin classes in colubrids, we recovered expression data of relevant toxins from published works with colubrid species (both proteomic and transcriptomic) [29,42,52,54,55,57,132]. We transformed the data into percentages to obtain comparable values for the proportions of each toxin class. Venoms were considered highly neurotoxic or proteolytic if >30% of the identified toxins were linked to those activities. We performed an ancestral state reconstruction analysis using the default parameters of the *fastAnc* function implemented in the Phytools package [138] on the R software using the phylogeny proposed by Zaher et al. [9] to estimate branch lengths using *Micrurus corallinus* and *Bothrops jararaca* as ourgroups. Then we built a tree using the *contMap* function in Phytools to represent the continuous variation in toxin proportions for each node using a color gradient. Compositional data for *Micrurus* and *Bothrops* were obtained from previous research [80,81]. As these data were obtained from various investigations that implemented different methodologies, some lacking proteomic confirmation of reported toxins, our comparisons are merely qualitative and should be interpreted with prudence. A more thoroughly built dataset is needed to draw a robust view of venom variation across colubrids.

Epidemiological data were obtained from manual inspection of National Public Health Surveillance System (Sistema Nacional de Vigilancia en Salud Pública: SIVIGILA, Spanish acronym) reports. Annual cases, clinical manifestations, species identification, and the use of antivenom were checked for all cases. A species was considered to be identified when the report included its full scientific name or a sufficiently specific common name that allowed for it to be classified at the family level. Annual cases were plotted using the PlotR packge developed by Guisande-Gonzalez [139], and linear regression models were performed following the procedures described by Guisande-Gonzalez et al. [140].

References

1. Mackessy, S.P. *Handbook of Venoms and Toxins of Reptiles.*; 1st ed.; CRC Press: Boca Raton, **2021**.
2. Junqueira-De-Azevedo, I.L.M.; Bastos, C.M.V.; Ho, P.L.; Luna, M.S.; Yamanouye, N.; Casewell, N.R. Venom-Related Transcripts from *Bothrops jararaca* Tissues Provide Novel Molecular Insights into the Production and Evolution of Snake Venom. *Mol. Biol. Evol.* **2015**, *32*, 754–766, doi:10.1093/molbev/msu337.
3. Lomonte, B.; Rey-Suárez, P.; Fernández, J.; Sasa, M.; Pla, D.; Vargas, N.; Bénard-Valle, M.; Sanz, L.; Corrêa-Netto, C.; Núñez, V.; et al. Venoms of Micrurus Coral Snakes: Evolutionary Trends in Compositional Patterns Emerging from Proteomic Analyses. *Toxicon* **2016**, *122*, 7–25.
4. Bucaretchi, F.; De Capitani, E.M.; Vieira, R.J.; Rodrigues, C.K.; Zannin, M.; Da Silva, N.J.; Casais-e-Silva, L.L.; Hyslop, S. Coral Snake Bites (*Micrurus* spp.) in Brazil: A Review of Literature Reports. *Clin. Toxicol.* **2016**, *54*, 222–234.
5. Oliveira, F.N.; Brito, M.T.; de Morais, I.C.O.; Fook, S.M.L.; de Albuquerque, H.N. Accidents Caused by *Bothrops* and *Bothropoides* in the State of Paraiba: Epidemiological and Clinical Aspects. *Rev. Soc. Bras. Med. Trop.* **2010**, *43*, 662–667, doi:10.1590/S0037-86822010000600012.
6. Kularatne, S.A.M.; Budagoda, B.D.S.S.; Gawarammana, I.B.; Kularatne, W.K.S. Epidemiology, Clinical Profile and Management Issues of Cobra (*Naja naja*) Bites in Sri Lanka: First Authenticated Case Series. *Trans. R. Soc. Trop. Med. Hyg.* **2009**, *103*, 924–930, doi:10.1016/J.TRSTMH.2009.04.002.
7. Otero-Patiño, R. Epidemiological, Clinical and Therapeutic Aspects of *Bothrops asper* Bites. *Toxicon* **2009**, *54*, 998–1011, doi:10.1016/j.toxicon.2009.07.001.
8. Uetz, P.; Freed, P.; Hošek, J. The Reptile Database Available online: https://reptile-database.reptarium.cz/ (accessed on 23 January 2024).
9. Zaher H, Murphy RW, Arredondo JC, Graboski R, Machado-Filho PR, Mahlow K, et al. Largescale molecular phylogeny, morphology, divergence-time estimation, and the fossil record of advanced caenophidian snakes (Squamata: Serpentes). *PLoS ONE (2019) 14*(5): e0216148. https://doi.org/10.1371/journal.pone.0216148 PMID: 31075128
10. Peñuela-Gómez, S.M. El Abandono En La Investigación Toxinológica de Los Colúbridos Opistoglifos. *Mem. Conf. Interna Med. Aprovech. Fauna Silv. Exót. Conv.* **2010**, *0*, 24–26.
11. Junqueira-de-Azevedo, I.L.M.; Campos, P.F.; Ching, A.T.C.; Mackessy, S.P. Colubrid Venom Composition: An -Omics Perspective. *Toxins (Basel).* **2016**, *8*, 1–24, doi:10.3390/toxins8080230.
12. Campbell, J.A.; Lamar, W.W.; Brodie, E.D.; others *The Venomous Reptiles of the Western Hemisphere*; Comstock Pub. Associates Ithaca [NY], **2004**; Vol. 1.
13. Kardong, K. V The Evolution of the Venom Apparatus in Snakes From Colubrids To Viperids & Elapids. *Mem. Inst. Butantan* **1982**, *4*, 106–118.
14. Kardong, K. V Colubrid Snakes and Duvernoy's "Venom" Glands. *J. Toxicol. - Toxin Rev.* **2002**, *21*, 1–19, doi:10.1081/TXR-120004739.
15. Jackson, T.N.W.; Young, B.; Underwood, G.; McCarthy, C.J.; Kochva, E.; Vidal, N.; van der Weerd, L.; Nabuurs, R.; Dobson, J.; Whitehead, D.; et al. Endless Forms Most Beautiful: The Evolution of Ophidian

Oral Glands, Including the Venom System, and the Use of Appropriate Terminology for Homologous Structures. *Zoomorphology* **2016**, *136*, 107–130, doi:10.1007/S00435-016-0332-9.
16. de Oliveira, L.; Jared, C.; da Costa Prudente, A.L.; Zaher, H.; Antoniazzi, M.M. Oral Glands in Dipsadine "Goo-Eater" Snakes: Morphology and Histochemistry of the Infralabial Glands in *Atractus reticulatus, Dipsas indica*, and *Sibynomorphus mikanii*. *Toxicon* **2008**, *51*, 898–913, doi:10.1016/J.TOXICON.2007.12.021.
17. Weinstein, S.A.; Warrell, D.A.; White, J.; Keyler, D.E. "Venomous? Bites from Non-Venomous Snakes: A Critical Analysis of Risk and Management of "Colubrid? Snake Bites (Livre Numérique Google). **2011**, 364.
18. Gaiarsa, M.P.; de Alencar, L.R. V; Martins, M. Natural History of Pseudoboine Snakes. *Pap. Avulsos Zool.* **2013**, *53*, 261–283, doi:10.1590/S0031-10492013001900001.
19. Giraudo, A.R.; Arzamendia, V.; Bellini, G.P.; Bessa, C.A.; Costanzo, M.B. Ecología de Una Gran Serpiente Sudamericana, *Hydrodynastes gigas* (Serpentes: Dipsadidae). *Rev. Mex. Biodivers.* **2014**, *85*, 1206–1216, doi:10.7550/rmb.43765.
20. Todd, B.D.; Willson, J.D.; Winne, C.T.; Semlitsch, R.D.; Gibbons, J.W. Ecology of the Southeastern Crowned Snake, *Tantilla coronata*. *Copeia* **2008** (2), 388–394, (*2008)*. doi:10.1643/CE-06-289.
21. Marques, O.A. V; Muniz-Da-Silva, D.F.; Barbo, F.E.; Cardoso, S.R.T.; Maia, D.C.; Almeida-Santos, S.M. Ecology of the Colubrid Snake *Spilotes pullatus* from the Atlantic Forest of Southeastern Brazil. *Herpetologica* **2014**, *70*, 407–416, doi:10.1655/HERPETOLOGICA-D-14-00012.
22. Corrêa, D.N. Ecologia Alimentar Das Serpentes Semi-Aquáticas *Erythrolamprus jaegeri jaegeri* (Günter, 1858) e *Erythrolamprus poecilogyrus sublineatus* (Cope, 1860) (Serpentes , Dipsadidae) Na Região Costeira Do Extremo Sul Do Brasil. An. Acad. Bras. Ciênc. **2016**, 88, (1), 293–308, https://doi.org/10.1590/0001-3765201520140570
23. Barbo, F.E.; Marques, O.A. V.; Sawaya, R.J. Diversity, Natural History, and Distribution of Snakes in the Municipality of São Paulo. *South Am. J. Herpetol.* **2011**, *6*, 135–160, doi:10.2994/057.006.0301.
24. Alencar, L.R. V; Gaiarsa, M.P.; Martins, M. The Evolution of Diet and Microhabitat Use in Pseudoboine Snakes. *S Am J Herpetol* **2013**, *8*, 60–66, doi:10.2994/SAJH-D-13-00005.1.
25. de Aguiar, L.F.S.; Di-Bernardo, M. Diet and Feeding Behavior of Helicops Infrataeniatus (Serpentes: Colubridae: Xenodontinae) in Southern Brazil. *Stud. Neotrop. Fauna Environ* **2010**, *39*, 7–14, doi:10.1080/01650520412331270927.
26. Serna-Botero, V.; Marín-Martínez, M.; Velásquez-Guarín, D.; Ramírez-Chaves, H.E. Boyaca Spiny Rat, *Proechimys chrysaeolus* (Mammalia: Echimyidae), a New Prey Item of the Banded Calico Snake, *Oxyrhopus petolarius* (Reptilia: Dipsadidae). *Herpetol. Notes* **2019**, *12*, 651–653.
27. Henderson, R.W. Trophic Relationships and Foraging Strategies of Some New World Tree Snakes (*Leptophis*, *Oxybelis*, *Uromacer*). *Amphibia-Reptilia* **1982**, *3*, 71–80, doi:10.1163/156853882X00185.
28. Modahl, C.M.; Mackessy, S.P. Venoms of Rear-Fanged Snakes: New Proteins and Novel Activities. *Front. Ecol. Evol.* **2019**, *7*, 1–18, doi:10.3389/fevo.2019.00279.

29. Modahl, C.M.; Mrinalini; Frietze, S.; Mackessy, S.P. Adaptive Evolution of Distinct Prey-Specific Toxin Genes in Rear-Fanged Snake Venom. *Proc. R. Soc. B Biol. Sci.* **2018**, *285*, doi:10.1098/rspb.2018.1003.
30. Sánchez, M.N.; Teibler, G.P.; López, C.A.; Mackessy, S.P.; Peichoto, M.E. Assessment of the Potential Toxicological Hazard of the Green Parrot Snake (*Leptophis ahaetulla marginatus*): Characterization of Its Venom and Venom-Delivery System. *Toxicon* **2018**, *148*, 202–212, doi:10.1016/j.toxicon.2018.04.027.
31. Sánchez, M.N.; Teibler, G.P.; Sciani, J.M.; Casafús, M.G.; Maruñak, S.L.; Mackessy, S.P.; Peichoto, M.E. Unveiling Toxicological Aspects of Venom from the Aesculapian False Coral Snake *Erythrolamprus aesculapii*. *Toxicon* **2019**, *164*, 71–81, doi:10.1016/j.toxicon.2019.04.007.
32. Torres-Bonilla, K.A.; Andrade-Silva, D.; Serrano, S.M.T.; Hyslop, S. Biochemical Characterization of Venom from *Pseudoboa neuwiedii* (Neuwied's False Boa; Xenodontinae; Pseudoboini). *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2018**, *213*, 27–38, doi:10.1016/J.CBPC.2018.06.003.
33. Torres-Bonilla, K.A.; Schezaro-Ramos, R.; Floriano, R.S.; Rodrigues-Simioni, L.; Bernal-Bautista, M.H.; Alice da Cruz-Höfling, M. Biological Activities of *Leptodeira annulata* (Banded Cat-Eyed Snake) Venom on Vertebrate Neuromuscular Preparations. *Toxicon* **2016**, *119*, 345–351, doi:10.1016/j.toxicon.2016.07.004.
34. Torres-Bonilla, K.A.; Panunto, P.C.; Pereira, B.B.; Zambrano, D.F.; Herrán-Medina, J.; Bernal, M.H.; Hyslop, S. Toxinological Characterization of Venom from *Leptodeira annulata* (Banded Cat-Eyed Snake; Dipsadidae, Imantodini). *Biochimie* **2020**, *174*, 171–188, doi:10.1016/J.BIOCHI.2020.04.006.
35. Salomão, M.D.G.; Albolea, A.B.P.; Almeida Santos, S.M. Colubrid Snakebite: A Public Health Problem in Brazil. *Herpetol. Rev.* **2003**, *34*, 307–312.
36. Angarita-Sierra, T.; Montañez-Méndez, A.; Toro-Sánchez, T.; Rodríguez-Vargas, A.; Angarita-Sierra, T.; Montañez-Méndez, A.; Toro-Sánchez, T.; Rodríguez-Vargas, A. A Case of Envenomation by the False Fer-de-Lance Snake *Leptodeira annulata* (Linnaeus, 1758) in the Department of La Guajira, Colombia. *Biomedica* **2020**, *40*, 20–26, doi:10.7705/biomedica.4773.
37. Hill, R.E.; Mackessy, S.P. Characterization of Venom (Duvernoy's Secretion) from Twelve Species of Colubrid Snakes and Partial Sequence of Four Venom Proteins. *Toxicon* **2000**, *38*, 1663–1687, doi:10.1016/S0041-0101(00)00091-X.
38. Hill, R.E.; Mackessy, S.P. Venom Yields from Several Species of Colubrid Snakes and Differential Effects of Ketamine. *Toxicon* **1997**, *35*, 671–678, doi:10.1016/S0041-0101(96)00174-2.
39. Mackessy, S.P. Biochemistry And Pharmacology Of Colubrid Snake Venoms. *J. Toxicol. Toxin Rev*, **2002**, *21*, 43–83, doi:10.1081/TXR-120004741.
40. Modahl, C.M.; Saviola, A.J.; Mackessy, S.P. Venom Genomics and Proteomics. *Venom Genomics and Proteomics* **2016**, 51–79, doi:10.1007/978-94-007-6416-3.
41. Peichoto, M.E.; Tavares, F.L.; Santoro, M.L.; MacKessy, S.P. Venom Proteomes of South and North American Opisthoglyphous (Colubridae

and Dipsadidae) Snake Species: A Preliminary Approach to Understanding Their Biological Roles. *Comp. Biochem. Physiol. - Part D Genomics Proteomics* **2012**, *7*, 361–369, doi:10.1016/j.cbd.2012.08.001.

42. Bayona-Serrano, J.D.; Viala, V.L.; Rautsaw, R.M.; Schramer, T.D.; Barros-Carvalho, G.A.; Nishiyama, M.Y.; Freitas-de-Sousa, L.A.; Mourada-Silva, A.M.; Parkinson, C.L.; Grazziotin, F.G.; et al. Replacement and Parallel Simplification of Nonhomologous Proteinases Maintain Venom Phenotypes in Rear-Fanged Snakes. *Mol. Biol. Evol.* **2020**, *37*, 3563–3575, doi:10.1093/MOLBEV/MSAA192.
43. Medeiros, C.R. de; Souza, S.N. de; Silva, M.C. da; Ventura, J. de S.; Piorelli, R. de O.; Puorto, G. Bites by Tomodon Dorsatus (Serpentes, Dipsadidae): Clinical and Epidemiological Study of 86 Cases. *Toxicon* **2019**, *162*, 40–45, doi:10.1016/j.toxicon.2019.03.005.
44. Diaz, F.; Navarrete, L.F.; Pefaur, J.; Rodriguez-Acosta, A. Envenomation by Neotropical Opistoglyphous Colubrid *Thamnodynastes cf. pallidus* Linné, 1758 (Serpentes:Colubridae) in Venezuela. *Rev. Inst. Med. Trop. Sao Paulo* **2004**, *46*, 287–290, doi:10.1590/S0036-46652004000500011.
45. Prado-Franceschi, J.; Hyslop, S. South American Colubrid Envenomations. *J. Toxicol. - Toxin Rev.* **2002**, *21*, 117–158.
46. Rebelato, M.M.; Ferri, V.Y.K.; Dalmolin, D.A.; Tozetti, A.M.; Verrastro, L. Envenomation by Opisthoglyphous Snake *Thamnodynastes hypoconia* (Cope, 1860) (Dipsadinae: Tachymenini) in Southern Brazil. *Toxicon* **2021**, *189*, 1–6, doi:10.1016/J.TOXICON.2020.10.022.
47. Gutiérrez, J.M.; Sasa, M. Bites and Envenomations by Colubrid Snakes in Mexico and Central America. *J. Toxicol. - Toxin Rev.* **2002**, *21*, 105–115, doi:10.1081/TXR-120004743.
48. Menegucci, R.C.; Bernarde, P.S.; Monteiro, W.M.; Bisneto, P.F.; Martins, M. Envenomation by an Opisthoglyphous Snake, *Erythrolamprus aesculapii* (Dipsadidae), in Southeastern Brazil. *Rev. Soc. Bras. Med. Trop.* **2019**, *52*, 0–2, doi:10.1590/0037-8682-0055-2019.
49. de Medeiros, C.R.; Hess, P.L.; Nicoleti, A.F.; Sueiro, L.R.; Duarte, M.R.; de Almeida-Santos, S.M.; França, F.O.S. Bites by the Colubrid Snake *Philodryas patagoniensis*: A Clinical and Epidemiological Study of 297 Cases. *Toxicon* **2010**, *56*, 1018–1024, doi:10.1016/j.toxicon.2010.07.006.
50. Oliveira, J.S.; Sant'Anna, L.B.; Oliveira Junior, M.C.; Souza, P.R.M.; Andrade Souza, A.S.; Ribeiro, W.; Vieira, R.P.; Hyslop, S.; Cogo, J.C. Local and Hematological Alterations Induced by *Philodryas olfersii* Snake Venom in Mice. *Toxicon* **2017**, *132*, 9–17, doi:10.1016/j.toxicon.2017.03.013.
51. Silva, K.V.; Said, R. do C.; Assy, J.G.P.L.; Duarte, M.R.; Torrez, P.P.Q.; França, F.O. de S. A Case of Envenomation Caused by *Oxybelis fulgidus* (Serpentes, Colubridae) in Brazilian Amazon. *Rev. Soc. Bras. Med. Trop.* **2019**, *52*, doi:10.1590/0037-8682-0426-2018.
52. Hofmann, E.P.; Rautsaw, R.M.; Mason, A.J.; Strickland, J.L.; Parkinson, C.L. Duvernoy's Gland Transcriptomics of the Plains Black-Headed Snake, *Tantilla nigriceps* (Squamata, Colubridae): Unearthing the Venom of Small Rear-Fanged Snakes. *Toxins 2021, Vol. 13, Page 336* **2021**, *13*, 336, doi:10.3390/TOXINS13050336.
53. Ching, A.T.C.; Paes Leme, A.F.; Zelanis, A.; Rocha, M.M.T.; Furtado, M.D.F.D.; Silva, D.A.; Trugilho, M.R.O.; Da Rocha, S.L.G.; Perales, J.; Ho,

P.L.; et al. Venomics Profiling of *Thamnodynastes strigatus* Unveils Matrix Metalloproteinases and Other Novel Proteins Recruited to the Toxin Arsenal of Rear-Fanged Snakes. *J. Proteome Res.* **2012**, *11*, 1152–1162, doi:10.1021/pr200876c.

54. Ching, A.T.C.; Rocha, M.M.T.; Paes Leme, A.F.; Pimenta, D.C.; de Fátima D. Furtado, M.; Serrano, S.M.T.; Ho, P.L.; Junqueira-de-Azevedo, I.L.M. Some Aspects of the Venom Proteome of the Colubridae Snake *Philodryas olfersii* Revealed from a Duvernoy's (Venom) Gland Transcriptome. *FEBS Lett.* **2006**, *580*, 4417–4422, doi:10.1016/J.FEBSLET.2006.07.010.
55. Calvete, J.J.; Bonilla, F.; Granados-Martínez, S.; Sanz, L.; Lomonte, B.; Sasa, M. Venomics of the Duvernoy's Gland Secretion of the False Coral Snake *Rhinobothryum bovallii* (Andersson, 1916) and Assessment of Venom Lethality towards Synapsid and Diapsid Animal Models. *J. Proteomics* **2020**, *225*, 103882, doi:10.1016/J.JPROT.2020.103882.
56. Torres-Bonilla, K.A.; Floriano, R.S.; Schezaro-Ramos, R.; Rodrigues-Simioni, L.; da Cruz-Höfling, M.A. A Survey on Some Biochemical and Pharmacological Activities of Venom from Two Colombian Colubrid Snakes, *Erythrolamprus bizona* (Double-Banded Coral Snake Mimic) and Pseudoboa Neuwiedii (Neuwied's False Boa). *Toxicon* **2017**, *131*, 29–36, doi:10.1016/J.TOXICON.2017.02.030.
57. Domínguez-Pérez, D.; Durban, J.; Agüero-Chapin, G.; López, J.T.; Molina-Ruiz, R.; Almeida, D.; Calvete, J.J.; Vasconcelos, V.; Antunes, A. The Harderian Gland Transcriptomes of Caraiba Andreae, Cubophis Cantherigerus and Tretanorhinus Variabilis, Three Colubroid Snakes from Cuba. *Genomics* **2019**, *111*, 1720–1727, doi:10.1016/J.YGENO.2018.11.026.
58. Modahl, C.M.; Frietze, S.; Mackessy, S.P. Transcriptome-Facilitated Proteomic Characterization of Rear-Fanged Snake Venoms Reveal Abundant Metalloproteinases with Enhanced Activity. *J. Proteomics* **2018**, *187*, 223–234, doi:10.1016/j.jprot.2018.08.004.
59. Pla, D.; Petras, D.; Saviola, A.J.; Modahl, C.M.; Sanz, L.; Pérez, A.; Juárez, E.; Frietze, S.; Dorrestein, P.C.; Mackessy, S.P.; et al. Transcriptomics-Guided Bottom-up and Top-down Venomics of Neonate and Adult Specimens of the Arboreal Rear-Fanged Brown Treesnake, *Boiga irregularis*, from Guam. *J. Proteomics* **2018**, *174*, 71–84, doi:10.1016/J.JPROT.2017.12.020.
60. Heyborne, W.H.; Mackessy, S.P. Venoms of New World Vinesnakes (*Oxybelis aeneus* and *O. fulgidus*). *Toxicon* **2021**, *190*, 22–30, doi:10.1016/J.TOXICON.2020.12.002.
61. Pla, D.; Sanz, L.; Whiteley, G.; Wagstaff, S.C.; Harrison, R.A.; Casewell, N.R.; Calvete, J.J. What Killed Karl Patterson Schmidt? Combined Venom Gland Transcriptomic, Venomic and Antivenomic Analysis of the South African Green Tree Snake (the Boomslang), *Dispholidus typus*. *Biochim. Biophys. Acta - Gen. Subj.* **2017**, *1861*, 814–823, doi:10.1016/J.BBAGEN.2017.01.020.
62. Kini, R.M.; Doley, R. Structure, Function and Evolution of Three-Finger Toxins: Mini Proteins with Multiple Targets. *Toxicon* **2010**, *56*, 855–867.
63. Modahl, C.M.; Mrinalini; Frietze, S.; Mackessy, S.P. Adaptive Evolution of Distinct Prey-Specific Toxin Genes in Rear-Fanged Snake Venom. *Proc. R. Soc. B* **2018**, *285*, doi:10.1098/RSPB.2018.1003.

64. Yamazaki, Y.; Morita, T. Structure and Function of Snake Venom Cysteine-Rich Secretory Proteins. *Toxicon* **2004**, *44*, 227–231, doi:10.1016/J.TOXICON.2004.05.023.
65. Tadokoro, T.; Modahl, C.M.; Maenaka, K.; Aoki-Shioi, N. Cysteine-Rich Secretory Proteins (CRISPs) from Venomous Snakes: An Overview of the Functional Diversity in a Large and Underappreciated Superfamily. *Toxins* **2020**, *12*, 175, doi:10.3390/TOXINS12030175.
66. Lu, Q.; Navdaev, A.; Clemetson, J.M.; Clemetson, K.J. Snake Venom C-Type Lectins Interacting with Platelet Receptors. Structure–Function Relationships and Effects on Haemostasis. *Toxicon* **2005**, *45*, 1089–1098, doi:10.1016/J.TOXICON.2005.02.022.
67. Rehorek, S.J. Squamate Harderian Gland: An Overview. *Anat. Rec.* **1997**, *248*, 301–306, doi:10.1002/(SICI)1097-0185(199707)248:3<301::AID-AR1>3.0.CO;2-S.
68. McDowell, S.B. Toxicocalamus, a New Guinea Genus of Snakes of the Family Elapidae. *J. Zool.* **1969**, *159*, 443–511, doi:10.1111/J.1469-7998.1969.TB03900.X.
69. Da Rocha, M.M.T.; Furtado, M.D.F.D. Analysis of Biological Activities from Philodryas Olfersii (Lichtenstein) and *P. patagoniensis* (Girard) Venoms (Serpents, Colubridae). *Rev. Bras. Zool.* **2007**, *24*, 410–418, doi:10.5380/rbz.v24i2.8468.
70. Pérez, O.A. De; Vila, L.L. De; Peichoto, M.E.; Maruñak, S.; Ruiz, R.; Teibler, P.; Gay, C.; Rey, L. Edematogenic and Myotoxic Activities of the Duvernoy ' s Gland Secretion of *Philodryas olfersii* from the North-East Region Of. *World Health* **2003**, *27*, 363–370.
71. Lemoine, K.; Salgueiro, L.M.; Rodríguez-Acosta, A.; Acosta, J.A. Neurotoxic, Hemorrhagic and Proteolytic Activities of Duvernoy's Gland Secretion from Venezuelan Opisthoglyphous Colubrid Snakes in Mice. *Vet. Hum. Toxicol.* **2004**, *46*, 10–14.
72. Estrella, A.; Sánchez, E.E.; Galán, J.A.; Tao, W.A.; Guerrero, B.; Navarrete, L.F.; Rodríguez-Acosta, A. Characterization of Toxins from the Broad-Banded Water Snake *Helicops angulatus* (Linnaeus, 1758): Isolation of a Cysteine-Rich Secretory Protein, Helicopsin. *Arch. Toxicol.* **2011**, *85*, 305–313, doi:10.1007/s00204-010-0597-6.
73. Cerda, P. A., Crowe-Riddell, J. M., Gonçalves, D. J., Larson, D. A., Duda Jr, T. F., & Davis Rabosky, A. R. . Divergent specialization of simple venom gene profiles among rear-fanged snake genera (*Helicops* and *Leptodeira*, Dipsadinae, Colubridae). **2022** *Toxins*, 14, 7, 489, doi: 10.3390/toxins14070489
74. Six, D.A.; Dennis, E.A. The Expanding Superfamily of Phospholipase A2 Enzymes: Classification and Characterization. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **2000**, *1488*, 1–19, doi:10.1016/S1388-1981(00)00105-0.
75. Campos, P.F.; Andrade-Silva, D.; Zelanis, A.; Leme, A.F.P.; Rocha, M.M.T.; Menezes, M.C.; Serrano, S.M.T.; Junqueira-De-Azevedo, I.D.L.M. Trends in the Evolution of Snake Toxins Underscored by an Integrative Omics Approach to Profile the Venom of the Colubrid *Phalotris mertensi*. *Genome Biol. Evol.* **2016**, *8*, 2266–2287, doi:10.1093/gbe/evw149.
76. Brust, A.; Sunagar, K.; Undheim, E.A.B.; Vetter, I.; Yang, D.C.; Casewell, N.R.; Jackson, T.N.W.; Koludarov, I.; Alewood, P.F.; Hodgson, W.C.; et

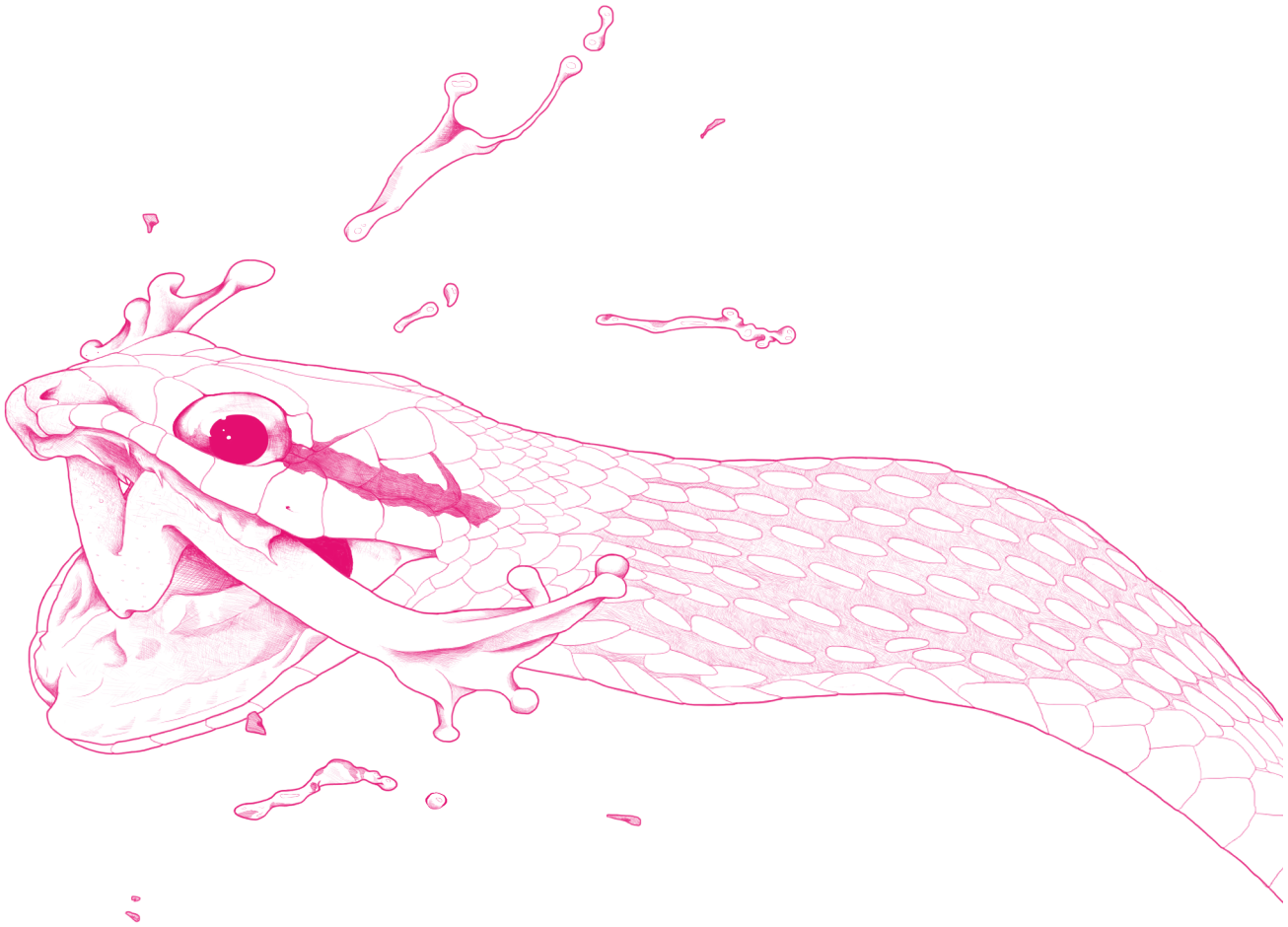
al. Differential Evolution and Neofunctionalization of Snake Venom Metalloprotease Domains. *Mol. Cell. Proteomics* **2013**, *12*, 651–663, doi:10.1074/mcp.M112.023135.
77. Casewell, N.R.; Wagstaff, S.C.; Harrison, R.A.; Renjifo, C.; Wüster, W. Domain Loss Facilitates Accelerated Evolution and Neofunctionalization of Duplicate Snake Venom Metalloproteinase Toxin Genes. *Mol. Biol. Evol.* **2011**, *28*, 2637–2649, doi:10.1093/molbev/msr091.
78. Sanz, L.; Calvete, J.J. Insights into the Evolution of a Snake Venom Multi-Gene Family from the Genomic Organization of *Echis ocellatus* SVMP Genes. *Toxins (Basel).* **2016**, *8*, doi:10.3390/toxins8070216.
79. Sanz, L.; Harrison, R.A.; Calvete, J.J. First Draft of the Genomic Organization of a PIII-SVMP Gene. *Toxicon* **2012**, *60*, 455–469, doi:10.1016/j.toxicon.2012.04.331.
80. Cidade, D.A.P.; Simão, T.A.; Dávila, A.M.R.; Wagner, G.; de L.M. Junqueira-de-Azevedo, I.; Lee Ho, P.; Bon, C.; Zingali, R.B.; Albano, R.M. *Bothrops jararaca* Venom Gland Transcriptome: Analysis of the Gene Expression Pattern. *Toxicon* **2006**, *48*, 437–461, doi:10.1016/J.TOXICON.2006.07.008.
81. Corrêa-Netto, C.; Junqueira-de-Azevedo, I. de L.M.; Silva, D.A.; Ho, P.L.; Leitão-de-Araújo, M.; Alves, M.L.M.; Sanz, L.; Foguel, D.; Zingali, R.B.; Calvete, J.J. Snake Venomics and Venom Gland Transcriptomic Analysis of Brazilian Coral Snakes, *Micrurus altirostris* and *M. corallinus*. *J. Proteomics* **2011**, *74*, 1795–1809, doi:10.1016/J.JPROT.2011.04.003.
82. Tasoulis, T.; Lee, M.S.Y.; Ziajko, M.; Dunstan, N.; Sumner, J.; Isbister, G.K. Activity of Two Key Toxin Groups in Australian Elapid Venoms Show a Strong Correlation to Phylogeny but Not to Diet. *BMC Evol. Biol.* **2020**, *20*, 1–13, doi:10.1186/S12862-020-1578-X/FIGURES/7.
83. True, J.R.; Carroll, S.B. Gene Co-Option in Physiological and Morphological Evolution. *Annu. Rev. Cell Dev. Biol.* **2002**, *18*, 53–80, doi:10.1146/annurev.cellbio.18.020402.140619.
84. Weinstein, S.A.; White, J.; Keyler, D.E.; Warrell, D.A. Non-Front-Fanged Colubroid Snakes: A Current Evidence-Based Analysis of Medical Significance. *Toxicon* **2013**, *69*, 103–113, doi:10.1016/J.TOXICON.2013.02.003.
85. Ribeiro, L.A.; Puorto, G.; Jorge, M.T. Bites by the Colubrid Snake *Philodryas olfersii*: A Clinical and Epidemiological Study of 43 Cases. *Toxicon* **1999**, *37*, 943–948, doi:10.1016/S0041-0101(98)00191-3.
86. Sevilla-Sánchez, M.J.; Ayerbe-González, S.; Bolaños-Bolaños, E. Aspectos Biomédicos y Epidemiológicos Del Accidente Ofídico En El Departamento Del Cauca, Colombia, 2009-2018. *Biomédica* **2021**, *41*, 314–337, doi:10.7705/BIOMEDICA.5853.
87. De Silva, H.A.; Ryan, N.M.; De Silva, H.J. Adverse Reactions to Snake Antivenom, and Their Prevention and Treatment. *Br. J. Clin. Pharmacol.* **2016**, *81*, 446–452, doi:10.1111/BCP.12739.
88. Koh, C.Y.; Kini, R.M. From Snake Venom Toxins to Therapeutics - Cardiovascular Examples. *Toxicon* **2012**, *59*, 497–506, doi:10.1016/j.toxicon.2011.03.017.
89. Pal, S.K.; Gomes, A.A.; Dasgupta, S.C.; Gomes, A.A. Snake Venom as Therapeutic Agents: From Toxin to Drug Development. *IJEB Vol.40(12) [December 2002]* **2002**.

90. Fox, J.; Serrano, S. Approaching the Golden Age of Natural Product Pharmaceuticals from Venom Libraries: An Overview of Toxins and Toxin-Derivatives Currently Involved in Therapeutic or Diagnostic Applications. *Curr. Pharm. Des.* **2007**, *13*, 2927–2934, doi:10.2174/138161207782023739.
91. Barua, A.; Mikheyev, A.S. Many Options, Few Solutions: Over 60 My Snakes Converged on a Few Optimal Venom Formulations. *Mol. Biol. Evol.* **2019**, *36*, 1964–1974, doi:10.1093/molbev/msz125.
92. J., A.; Ohno, S. Evolution by Gene Duplication. *Popul. (French Ed.* **1971**, *26*, 1176, doi:10.2307/1530208.
93. Vonk, F.J.; Casewell, N.R.; Henkel, C. V.; Heimberg, A.M.; Jansen, H.J.; McCleary, R.J.R.; Kerkkamp, H.M.E.; Vos, R.A.; Guerreiro, I.; Calvete, J.J.; et al. The King Cobra Genome Reveals Dynamic Gene Evolution and Adaptation in the Snake Venom System. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 20651–20656, doi:10.1073/pnas.1314702110.
94. Bazaa, A.; Juárez, P.; Marrakchi, N.; Lasfer, Z.B.; Ayeb, M. El; Harrison, R.A.; Calvete, J.J.; Sanz, L. Loss of Introns along the Evolutionary Diversification Pathway of Snake Venom Disintegrins Evidenced by Sequence Analysis of Genomic DNA from Macrovipera Lebetina Transmediterranea and *Echis ocellatus*. *J. Mol. Evol.* **2007**, *64*, 261–271, doi:10.1007/s00239-006-0161-4.
95. Stenseth, N.C. Where Have All the Species Gone? On the Nature of Extinction and the Red Queen Hypothesis. *Oikos* **1979**, *33*, 196, doi:10.2307/3543998.
96. Kerfoot, W.C.; Weider, L.J. Experimental Paleoecology (Resurrection Ecology): Chasing Van Valen's Red Queen Hypothesis. *Limnol. Oceanogr.* **2004**, *49*, 1300–1316, doi:10.4319/lo.2004.49.4_part_2.1300.
97. Holding, M.L.; Drabeck, D.H.; Jansa, S.A.; Gibbs, H.L. Venom Resistance as a Model for Understanding the Molecular Basis of Complex Coevolutionary Adaptations. *Integr. Comp. Biol*, **2016**, *56*, 1032–1043, doi:10.1093/icb/icw082
98. Voss, R.S.; Jansa, S.A. Snake-Venom Resistance as a Mammalian Trophic Adaptation: Lessons from Didelphid Marsupials. *Biol. Rev.* **2012**, *87*, 822–837, doi:10.1111/j.1469-185X.2012.00222.x.
99. Khan, M.A.; Dashevsky, D.; Kerkkamp, H.; Kordiš, D.; de Bakker, M.A.G.; Wouters, R.; van Thiel, J.; op den Brouw, B.; Vonk, F.; Manjunatha Kini, R.; et al. Widespread Evolution of Molecular Resistance to Snake Venom α-Neurotoxins in Vertebrates. *Toxins* **2020**, *12*, 638, doi:10.3390/TOXINS12100638.
100. Gan, Z.R.; Gould, R.J.; Jacobs, J.W.; Friedman, P.A.; Polokoff, M.A. Echistatin. A Potent Platelet Aggregation Inhibitor from the Venom of the Viper, *Echis carinatus*. *J. Biol. Chem.* **1988**, *263*, 19827–19832, doi:10.1016/s0021-9258(19)77710-2.
101. Markland, F.S. Snake Venoms and the Hemostatic System. *Toxicon* **1998**, *36*, 1749–1800, doi:10.1016/S0041-0101(98)00126-3.
102. Scarborough, R.M.; Rose, J.W.; Naughton, M.A.; Phillips, D.R.; Nannizzi, L.; Arfsten, A.; Campbell, A.M.; Charo, I.F. Characterization of the Integrin Specificities of Disintegrins Isolated from American Pit Viper Venoms. *J. Biol. Chem.* **1993**, *268*, 1058–1065, doi:10.1016/s0021-9258(18)54041-2.

103. Saviola, A.J.; Peichoto, M.E.; Mackessy, S.P. Rear-Fanged Snake Venoms: An Untapped Source of Novel Compounds and Potential Drug Leads. *Toxin Rev.* **2014**, *33*, 185–201, doi:10.3109/15569543.2014.942040.
104. Badari, J.C.; Díaz-Roa, A.; Teixeira Rocha, M.M.; Mendonça, R.Z.; Silva Junior, P.I. da Patagonin-CRiSP: Antimicrobial Activity and Source of Antimicrobial Molecules in Duvernoy's Gland Secretion (Philodryas Patagoniensis Snake). *Front. Pharmacol.* **2021**, *11*, 2374, doi:10.3389/FPHAR.2020.586705/BIBTEX.
105. Peichoto, M.E.; Mackessy, S.P.; Teibler, P.; Tavares, F.L.; Burckhardt, P.L.; Breno, M.C.; Acosta, O.; Santoro, M.L. Purification and Characterization of a Cysteine-Rich Secretory Protein from *Philodryas patagoniensis* Snake Venom. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2009**, *150*, 79–84, doi:10.1016/J.CBPC.2009.03.002.
106. Angarita-Sierra, T.; Montañez-Méndez, A.; Toro-Sánchez, T.; Rodriguez-Vargas, A. A Case of Envenomation by the False Fer-de-Lance Snake *Leptodeira annulata* (Linnaeus, 1758) in the Guajira Department, Colombia. *Biomédica* **2020**, *40*. 20-26. doi: 10.7705/biomedica.4773.
107. Angarita-Corzo, K.; García-Peluffo, J.D.; Franco-Gutiérrez, M. Case Report of Human Envenomation by a False Coralsnake, *Erythrolamprus bizona* Jan, 1863. *Herpetol. Notes* **2023**, *16*, 627–631.
108. Barrio-Amorós, C.L. On the Taxonomy of Snakes in the Genus *Leptodeira*, with an Emphasis on Costa Rican Species. *Reptil. Amphib.* **2019**, *26*, 1–15, doi:10.17161/RANDA.V26I1.14321.
109. Gorzula, S. *Leptodeira annulata ashmedeadii* Envenomation. *Herpetol. Rev.* **1982**, *13*, 47.
110. Rodríguez-González, L.A.; Portillo-Portillo, P.A. Evaluación de La Actividad Neurotóxica de La Secreción Salival de *Erythrolamprus bizona* y *Leptophis ahaetulla* Serpientes Opistoglifas En Nervio Ciático de Bufo Marinus, Universidad del Quindio, **2006**.
111. Heyborne, W.H.; Mackessy, S.P. Identification and Characterization of a Taxon-Specific Three-Finger Toxin from the Venom of the Green Vinesnake (*Oxybelis fulgidus*; Family Colubridae). *Biochimie* **2013**, *95*, 1923–1932, doi:10.1016/J.BIOCHI.2013.06.025.
112. Crimmins, M.L. A Case of Oxybelis Poisoning in Man. *Copeia* **1937**, *1937*, 233, doi:10.2307/1436270.
113. Babo Martins, S.; Bolon, I.; Chappuis, F.; Ray, N.; Alcoba, G.; Ochoa, C.; Kumar Sharma, S.; Nkwescheu, A.S.; Wanda, F.; Durso, A.M.; et al. Snakebite and Its Impact in Rural Communities: The Need for a One Health Approach. *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007608, doi:10.1371/journal.pntd.0007608.
114. Lette-Pinto, R.N.; Da Silva, N.J.; Aird, S.D. Human envenomation by the South American opisthoglyph *Clelia clelia* plumbea (Wied). *Toxicon* **1991**, *29*, 1512–1516, doi:https://doi.org/10.1016/0041-0101(91)90008-f.
115. de Araújo, M.E.; dos Santos, A.C.M.C.A. Cases of Human Envenoming Caused by *Phylodryas olfersii* and *Philodryas patagoniensis* (Serpentes: Colubridae). *Rev. Soc. Bras. Med. Trop.* **1997**, *30*, 517–519, doi:10.1590/S0037-86821997000600013.
116. Fowler, I.R.; Salomão, M. da G. Activity Patterns in the Colubrid Snake Genus *Philodryas* and Their Relationship to Reproduction and Snakebite. *Bull. Chicago Herpetol. Soc.* **1994**, *29*, 229–232.

117. Prado-Franceschi, J.; Hyslop, S. South American colubrid envenomations. *Journal of Toxicology: Toxin Rev* **2002**, *21*, 117–158, doi:10.1081/TXR-120004744.
118. Salomao, M.A.; Pinto-Albolea, A.B.; Almeida-Santos, S.M. Colubrid Snakebite: A Public Health Problem in Brazil. *Herpetol. Rev.* **2003**, *34*, 307–312.
119. Mandelbaum, F.R.; Reichl, A.P.; Assakura, M.T.; Serrano, S.M.T. *Philodryas* Venom Metalloproteinases. *Handb. Proteolytic Enzym. Second Ed.* **2004**, *1*, 701–702, doi:10.1016/B978-0-12-079611-3.50210-X.
120. Peichoto, M.E.; Teibler, P.; Mackessy, S.P.; Leiva, L.; Acosta, O.; Gonçalves, L.R.C.; Tanaka-Azevedo, A.M.; Santoro, M.L. Purification and Characterization of Patagonfibrase, a Metalloproteinase Showing α-Fibrinogenolytic and Hemorrhagic Activities, from *Philodryas patagoniensis* Snake Venom. *Biochim. Biophys. Acta - Gen. Subj.* **2007**, *1770*, 810–819, doi:10.1016/J.BBAGEN.2006.12.014.
121. Acosta de Pérez, O.; Leiva de Vila, L.; Peichoto, M.; Maruñak, S.L.; Ruíz, R.; Teibler, P.; Gay, C.; Rey, L. Edematogenic and Myotoxic Activities of the Duvernoy ' s Gland Secretion of *Philodryas olfersi* from the North-East Region Of. *BIOCELL* **2003**, *27*, 363–370.
122. Assakura, M.T.; Reichl, A.P.; Mandelbaum, F.R. Isolation and Characterization of Five Fibrin(Ogen)Olytic Enzymes from the Venom of *Philodryas olfersii* (Green Snake). *Toxicon* **1994**, *32*, 819–831, doi:10.1016/0041-0101(94)90007-8.
123. Assakura, M.T.; Salomão, M. da G.; Puorto, G.; Mandelbaum, F.R. Hemorrhagic, Fibrinogenolytic and Edema-Forming Activities of the Venom of the Colubrid Snake *Philodryas olfersii* (Green Snake). *Toxicon* **1992**, *30*, 427–438, doi:10.1016/0041-0101(92)90539-H.
124. Prado-Franceschi, J.; Hyslop, S.; Cogo, J.C.; Andrade, A.L.; Assakura, M.; Cruz-Höfling, M.A.; Rodrigues-Simioni, L. The Effects of Duvernoy's Gland Secretion from the Xenodontine Colubrid *Philodryas olfersii* on Striated Muscle and the Neuromuscular Junction: Partial Characterization of a Neuromuscular Fraction. *Toxicon* **1996**, *34*, 459–466, doi:10.1016/0041-0101(95)00146-8.
125. Prado-Franceschi, J.; Hyslop, S.; Cogo, J.C.; Andrade, A.L.; Assakura, M.T.; Reichl, A.P.; Cruz-Höfling, M.A.; Rodrigues-Simioni, L. Characterization of a Myotoxin from the Duvernoy's Gland Secretion of the Xenodontine Colubrid *Philodryas olfersii* (Green Snake): Effects on Striated Muscle and the Neuromuscular Junction. *Toxicon* **1998**, *36*, 1407–1421, doi:10.1016/S0041-0101(98)00075-0.
126. Assakura, M.T.; Reichl, A.P.; Mandelbaum, F.R. Isolation and Characterization of Five Fibrin(Ogen)Olytic Enzymes from the Venom of *Philodryas olfersii* (Green Snake). *Toxicon* **1994**, *32*, 819–831, doi:10.1016/0041-0101(94)90007-8.
127. Peichoto, M.E.; Tavares, F.L.; Santoro, M.L.; MacKessy, S.P. Venom Proteomes of South and North American Opisthoglyphous (Colubridae and Dipsadidae) Snake Species: A Preliminary Approach to Understanding Their Biological Roles. *Comp. Biochem. Physiol. Part D Genomics Proteomics* **2012**, *7*, 361–369, doi:10.1016/J.CBD.2012.08.001.
128. Oliveira, J.S.; Sant'Anna, L.B.; Oliveira Junior, M.C.; Souza, P.R.M.; Andrade Souza, A.S.; Ribeiro, W.; Vieira, R.P.; Hyslop, S.; Cogo, J.C.

Local and Hematological Alterations Induced by *Philodryas olfersii* Snake Venom in Mice. *Toxicon* **2017**, *132*, 9–17, doi:10.1016/J.TOXICON.2017.03.013.
129. Torres-Bonilla, K.A.; Andrade-Silva, D.; Serrano, S.M.T.; Hyslop, S. Biochemical Characterization of Venom from *Pseudoboa neuwiedii* (Neuwied's False Boa; Xenodontinae; Pseudoboini). *Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol.* **2018**, *213*, 27–38, doi:10.1016/j.cbpc.2018.06.003.
130. Lemoine, K.; Girón, M.E.; Aguilar, I.; Navarrete, L.F.; Rodríguez-Acosta, A. Proteolytic, Hemorrhagic, and Neurotoxic Activities Caused by *Leptodeira annulata ashmeadii* (Serpentes: Colubridae) Duvernoy's Gland Secretion. *Wilderness Environ. Med.* **2004**, *15*, 82–89, doi:10.1580/1080-6032(2004)015[0082:PHANAC]2.0.CO;2.
131. Diaz, F.; Navarrete, L.F.; Pefaur, J.; Rodriguez-Acosta, A. Envenomation by Neotropical Opistoglyphous Colubrid *Thamnodynastes cf. pallidus* Linné, 1758 (Serpentes:Colubridae) in Venezuela. *Rev. Inst. Med. Trop. Sao Paulo* **2004**, *46*, 287–290, doi:10.1590/S0036-46652004000500011.
132. Ching, A.T.C.; Leme, A.F.P.; Zelanis, A.; Rocha, M.M.T.; Furtado, M. de F.D.; Silva, D.A.; Trugilho, M.R.O.; da Rocha, S.L.G.; Perales, J.; Ho, P.L.; et al. Venomics Profiling of *Thamnodynastes strigatus* Unveils Matrix Metalloproteinases and Other Novel Proteins Recruited to the Toxin Arsenal of Rear-Fanged Snakes. *J. Proteome Res.* **2012**, *11*, 1152–1162, doi:10.1021/PR200876C.
133. Walls Gordon L. (Gordon Lynn) *The Vertebrate Eye and Its Adaptive Radiation*; Bloomfield Hills, Mich.,Cranbrook Institute of Science, **1942**.
134. Bhaumik, S.; Jagadesh, S.; Lassi, Z. Quality of WHO Guidelines on Snakebite: The Neglect Continues. *BMJ Glob. Heal.* **2018**, *3*, doi:10.1136/bmjgh-2018-000783.
135. Organization, W.H. *Snakebite Envenoming: A Strategy for Prevention and Control: Executive Summary*; World Health Organization, **2019**.
136. Lynch, J.D. El Contexto de las Serpientes de Colombia Con Un Análisis De Las Amenazas En Contra De Su Conservación. *Rev. acad. colomb. cienc. exact. fis. nat.* , **2012**, 36 (140), 435-449
137. Lynch, J.D.; Angarita, T.; Ruiz, F.J. *Programa Nacional Para La Conservación de Las Serpientes Presentes En Colombia*; Ministerio de Ambiente y Desarrollo Sostenible, **2014**.
138. Revell, L.J. Phytools: An R Package for Phylogenetic Comparative Biology (and Other Things). *Methods Ecol. Evol.* **2012**, *3*, 217–223, doi:10.1111/j.2041-210X.2011.00169.x.
139. Guisande-González, C. Plots with R: Package ' PlotsR ' **2020**, 368.
140. Guisande-Gonzáles, C.; Vaamonde-Liste, B.; Barreiro-Felpeto, A. Rwizard **2014**, 1–452.



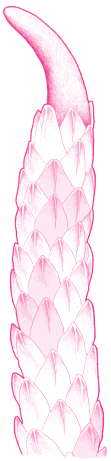
Chapter 5

Intricate mixtures: Diving inside the venoms

Ariadna Rodriguez-Vargas, Andres Pereañez, Camila Figueredo-Salinas, Juan Carlos Vega-Garzon, Adrian Marcelo Franco-Vasquez, Teddy Angarita-Sierra

Abstract: Since snake venoms have evolved to subdue or immobilize their prey and to favor their digestive processes and deter predators, snake venom toxins exhibit high specificities for precise functional and metabolic targets. For instance, metalloproteinases act mainly by altering endothelial junctions, phospholipases A_2 injure skeletal muscle tissues, and three-finger toxins affect transmission at neuromuscular synaptic junctions. Therefore, snakebite envenoming can be characterized according to the occurring hemocytotoxic, myotoxic, and neurotoxic pathophysiological processes. Currently, hypotheses about toxic action mechanisms and potencies of the individual components of the venom remain open, as do hypotheses about the main drivers in the composition variability of snake venoms. These are fundamental topics because they play a main role in the outcome of the snakebite envenomation. This chapter addresses a comprehensive review about the complex composition of snake venoms, detailing the biochemical and functional nature of the toxins while highlighting their activity during snakebite envenomation.

Keywords: Snake venoms, toxins, envenomation, functional activity, 3D models.



Citation: Rodriguez-Vargas A; Pereañez A; Figueredo-Salinas C; Vega-Garzon, JC; Franco-Vasquez, AM; Angarita-Sierra T. Chapter 5. Intricate mixtures: Diving inside the venoms. In Book: *Bites, venoms, and venomous snakes of Colombia*; Angarita-Sierra, T., Ruiz-Gomez, FJ, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 201–274. doi:10.33610/848004dipgzd

Copyright: © 2024 by the authors. Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0) license (http://creativecommons.org/licenses/by/4.0/).

Illustrations by:
Oscar A. Ramirez Ruiz

1. On snake venom basics

1.1 Venom delivery systems

Snakes are known for their remarkable traits, including their adaptations for overpowering their prey, such as constriction or envenomation. Particularly, venom delivery systems have received great attention due to their sophisticated specialization, evolutionary pathways, and ecological convergences [1–3]. The delivery mechanisms of toxins have been mainly studied in highly venomous families such as Elapidae (e.g., coral snakes, cobras) and Viperidae (e.g., pitvipers, vipers), and most recently in Colubridae (e.g., grass snakes, racer snakes), the largest family of snakes in the world [1].

The venomous apparatus of snakes belonging to the Viperidae, Elapidae, and Colubridae families, almost like any other system that requires similar efficiency, depends on four basic tools for its performance. First, venom must be produced and stored in a reservoir. Then, it needs to be transported to the intended site for discharge. Afterwards, a mechanism is required to release the venom towards a specific organism, minimizing the risk of loss. The fourth component involved is related to the mechanisms surrounding the reservoir, responsible for emptying it and initiating the flow of venom through the system, from the initial storage site to the target [4].

Specifically, the venomous glands and accessory glands, the connecting ducts, the fangs, and the striated muscles that surround the glands represent the global venomous apparatus. The first three components present in pairs, one on each side of the head, towards the temporal region and ending in the maxillary region. The muscle fibers cover a slightly wider area of the head of the snake and are part of the compression system for ejecting the venom. The snake's biting and venom dispensing mechanisms involve the digastric, pterygoid, anterior temporal, and posterior temporal muscle groups that are located on each side of the head [5].

The venomous apparatus of snakes is characterized by its fangs that are always located on the maxilla of the upper jaw and never on any other tooth-bearing bone [6]. Among fanged snakes, the structure and position of the fangs allow the recognition of three types of dental architecture (Figure 1): short front fangs with enclosed venom-conducting canals and a visible suture line connecting the orifices (proteroglyphous: Elapidae and Atractaspididae like coral snakes or African asp snake; Figure 1C); enlarged and tubular front fangs with enclosed venom-conducting ducts and a smooth surface between the orifices (solenoglyphous: Viperidae like rattlesnakes or bushmasters; Figure 1D); and enlarged, solid grooved fangs located at the posterior end of the maxilla (opisthoglyphous: rear fangs of false water cobras: Figure 1B) exhibited by numerous lineages of colubrids (the grooves can be present on anterior, posterior, lingual or labial sides of the fang). The frontal position of the fangs in proteroglyphous and solenoglyphous structures differs not only in fang structure but also in the high level of kinematic freedom of the maxillary bone in solenoglyphous species. This flexibility allows them to position the fangs almost parallel to the roof of the mouth when closed and to rotate them

over 120 degrees along the anteroposterior axis, orienting them directly toward the target [4].



Figure 1. Types of types of dental architecture in snakes. (A) Aglyphus (Green anaconda *Eunectes murinus* UF 84822). (B) Opistoglyphus (Coastal House Snake *Thamnodynastes pallidus*: UMMZ 246849). (C) Proteroglyphus (coral snake *Micrurus nigrocinctus* UMMZ 131984). (D) Solenglyphus (South American rattlesnake *Crotalus durissus*: UMMZ 119571). All images were retrieved and accessed on 5/08/2023 from Morphosource under CC BY-NC-ND 4.0 licence.

The evolutionary origin of fangs, as well as other elements of the venomous apparatus has been the subject of controversy and involves several underlying forces that have driven fang diversification [2]. Despite the unresolved evolutionary questions, most studies agree that the front-fangs exhibited in the Viperidae and Elapidae both independently derived from rear-fang snakes, reflecting convergent evolution [4]. The evolution of the venom delivery system has been frequently associated with dietary and ecological specializations. For instance, among venomous snakes, two predominant predatory strategies exist: rear-fanged snakes and elapids usually exhibit a bite-and-hold strategy, while most vipers and Atractaspis use a bite-and-release strategy, particularly for larger prey [2,7]. Thus, the lengthening of the fang suggests that it has been driven by the strike behavior rather than by increased stress (stretching force) associated with structural changes in the venomous apparatus [2].

The venomous apparatus rapidly discharges venom into the prey to ensure a food source or as a form of defense against predators, since snakes move slowly and have a lower metabolism [5]. There are two general mechanisms of venom delivery. The first exhibited in viperid and elapid snakes, is a high-pressure system in which a pulse of venom is delivered quickly by a sudden pressure surge. The second type of venom delivery system is found in rear-fanged colubrids, where the release of oral secretions is more protracted [1]. In this low-pressure system, venom delivery is caused by the mechanical force obtained when the fangs penetrate the prey. In the high-pressure system, the direct action of the jaw muscles on the venom gland generates a pressure that causes quick delivery of venom. The high-pressure system establishes a tight seal between the duct and fang, ensuring that pressure is maintained throughout the conduction channel and driving the venom into the prey. The low-pressure system lacks this tight seal, resulting in a lower pressure of venom flow [1].

Snakes from the Colubridae family possess a different “venomous” apparatus (see Chapter 4). The Duvernoy’s gland, which is homologous to the venomous gland and located in a similar position, lacks a large lumen reservoir and compression muscles around it. This system uses relatively lower pressures than the one mentioned above, and releases secretion slowly into the oral epithelium adjacent to the teeth that may have grooves but not hollows such as the fangs [1,2,7,8]. The Duvernoy’s gland structure varies greatly among species of Colubridae, with all the transitions between the absence of the gland to the presence of a purely serous well-differentiated Duvernoy’s gland [3]. Similarly, Duvernoy’s secretions exhibit a significant degree of compound variability, producing toxins of an enzymatic digestive or pancreatic origin, as well as phospholipases, phosphodiesterases, and proteases [3] (see Chapter 4).

Initial research on snake venoms focused on their toxic properties and oral secretions. It is evident that the components of venoms, including those of the Duvernoy’s glands, have multiple biological functions, and

even biotechnological purposes [5] (see Chapters 4 and 10). The venomous systems of elapids and vipers differ in size and morphology, but they all share a similar basic design of composition and function.

1.2. Venom definition

To define venom accurately, we refer to a statement of Arbuckle et al. [10], defining venom as "a biological substance produced by an organism that contains molecules (toxins) that interfere with physiological or biochemical processes in another organism. The venomous organism has evolved this substance to provide benefits to itself once introduced into the other organism. The venom is produced and stored in a specialized structure and actively transferred to another organism through an injury caused by a specialized delivery system".

In a biological context, the term "toxic" refers to the lethal property of a chemical expressed as the median lethal dose (LD_{50}) or absolute lethal dose (LD_{100}), usually identified and characterized under defined laboratory conditions. The term "venomous" refers to the function or biological role of the secretion of a substance produced by an animal that is used for defense or the procurement of another animal as prey [6]. The observation of the animal in its natural habitat is usually the basis for concluding whether a secretion is used as venom. The two terms rest on different concepts so more is at issue than mere semantics [3].

Venoms are mixtures of proteins, enzymes, peptides, ions, carbohydrates, and traces of other molecules produced in glands and secreted through specialized systems to subdue prey and digest, or deter predators (e.g., snake, scorpion, or spider venoms, among others). In contrast, poisons are substances that are concentrated in the bodies of certain organisms or anatomical regions of those organisms. They can cause adverse effects in other organisms that handle or consume them (e.g., lionfish spines or skin of poisons frogs found in the family Dendrobatidae). Toxins are substances produced by living organisms that can disrupt the normal homeostasis of other organisms that are exposed to them [10].

Both venoms and poisons are composed of toxins, and toxinology is the study of these substances. Toxinology is a sub-area of toxicology that is responsible for studying molecules produced by living organisms. These molecules are either distributed as venoms or allocated as poisons in the tissues of animals, plants, fungi, and bacteria; and they may cause damage to the target organism [4,11].

Venomous is an ecological trait that behaves as an intermediary in the interactions between two or more organisms. However, it is important to know that the term "venomous" does not necessarily mean "dangerous" (Figure 2). The level of danger depends on the susceptibility of the target organism to the venom, as well as the amount of venom that is injected. In some cases, such as with a dry bite (a bite without venom inoculation) [12], the venom may not even reach the organism. Venom is a common trait among medically

important snakes that are typically found in Elapidae and Viperidae snake families [13]. However, some non-venomous species, such as certain rear fanged colubrids [19] (see Chapter 4), have been known to cause mild envenoming or even life-threatening symptoms with their secretions.

The composition and activity of snake venoms evolved in parallel with the physiology of their prey and, in some cases, their natural predators. Although it was previously believed that humans have not exerted significant defensive selective pressure on snake venoms, and that envenoming was considered collateral damage in the chemical weapons race between venomous snakes and their prey [15], recent research suggests that the venom and delivery mechanism of spitting cobras (e.g., *Hemachatus haemachatus*, *Naja sumatra*) evolved to cope with hominins and mammals 2.5 million years ago. The venom was directed to produce severe pain in the hominins that posed a possible threat to them [18]. Thus, there are several dimensions of snake venom evolution that remain unexplored and require further research.

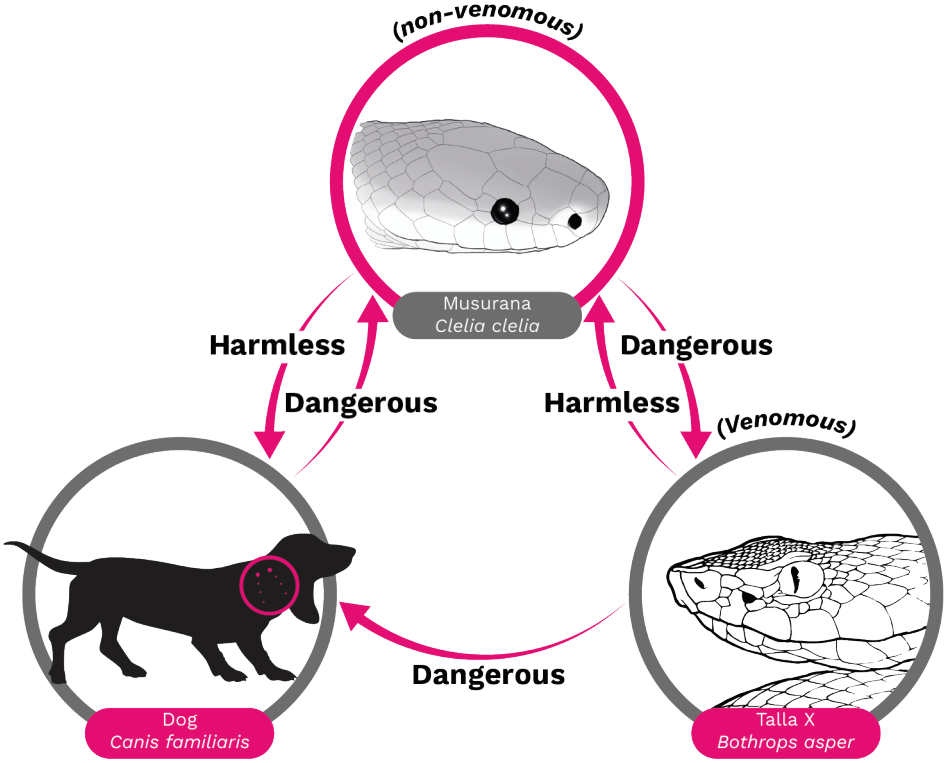


Figure 2. Venomous = Dangerous? A clinical toxicologist would describe a *Bothrops asper* as venomous because its bite has dire consequences for humans and many mammals such as dogs. However, to a *Clelia clelia* snake, a *B. asper* is a delicious meal and not venomous according to a definition requiring death or adverse pharmacological activity [20–23].

1.3. Interspecific and intraspecific variation

Snake venoms exhibit significant variation in composition and biological activity, both within and between species, as well as at higher taxonomic levels. This variation is considered adaptive because it enables the snake to have prey readily available or to deter predators [15]. Venom variability has significant implications for both primary venom research and snakebite management, including the selection of antivenoms, specimens and species for antivenom production to address snakebite pathophysiology [15,19,20].

Snake venom exhibits variability in multiple dimensions (see Chapter 3) including: interfamily, intergenera, interspecies, and intraspecies variations, as well as geographical variation between individual specimens, across individual ontogeny, seasonal changes, diet, habitat, and sexual dimorphism [21].

At the taxonomic family level, the venoms of elapids and vipers differ. Certain families of toxins have been recruited into each venom and are found exclusively in the venoms of one lineage and not the other [22]. Similarly, there are differences in venom compositions between genera within each family and between species within each genus. The therapeutic approach should be based on the understanding that antivenoms have been developed based on families, genera, species, and regions [15] (see Chapter 6).

Recently, it has been recognized that venom varies within species, including regional variations, as well as variations between ages and sizes (see Chapter 3). Venom is an ecological trait that evolves dynamically. Composition of a snake's venom is also influenced by the variety of its diet. Juvenile specimens tend to consume different prey than adults of the same species, exhibiting different feeding and behavioral strategies. For instance, juveniles may be nocturnal while adults are more diurnal. As a result, juveniles may employ a bite and hold strategy, while adults may bite and release [15].

Regional variation may be related to ecological differences between populations and to neutral evolution that is present in venomous systems and works in association with positive selection [23]. This suggests that for a trait to evolve rapidly, there must be significant heritable diversity within populations. This supports the hypothesis that variations would occur among the venoms of adult members of a single population [15].

The molecular-level documentation on the dynamism of the evolution of venom is well-established. Transcriptomic and proteomic analyses of *Bothrops atrox* venom reveal that transcription and translation mechanisms facilitate a venom's phenotypic variation. The evidence reveals that venoms contain two types of proteins: conserved core function proteins, which are essential for proper functioning and adaptive proteins, which are less conserved, vary in expression, and can be personalized in their function [19]. These observations suggest that the genetic mechanisms controlling venom variability extend beyond the selection of gene copies or mutations in structural genes. They also include the selection of mechanisms that control gene expression, contributing to the plasticity of venomous snake phenotypes [19].

Venom variability occurs due to evolutionary trends of venomous snakes and the specialization of some of its toxins. For instance, the venoms of both the Elapidae and the Viperidae are dominated by two or three families of proteins: phospholipases A_2 (PLA_2) and three-finger toxin (3FTx) for elapids, and metalloproteinases (SVMP), PLA_2, and serine proteinases (SVSP) for vipers. On average, these protein families make up 83% and 67% of the venom proteome for elapids and vipers, respectively [24].

Snake venoms from the Elapidae are mainly neurotoxic, while those from the Viperidae family induce coagulopathies, bleeding, and local tissue damage [25,26]. This is due to the prevalence of 3FTx and PLA_2 proteins in elapid venom that can cause myasthenia gravis-like weakness [27]. This weakness can affect any muscle and if the neuromuscular blockade involves the muscles of respiration, it can lead to death [26,27]. On the other hand, viperid venom has mainly hemotoxic and myotoxic effects. The agents identified in viper's venom include thrombin-like proteinases that lead to coagulopathy [28], hyaluronidases that alter extracellular matrices, phospholipases A_2 that cause local inflammation and pain [29], and metalloproteinases that contribute to hemorrhage [30,31]. The result is local tissue destruction worsened by coagulopathy [26].

There is variation in venom profiles between species within the same genus [32–34]. For instance, the venoms of two species of viperids from the *Bothriechis* genus*, B. lateralis* and *B. schlegelli* that have been compared using proteomics show that the venoms of these two species contain bradykinin-enhancing peptides (BPP) and PLA_2 proteins, serine proteinases, L-amino acid oxidases (LAAO), cysteine-rich secretory proteins (CRISP) and Zn^{2+}-dependent metalloproteinases (SVMP). However, each species has a different relative abundance of each protein family. Additionally, each venom contains distinct components. For instance, *B. lateralis* includes vascular endothelial growth factor (VEGF) and C-type lectin-like molecules, while *B. schlegelii* has Kasal-type protease inhibitors [35].

The venom proteins of both *Bothriechis* species are less than 10% similar, indicating a significant divergence in venom composition. Despite both species adapting to arboreal habits, the biochemical characteristics of their venom are likely related to the characteristics of their consumed prey [32,35]. The composition of venoms can provide clues to rationalize the various signs of envenoming caused by *B. schlegelii* and *B. lateralis* [35].

There is also variation in the profile of snake venom between individuals of the same species, with intraspecific differences found in geographical locations [28,36,37]. For instance, the composition and toxicological profile of the venom of the rattlesnake *Crotalus simus* in Mexico were analyzed at the subspecies level. The venoms of the subspecies *C. s. simus, C. s. culminatus,* and *C. s. tzabcan* differ in the expression of the neurotoxic complex "crotoxin". *Crotalus s. simus* has the highest concentration of crotoxin followed by *C. s. tzabcan*, while the venom of *C. s. culminatus* is almost devoid of this neurotoxic PLA_2. Proteomic analysis closely correlates with toxicological profiles. For instance, *C. s. simus* venom contains high amounts of crotoxin and serine proteinases, while *C. s. culminatus* venom has higher amounts of metalloproteinases and crotamine. This suggests that the geographical variation in venom composition may reflect natural selection to feed on local prey [38]. The rising occurrence of crotamine in populations of *Crotalus* is sufficient reason to caution the necessity of developing an antivenom that can neutralize this toxin [36].

There is variation in venom composition between young and adult snake species due to differences in diet [39]. Typically, young pit vipers feed on ectothermic animals while adults feed primarily on small mammals [38,40,41]. Consequently, studies focused on the ontogeny of these animals for some species are described [42,43]. Ontogenetic studies on *Bothrops* venoms obtained from adults and newborns revealed different plasma coagulation activities. The research identified two distinct venom profiles: young individuals with little PLA_2 K-49 myotoxin and more proteases, while adults showed a lot of PLA_2 K-49 but fewer proteases [30,50]. Newborn venom is less inflammatory, less hemorrhagic, but more myotoxic and procoagulant compared to adult venom. On the other hand, adult venom exhibits more hemorrhagic activity *in vivo* than young individuals [45–47].

It has been established that certain venom profiles should vary between sexes and adapt to specific prey species [38,41,48,49]. In contrast, electrophoresis analysis of the venoms of 30 offspring from a single litter of *Bitis gabonica* (Gaboon viper) revealed individual variations, but no significant differences based on sex were noted [49]. In Colombian snakes, the relationships between venom variability and life history traits represent an underexplored field that requires greater research efforts.

The venom's action and proportionate mix of actions are determined, at least in part, by the type of prey sought by a population of snakes [20,50]. For instance, it is important to note that CRISP toxins are abundant in colubrid venoms from reptile-feeding species like *Telescopus dhara* (Arabian cat snake) and *Trimorphodon biscutatus* (Western lyre snake). The toxic hypothermic effect of this toxin is useful for slowing down the movement of ectothermic prey ("cold blood") [51]. The source of variation in snake venom profiles is an exciting and unresolved research field that requires a multidisciplinary approach and significant effort to unveil the mechanisms that generate the intricate mixtures of snake venoms.

2. On venom essentials

2.1. Snake venom metalloproteinases

Snake venom metalloproteinases (SVMPs) cause hemorrhage in the microvasculature of their prey, which contribute to the prey's immobilization, as observed in snakebite accidents. Additionally, SVMPs induce myotoxicity, edema, dermonecrosis, blister formation, and coagulation disorders [52,53].

The venom mixture contains various toxins, including SVMPs, which can break down proteins into smaller polypeptides or amino acids. SVMPs are a type of zinc-dependent endopeptidase that belongs to the ADAMs/Adamalysin/reprolysins subfamiliy that is part of the metzincins family [54,55]. Metzincins are metallopeptidases that have globular catalytic domains. Many of these domains are multidomain and contain a consensus zinc-binding amino acid sequence, HEXXHXXGXX, and a β-1-4 turn that has a highly conserved methionine residue (Met-turn). This residue forms a hydrophobic base for the zinc cofactor and the three histidine residues

involved in catalysis [56]. Therefore, these toxins are composed of five domains or functional and/or structural units. First, a signal peptide which is a short peptide (~ 16-32 amino acids) that prompts a cell to translocate the protein, then SVMPs that can contain pro-domain, metalloproteinase, desintegrin, and rich in cysteine [56].

Nevertheless, toxins in venom secretion-mature proteins can be in different combinations of the last three domains and are classified into three types, based on their molecular mass and domain composition. Type P-I SVMPs have a molecular mass between 20 and 30 kDa and exhibit only a metalloproteinase domain. Type P-II SVMPs have a molecular mass between 30 and 60 kDa and contain both metalloproteinase and disintegrin domains. Type P-III SVMPs have a molecular mass between 60 and 100 kDa and are rich in cysteine domains in addition to metalloproteinase and disintegrin domains [54,57,58]. The last subclass is determined based on post-translational modifications, such as proteolytic processing between the proteinase, disintegrin domains (class P-IIIb), dimerization (P-IIIc), protein addition type C-type lectins (P-IIId), and the initially described canonical P-IIIs known as P-IIIa [54,58].

The metalloproteinase domain structure, the most characteristic domain in SVMPs, is formed by five β-sheets, four α-helices and a short α-helix in the N-terminal. The β1, β2, β3 and β5-sheets are parallel to each other, while the β4-sheet is anti-parallel to the other β-sheets. The domain is divided into two parts (M and S sub-domains) by the substrate binding cleft [65]. Within this cleft, Zn^{2+} is coordinated by the Nε2 atom of histidine residues located at positions 142, 152, and 156, as well as catalytic water molecules.

Below the active site lies the side chain of a highly conserved methionine residue that forms a hydrophobic base for the zinc cofactor and constitutes the Met-turn, a highly conserved characteristic within the metzincins family [59]. Additionally, these enzymes require a Ca^{2+} ion to stabilize their structure, and the location of this cation is structurally opposite the substrate binding cleft [58,59]. The active site of the metalloproteinase domain includes a glutamate residue (Glu143) that is involved in initiating the catalytic cycle by deprotonating the catalytic water (Figure 3).

After deprotonating the water (denotation of a hydrogen cation), the resulting hydroxyl group acts as a nucleophile and attacks the carbonyl of the peptide bond which is then hydrolyzed. An oxyanion subsequently is formed and stabilized by the Zn^{2+} cofactor. Finally, the dislocated electrons on the oxygen return to their original bond, and the reaction products are released with the help of new water molecules [60].

SVMPs have a substrate binding cleft that plays a crucial role in binding and stabilizing the substrate for further catalytic reaction. Additionally, the catalytic residues and zinc coordination also contribute to the reaction. The cleft contains subsites, including S1, S2, S3, S1', S2' and S3' that interact with the substrate side chains. The enzyme's active site serves as a center,

with S1 to S3 located from the active site to the N-terminal, and S1' to S3' is located from the active site to the C-terminal. The peptide bond to be hydrolyzed is located in the center of the active site, and the substrate has complementary sites as follows: P1 to P3 sites interact with S1 to S3 subsites on the enzyme and P1' to P3' sites interact with S1' to S3' subsites on the enzyme. This nomenclature allows for an exact description of the interaction between the enzyme and substrate or inhibitors [60,61] (Figure 4).

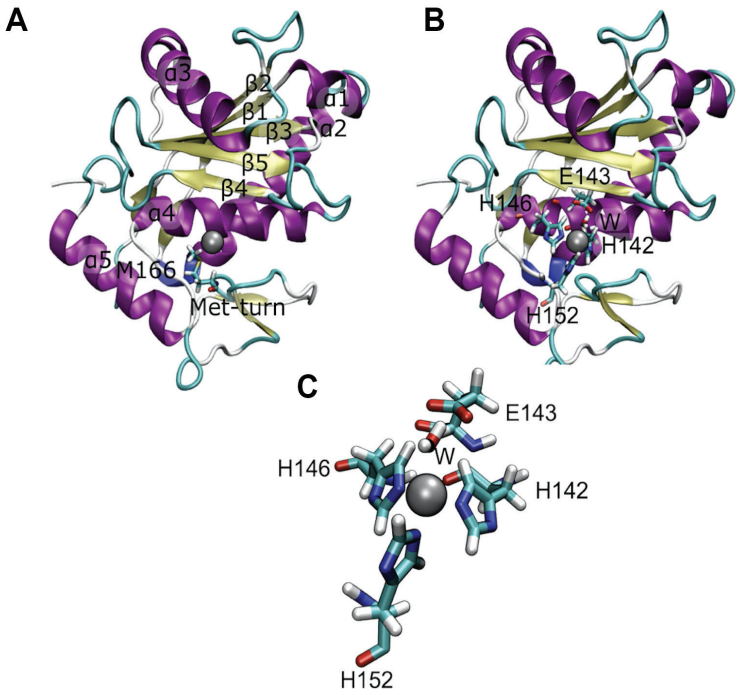


Figure 3. Structure of a P-I SVMP: (A) The figure displays the arrangement of secondary structure, including Met166 and the Met-turn. (B) Shows the position of the zinc ion (gray sphere), its coordination, and the catalytic residues. (C) Depicts the active site of the enzyme. The figure was prepared from the structure with PDB code 2W15 using VMD (Visual molecular dynamics). Figure made by by Lina María Preciado Rojo.

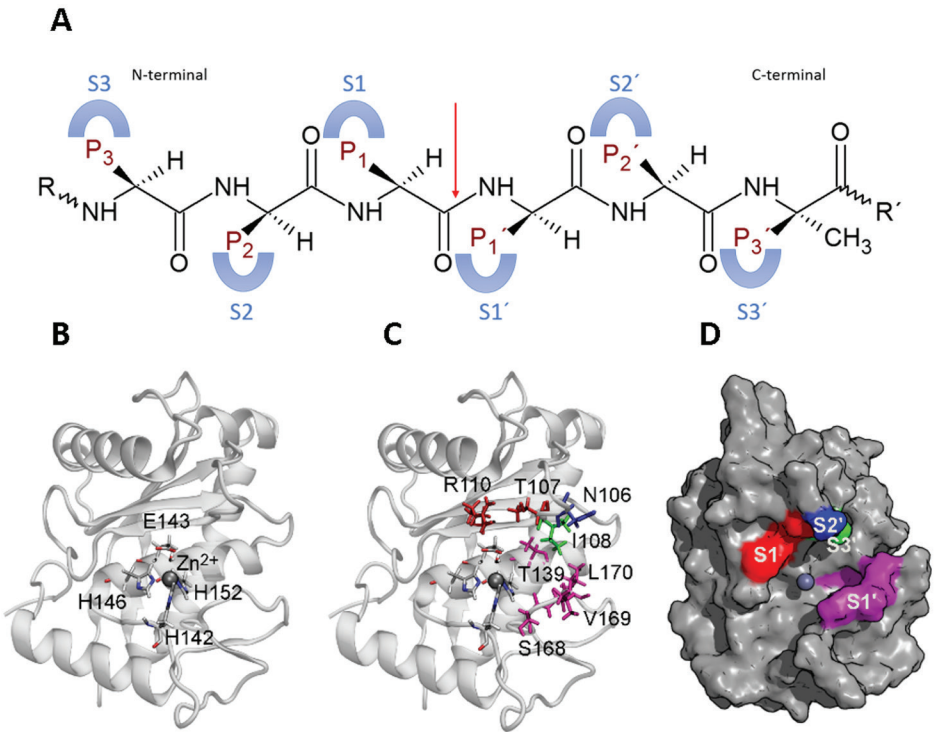


Figure 4. Subsites of a P-I SVMP. (A) Schematic representations of subsites in the enzyme and their interaction with sites in the substrate. The red arrow points out the peptide bond to be hydrolyzed. (B) Active site of the enzyme and the catalytic residues. (C) Side chain of the residues involved in the subsites in the enzyme by a color key. Red: S1, blue: S2', light green: S3', and purple: S1'. (D) Surface of the enzyme and the location of the subsites with the same color key as C. The figure was prepared from the structure with PDB code 2W15 using VMD (Visual molecular dynamics). Figure made by by Lina María Preciado Rojo.

2.2. Biological effects induced by SVMPs

Hemorrhage

This activity can be either local or systemic and is catalytic-dependent. A two-steps hypothesis has been proposed to explain how these toxins induce hemorrhage [52]. Initially, SVMPs hydrolyze the substrate at the basement membrane (BM), such as type IV collagen, laminin, nidogen, and perlecan. This cleavage weakens the mechanical stability of the BM and increases the distensibility of the micro vessel wall. Furthermore, SVMPs can degrade other proteins, including those involved in binding BM to the extracellular matrix of muscles, such as non-fibrillar collagens VI, XII, XIV, XV, XVI, and XIX, as well as fibrillar collagen type V [53,62,63].

In the second step, hemodynamic forces act on the microvasculature including shear stress and hydrostatic pressure on the micro vessel wall. These forces potentiate the distention of the wall, leading to its disruption and the extravasation of red blood cells [58] (Figure 5).

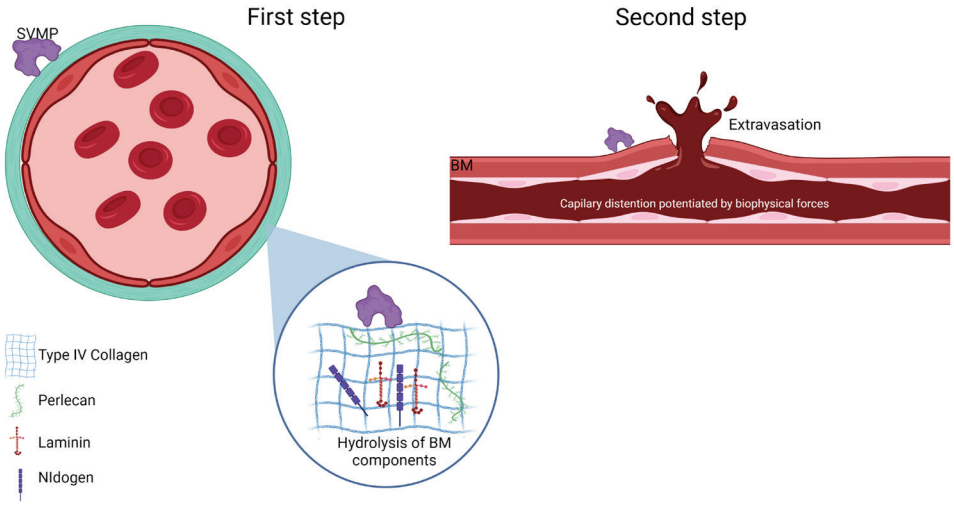


Figure 5. Schematic representation of the two-step model for hemorrhage induced by SVMPs. The first step involves the hydrolysis of components of the basement membrane (BM) by SVMPs that destabilizes the interaction between endothelial cells and BM. In the second step, biophysical forces contribute to the potentiation of capillary distention of the microvessel wall, leading to its disruption. For further details, please refer to the text. Image created with Bio Render.

There are two types of hemorrhage, per rhexis and per diapedesis. Per rhexis is characteristic of the capillary microvasculature. In this case, extravasation occurs through gaps formed in the vessel wall, as described above. Additionally, the thickness of the endothelial cell is decreased, making the capillary wall weaker [52,53,64]. Although hemorrhage induced by SVMPs is primarily caused by the per rhexis mode, per diapedesis mechanism is observed in venules where erythrocytes escape through widened intercellular junctions instead of in gaps in endothelial cells [65].

Additionally, SVMPs induce apoptosis (a cell death mechanism) of endothelial cells [66–70]. In certain cases, the levels of anti-apoptotic proteins from the BCL-2 family remain unaffected, while in other cases the levels of BCL-XL, another anti-apoptotic protein are decreased [67,70]. In addition, caspase 3 and 8 activation has been observed [67,70]. These proteases participate in the intracellular signaling cascade that leads to cell apoptosis. SVMPs can increase the expression levels of several matrix

metalloproteinases (MMPs) [71], which can increase their hemorrhagic effect by degrading the extracellular matrix and a further weakness of bone marrow derived endothelial cells interaction.

There is a clear difference in the potency of SVMPs inducing hemorrhage. Specifically, P-III and P-II SVMPs are more hemorrhagic than P-I SVMPs. The molecular mechanism underlying this phenomenon has not yet been fully elucidated, but some hypotheses provide insights into this topic. Firstly, P-II and P-III SVMPs contain disintegrin, cysteine-rich domains, and in some cases, a C-type lectin domain (P-III). These domains can direct toxins to specific targets in the microvasculature, concentrating them in the areas where they can cause the most harm [58,61].

For instance, the disintegrin domain can direct to integrins expressed on the cell membrane of endothelial cells [72]. Therefore, P-II and P-III SVMPs exhibit a co-localization pattern with type IV collagen on the vessel wall. In contrast, P-I SVMPs, which lack additional domains, are widely distributed in the extracellular matrix and are not concentrated in the vessels [73,74].

Furthermore, studies have shown that the plasma proteinase inhibitor α_2-macroglobulin inhibits the proteolytic and hemorrhagic activities of several P-I SVMPs, but it does not have inhibitory capacity against P-II and P-III SVMPs [75–77]. The structural basis for these differences is still unknown. However, one hypothesis is that the additional domains found in P-II and P-III create obstacles for the binding of α_2-macroglobulin, resulting in failure to recognize. This discovery may also explain the ability of P-II and P-III SVMPs to cause systemic hemorrhage, as they are not impeded by α_2-macroglobulin once they enter the systemic circulation, whereas P-I SVMPs are rapidly inhibited by this protease inhibitor [53].

Another important aspect related to SVMPs is that P-I SVMPs differ significantly in their ability to induce hemorrhage [78–80]. Although they have similar proteolytic activity towards several substrates *in vitro*, some of these enzymes cause hemorrhage while others do not [63,79,80]. Significant differences were observed between hemorrhagic and non-hemorrhagic SVMPs in their ability to hydrolyze BM components in vivo. The P-I SVMP toxin BaP1 hydrolyzes type IV collagen and perlecan to a higher extent than the non-hemorrhagic P-I SVMP leucurulysin-A. Proteomic analysis of exudates collected from muscle tissue injected with these SVMPs also revealed differences in the types of extracellular matrix components present [63].

Nevertheless, the detailed structural determinants of hemorrhagic activity of P-I SVMPs remain largely unknown. Therefore, the possibility to predict the hemorrhagic potential of SVMPs based on structure or sequence analysis remains uncertain. However, a recent hypothesis suggests that differences in the dynamics of a loop located near the catalytic site may explain the variable hemorrhagic activity [82].

Another important finding was the differences in the electrostatic surfaces between these types of proteins [83]. Bioinformatic tools have re-

cently been used to predict the interaction of hemorrhagic P-I or non-hemorrhagic P-I SVMPs with their natural substrates such as type IV collagen, perlecan and laminin domains [84]. Hemorrhagic P-I SVMPs can form catalytic complexes with their substrates, while non-hemorrhagic P-I SVMPs cannot. Additionally, non-hemorrhagic P-I SVMPs may have a greater volume area of the substrate binding cleft than hemorrhagic P-I SVMPs. Despite all the results described, more studies are needed to clarify the hemorrhagic activity among types of P-I SVMPs.

Myonecrosis and impairment muscle regeneration

The inoculation of snake venom metalloproteinases (SVMPs) results in necrotic muscle damage (myonecrosis) that is caused by hemorrhage induced by the same toxins. Bleeding affects the blood supply to the affected tissue, leading to ischemia. Ischemia may cause hypoxia and muscle cell damage [30,80,85]. This effect can contribute to the myotoxic activity induced by other snake venom toxins, mainly phospholipases A_2s (PLA_2s).

The process of muscle regeneration after an injury requires three conditions: intact blood supply, restitution of neuromuscular junction, and intact BM [86]. Additionally, a synchronized interplay among several inflammatory substances and cells is involved. SVMPs affect two of these requirements by degrading the components of BM, resulting in a weakening of the microvasculature wall and extravasation of vessel content. Smooth muscle necrosis in the wall of intramuscular arteries caused by SVMPs can affect muscle regeneration by decreasing the blood supply, [87,88]. Oxygen and nutrients to the affected tissue decrease, and the muscle regeneration process is affected [85].

Blister formation and skin death (dermonecrosis)

Blister formation induced by SVMPs is caused by their catalytic activity against the BM components located at the dermal-epidermal junction [71,89,90]. The SVMPs hydrolyze collagen type IV, laminin, and nidogen, among other proteins, in the blister fluids [89,90]. Following this hydrolysis, the epidermis separates from the dermis, resulting in the formation of a blister which accumulates mainly plasma fluid, favored by the inflammatory process, as well as fibrinogen and fibrin that are degraded by various SVMPs. Dermonecrosis may occur when cells detached and then die, and subsequently an inefficient regenerative process takes place, either with or without scar formation [96]. However, further studies are needed to obtain more insight into this issue.

Edema and inflammation

A hypothesis was that the edema-forming activity of SVMPs might be initially related to the extravasation of blood vessels contents into the interstitial space. However, this hypothesis was partially refuted when injections of sub-hemorrhagic doses of SVMPs provoked edema, suggesting that this effect is independent of extravasation and is induced by multicomponent events [86], this defines a second mechanism of edema formation mediated by proinflammatory activity. First, leukocyte infiltration occurs in the affected organ. Then, mast cell degranulation and histamine

release occur that can also lead to macrophage activation [71,91–93]. Similarly, SVMPs stimulate the release of IL-1 and IL-6, leading to an increased tumor necrosis factor (TNF) mRNA level, resulting in the overexpression of this protein responsible for inflammation processes [71,93,95].

2.3. Snake venom phospholipases A_2

Venomous snakes employ phospholipases A_2 (PLA_2s), found in their venoms to cause myonecrosis (death of muscle cells), neurotoxicity (paralysis of breathing muscles), inflammation, pain, and disruption of normal coagulation. All these effects contribute to the immobilization of the prey and can be observed in cases of snakebite envenomation.

General aspects: classification, catalyzed reaction, and overall structure

There are sixteen groups of PLA_2s (IA, IB, IIA, IIB, IIC, IID, IIE, IIF, III, V, IX, X, XIA, XIB, XII, XIV) that are classified based on their sequence, molecular mass, origin, disulfide position, calcium requirement, and other characteristics [95]. The PLA_2s can hydrolyze the ester bond from the sn-2 position from glycerophospholipids, producing a fatty acid and a lysophospholipid (Figure 6).

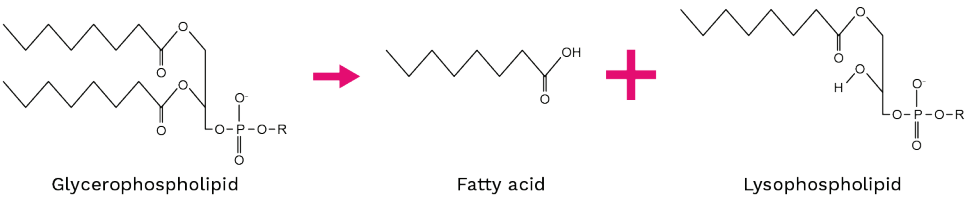


Figure 6. Catalyzed reaction by PLA_2s.

The PLA_2 enzyme family is present in a wide variety of organisms, including animals, plants, fungi, and bacteria. However, among the sixteen types of PLA_2, those found in snake venoms belong to groups IA, IIA, and IIB [102]. Group IA PLA_2s are present in the venoms of the Elapidae that in the Americas is represented by the coralsnakes (*Micrurus* spp.). These PLA_2s typically have a molecular mass around 13-15 kDa and exhibit seven disulfide bridges, one of which is exclusive to this group and is located between 11 and 77 cysteines.

Group IIA PLA_2s are found in the venoms of the Viperidae, such as *Crotalus, Lachesis, Bothrops,* and *Porthidum* snakes. The molecular mass of group IIA PLA_2s is like that of group IA PLA_2s, also present in Viperidae venoms, both groups with seven disulfide bridges. The exclusive disulfide bridge in group IIA PLA_2s is located between 50 and 137 cysteines. Group IIA comprise another set of snake venom PLA_2s, including an enzyme also isolated from the venom of Russell's viper (*Daboia russellii*) from Asia (India, Pakistan, Nepal, Sri Lanka, Bangladesh, and Bhutan). A unique characteristic of this protein is that it has one less disulfide bridge than PLA_2s mentioned above [95,96].

Elapid and viperid PLA_2s have human counterparts. For instance, pancreatic PLA_2 group IB is homologous to the enzymes of group IA. Synovial

PLA_2 belongs to group IIA and has a structure like snake venom proteins in the same group. However, it is well known that human PLA_2s do not cause the harmful effects observed in snake venoms. These effects include pre and/or post-synaptic neurotoxicity, local and/or systemic myotoxicity, anticoagulation, cardiotoxicity, modulation of platelet aggregation, hemolytic, edema, hypotensive activities, and direct damage to organs such as the kidney, lung, and liver [97].

Snake venom PLA_2s are classified as basic or acidic proteins based on their isoelectric point. Most PLA_2 are basic. Though acidic PLA_2s are known to have catalytic activity, they are generally devoid of biological effects. However, in some cases, they show myotoxic and edema-forming activities [98,99]. The acidic proteins have also been identified in Colombian venoms, including species such as *Porthidium nasutum*, *P. lansbergii,* and *Bothrops asper* [100–102].

To explain the variety of effects and differences in the susceptibility among various tissues, a model was proposed that deals with "target sites" on the surface of cells or target tissues [103]. The model suggests that specific "pharmacological sites" responsible for inducing each activity are recognized in these areas that are found in the three-dimensional structure of PLA_2s. The model proposes that pharmacological sites may or may not overlap with the active site. The pharmacological effects induced by PLA_2s are determined by the high affinity between the target molecule and the pharmacological site. This is assumed to be due to the ubiquitous presence of phospholipids in cell membranes. It is unlikely that these are the target molecules that the model affirms. In contrast, it is plausible that receptors are glycoproteins or other proteins that are differentially expressed [103].

PLA_2s from snake venoms are calcium (Ca^{2+}) dependent enzymes that structurally consist of three α-helices, two antiparallel β-sheets, and a calcium-binding loop (Figure 7). These proteins have a variable length, ranging from 119 to 134 amino acids. The hydrophobic channel involved in driving the substrate towards the active side is defined by residues located in two of the α-helices (α-helix two, residues 37-57, and α-helix three, residues 90-109, respectively), in addition to the N-terminal helix (α-helix one) and the residues Leu2, Val3, Phe5, His6, Ile9, Trp19, Val31, Lys69, Ala102, and Ala103. The active site is formed by His48, Asp49, Tyr52, and Asp99. Then, the calcium-binding loop coordinates the calcium required for catalysis and is formed by Tyr28, Gly30, Gly32, and Asp49 [104] (Figure 7).

The PLA_2s catalyze reactions that can result in chemical incoherence due to the enzymes being water-soluble, while their substrate is amphipathic (a region of the molecule is hydro soluble, and the other is liposoluble). To overcome this issue, PLA_2s have an interfacial binding surface (i-face), that mediates their adsorption at the lipid-water interface outside the cell membrane [104] (Figure 8A).

For a catalytic reaction to occur at the interface, a series of events must take place. These include the phospholipid exiting the membrane

and reaching the active site of PLA_2. The phospholipid passes through a hydrophobic channel via a diffusion process that requires no energy. After locating the substrate in the enzyme's active site, the phospholipid must be anchored to reduce decrease the degrees of freedom of the ester bond at the sn-2 position. This fixation is accomplished by the amino acid at position 69 (Lys or Tyr) through a hydrogen bond usually with the phosphate of the sn-3 position [104] (Figure 8B).

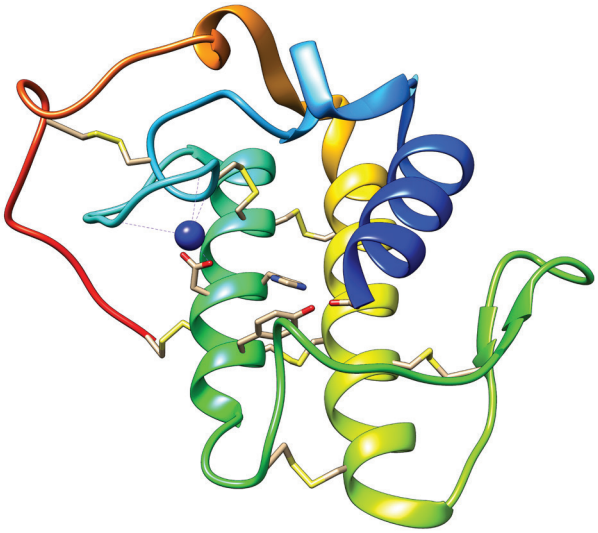


Figure 7. Overall structure of snake venom PLA_2s. The alpha-helices one, two and three are shown in blue, green, and yellow. The calcium-binding loop is shown in cyan (blue-green), and Ca^{2+} ion is represented as a blue sphere. The disulfide bridges are presented as yellow sticks. Moreover, catalytic residues are also shown in sticks (His48, Asp49, Tyr52 and Asp99) (Figure was prepared from the structure with PDB code 2QOG, chain B).

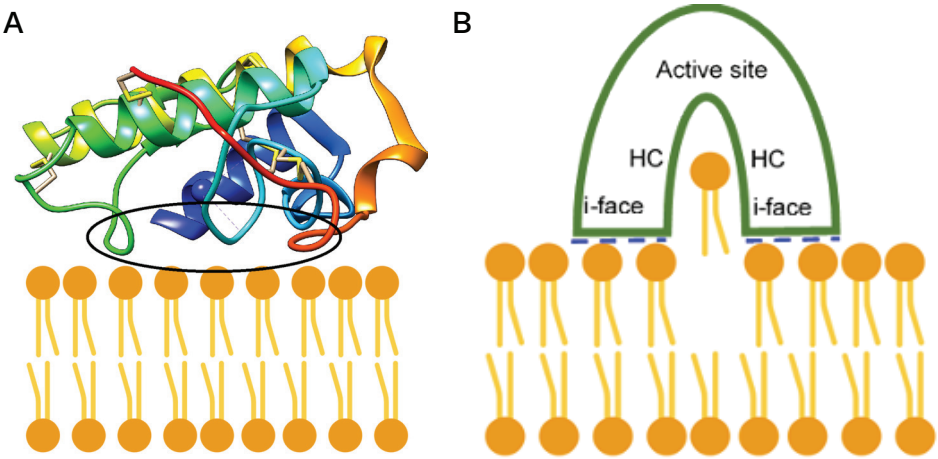


Figure 8. (A) Schematic representation of i-face. The black circle indicates the regions involved in membrane recognition and enzyme adsorption onto the lipid-water interface. (B) Schematic representation of the driving of glycerophospholipid to the active site through the hydrophobic channel (HC). The blue dash lines represent hydrophobic and electrostatic interactions between the amino acids of the enzyme and glycerophospholipids. The glycerophospholipids were extracted from Bio Render.

Following the recognition of the substrate and its displacement through the hydrophobic channel, the catalytic cycle begins. His48 is protonated by extracting a hydrogen ion from water, generating a hydroxyl group (OH-) that acts as a nucleophile. The OH- then attacks the sn-2 ester bond, leading to the formation of an oxyanion that is stabilized by the Ca^{2+} ion. Finally, the dislocated electrons on oxygen return to their original bond, and the reaction products are released [104,105].

Snake venom PLA_2s require catalytic activity to induce most of their biological effects. However, since 1984 [106], a sub-group of these toxins

have been discovered that have substituted their catalytic residue Asp49 for other amino acids (mainly Lys, but including Gln, Ser, and Arg). This replacement renders the new proteins enzymatically inactive, and they are known as PLA_2-homologues or PLA_2-like myotoxins. Although they cannot hydrolyze membrane glycerophospholipids, they can cause myotoxicity and edema [22,107].

Biological effects induced by snake venoms PLA_2s

Myotoxicity

Snake venom PLA_2s may induce myonecrosis through myotoxicity. Although the molecular events are not fully understood, there is a detailed description of the cellular processes that involve the damage of these toxins to muscle cells. Firstly, the myotoxin is suggested as binding to the target cell. Some authors have identified a protein from rabbit muscle named type-M receptors [108]. The receptor has a molecular mass of 180 kDa and contains tandem repeats of regions with homologies to carbohydrate recognition domains (CDR).

A recent report suggests that nucleolin, a nuclear protein also expressed in the cell membrane, can interact with and mediate the internalization of a Lys49 PLA_2 [109]. Subsequently, PLA_2s disrupt the muscle membrane. Asp49 PLA_2 catalyzes this process, while PLA_2-homologues destabilize the protein through hydrophobic and electrostatic interactions with glycerophospholipids.

Fernandes et al. [110] proposed that these toxins have an oligomeric state that may be allosterically activated by a small molecule, probably a fatty acid. The protein is then docked in the membrane at a specific site called the Membrane Docking Site (MDoS). Finally, PLA_2s provoked the destabilization of membrane glycerophospholipids through the action of the Membrane Disrupting Site (MDiS) [111]. These events induce changes in the selectivity and permeability of the muscle membrane, leading to massive calcium influx and cellular depolarization. Additionally, creatine kinase (CK), lactic dehydrogenase (LDH), and other molecules are released that are used as markers of myotoxicity.

The elevated concentration of calcium ions in the cytoplasm of muscle cells leads to hypercontraction of the sarcomere, resulting in mechanical damage. Calcium ions also induce an overload of mitochondria, leading to damage in the electron transport chain and ATP synthesis. Additionally, calcium can activate cytosolic PLA_2s that can increase intracellular damage, particularly in membrane organelles; and it can also stimulate the proteolytic action of calpains, triggering cytoskeletal disorganization. Finally, snake venom PLA_2s can be internalized, leading to increased internal damage. These events ultimately result in cell death by necrosis (Figure 9), which is a significant factor in snakebite envenoming by viperids, including those in Colombia (see Chapter 9) [111,112].

Systemic myotoxicity is another known biological effect of some snakebites, noted in Colombia in the case of rattlesnake accidents involving

Crotalus durissus (see Chapter 9) [113]. Although the molecular determinants for this effect are currently unknown, it is suggested that the toxin might bind to low-affinity sites in the injected area and then be distributed to other anatomical regions of the body. This distribution can induce myotoxicity through the events described above [119]. Snake venom PLA_2s can cause myotoxic activity leading to rhabdomyolysis that can affect renal function by blocking glomerular filtration due to excess myoglobin in the blood. This can ultimately result in Acute Kidney Failure (AKI) [114,115].

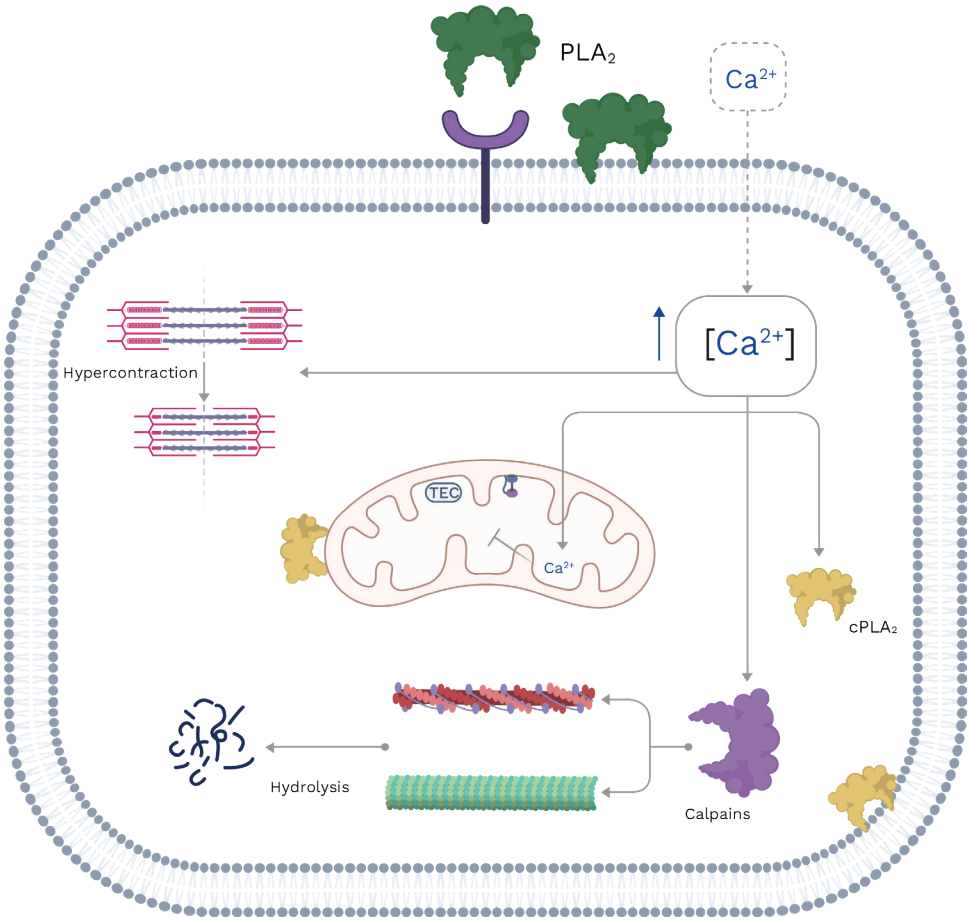


Figure 9. Schematic representation of the cellular events that occur during myotoxicity induced by snake venom PLA_2s. TEC: transport electron chain; $cPLA_2$: cytosolic PLA_2s. The muscle cell membrane is disrupted by catalytic or non-catalytic mechanisms of PLA_2s, leading to a massive influx of Ca^{2+}. This results in hypercontraction of the sarcomere, activation of calpain, and $cPLA_2$, all of which contribute to increased damage. For more details, see the text. Image created with BioRender and edited by Oscar A. Ramirez Ruiz.

Edema

Intramuscular injection of snake venom PLA_2s induces inflammation, characterized by increased vascular permeability, edema formation, leukocyte recruitment to affected tissues, and the release of inflammatory mediators [116]. However, it should be noted that the mechanisms by which these toxins induce this effect have not been entirely elucidated. Asp49 PLA_2s can generate arachidonic acid that serves as the starting point for producing eicosanoids that amplify the inflammatory response. In contrast, PLA_2-homologues induce edema and degranulation of mast cells in a catalytic-independent manner. It is evident that enzymatic activity is not strictly necessary to cause these effects. A variety of endogenous molecules including histamine, 5-hydroxytryptamine, bradykinin, tachykinins, arachidonic acid metabolites, pro-inflammatory cytokines,

and nitric oxide (NO) have been implicated in venom PLA_2-inflammatory activity [116,117]. Other structural characteristics of PLA_2s are also involved inducing inflammation. However, further studies are necessary to elucidate these molecular regions [116,117].

Neurotoxicity

Presynaptic neurotoxicity or β-neurotoxicity is another relevant effect observed in some snakebites. For instance, these are inflicted by South American rattlesnakes (*Crotalus durissus*) and coralsnakes (*Micrurus* species; see Chapter 9) [113]. The toxins responsible for this effect are named β-neurotoxins, and their structures possess one, two, three and up to five subunits that can interact either by covalent or non-covalent bonds. One of their subunits is a catalytically active PLA_2 [118]. Toxicity arises from the inhibition of acetylcholine (Ach) release, leading to a flaccid paralysis of respiratory muscles, including the diaphragm. The inhibition of Ach release occurs in three steps: first, there is a slight, transient inhibition Ach release that is related to the binding of PLA_2 to the presynaptic membrane; second, there is a significant increase in Ach release; finally, there is a sustained inhibition of Ach release [112,119,120].

Cellular events involved in presynaptic neurotoxicity induced by snake venom PLA_2s include the binding of the toxin to a target cell (motoneuron). Although the precise identification of the receptor has not yet been performed, Lambeau et al. [121] found a high-affinity protein in the rat brain named N-type due to its neuronal origin. These receptors may be located near the region where Ach is released. After binding, PLA_2 hydrolyzes glycerophospholipids at the presynaptic membrane, leading to an accumulation of fatty acids and Lysophospholipids [119,122].

The presynaptic membrane's outer layer has a high concentration of lysophospholipids that induce a curvature and promote synaptic vesicle membrane fusion while inhibiting endocytosis. An experiment that added mixtures of fatty acids and lysophospholipids on neuromuscular junctions supported this observation. The effects were similar to those induced by neurotoxic PLA_2s [123]. The destabilization of the neuron membrane induces an influx of Ca^{2+} ions which can activate endogenous PLA_2s and calpains. This activation can cause internal membrane degradation and cytoskeleton disorganization. Additionally, the high concentration of calcium in the cytosol can destabilize mitochondria and disrupt their biochemical processes [124]. Finally, the toxin is internalized by neurons.

However, the mechanism by which β-neurotoxins are transported into nerve cells remains unknown. These toxins could be internalized through pores generated after presynaptic membrane hydrolysis, or proteins could use endocytosis of synaptic vesicles as a vehicle to enter nerve cells [125]. Once PLA_2 enters the neuron, it binds to certain proteins such as calmodulin, protein disulfide isomerase, and proteins named 14-3-3; but the specific implications for the toxin's mode of action are unclear. Similarly, calmodulin stabilizes the PLA_2's ability to hydrolyze glycero-

phospholipids [126]. Additionally, proteins 14-3-3 play an important role in directing PLA_2 to synaptic vesicles to carry out their catalytic activity and subsequently inhibit vesicle endocytosis [127]. Ultimately, these events contribute to the failure of the neuromuscular junction, resulting in the inhibition of Ach release and subsequent flaccid paralysis [118–121,128,129] (see Figure 10).

Crotoxin (CTX) is a β-neurotoxin derived from *Crotalus durissus*, the South American rattlesnake [138]. It consists of a basic PLA_2 subunit known as CB and an acidic subunit called crotapotin (subunit A or CA). The latter acts as a chaperon, guiding the CB subunit to target cells (motoneurons) and preventing the binding of the PLA_2 to non-specific sites [131,132]. Additionally, CA increases the neurotoxic activity of CB but decreases its enzymatic activity [133,134]. Therefore, the crotoxin complex is responsible for the neurotoxicity observed in envenomation by Colombian rattlesnake. Other toxins found in Colombia, classified as β-neurotoxins include the PLA_2s isolated from the venoms of the coral snakes *Micrurus mipartitus* and *M. dumerilii* [135].

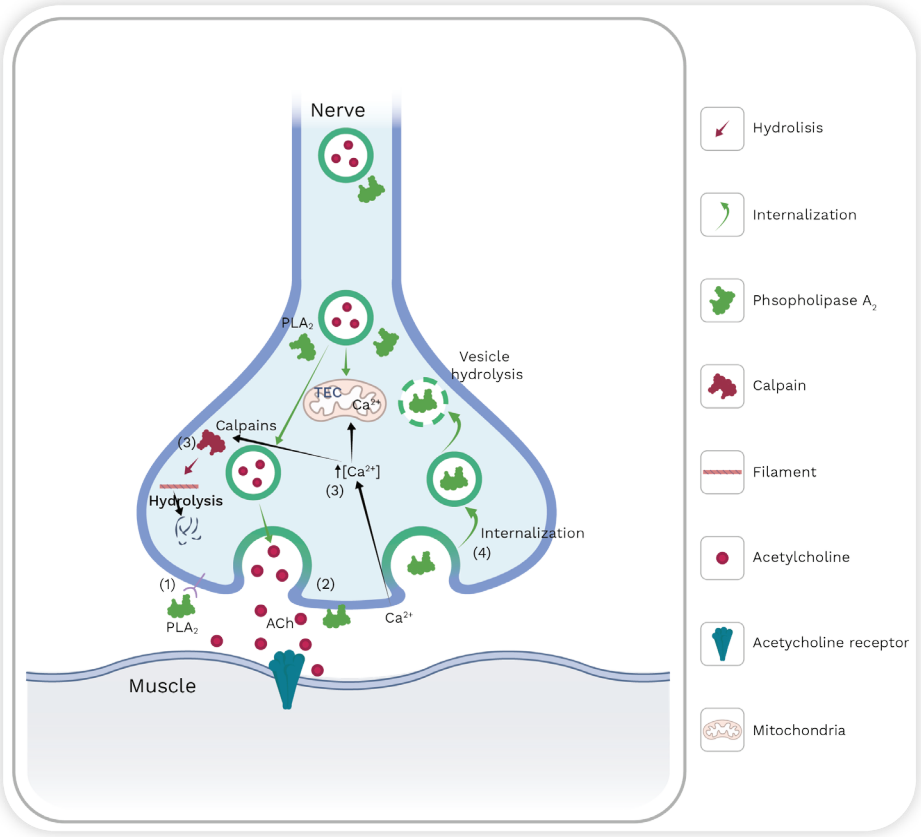


Figure 10. Schematic representation of the cellular events that occur during neurotoxicity induced by snake venom PLA_2s. TEC: transport electron chain. PLA_2s can enter presynaptic vesicles and hydrolyze them (step 1 and 2). Membrane destabilization can provoke a massive influx of calcium (step 3) that can activate calcium-dependent enzymes, such as calpains that promote cytoskeleton hydrolysis. PLA_2s are internalized into the nerve terminal to perpetuate the damage (step 4). For more details see the text. Image created with BioRender and edited by Oscar A. Ramirez Ruiz.

Anticoagulant activity

Based on their anticoagulant potency, PLA_2 enzymes are classified into strong, weak, and non-anticoagulant enzymes. Strongly anticoagulant PLA_2 enzymes inhibit blood coagulation at low concentrations (<2 µg/mL),

while weakly anticoagulant PLA_2 enzymes show effects between 3 and 10 µg/ml. Some venom PLA_2 enzymes do not significantly prolong clotting times, even at 15 µg/ml; and they are, therefore, classified as non-anticoagulant enzymes [138].

Early studies on PLA_2 suggest that catalytic activity is necessary for its anticoagulant effects [137]. However, recent studies propose that strong anticoagulant PLA_2s act through both enzymatic and non-enzymatic mechanisms, the latter being mediated by an "anticoagulant site". Kini et al. [138] proposed the position of this site in the three-dimensional structure of PLA_2s. They suggest that in strong anticoagulant PLA_2s, the region between the 53 and 76 residues is positively charged, whereas PLA_2s with weak or non-anticoagulant activity have a predominance of negative or neutral charges in this region [136,138]. This region is commonly referred to as the "anticoagulant site".

Nonetheless, Stefansson et al. [139] proposed a non-enzymatic mechanism for anticoagulant PLA_2s. The study demonstrates that a PLA_2 from Black-necked spitting cobra (*Naja nigricollis*) venom that has strong anticoagulant properties, binds to coagulation factor Xa, blocking the further activation of prothrombin. Faure et al. [149] proposed a structural model for the interaction between PLA_2s and coagulation factor Xa. The authors proposed various bioinformatic approaches and binding studies to propose that specific residues 2, 3, and 7 from helix 1; 16, 18, 19, 23, 24, 31–34 from Ca^{2+} binding loop; 53, 59, 60, 69, 70 from helix 3; and 118, 119, 121–124, 129–131, and 133 from β-sheets bind together. Currently accepted knowledge proposes that snake venom PLA_2s can elicit their anticoagulant activity through both enzymatic and non-enzymatic mechanisms [141,142].

Other biological activities induced by snake venom PLA_2s

Venom PLA_2s can modulate platelet aggregation, either inducing or inhibiting it. These are classified into three distinct classes: A, B, and C [143]. Class A enzymes initiate platelet aggregation [144,145]; class B PLA_2s cause the inhibition of platelet aggregation [146,147]; and class C PLA_2s show biphasic effects that induce platelet aggregation at low doses or short incubation times, while they inhibit platelet aggregation at higher concentrations or prolonged incubation [148].

Additionally, these toxins have hypotensive effects. However, the molecular and cellular mechanisms responsible for the reduction of blood pressure are not fully understood [149]. For instance, BthA-I-PLA_2, isolated from the jararacussu pitviper snake (*Bothrops jararacussu)*, reduces blood pressure due to its phospholipase activity. This was supported by the alkylation of His48 with p-bromophenacyl bromide that blocks the enzyme's catalytic mechanism [150]. On the other hand, the PLA_2 toxins OSC3a and OSC3b isolated from Papuan Taipan (*Oxyuranus scutellatus)*, produce hypotensive effects by generating cyclooxygenase metabolites (dilator prostaglandins or prostacyclin) that may be involved in the release of endogenous mediators, such as BmooPLA$_2$-I, from the Brazilian lancehead (*Bothrops moojeni)*, has not been reported [152].

Snake venom PLA_2 has been reported to exhibit cytotoxic, bactericidal, and anti-viral activities. Some toxins elicit these activities in a catalytic-dependent manner, while PLA_2-homologues have also been reported to have these effects, suggesting that catalytic activity is not fully involved in inducing the mentioned biological effects [153,154]. Further studies are needed to reveal the molecular mechanisms involved in inducing these effects.

2.4. Snake venom L-Amino Acid Oxidases (LAAOs)

The L-Amino acid oxidases are widely present in snake venoms, as well as in other organisms [155–157]. These flavoproteins are responsible for the yellow color in snake venoms. They catalyze the stereospecific oxidative deamination of L-amino acids producing the corresponding alpha-keto acid, ammonia, and hydrogen peroxide (H_20_2) (Figure 11). LAAOs are homodimeric proteins and consist of three domains (Table 1). Each monomer has a molecular mass of 57-68 kDa. The enzyme typically makes up 1%-4% of the venom by weight. However, in certain species, such as the Malayan pit viper (*Callosellasma rhodostoma),* the enzyme can represent up to 30% of the dried venom by weight [167]. The presence of LAAOs in snake venoms and their biological activities can enhance the actions of other major toxins and contribute to either prey immobilization or increased toxicity in snakebites.

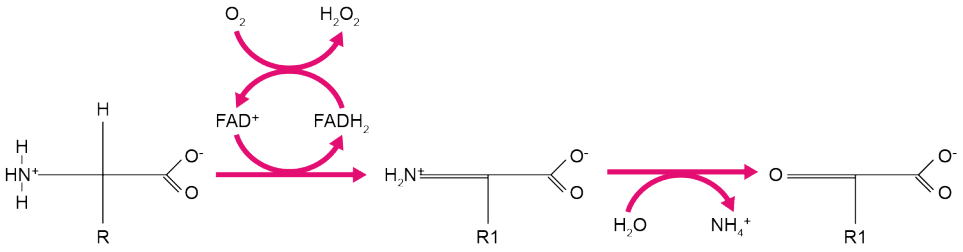


Figure 11. Catalyzed reaction of snake venom LAAOs.

Table 1. Domains of snake venom LAAOs

Domains	Amino acid residues range
FAD-binding domain	35-64, 241-318, 446-471
Substrate-binding domain	5-25,73-129, 232-235,323-420
Helical domain	130-230

The Flavin Adenine Dinucleotide (FAD)-binding domain stabilizes the FAD^+ molecule necessary for catalysis. This domain's secondary structure consists of six β-sheets and five α-helices with the addition of two short β-sheets and one α-helix. Four of the six β-sheets are parallel, and the remaining two are antiparallel. Both short β-sheets are parallel to each other. This domain contains the consensus sequence of glycine residues (G40XG42XXG45) that are involved in access to the negatively charged phosphate group of the coenzyme. Additionally, several salt-bridges stabilize this domain [159,160]. The substrate-binding domain interacts with the amino acid to be oxidized, and it contains six α-helices and eleven β-sheets [159,160]. Finally, the helical domain is the only one with a continuous sequence. The domain's secondary structure consists of six α -helices with one short α -helix, and several loops [159,160] (see Figure 12).

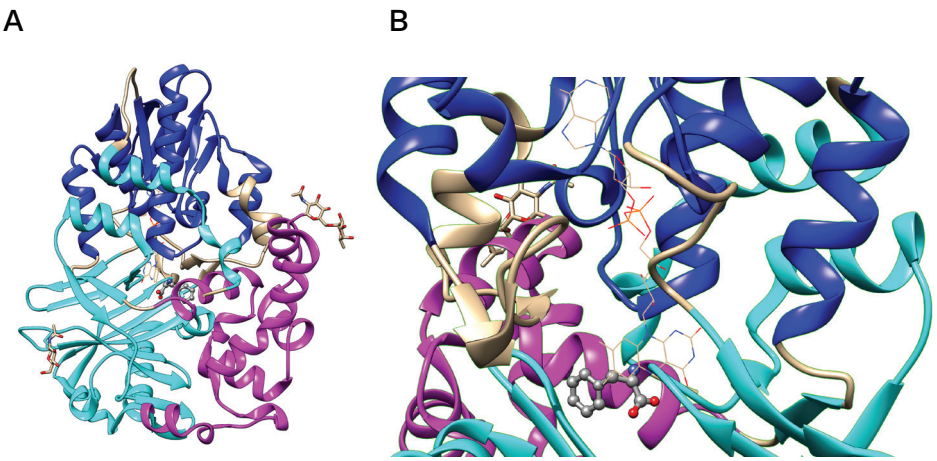


Figure 12. Structure of snake venom LAAO. (A) the FAD-binding domain is shown in blue. The substrate-binding domain is displayed in cyan (blue-green), and helical domain is presented in magenta. Glycosilations are depicted as sticks. (B) A phenylalanine residue is shown in balls and sticks in the active site, while FAD is depicted as wire. Figure prepared from PDB code 2IID.

The most important residue for catalysis is His223, which is conserved in all LAAOs found in snake venoms [166,168]. This residue acts as a nucleophile by deprotonating the α-amino of the substrate (amino acid) [157,159]. Additionally, this amino acid has two conformations, A and B. During catalysis, His223 spends 40% of its time in conformation A and 60% in B. As conformation A, this residue impedes the entry of oxygen; whereas, in conformation B, oxygen can freely enter and participate in the final formation of hydrogen peroxide to recover the FAD^+ [161]. Although LAAOs oxidize all L-amino acids, they prefer aromatic and hydrophobic amino acids, such as phenylalanine and leucine [159,160,162].

It is important to note that these toxins are glycoproteins. In some cases, the removal of carbohydrates is known to reduce the enzymatic and biological effects caused by snake venom LAAOs [163,164]. However, deglycosylation does not affect the enzymatic activities of LAAOs from lancehead pitvipers such as *Bothrops pauloensis*, *B. jararaca*, *B. alternatus*, and *B. moojeni* [165–167]. Therefore, further studies are needed to determine the precise role of glycosylation in snake venom LAAOs and their implications on the biological effects induced by these enzymes. Some LAAOs are known in the venom of medically important Colombian species, including *Crotalus durissus, Bothriechis schlegelii*, and *Micrurus mipartitus* [135,168,169]. These enzymes induce several biological effects, which are described below [135,168–170].

Biological activities induced by snake venom LAAOs

The biological effects of LAAOs are attributed to the hydrogen peroxide (H_20_2) generated in the catalytic reaction. The effects are inhibited by H_20_2 scavengers such as catalase [171–174]. However, several studies demonstrate that biological effects are not completely recovered by incubation with catalase, suggesting that the effects produced by LAAOs are not solely due to the production of H_20_2 [157,175,176].

Hemorrhage

Some LAAOs found in snake venoms can induce hemorrhage by themselves [165,177–179]. This effect is attributed to the capacity of snake

venom LAAOs to induce apoptosis in endothelial cells. This leads to the rupture of the endothelium and the extravasation of red blood cells. The accumulation of H_20_2 in blood vessels may be responsible for the apoptotic effect on endothelial cells [177]. Thus, it is suggested that this effect contributes to the hemorrhagic activity provoked by SVMPs.

Edema

Some snake venom LAAOs can induce edema. However, the precise mechanism that produces this effect is not entirely understood. A LAAO from the Japanese snake mamushi (*Gloydius blomhoffii*) is reported to stimulate lymphocytes and monocytes to release proinflammatory cytokines, IL-6, IL-2, and IL-12, which may explain the inflammatory activity of this enzyme [180]. However, Izidoro et al. [171] suggested that edema formation is due to the activation of the inflammatory response by the H_2O_2 generated, as administration of glutathione (an antioxidant) to the mouse paw inhibits the edema-inducing activity of the enzyme [181].

Modulation of platelet aggregation

The effect of snake venom LAAOs on platelet aggregation is inconclusive. Some enzymes induce platelet aggregation, whereas other toxins produce inhibition of these cells' aggregation [162]. Du and Clemetson [182] proposed that hydrogen peroxide generated in the catalytic reaction of LAAOs is responsible for inducing and inhibiting platelet aggregation. The inhibitory activity may be related to the hydrogen peroxide that impedes the interaction between fibrinogen and its receptor on the surface of the platelet (GPIIb/IIIa) or reduces the binding of ADP to the platelets [183,184]. On the other hand, platelet activation may be a consequence of thromboxane A_2 production elicited by H_2O_2 [172]. Despite this evidence, further systematic studies are required to determine why LAAOs can either elicit or inhibit platelet aggregation.

Other biological activities that are not related to snakebite envenoming

LAAOs induce several activities that make them attractive for searching for therapeutic alternatives for protozoal, viral, bacterial infections, and cancer due to their cytotoxicity (see Chapter 10). Several snake venom LAAOs have antileishmanial activity [171–173,185–187]. This effect is attributed to the H_2O_2 generated by the catalytic cycle of the enzymes.

Likewise, a snake venom LAAO isolated from *Trimeresurus stejnegeri* (Chinese green tree viper) has antiviral activity against HIV-1 [179]. LAAOs have also demonstrated antibacterial activity against both gram-negative and gram-positive bacteria [162,168,181,188]. The mode of action of snake venom LAAOs for inducing this effect is attributed to H_2O_2. However, the binding of the enzyme to the bacterial surface and further concentration of H_2O_2 seems to be essential for this effect [189]. Several snake venom LAAOs show cytotoxic activity against tumor cell lines [199]. They can induce apoptosis in these cells. This effect involves H_2O_2, but it is also speculated that glycan moieties are crucial for the interaction with the target cell [191].

2.5. Snake venom disintegrins

The term "disintegrin" was first introduced to toxinology in 1987 by Huang et al. [192]. They described the isolation and characterization of Trigamin from the venom of *Craspedocephalus gramineus* (Common bamboo viper). However, today this term is used to define toxins with a length between 40-100 amino acids present in the snake venom of the Viperidae. These toxins are produced by proteolytic processing of P-II and P-III SVMPs. Initially, the Arg-Gly-Asp (RGD) motif was described as an essential structural characteristic for their main activity, which is to inhibit platelet aggregation by blocking β1 and β3 integrins [198].

Structure of snake venom disintegrins

Snake venom disintegrins can be classified into four groups based on their number of amino acids and disulfide bonds [193,194]. The first group comprises short disintegrins from 41 through 51 residues and four disulfide bonds. The second group consists of medium size disintegrins that contain about 70 amino acids and six disulfide bonds. The third group comprises long peptides with approximately 84 amino acids, and seven cysteine bonds cross-link them. The fourth group incorporates homo and heterodimers. These disintegrins are composed of 67 amino acids and ten cysteine residues, involved in stabilizing the structure by forming intra and interchain disulfide bonds.

The RDG motif is the most common in snake venom disintegrins. Nevertheless, other motifs have also been identified. Table 2 describe the diversity of these motifs and their molecular targets (mainly integrins). Structural studies of short, medium, large size, and dimeric disintegrins revealed a mobile loop that contains the active motif, which protrudes 14–17 Å from the protein core [193,194,195] (see Figure 13).

Table 2. Integrins, their function, and snake venom disintegrins that inhibit them.

Integrin	Function	Disintegrins that inhibit it*	References
α5β1	The major fibronectin receptor	RGD, VGD and MDG	[145,147, 148,193, 197,198]
α8 β1	Tenascin receptor	RGD	
αvβ1	Vitronectin receptor	RGD	
αvβ3	Vitronectin receptor	RGD, WGD	
αIIbβ3	Platelet fibrinogen receptor involved in platelet aggregation	RGD, WGD and KGD	
α4β1	Fibronectin and VCAM-1 receptor	MLD	
α4β7	Fibronectin, VCAM-1 and MdCAM receptor	MLD	
α3β1	Laminin receptor	MLD	
α6β1	Laminin receptor	MLD	
α7β1	Laminin receptor	MLD	
α9β1	Tenascin receptor	MLD	
α1β1	Collagen IV receptor	KTS and RTS	

*Standard Amino Acid 1-letter Code.

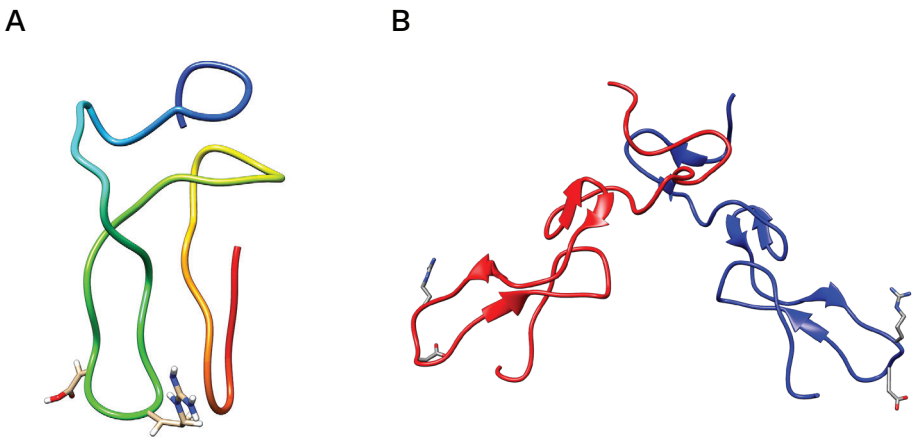


Figure 13. Structure of snake venom disintegrins. (A) Monomeric disintegrin; (B) Heterodimeric disintegrin. The blue and red chains represent different subunits. The RGD motif is displayed in the sticks. N-term and C-term represent the N-terminal and C-terminal of each chain, respectively. This image was prepared from PDB structures with codes 2MOP (monomeric) and 1TEJ (heterodimeric).

Biological activities induced by snake venom disintegrins

Due to their capacity to block integrins, the most important activity induced by snake venom disintegrins is the inhibition of platelet aggregation. This can contribute to the hemostatic disorders caused by SVSPs, SVMPs and other toxins in snakebite envenoming [199–203]. In fact, the anti-platelet aggregation activity of disintegrins has made them essential templates for the development of drugs used as antithrombotic agents. For instance, Tirofiban is derived from echistatin found in the venom of *Echis carinatus* (Phoorsa snake) [204] and, Eptifibatide obtained from barbourin present in the venom of *Sistrurus miliarius barbouri* [200,201,205] (see Chapter 10).

Additionally, snake venom disintegrins have shown potential in anticancer therapy. Disintegrins can target a wide variety of integrins; and, thus, it is plausible that they have the potential to interfere in essential processes involved in carcinogenesis, tumor growth, invasion, and migration (see Chapter 10). Thus, disintegrins can be used as tools and potential drugs for the treatment of this health problem [197,206–208].

2.6. Three-finger toxins (3FTx)

Structure

The protein family known as three-finger toxins consists of non-enzymatic polypeptides ranging from 60 to 62 amino acid residues (short chain) and between 66 and 74 amino acid residues (long chain). They are typically monomers and contain 4 to 5 highly conserved disulfide bridges. Their folding is characterized by three loops with beta strands that run from a hydrophobic core that is in turn crossed by four disulfide bonds. Venoms from elapids, hydrophids, and colubrids snakes contain these proteins [209].

The primary structure of a protein is determined by its sequence of amino acids that are represented as beads on a necklace. To have a complete and functional structure, specific interactions between amino acids are necessary for proper folding. Figure 14 shows the primary and tertiary

structure of mipartoxin-I, a toxin found in the venom of *Micrurus mipartitus* (Redtail coralsnake).

Although the members of the 3FTx family share conserved structural features that enable them to fold and maintain their integrity, some modifications also occur. These modifications may be related to the diversity of their functions and their affinity for certain molecular targets [210,211]. In addition to the eight cysteine residues present in the central region that favor the formation of disulfide bridges, the aromatic residues of tyrosine or phenylalanine in positions 25 and 27 are also conserved and contribute to the adequate folding and stability of the beta sheet structure in this toxin. The stability of native conformation is also contributed by arginine residues at position 39 and aspartic acid at position 60, as they form salt bridges with any of the terminal ends [209,212]. Table 3 lists the reported variations described for the 3FTx structures, including the main ones.

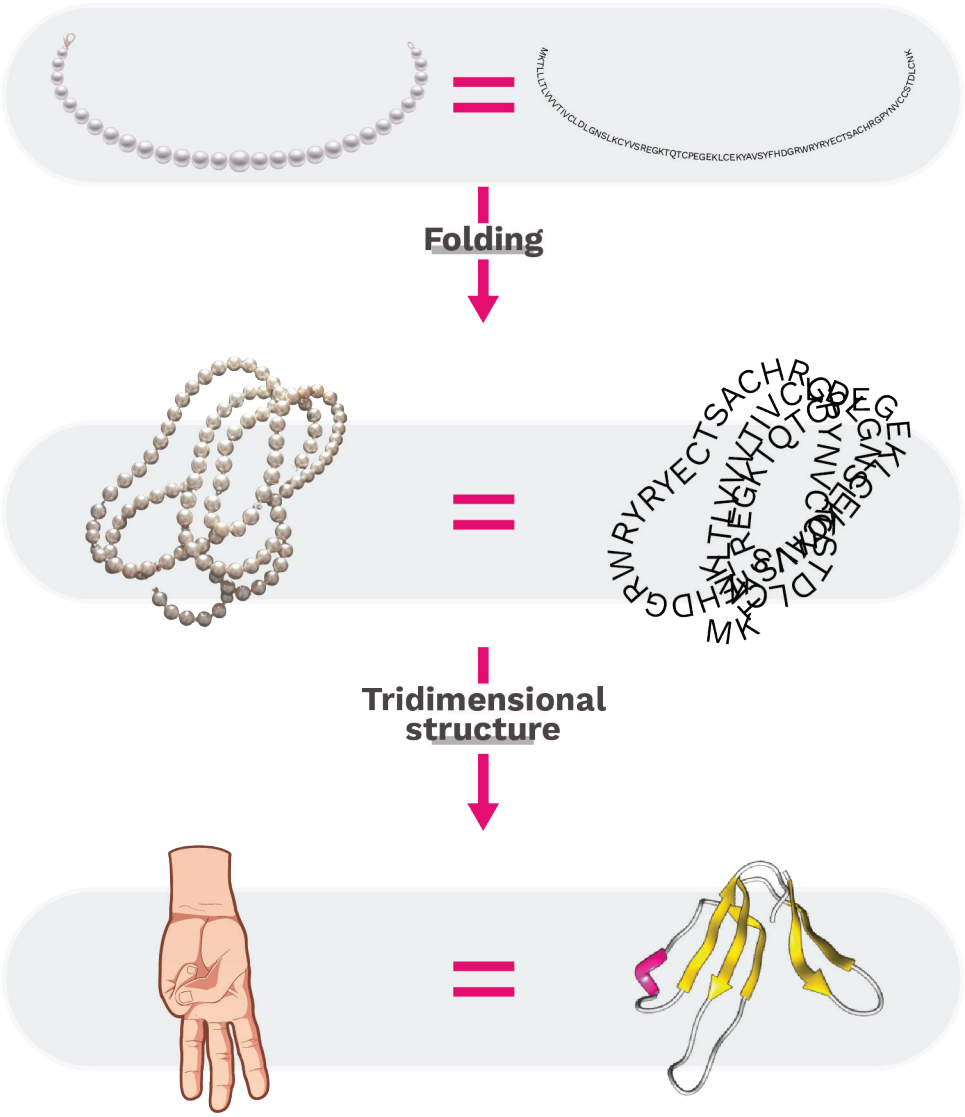
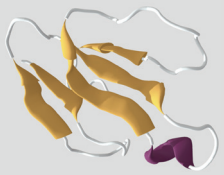
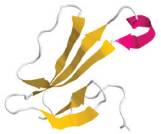
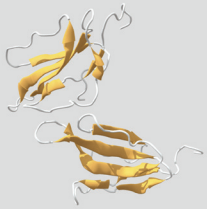


Figure 14. Primary structure of mipartoxin-I from the venom of *Micrurus mipartitus* (GenBank code: AVI57319.1) is represented as a long chain. Interactions between amino acid residues facilitate folding at various sites of the chain, contributing to secondary and tertiary structure conformation. Mipartoxin-I, has a tridimensional structure that resembles the three central fingers of a hand. That is why it belongs to the protein family known as three-finger toxins (3FTx) [210].

Table 3. Some examples of the structural variations found in the family of three-finger toxins.

Variation / Tridimensional structure	Toxin name	Snake species	Annotations	References
Short chain 	Erabutoxin (PDB code 1QKE)	*Laticauda semifasciata* Chiness sea snake (Elapidae)	62 amino acids	[213]
Long chain 	Bungaro-toxin (PDB code 1IKC)	*Bungarus multicinctus* Krait with many bands from Taiwan (Elapidae)	74 amino acids	[214]
Presence of a fifth disulfide bond in loop I 	Recombi-nant WTX (PDB code 2MJ0)	*Naja kaouthia* South and Southeast Asian monocled Cobra (Elapidae)	Although it was previously classified as a weak neurotoxin, recent studies show that toxins with a fifth disulfide bond in that position are highly toxic and bind to nicotinic acetylcholine receptors at concentrations in the nanomolar range. These toxins are referred to as “non-conventional 3FTx” due to their differing selectivity and specificity from canonical neurotoxins. An additional example is the BM14 toxin found in the venom of *Bungarus multicintus* from Taiwan. This toxin exhibits a higher affinity for the muscarinic receptor of acetylcholine M2 than for M1	[215,216]
Presence of a fifth disulfide bond in loop II 	NTX-1 (PDB code 1NTN)	*Naja oxiana* Central Asian cobra (Elapidae)	The fifth disulfide bond in loop II provides a twist and forms a short helical segment at the tip of the loop. This additional binding plays an important role in specificity on a7 nicotinic acetylcholine receptor blockade. However, toxins such as candoxin and haditoxin that lack this variation, also bind to this receptor	[217]

Extensions in N-terminal or C-terminal 	Denmotoxin (PDB code 2H5F)	*Boiga dendrophila* Mangrove snake or the Gold-ringed cat snake from Malaysia (Colubridae)	The protein has an extended N-terminal segment with seven additional residues, which are covered by a pyroglutamic acid. The effect of this structural variation on its function is currently unknown. Typically, long-chain neurotoxins have 2-9 additional residues at the C-terminus	[218]
Dimers with non-covalent bonds 	Haditoxin (PDB code 3HH7)	*Ophiophagus hannah* King Cobra from India to Southeast Asia (Elapidae)	Most 3FTx are monomers, but some can exist naturally as dimers. Haditoxin is a homodimer, with two short-chain monomers that are non-covalently bonded and oriented in opposite directions. This gives haditoxin the ability to function as an antagonist of muscle (abgd) and neuronal (a7, a3b2 and a4b2) nicotinic acetylcholine receptors	[219]
Covalently bonded dimers 	Irditoxin (PDB code 2H7Z)	*Boiga irregularis* Brown tree snake of Australia, Papua New Guinea, and islands of northwestern Melanesia (Colubridae)	The heterodimer consists of two non-conventional 3FTx subunits, each containg an extra cysteine that forms an interchain disulfide bond. Additionally, the N-terminal has a seven amino acid residue extension. This results in a potent postsynaptic neurotoxin Other examples include the a-cobrotoxin/cytotoxin heterodimer, which binds to the muscle nicotinic acetylcholine receptor a7 but has no cytotoxic activity, and the homodimer of a-cobrotoxin that recognizes the neuronal nicotinic acetylcholine receptor a3b2 like k-bungarotoxin	[218]
Synergistic toxins 	SynTx (PDB code 7C28)	*Dendroaspis jamesoni* Congo green mamba (Elapidae)	The molecule is a homodimer connected by an interchain disulfide bond. Dimerization occurs through the loops II and III of the monomers. Although this group contains molecules with a similar amino acid sequence to other neurotoxins and cytotoxins of the 3FTx family. Its toxicity may be low or null. However, its importance lies in the fact that it increase the toxicity of other venom proteins (synergism), such as angusticeps-like toxins, which, in turn, behave as cholinesterase inhibitors.	[5,221,222]

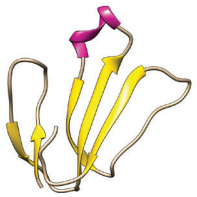
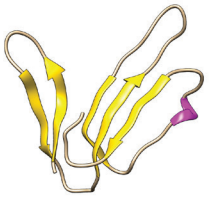
Tridimensional models taken from PDB and prepared in UCSF Chimera v.1.17.3. All these toxins have a 3D model view available in https://serpientes.ins.gov.co/

Recently, some authors proposed a guideline to systematically separate and study three-finger toxins due to the high variability between the amino acid sequences of these proteins [223–225]. They assembled a database with selected 3FTx sequences known for *Micrurus* species from Brazil and classified the proteins based on their similarity and structural identity. Accordingly, the authors established parameters for proposing a reclassification based on key functional amino acids, disulfide bridges, charge distribution on the surface, and the phylogenetic relationship of the sequence [223–225]. The functionality of the protein can be attributed to nine groups based on sequence homology. This classification only serves as a reference for the three-finger toxins found in *Micrurus* species from South America.

The models predicted from the primary sequences of Brazilian specimens were based on homologous sequences of Old-World elapids, including *Dendroaspis polylepis, Naja atra, Bungarus multicinctus, B. candidus, Ophiophagus hannah, Dendroaspis jamesoni* and *Hemachatus haemachatus* (Ring-necked spitting cobra). Only one sequence was built from fulditoxin of *Micrurus fulvius* venom (PDB code 4RUD). The newly conformed nine groups included representative local specimens such as *Micrurus altirostris, Micrurus carvalhoi, Micrurus corallinus, Micrurus frontalis, Micrurus lemniscatus, Micrurus paraensis, Micrurus spixii,* and *Micrurus surinamensis* [224].

Only a small number of three-finger toxins have been purified and characterized from Colombian *Micrurus* venoms. Currently, only three toxins have three-dimensional models predicted from sequences reported in the literature. Two of these toxins belong to Colombian medically important snake species (*M. mipartitus* and *M. dumerilii*; Table 4) [225]. Moreover, they exemplify the bicolor and monadal pattern groups, respectively [235]. Another species in the monadal pattern group, *M. clarki* has also had 3FTx research conducted on it [227].

Table 4. Three finger toxins structures purified from some *Micrurus* venoms in Colombia. Each sequence is assigned to a Uniprot accession number

Three finger toxins name and UniProt accession number	**Snake species**	**Sequence**	**Predicted 3D model**	**References**
Clarkitoxin-I-Mdum C0HLK5	*Micrurus dumerilii* Capuchin coral snake Elapidae	RICDDSSIPFLRTPQLCPKGQDVCYK- KTPIVKKFKWLQKKGCASSCPKDG- FIKIFKIECCTKDNCI		[226]
Mipartoxin-1 B3EWF8	*Micrurus mipartitus* Redtailed coral snake	MKTLLLTLVVVTIVCLDLGNSLKCYVS- REGKTQTCPEGEKLCEKYAVSYFH- DGRWRYRYECTSACHRGPYNVCCST- DLCNK		[226]
Clarkitoxin-1 C0HK04	*Micrurus clarki*	RICDDSSIPFLRTPQLCPKGQDVCYK- KTPIVKKFKWLQKKGCASSCPKNG- FIKIFKIECCTKDNCI		[227]

Sequences used to predict the three-dimensional structures presented using the SWISS MODEL Software [239,240]. Molecules adjusted and prepared in UCSF Chimera v.1.17.3

Biological activities

Although 3FTx family toxins share a structural similarity, they exhibit a wide variety of pathophysiological activities and mechanisms of action, including postsynaptic neurotoxicity, cytotoxicity, cardiotoxicity, anticoagulant, and antiplatelet activities. In addition, they act as L-type Ca^{2+} channel antagonists and can synergistically promote hypotension with muscarinic toxins [223]. The 3FTx have a small, compact, and stable structure with multiple interaction sites to achieve various functions that have been evolutionarily optimized [230].

Neurotoxins

Neurotoxicity is possibly the best-known effect of 3FTx due to the drastic effect of flaccid paralysis and respiratory failure in snakebite accidents. Interference with the signal transmission can occur by different routes. In the case of altered cholinergic transmission in the central and peripheral nervous systems, neurotoxicity can occur at various sites in the postsynaptic region, affecting both nicotinic and muscarinic receptors [15] (Figure 15). The following are examples of toxins and their mechanisms of action that lead to neurotoxicity:

Curare-like toxins or α-neurotoxins.— These are similar to the alkaloid curare (from the plant extract from indigenous peoples in Central and South America). They are long and short chain neurotoxins that bind to the muscle acetylcholine receptor a1 and prevent effective neuromuscular transmission. In contrast, long-chain neurotoxins bind with high affinity to the neuronal acetylcholine receptor α7.

Muscarinic toxins.— These comprise toxins that act as agonists or antagonists on muscarinic receptors. Muscarinic toxin α (MTα) isolated from mamba venom (*Dendroaspis jamesoni*) is a potent α2B-adrenoceptor antagonist [212,223].

α-neurotoxins.— These specifically bind to the neuronal nicotinic acetylcholine receptor (α3β4). They resemble long-chain a-neurotoxins in structure but occur as dimers. They do not recognize a1 acetylcholine receptor.

Recently, micrurotoxins 1 and 2 (MmTX1 and MmTX2), isolated from the venom of *Micrurus mipartitus* (Redtailed coralsnake), have been described as agonists at the ionotropic $GABA_A$ receptor that enhances receptor opening and desensitization.

Acetylcholinesterase inhibitors interfere with neuromuscular transmission by inhibiting binding to the peripheral site of the acetylcholinesterase enzyme (AChE) present at the neuromuscular junction. This prevents the AChE from capturing and degrading acetylcholine, leading to muscle twitching due to the accumulation of acetylcholine in the synaptic cleft. These inhibitors are known as fasciculins and have similar structures to short-chain neurotoxins [209,212].

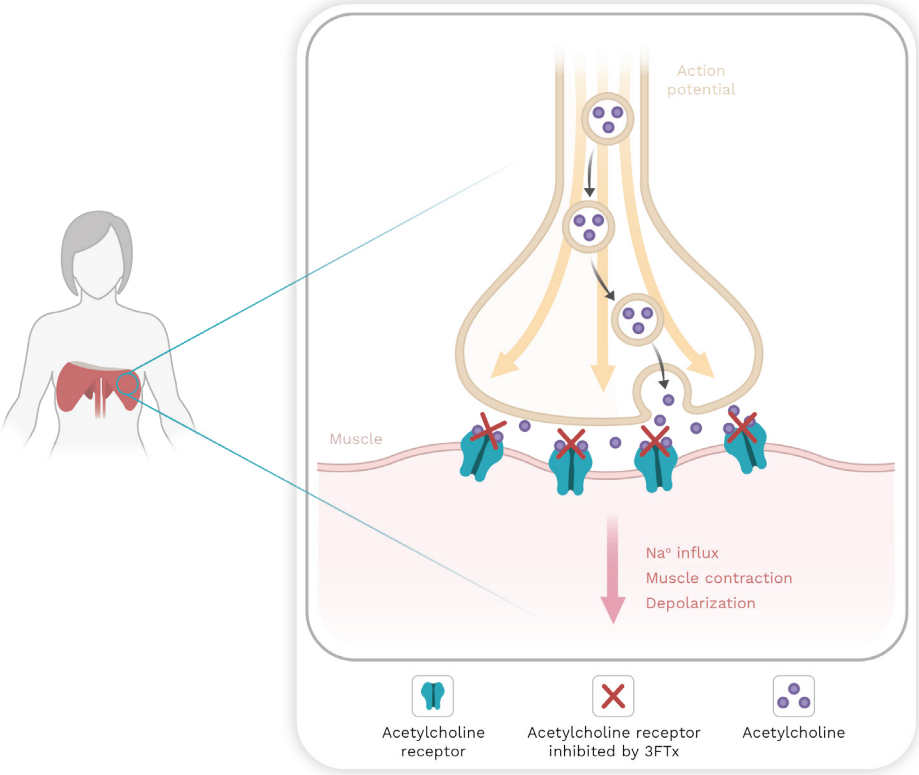


Figure 15. Three-finger toxins block acetylcholine receptors on the effector cell in the neuromuscular junction, leading to flaccid paralysis in myocytes, and respiratory failure when diaphragm is affected. Image created with BioRender and edited by Oscar A. Ramirez Ruiz.

Cardiotoxins

Cardiotoxins are a group of toxins that have been found exclusively in cobra venoms, particularly in species of *Naja*. These toxins are similar to short-chain neurotoxins, contain four disulfide bonds, and they represent the second largest group after neurotoxins. They are responsible for increasing the heart rate and can lead to cardiac arrest in a dose-dependent manner. Additionally, many toxins in this group also have cytolytic effects, forming pores in lipid membranes; they are also known as cytolysins. Finally, another subgroup is recognized within this classification, the b-cardiotoxins and related toxins. This subgroup exhibits blocking activity of cardiac β1 and β2 adrenergic receptors [212].

L-type calcium channel blockers

Structurally, these channel blockers are polypeptides similar to short-chain neurotoxins. They bind to the 1,4-dihydropyridine binding site of L-type calcium channels and physically block calcium currents, leading to relaxation of smooth muscle and inhibition of cardiac contractions [212].

Hemostasis disorders

Only a few 3FTx have the amino acid sequence RGD (Arginine-Glycine-Aspartic Acid) in an accessible site for interaction. The RGD tripeptide participates in the adhesive function of several proteins, interfering with the interaction between fibrinogen and its receptor complex for glycoprotein IIB-IIIa ($\alpha_{IIb}\beta_3$) inhibiting platelet aggregation [223].

The KT-6.9, toxin isolated from the venom of the cobra *Naja kaouthia* inhibits platelet aggregation induced by adenosine diphosphate (ADP), thrombin, and arachidonic acid. As a result, it inhibits platelet aggregation (primary hemostasis) in a dose-dependent manner [223]. In secondary hemostasis, the cobra venom of the species *Hemachatus haemachatus* (Ring-necked spitting Cobra) contains ringhalexin and exactin that inhibit the activation of coagulation factor X, causing coagulation alterations [223].

Interaction with sodium channels

Some 3FTxs interact with acid-sensitive ion channels (ASIC) that are normally selective ion channels for Na^+ and are activated by protons. ASICs are widely distributed throughout the peripheral and central nervous system of vertebrates. These channels are involved in synaptic plasticity, neurodegeneration, and pain perception [223].

2.7. Snake Venom Serine Proteinase (SVSP)

Structure

Serine proteinases are glycosylated proteins with enzymatic activity. Their molecular weight ranges between 26 and 67 kDa, depending on the degree of N- or O-glycosylation. The subgroup known as thrombin-like serine proteinases is responsible for degrading fibrinogen and amplifying bleeding conditions during envenomation [244]. In *Bothrops asper* venom (common lancedhead pitviper) serine proteinases are abundant constituents representing between 5%-18% of the proteins, depending on the age of the snake and their geographic region [235]. These proteases, along with hyaluronidases, metalloproteinases, and phospholipases A_2 belong to the group of hemotoxic enzymes that are important to viper venoms [232].

These proteases are characterized by a typical chymotrypsin fold and two barrels of 6-stranded β sheets (Figure 16), their active site is located in the cleft between the latter and includes a canonical catalytic triad of His-Asp-Ser. Although they share between 50%-80% identity in their amino acid sequences, they differ significantly in their substrate specificity, and therefore, in their activity [233]. Several SVSPs have pharmacologic activity (see Chapter 10). For instance, Mambalgins are toxins found in black mamba venom (*Dendroaspis polylepis*). They have analgesic effects by blocking channels with the ASIC1a and ASIC2a subunits in the central nervous system, as well as channels that include the ASIC1b subunit in nociceptors [229].

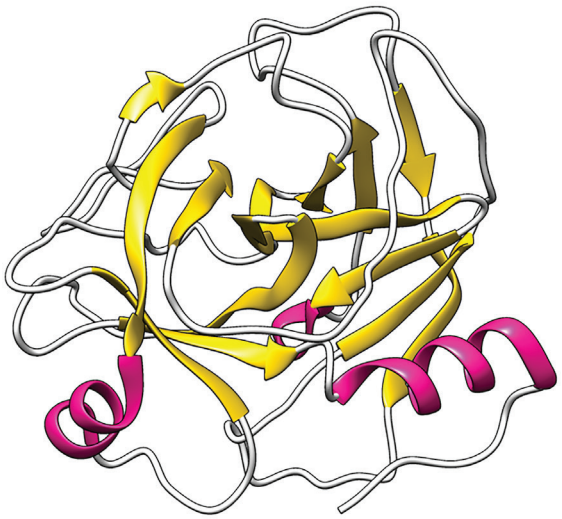


Figure 16. AaV-SP-I, a glycosylated serine proteinases found in the snake venom of *Agkistrodon acutus*. Tridimensional model taken from PDB (code 1OP0) and prepared in UCSF Chimera v.1.17.3

Biological activities

Hemotoxic and coagulopathic

The human body can counteract the hemorrhagic effect produced by metalloproteinases. However, the thrombin-like serine proteinases present in the venom of viperids prevent the formation of an effective fibrin plug. Under physiological conditions, endogenous thrombin cleaves specific regions of fibrinogen in the circulation before bleeding. This exposes the site for binding other fibrinogen molecules undergoing the same hydrolysis, forming a fibrin mesh that stabilizes the clot and promotes hemostasis.

Thrombin-like serine proteinases simulate thrombin activity, but they hydrolyze different sites on the fibrinogen molecule. This alters the binding between fibrinogen molecules, resulting in irregular consumption of fibrinogen and unstable fibrin clot formation (Figure 17) [234–237]. While many serine proteinases act on the α chain of fibrinogen, some purified from *Agkistrodon* species act on both α and β chains [238]. In addition, this subgroup of enzymes has the ability to activate coagulation factors VIII and XIII, as well as thrombin. They are also responsible for the activation of protein C, platelet aggregation, activation of plasminogen, and factor V. These actions can result in consumptive coagulopathy (Figure 17) [238,239].

Asperase was the first serine proteinases isolated from the venom of *Bothrops asper*. It is a glycosylated enzyme with a molecular mass of 30 kDa. This protein promotes defibrinogenation in murine models and causes behavioral alterations, such as loss of the righting reflex, opisthotonos (spasm of the muscles causing backward arching of the head, neck, and spine), and intermittent rotations along the longitudinal axis of the body. This characteristic is similar to that of Gyroxin, a thrombin-like enzyme found in the venom of *Crotalus durissus* from Brazilian populations [236,240].

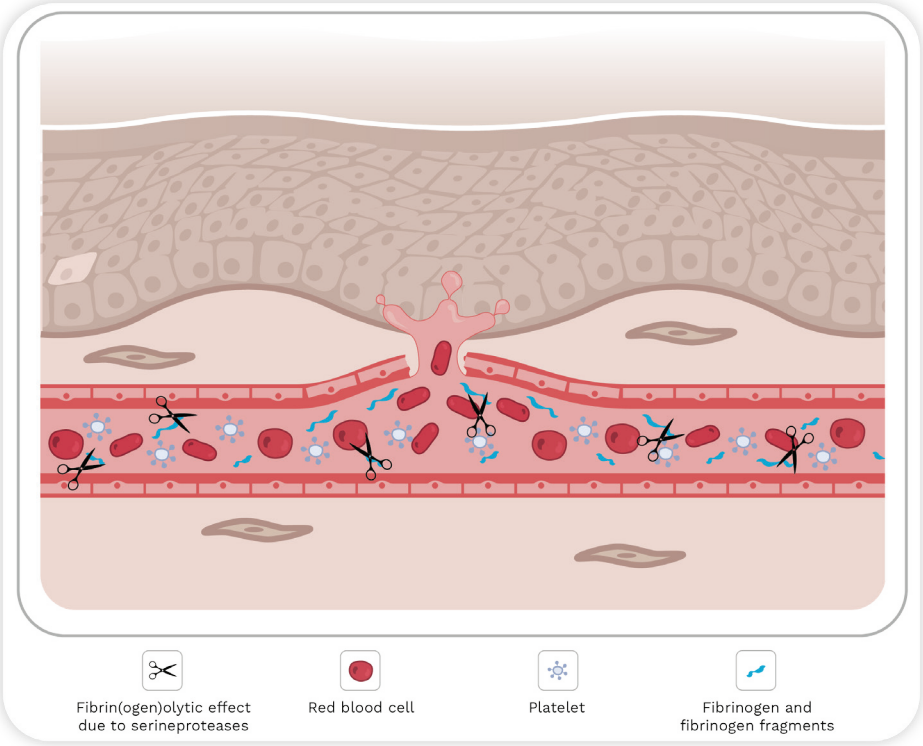


Figure 17. Hemostatic alteration model. Circulating fibrinogen molecules (represented in blue) undergo inadequate hydrolysis by thrombin-like serine proteinases, favoring their erratic and excessive consumption and resulting in deficient fibrin clot formation and unsatisfactory hemostasis. Additionally, platelets (structures in white) aggregate inappropriately at sites other than the injury site, leading to inappropriate consumption, uncontained bleeding, and the presence of microthrombi. The image was created with BioRender and edited by Oscar A. Ramirez Ruiz.

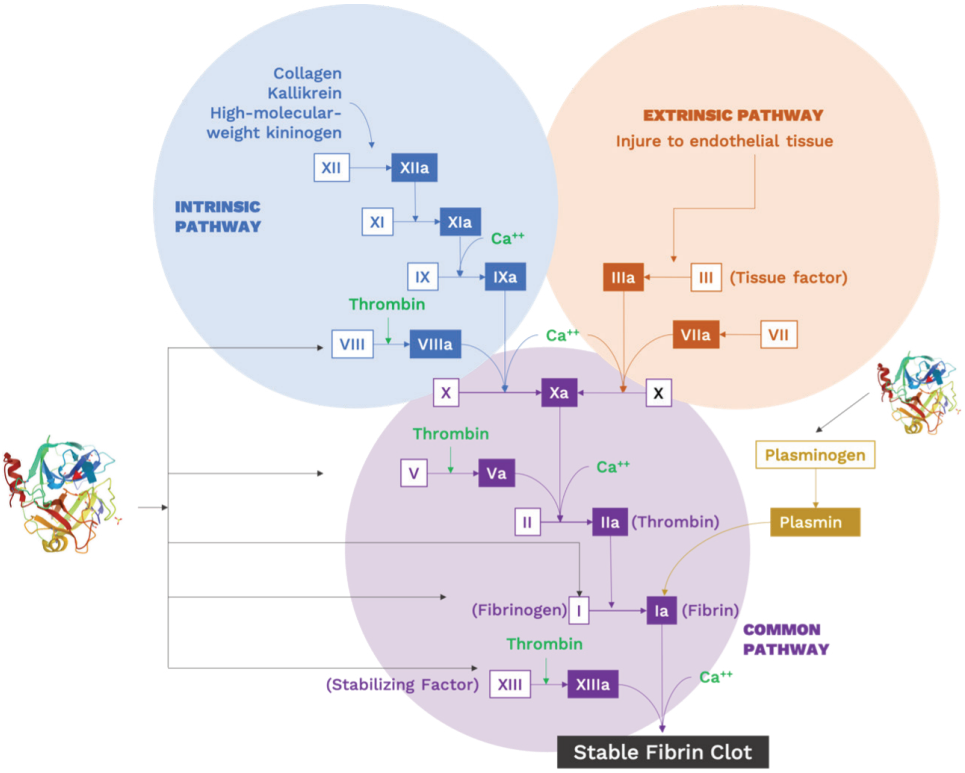


Figure 18. Schematic of the blood coagulation cascade pathways under physiological conditions indicating the respective sites of action of snake venom serine proteinases (SVSP). Red arrows indicate activation by SVSPs. The black arrow indicates fibrinogenase activity, causing inadequate consumption of fibrinogen, and the dotted arrow highlights the thrombin-like activity (see more information within the text). Additionally, SVSPs act as platelet activation enzymes, allowing excessive and inappropriate aggregation of platelets. Moreover, SVSPs activate protein C (not shown in the figure) and act as an anticoagulant by irreversibly inactivating factor Va and factor VIIIa. SVSPs can also act as kallikrein-like enzymes, converting kininogen into bradykinin and exacerbating the manifestations associated with hemodynamic disorders.

Hypotension
The production of bradykinin (BK) by kallikrein-like serine proteinases, both with the inhibition of BK degradation by bradykinin potentiating peptides (BPP), results in an overall increase in BK levels. As a result, stimulation of the bradykinin B_2 receptor induces vasodilation, as well as anti-fibrotic and anti-inflammatory effects, through various intracellular mechanisms [149,235].

Some isolated serine proteinases, mainly from viperid venoms but also in elapids such as the sea snake *Hydrophis hardwickii* (Spine-bellied sea snake), exhibit hypotensive activity similar to kallikrein that releases bradykinin and causes vasodilation. These proteases degrade angiotensin I into angiotensin II that is then converted into tetrapeptides that do not have hypertensive activity. Afterwards, fibrinogen levels in the blood decrease, altering blood flow, resulting in the release of BK and Met-Lys-bradykinin from kininogen that act on β_1 and β_2 receptors, causing vasodilation and subsequent hypotension [149].

2.8. C-type lectins (CTL) and C-type lectin-like proteins (CLP)

Structure
Snake venom lectins have properties that are intermediate between the two important families of animal lectins, designated *S* and *C* type lectins. They are extracellular and have this name because they require calcium ions for their sugar-interacting activity. S-type lectins owe their name to the fact that they require thiol group-reducing agents for full activity, both intra and extracellular, and exhibit specificity for galactosides [241].

Several C-type lectins and related proteins have been isolated and characterized from snake venoms. These proteins can be broadly classified into two main groups based on their structural and functional properties: true C-type lectins, and a more complex group of proteins structurally related to C-type lectins, but with different functions, called C-type lectin-like proteins (CLP) [242].

The C-type lectins are calcium-dependent. They are carbohydrate-binding proteins with canonical structures like selectins and mannose-binding proteins. They are homodimers (or higher multimers) with sugar binding properties (mainly galactosides; Figure 19A) [242]. The C-type lectins from snake venom (SVgalLs) that bind to galactosides are an example of true C-type lectins. One example of them is known from *Lachesis stenophrys* venom (Central American Bushmaster) but is also present in venoms of vipers such as *Crotalus atrox* (Western diamond-backed rattlesnake) [243].

The carbohydrate recognition domain (CRD) in SVgalLs is generally represented by three amino acid residues, Gln-Pro-Asp, considered determinants in their calcium-mediated affinity for galactose. The specificity for this carbohydrate is related to the interaction of hydroxyl groups 3 and 4, with residues Q96 and D98 through hydrogen bonds [243].

C-type lectin-like proteins have amino acid sequences similar to C-type lectins, but they do not possess carbohydrate-binding properties. Additionally, any association with Ca^{2+} does not occur through the classical C-type lectin pathway with the binding loop. They are heterodimers, consisting of closely related α and b subunits, generally linked by a disulfide bond, and form an integrated structure by exchanging loops. They can also form larger structures through a higher order multimeric association linked by disulfide bonds or by non-covalent interactions [242]. C-type lectin-like proteins (CLP) are named for their high sequence similarity (15%-40%) with the carbohydrate recognition domains of C-type lectins. The α and β subunits have molecular weights of 14-15 and 13-14 kDa, respectively. Multimerization of these heterodimers can occur through covalent and non-covalent interactions, resulting in αβ, $(αβ)_2$, and $(αβ)_4$ structures. CLP αβ heterodimers are formed by domain swapping, where a domain from one subunit replaces the corresponding domain of the other (Figure 19B) [244].

CLPs, also known as snaclecs, exhibit various activities such as anticoagulant effects and induction or inhibition of platelet aggregation [260], depending on their structure and function. Pharmacological characterization shows that snaclecs can either enhance or inhibit the function of clotting factors, highlighting their potential in drug discovery for blood-related diseases [244].

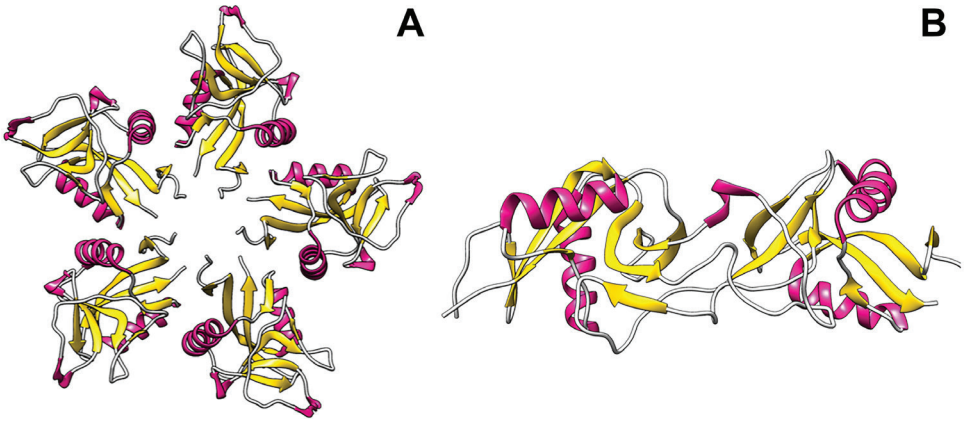


Figure 19. (A) Structure of the C-type lectin purified from *Crotalus atrox* snake venom. PDB code 1MUQ. (B) Structure of a C-type lectin-like protein called flavocetin-A, purified from *Trimeresurus flavoviridis* snake venom. PDB code 1C3A. Molecules prepared in UCSF Chimera v.1.17.3

Biological activities

Haemostatic disturbances

Platelets are essential for hemostasis, and their aggregation is the first step in the blood coagulation cascade. This process depends on the interaction of many platelet surface receptors, including ADP, collagen, and other factors. Venoms contain components that disrupt this interaction leading to continuous bleeding in the prey (Figure 20).

C-type lectins and related proteins target specific glycoprotein or integrin receptors to inhibit or promote platelet aggregation. Most venom lectins achieve their effects by targeting the platelet surface glycoprotein receptor GPIb, and some also target GPIV that are involved in collagen-platelet interaction. Convulxin, isolated from the venom of *Crotalus durissus* from Brazil-

ian populations, appears to participate in the pathway involving GPIV. Snake venom C-type lectins also bind to integrins, as well as von Willebrand factor (vWF), thrombin, and certain unidentified platelet surface receptors [246].

The effects of snaclecs include anticoagulant activity by inhibiting factors Xa and IXa and blocking the interaction between factor Xa and its cofactors in the prothrombinase complex that is dependent on Ca^{2+} [244,247]. For instance, thrombin inhibition that is dependent on calcium [268], and platelet activation, where C-type lectins such as botrocetin and bitiscetin from *Bothrops jararaca* and *Bitis arietans*, preferentially bind to the von Willebrand factor and enlarge the binding surface with the platelet receptor GPIb, stimulate platelet aggregation [262,267]. In addition, the lectin echicetin found in the venom of *Echis carinatus* has antithrombotic properties, it acts on the platelet receptor GPIb, preventing its aggregation [244].

Inflammatory response

The role of SVgalL in the inflammatory response has been investigated using different animal models and approaches to evaluate the participation of leukocytes and the recognition of extracellular matrix (ECM) glycoconjugates in the inflammatory response. Lectins from *Bothrops godmani* (BgL) and *Bothrops jararacussu* (BJcuL) have the ability to induce increased vascular permeability in the mouse leg, resulting in moderate acute edema. This relationship may be associated with the ability of lectins to stimulate mast cell degranulation and the release of histamine and serotonin to trigger local inflammatory responses [243].

Other lectin-related inflammatory events have been observed experimentally. For instance, BJcuL increases leukocyte adherence in microvessels of the cremaster muscles of mice. This toxin can bind to fibronectin and vitronectin glycoproteins, suggesting that the migration of peripheral blood leukocytes to the inflammatory site involves cell adhesion to lectin-mediated extracellular matrix (ECM) proteins. BJcuL not only recognizes glycoligands on the surface of the neutrophil cell, but also promotes polarization and migration and improves adhesion to fibronectin and Matrigel that are components of the ECM. This lectin induces functional activation of neutrophils by increasing phagocytosis and superoxide production. Galatrox, a lectin from *Bothrops atrox* venom, promotes acute neutrophil migration and the release of IL-1α and IL-6 cytokines. In addition, it recognizes neutrophil cell membrane glycoconjugates, interacts with ECM laminin, and induces neutrophil chemotaxis *in vitro* [243].

Effects on kidney function

In isolated rat kidneys, the lectins BiL (from *Bothrops insularis*), BpirL (from *Bothrops pirajai)*, and BmLec (from *Bothrops moojeni*) induced alterations in renal function parameters, including perfusion pressure, vascular resistance, urinary flow, glomerular filtration rate, and ion transport in the tubules. The histological analysis of the kidneys of animals that received BiL suggests a direct lesion of the glomerular and tubular renal cells. The mechanism involves direct cytotoxicity in the glomerular region induced by lectins and the presence of systemic pro-inflammatory mediators such as prostaglandins [242].

EFFECTS OF SNAKE VENOM ON THE HUMAN BODY

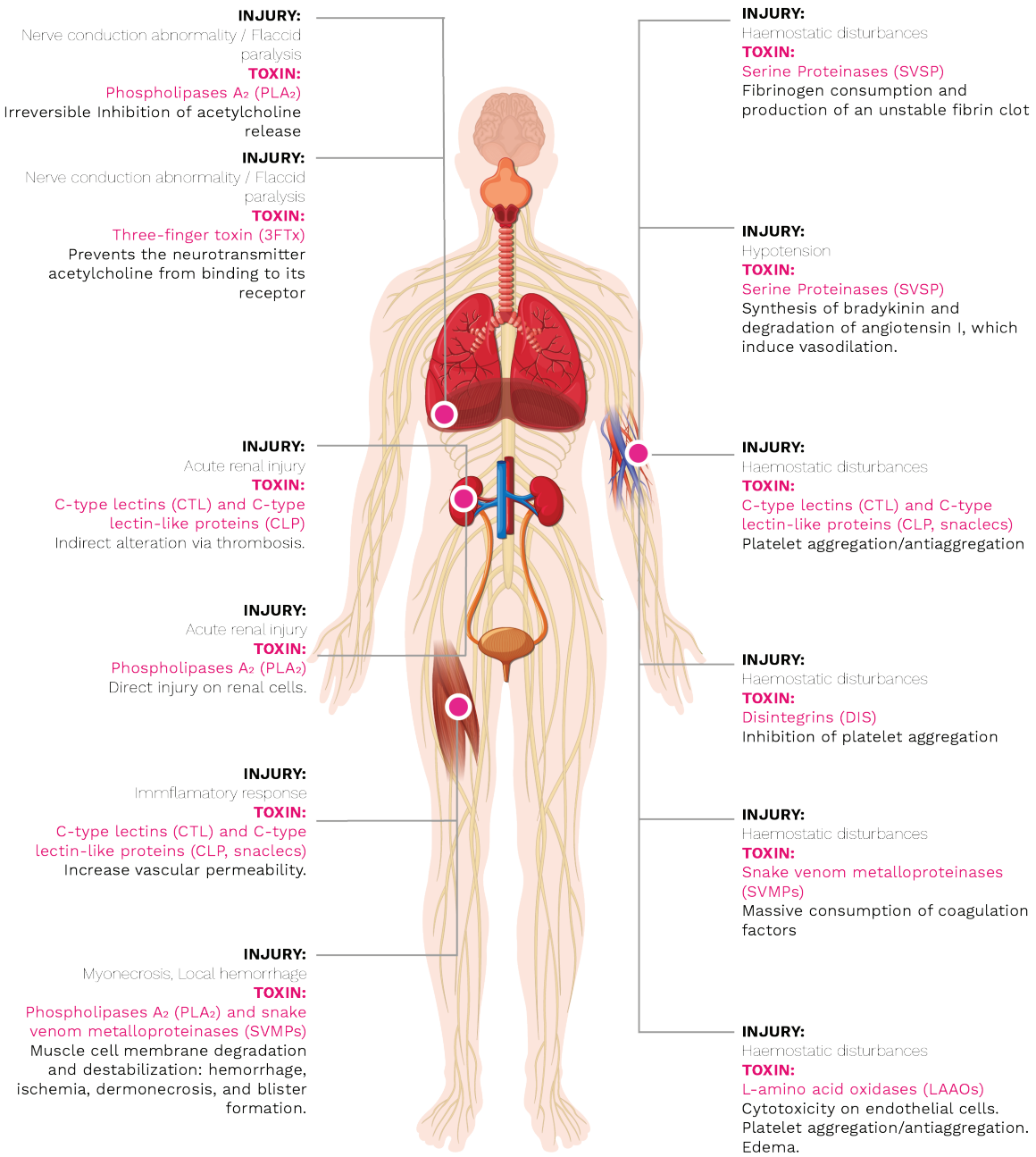


Figure 20. Effects of snake venom on the human body.

2.9. Minor proteins and peptides in snake venoms

The omic (proteomic, transcriptomic, and genomic) techniques, along with recent advances in chromatographic methods and bio-guided venom fractionation, have enabled the identification of peptides and proteins that are present in low quantities in snake venom. These substances include enzymes, proteins without enzymatic activity and peptides. The following sections will describe the role of some of these minor toxins in snakebite envenomation, as well as their potential biological activities.

Hyaluronidases

Hyaluronidases are enzymes found in snake venom that degrade hyaluronan, the principal glycosaminoglycan from the extracellular matrix (ECM) [7,248]. This degradation, combined with the cleavage of other components of ECM such as collagen fibers, laminin, nidogen, and perlecan by SVMPs, promotes the spreading of the other toxins at the bite site. This shows that, for example, a hyaluronidase from *Naja naja* increases the myotoxic activity of a PLA_2 and the hemorrhagic effect of a SVMP [249].

Snake venom hyaluronidase has the potential to generate low molecular mass fragments of hyaluronan that can activate the host's inflammatory response [250,251]. These same fragments may activate matrix metalloproteinases (MMPs) and increase damage to the extracellular matrix (ECM). Studies have shown that these moieties upregulate the expression of MMPs [252].

Acetylcholinesterases

Acetylcholinesterases are toxins that hydrolyze acetylcholine into choline and acetate. Human enzymes degrade the neurotransmitter in the synapses that is one mechanism for ending the signaling processes. Elapid snake venoms typically contain these enzymes, except for mambas that contain fasciculins, inhibitors of acetylcholinesterases [253,254]. These enzymes could contribute to the neurotoxicity induced by three-finger toxins and PLA_2s (as discussed earlier in this chapter) by reducing the levels of acetylcholine in synapses and promoting the action of the toxins. However, this hypothesis requires further confirmation through additional research.

Nucleotidases

Nucleotidases are enzymes that degrade nucleotides into nucleosides. One sub-group of nucleotidases are the 5' nucleotidases that specifically catalyze the hydrolysis of phosphate esterified at carbon 5' of the ribose of the nucleotides. These enzymes have been isolated from snake venoms, and their principal biological activity is the inhibition of platelet aggregation [255–258]. Thus, this activity can contribute to platelet inhibition induced by other toxins, such as disintegrin, C-type lectins, PLA_2s, and three-finger toxins. Adenosine is probably one of the nucleosides released. It can potentiate other activities, such as prey immobilization by activating neuronal adenosine A_1 receptors, suppressing acetylcholine release from motor neurons and excitatory neurotransmitters from central sites. Adenosine also exacerbates venom-induced hypotension by acti-

vating A_2 receptors in the vasculature. In addition, adenosine activates A_3 receptors on the mast cells that lead to the release of vasoactive substances and increase vascular permeability [259].

Phospholipase B (PLB)

Phospholipase B hydrolyzes glycerophospholipids at the sn-1 and sn-2 positions [260]. These enzymes have been identified in snake venom proteomic studies and venom gland transcriptomic analyses, including from Colombian medically important species such as *Micrurus dumerillii, Bothrocophias myersi, Porthidium lansbergii,* and *Bothrops asper* [261–264]. The first report of these enzymes in snake venoms indicates that they induce hemolytic activity [265]. The PLBs possibly contribute to the lysis of red blood cells caused by other toxins. However, due to their low quantities in venoms, it has not been possible to identify other biological activities.

Cystein-rich secretory proteins (CRISPs)

Several snake venoms contain cystein-rich secretory proteins that target ion channels such as BKCa channels (large-conductance calcium-activated potassium channels) and cyclic nucleotide-gated (CNG) ion channels [266,267]. In addition, CRISPs increase vascular permeability and inflammatory responses by activating leukocyte and neutrophil infiltration [289]. Therefore, CRISPs contribute to the edema-forming activities of other toxins, mainly PLA_2s and SVMPs.

Growth factors

Vascular endothelial growth factors (VEGFs) and nerve growth factors (NGFs) have been identified in snake venoms [269,270], but their role in snakebites is yet not fully elucidated. Studies have shown that NFGs and VEGF can induce changes in different cell lines. For instance, a NGF isolated from cobra venom was able to delay the growth of Ehrlich ascites carcinoma [292]. Additionally, another NGF from the cobra *Naja sputatrix* upregulates endogenous expression of NGF in PC12 cells, as well as pro-survival cell surface receptors and ion channels [293]. On the other hand, snake venom VEGFs induce hypotension, vascular permeability, and may lead to endothelial cell differentiation [266,273].

Proteinase inhibitors

Several types of proteinases have been identified in snake venoms, including kazal, Kunitz, and tripeptide inhibitors; but their roles in snakebites have not been fully clarified. Kunitz-type proteinase inhibitors have antibleeding activity by inhibiting plasmin [274,275]. In addition, Kunitz inhibitors have been reported to exhibit antiangiogenic and anti-tumor activities [276]. On the other hand, a kazal-type inhibitor isolated from the venom of *Bothriechis schelegelii* did not display cytotoxic, hemorrhagic, or myotoxic effects. However, it did induce edema in the footpad of mice and inhibited the enzymatic activity of trypsin. This inhibitor may reduce the proteolytic activity while the venom is stored in the venom gland [277]. Some snake venoms contain tripeptide inhibitors that are potent SVMP inhibitors that may attenuate the proteolytic activity of metalloproteinases in the venom gland [278]. Other components identified in snake venoms include phosphodiesterases

[259,279,280], aminopeptidases [281], and Vesprins [282,283], among others. However, their roles in snakebites remain poorly understood.

6. Colombian snake proteomes

Venom in snakes facilitates their survival in an exclusive environment. Understanding snake venoms is a challenge due to the complexity of the toxins, their interrelationships, and the pathophysiological consequences of snakebite envenomation. Although venomous snake families share protein groups, each group has related proteins that vary in their amino acid sequence and abundance, contributing to differences in the overall biological activity of individual venoms. For instance, viperid venoms have high enzymatic activity that results in blood coagulation disturbances, while elapid venoms show predominantly neurotoxicity [57].

The biochemical characterization of venoms show that they are essentially mixtures of proteins and peptides that exhibit various biological activities [57]. The understanding of venoms and their properties has become more precise with the advancement of techniques and tools used for their study.

The analysis of venom initially involved chromatographic studies added to one-dimensional polyacrylamide gel electrophoresis, revealing large protein groups. This provided a baseline for more specific studies. With the advent of liquid chromatography associated with mass spectrometry techniques, two-dimensional electrophoresis, and sequencing methods, a global approach was carried out. This allowed for the visualization of the complexity of the venoms and the individual characterization of proteins.

There are effective methods for handling raw venoms. One such involves simplifying the venom prior to conducting mass spectrometry and integrating it with Edman¢s degradation sequence analysis [32]. Another way to analyze the venom is through direct or shotgun approaches, using liquid chromatography coupled to tandem mass spectrometry. Alternatively, indirect approaches involve analyzing the chromatographic fractions obtained from the crude venom or from the spots resulting from two-dimensional (2-D) electrophoresis.

The method of choice depends on the research question. The total number of unique proteins in the 2-D electrophoresis gel is typically higher than the number determined by direct analysis of the venom through mass spectrometry. Each spot may contain more than one protein. This is related due to the complexity of venoms, the presence of multiple isoforms, and post-translational modifications of proteins, rather than the presence of many different proteins [57].

An example of a methodology for separating venom components is proposed below using crotamine of the venom of *C. d. cumanensis* as a model (Figure 21). In step 1, the venom is separated in each component by using chromatographic methods; then a fraction is selected and taken to enzy-

matic digestion in step 2. Using different techniques like mass spectrometry or Edman sequencing the resulting peptides are overlapping to obtain the primary structure. The study of the resulting peptides can also provide information on different forms (isoforms or proteoforms) of a protein. By using specialized bioinformatics servers, the sequence can be used to predict a tridimensional model of the protein (step 3). Step 4 stands for an alternative way to identify the sequence by selecting the fraction and submitting it to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to verify its purity. Thus, the band is excised from the gel to be digested enzymatically (step 5), and the identification and modelling are done as previously described in the steps 2 and 3. Step 6 remarks the use of the separated fraction to directly determine molecular mass by mass spectrometry.

An alignment using the Basic Local Alignment Search Tool (BLAST) was performed using the sequences from peptides obtained after enzymatic digestion that allowed for alignment with the crotamine from *Crotalus durissus terrificus* (Brazilian populations). This sequence was then used to predict the three-dimensional structure using SWISS MODEL Software [228]. Additionally, these techniques have been used to compare venoms, determine genetic, ecological, and geographic variations, and even to build phylogenetic hypothesis of the species that produce specific toxins [57,284,285].

Reverse phase liquid chromatography is the most used technique for intact protein separation. However, it cannot isolate various forms of proteins (proteoforms) due to the variation in their physicochemical properties. Therefore, other separation methods based on hydrophobicity, hydrophilic interaction, charge, size and affinity, are also employed [286]. Even other techniques, such as electrophoresis and isoelectric focusing, have been described as possible alternatives [287].

However, these separation techniques may not always effectively resolve complex proteomes. In contrast, top-down proteomic strategies can be used to identify and characterize proteins by fragmenting intact proteins, rather than measuring peptides produced from proteins through tryptic digestion procedures [198]. Direct analysis of intact proteins provides enriched data, covering nearly the complete protein sequence, and enables identification of proteoforms and the localization of modifications [288]. Currently, this technique is limited to low and medium-size proteins. However, it will probably be used for larger protein complexes in the near future.

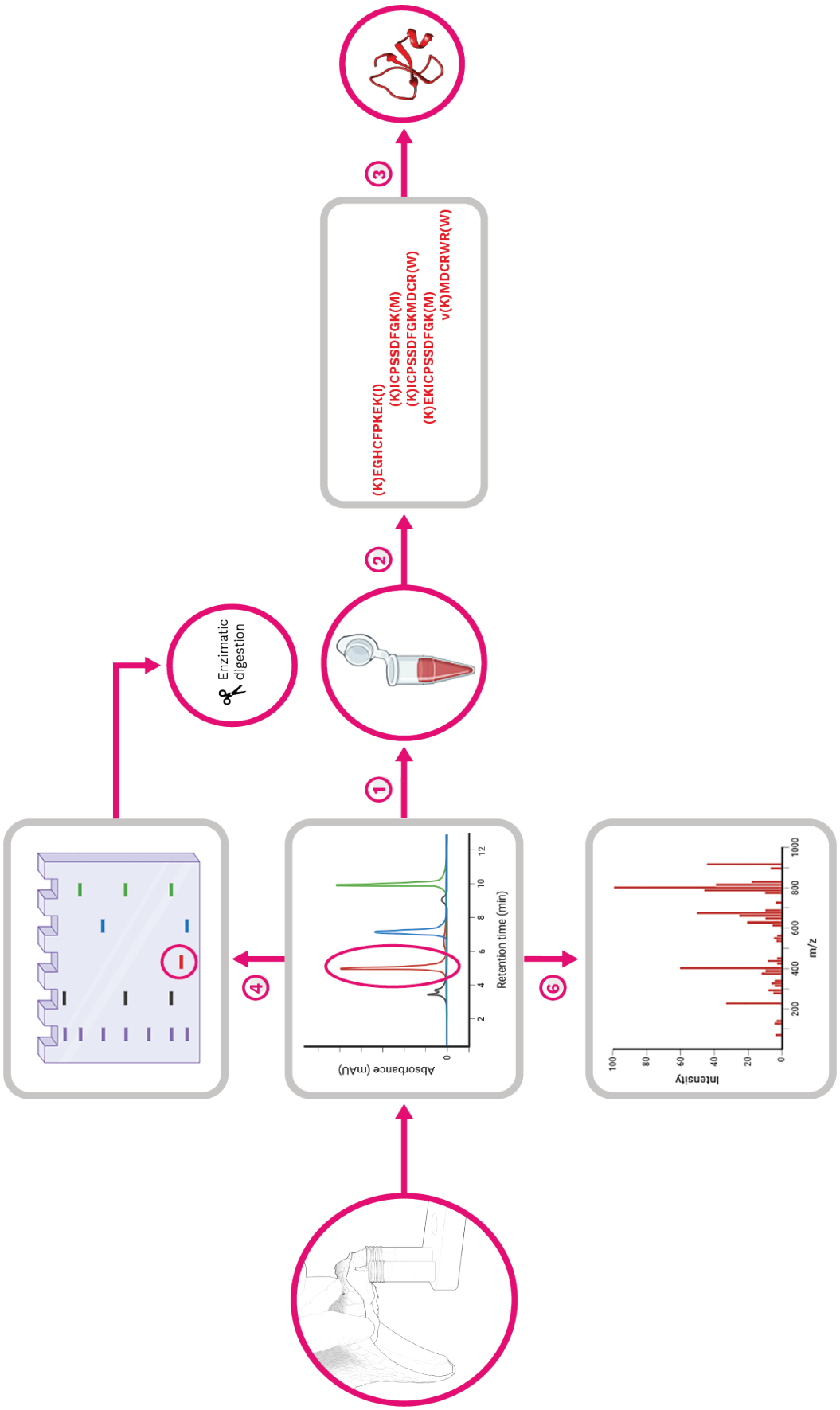


Figure 21. Schematic representation of a complete venom approach, using the venom of *Crotalus durissus* (Colombian populations) and its crotamine as an example. The venom initially obtained is separated into fractions using RP-HPLC technique.

Snake venom proteomes are typically represented graphically as pie charts, displaying the abundance percentage of each protein family and its relation to the venom's overall activity (See Chapter 3). In Colombia, the venom of vipers (Figure 22), shows a higher concentration of metalloproteinases in juvenile *Bothrops asper* specimens that tends to decrease in adult individuals. This difference is compensated by a slight increase in the presence of PLA_2, serine proteinases, and L-amino acid oxidases [289]. In the venom of *Bothrops atrox*, metalloproteinases represent almost 50% of the proteome. While PLA_2 is also present, it is worth noting that the venom contains duplicate amounts of serine proteinases and L-amino acid oxidases compared to its Brazilian counterpart [290].

Porthidium lansbergii venom maintains the 2:1 ratio between metalloproteinases and PLA_2, but it also contains a significant amount of disintegrins and C-type lectins (13% and 7%, respectively) [291]. Although the venom of *P. lansbergii* is rich in these components, studies of their biotechnological applications are still in their early stages [292]. In contrast, the venom of *Bothrocophias myersi* (Equis red snake) is mainly composed of PLA_2 and SVMPs (54% and 21.4%, respectively) [284]. The venoms of other vipers, such as *Bothrocophias campbelli* (Chocoan toadhead pitviper) contain less than 1% disintegrins [198] or the venom from *Crotalus durissus*, which have 1.18% of C-type lectins in its proteome [293].

Particularly, the venom of *C. durissus* is composed of over 60% crotoxin, a neurotoxic PLA_2, and 6% crotamine, a low molecular weight myotoxin that is typical of rattlesnakes [293] (Figure 22). In contrast, *Lachesis acrochorda* (Chocoan Bushmaster) has a predominance of serine proteinases, followed by metalloproteinases and vasoactive peptides, including uncommon toxins like bradykinin-potentiating peptides (BPP) and C-type natriuretic peptide (C-NP) [294].

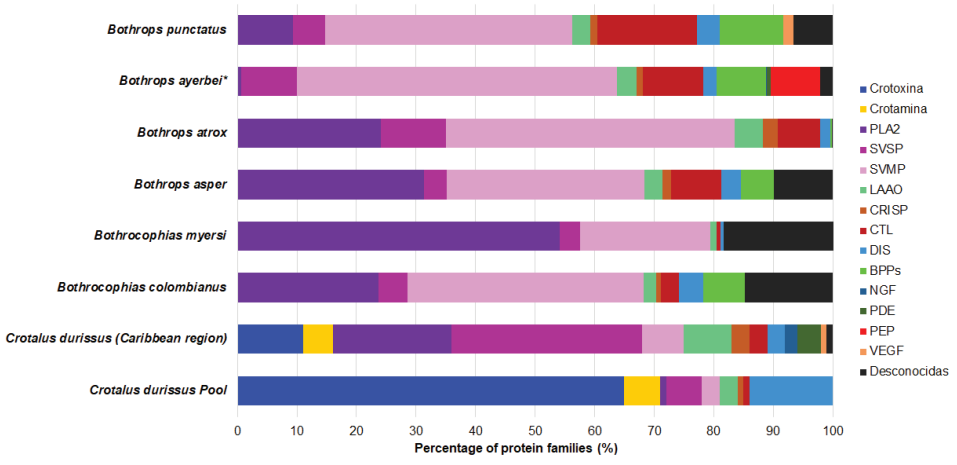


Figure 22. Toxin representativeness in the venom proteomes of Colombian pitviper snakes with available data. BPPs: bradykinin-potentiating peptides; PLA_2: phospholipase A_2; SVSP: snake venom serine proteinase; SVMP: snake venom metalloproteinase; LAAO: L-amino acid oxidase; CRISP: cysteine-rich secretory protein; CTL: C-type lectins and lectin-like; DIS: disintegrin; NGF: nerve growth factor; VEGF: vascular endothelium growth factor; PEP: other peptides; UNK: unknown; Crotoxin: A crystalline phospholipase A_2 type neurotoxin obtained from rattlesnake venom of the genus *Crotalus*; Crotamine: A low molecular weight myotoxin from rattlesnake venom of the genus *Crotalus*. Species venom proteomes were retrieved from [81,290,293,295–297].

Only eight species of Colombian elapids have proteomic characterizations available. When considering the dichotomy of 3FTx-PLA_2, in the venom of *Micrurus mipartitus,* 3FTxs predominate with 61% [285], while for *M.*

dumerilii, M. helleri, M. medemi, and M. sangilensis 52%, 41%, 43% and 30% of the proteome is represented by PLA_2, respectively [264], which includes significant proportions of Kunitz peptides for *M. dumerilii* and metalloproteinases for *M. helleri, M. medemi*, and *M. sangilensis* (Figure 23). The venom of the pelagic yellow-bellied sea snake *Hydrophis platurus* from the Costa Rican population contains approximately 50% 3FTx, compared to 33% PLA_2 [297], However, no Colombian population has been yet characterized.

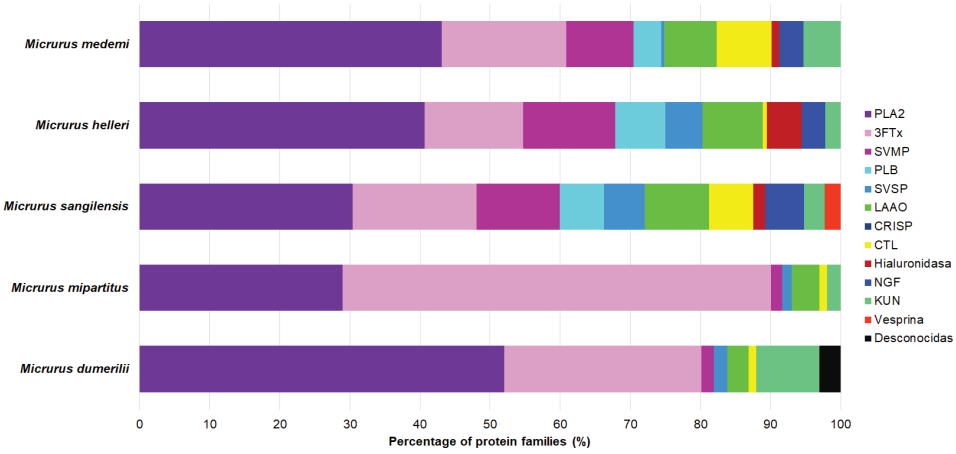


Figure 23. Toxin representativeness in the venom proteomes of Colombian Coral snakes with available data. PLA_2: phospholipase A_2; SVSP: snake venom serine proteinases; SVMP: snake venom metalloproteinases; LAAO: L-amino acid oxidase; CRISP: cysteine-rich secretory protein; CTL: C-type lectins and lectin-like; 3FTx: three-finger toxins; KUN: Kunitz peptides. Species venom proteomes were retrieved from [264,285].

Although the characterization of venom proteomes from Colombian snake species has increased, further studies are needed. Due to low encounter rates, cryptic habitats, and high species richness, most venoms from medically important snakes in Colombia lack proteomic characterization, with coral snakes (*Micrurus*) showing the greatest knowledge gap. In this way, identifying the main toxins from the venom of each species will allow us to understand the pathophysiological activities of each component and their potential applications in biotechnology and medicine.

Potential uses of snake venoms

Snake venoms are complex mixtures containing toxins that target several physiological processes. They have been recognized as sources of bioactive molecules since the discovery and development of captopril (see Chapter 10). There have been successful cases in which drugs have been produced from snake toxins. For instance, Tirofiban is a drug used to inhibit platelet aggregation in acute coronary syndromes. The disintegrin identified in the venom of *Echis carinatus* was modified and formulated to develop this drug [298].

Snake venoms are also useful for studying biological processes. For instance, batroxobin is used for diagnostic purposes in clinical chemistry. Reptilase (Reptilase®, Pentapharm, Basel, Switzerland) is an alternative to thrombin time in samples that contain heparin or to diagnose hypofibrinogenemia [299]. Venoms are important sources of bioactive compounds that have potential as lead compounds for drug development or as molecules for studying some biological processes (see Chapter 10).

Appendix: Material and Methods

Visualization of the Secondary Structure of Toxins in 3D
In this chapter, the study of toxins is approached from their secondary structure. For accurate visualization, regions with secondary structures are selectively colored: alpha helices (in magenta), beta sheets (in green/yellow), turns, and random structures (in white).

A schematic visualization mode was chosen to showcase the Richardson rep-resentation (MolScript). This method resembles the ribbon model but uses ar-rowheads to indicate the orientation of strands and helices, while segments lacking secondary structure are depicted as cords rather than ribbons [301,302].

In some proteins, or specific regions within them, there are insufficient interac-tions to distinguish any level of organization beyond the primary structure. In such cases, this is referred to as a random coil conformation.

When the main chain or backbone of a polypeptide folds into a right-handed helical shape, it adopts a conformation known as an alpha helix. Conversely, when the polypeptide's backbone is stretched to its maximum covalent bond length, it assumes a spatial configuration known as a beta structure (beta sheet), typically represented by arrows [302]. Beta sheets can be parallel (when strands run in the same direction) or anti-parallel (when strands run in opposite directions).

Polypeptide chain sequences with alpha or beta structures are often con-nected by beta turns. These are short sequences with a characteristic confor-mation that imposes a sharp 180-degree turn on the polypeptide backbone.

The 3D toxin models were obtained from the Protein Data Bank (PDB), down-loaded in Wavefront format (.obj), and subsequently optimized using Blend-er[303,304]. They were then imported into Jmol to generate the schematic-style pattern and exported in 3D VRML format [305]. Finally, the models were imported into the Sketchfab platform to generate the HTML visualization code [306].

References

1. Kardong, K.; Lavin-Murcio, P. Venom Delivery of Snakes as High-Pressure and Low-Pressure Systems. *Copeia* **1993**, 3, 644-650, doi:10.2307/1447225
2. Broeckhoven, C.; Du Plessis, A. Has Snake Fang Evolution Lost Its Bite? New Insights from a Structural Mechanics Viewpoint. *Biol Lett* **2017**, *13*, doi:10.1098/rsbl.2017.0293.
3. Vidal, N. Colubroid Systematics: Evidence for an Early Appearance of the Venom Apparatus Followed by Extensive Evolutionary Tinkering. *J Toxicol Toxin Rev* **2002**, *21*, 21–41.
4. Kardong, K. V The Evolution of the Venom Apparatus in Snakes from Colubrids to Viperids & Elapids. *Mem. Inst. Butantan* **1982**, *4*, 106–118.
5. Weinstein, S.A.; Smith, T.L.; Kardong, K. V Reptil Venom Glands: Form, Function, and Future. In *Handbook of venoms and toxins of reptiles*; Mackessy, S.P., Ed.; CRC Press: Boca Raton, **2010**; pp. 65–91 ISBN 978-0-8493-9165-1.
6. Vonk, F.J.; Admiraal, J.F.; Jackson, K.; Reshef, R.; De Bakker, M.A.G.; Vanderschoot, K.; Van Den Berge, I.; Van Atten, M.; Burgerhout, E.; Beck, A.; et al. Evolutionary Origin and Development of Snake Fangs. *Nature* **2008**, *454*, 630–633, doi:10.1038/nature07178.
7. Deufel, A.; Cundall, D. Functional Plasticity of the Venom Delivery System in Snakes with a Focus on the Poststrike Prey Release Behavior. *Zool Anz* **2006**, *245*, 249–267, doi: 10.1016/j.jcz.2006.07.002.
8. Glaudas, X.; Kearney, T.C.; Alexander, G.J. To Hold or Not to Hold? The Effects of Prey Type and Size on the Predatory Strategy of a Venomous Snake. *J Zool* **2017**, *302*, 211–218, doi:10.1111/jzo.12450.
9. Mackessy, S. *Handbook of Venoms and Toxins of Reptiles*; Mackessy, S., Ed.; CRC Press, **2016**; ISBN 9780429186394.
10. Arbuckle, K. Evolutionary Context of Venom in Animals. In *Evolution of Venomous Animals and Their Toxins*; Gopalakrishnakone, P., Malhotra, A., Eds.; Springer: Dordrecht, **2017**; pp. 3–31 ISBN 978-94-007-6458-3.
11. Bock, W.J. The Definition and Recognition of Biological Adaptation. *Am Zool* **1980**, *20*, 217–227.
12. Wexler, P.; Fonger, G.C.; White, J.; Weinstein, S. Toxinology: Taxonomy, Interpretation, and Information Resources. *Sci Technol Libr (New York, NY)* **2015**, *34*, 67–90, doi:10.1080/0194262X.2014.993788.
13. Mebs, D. *Venomous and Poisonous Animals: A Handbook for Biologists, Toxicologists and Toxinologists, Physicians and Pharmacists*; Medpharm Scientific Publ., **2002**; ISBN 9783804750234.
14. Vetter, R.S.; Schmidt, J.O. Semantics of Toxinology. *Toxicon* 2006, *48*.
15. Casewell, N.R.; Jackson, T.N.W.; Laustsen, A.H.; Sunagar, K. Causes and Consequences of Snake Venom Variation. *Trends Pharmacol Sci* **2020**, *41*.
16. Hiestand, P.C.; Hiestand, R.R**.** *Dispholidus typus* (Boomslang) Snake Venom: Purification and Properties of the Coagulant Principle. *Toxicon* **1979**, *17*, doi:10.1016/0041-0101(79)90282-4.
17. Lomonte, B.; Cerdas, L.; Solórzano, A.; Martinez, S. El Suero de Neonatos de *Clelia clelia* (Serpentes: Colubridae) Neutraliza La Acción Hemorrágica Del Veneno de *Bothrops asper* (Serpentes: Viperidae). *Revista Biol. Trop.* **1989**, *38*, 325–326.

18. Kazandjian, T.D.; Petras, D.; Robinson, S.D.; van Thiel, J.; Greene, H.W.; Arbuckle, K.; Barlow, A.; Carter, D.A.; Wouters, R.M.; Whiteley, G.; et al. Convergent Evolution of Pain-Inducing Defensive Venom Components in Spitting Cobras. *Science (1979)* **2021**, *371*, 386–390, doi:10.1126/science. abb9303.
19. Amazonas, D.R.; Portes-Junior, J.A.; Nishiyama-Jr, M.Y.; Nicolau, C.A.; Chalkidis, H.M.; Mourão, R.H.V.; Grazziotin, F.G.; Rokyta, D.R.; Gibbs, H.L.; Valente, R.H.; et al. Molecular Mechanisms Underlying Intraspecific Variation in Snake Venom. *J Proteomics* **2018**, *181*, 60–72, doi:10.1016/j.jprot.2018.03.032.
20. Chippaux, J.P.; Williams, V.; White, J. Snake Venom Variability: Methods of Study, Results and Interpretation. *Toxicon* **1991**, *29*, 1279–1303.
21. Chippaux, J.P.; Goyffon, M. Venoms, Antivenoms and Immunotherapy. *Toxicon* **1998**, *36*, 823–846, doi:10.1016/S0041-0101(97)00160-8.
22. Lomonte, B.; Rangel, J. Snake Venom Lys49 Myotoxins: From Phospholipases A_2 to Non-Enzymatic Membrane Disruptors. *Toxicon* **2012**, *60*, 520–530, doi: 10.1016/j.toxicon.2012.02.007.
23. Vélez, S.M.; Salazar, M.; Acosta de Patiño, H.; Gómez, L.; Rodriguez, A.; Correa, D.; Saldaña, J.; Navarro, D.; Lomonte, B.; Otero-Patiño, R.; et al. Geographical Variability of the Venoms of Four Populations of *Bothrops asper* from Panama: Toxicological Analysis and Neutralization by a Polyvalent Antivenom. *Toxicon* **2017**, *132*, 55–61, doi: 10.1016/j.toxicon.2017.04.002.
24. Tasoulis, T.; Isbister, G.K. A Review and Database of Snake Venom Proteomes. *Toxins (Basel)* **2017**, doi:10.3390/toxins9090290.
25. Aminoff, M.J.; So, Y.T. Effects of Toxins and Physical Agents on the Nervous System. In *Neurology in Clinical Practice*; Elsevier, **2012**; pp. 1353–1376.
26. Thornton, S.L. Snakes. In *Encyclopedia of Toxicology: Third Edition*; Academic Press, **2014**; pp. 310–312 ISBN 9780123864543.
27. Rolan, T.D. Neurotoxic Snakes of the Americas. *Neurol Clin Pract* **2015**, *5*, 383–388, doi:10.1212/CPJ.0000000000000180.
28. Castro, E.N.; Lomonte, B.; del Carmen Gutiérrez, M.; Alagón, A.; Gutiérrez, J.M. Intraspecies Variation in the Venom of the Rattlesnake Crotalus Simus from Mexico: Different Expression of Crotoxin Results in Highly Variable Toxicity in the Venoms of Three Subspecies. *J Proteomics* **2013**, *87*, 103–121, doi:10.1016/j.jprot.2013.05.024.
29. Sunagar, K.; Undheim, E.A.B.; Scheib, H.; Gren, E.C.K.; Cochran, C.; Person, C.E.; Koludarov, I.; Kelln, W.; Hayes, W.K.; King, G.F.; et al. Intraspecific Venom Variation in the Medically Significant Southern Pacific Rattlesnake (*Crotalus oeganus helleri*): Biodiscovery, Clinical and Evolutionary Implications. *J Proteomics* **2014**, *99*, 68–83, doi:10.1016/j.jprot.2014.01.013.
30. Gutiérrez, J.M.; Rucavado, A. Snake Venom Metalloproteinases: Their Role in the Pathogenesis of Local Tissue Damage. *Biochimie* **2000**, *82*, 841–850, doi:10.1016/s0300-9084(00)01163-9.
31. Estêvão-Costa, M.I.; Diniz, C.R.; Magalhães, A.; Markland, F.S.; Sanchez, E.F. Action of Metalloproteinases Mutalysin I and II on Several Components of the Hemostatic and Fibrinolytic Systems. *Thromb Res* **2000**, *99*, 363–376, doi:10.1016/S0049-3848(00)00259-0.

32. Calvete, J.J.; Escolano, J.; Sanz, L. Snake Venomics of *Bitis* Species Reveals Large Intragenus Venom Toxin Composition Variation: Application to Taxonomy of Congeneric Taxa. *J Proteome Res* **2007**, *6*, 2732–2745, doi:10.1021/pr0701714.
33. Mackessy, S.P. Evolutionary Trends in Venom Composition in the Western Rattlesnakes (*Crotalus viridis* sensu lato): Toxicity vs. Tenderizers. *Toxicon* **2010**, *55*, 1463–1474, doi: 10.1016/j.toxicon.2010.02.028.
34. Salazar, A.M.; Guerrero, B.; Cantu, B.; Cantu, E.; Rodríguez-Acosta, A.; Pérez, J.C.; Galán, J.A.; Tao, A.; Sánchez, E.E. Venom Variation in Hemostasis of the Southern Pacific Rattlesnake (*Crotalus oreganus helleri*): Isolation of Hellerase. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology* **2009**, *149*, 307–316, doi: 10.1016/j.cbpc.2008.08.007.
35. Lomonte, B.; Escolano, J.; Fernández, J.; Sanz, L.; Angulo, Y.; Gutiérrez, J.M.; Calvete, J.J. Snake Venomics and Antivenomics of the Arboreal Neotropical Pitvipers *Bothriechis lateralis* and *Bothriechis schlegelii*. *J Proteome Res* **2008**, *7*, 2445–2457, doi:10.1021/pr8000139.
36. Boldrini-França, J.; Corrêa-Netto, C.; Silva, M.M.S.; Rodrigues, R.S.; De La Torre, P.; Pérez, A.; Soares, A.M.; Zingali, R.B.; Nogueira, R.A.; Rodrigues, V.M.; et al. Snake Venomics and Antivenomics of *Crotalus durissus* Subspecies from Brazil: Assessment of Geographic Variation and Its Implication on Snakebite Management. *Proteomics* **2010**, *73*, 1758–1776, doi: 10.1016/j.jprot.2010.06.001.
37. Fry, B.G.; Wickramaratna, J.C.; Hodgson, W.C.; Alewood, P.F.; Kini, R.M.; Ho, H.; Wüster, W**.** Electrospray Liquid Chromatography/Mass Spectrometry Fingerprinting of Acanthophis (Death Adder) Venoms: Taxonomic and Toxinological Implications. *Rapid Communications in Mass Spectrometry* **2002**, *16*, 600–608, doi:10.1002/rcm.613.
38. Daltry, J.C.; Wüster, W.; Thorpe, R.S. Diet and Snake Venom Evolution. *Nature* **1996**, *379*, 537–542, doi:10.1038/379537a0.
39. Sasa, M. Diet and Snake Venom Evolution: Can Local Selection Alone Explain Intraspecific Venom Variation? *Toxicon* **1999**, *37*, 249–252, doi:10.1016/S0041-0101(98)00121-4.
40. Hartmann, P.A.; Hartmann, M.T.; Martins, M. Ecology of a Snake Assemblage in the Atlantic Forest of Southeastern Brazil. *Pap Avulsos Zool* **2009**, *49*, 343–360, doi:10.1590/s0031-10492009002700001.
41. Daltry, J.C.; Ponnudurai, G.; Shin, C.K.; Tan, N.H.; Thorpe, R.S.; Wüster, W. Electrophoretic Profiles and Biological Activities: Intraspecific Variation in the Venom of the Malayan Pit Viper (*Calloselasma rhodostoma*). *Toxicon* **1996**, *34*, 67–79, doi:10.1016/0041-0101(95)00122-0.
42. Calvete, J.J.; Sanz, L.; Pérez, A.; Borges, A.; Vargas, A.M.; Lomonte, B.; Angulo, Y.; Gutiérrez, J.M.; Chalkidis, H.M.; Mourão, R.H.V.; et al. Snake Population Venomics and Antivenomics of *Bothrops atrox*: Paedomorphism along Its Transamazonian Dispersal and Implications of Geographic Venom Variability on Snakebite Management. *J Proteomics* **2011**, *74*, 510–527, doi:10.1016/j.jprot.2011.01.003.
43. Silva, F.M. da; Oliveira, L.S. de; Nascimento, L.R. de S.; Machado, F.A.; Prudente, A.L. da C**.** Sexual Dimorphism and Ontogenetic Changes of Amazonian Pit Vipers (*Bothrops atrox*). *Zool Anz* **2017**, *271*, 15–24, doi:10.1016/j.jcz.2017.11.001.

44. Gutiérrez, J.; Lomonte, B. Phospholipase A2 Myotoxins from *Bothrops* Snake Venoms. *Toxicon* **1995**, *33*, 1405–1424.
45. López-Lozano, J.L.; de Sousa, M.V.; Ricart, C.A.O.; Chávez-Olortegui, C.; Flores Sanchez, E.; Muniz, E.G.; Bührnheim, P.F.; Morhy, L. Ontogenetic Variation of Metalloproteinases and Plasma Coagulant Activity in Venoms of Wild *Bothrops atrox* Specimens from Amazonian Rain Forest. *Toxicon* **2002**, *40*, 997–1006, doi:10.1016/S0041-0101(02)00096-X.
46. da Silva Aguiar, W.; da Costa Galizio, N.; Sant'Anna, S.S.; Silveira, G.P.M.; de Souza Rodrigues, F.; Grego, K.F.; de Morais-Zani, K.; Tanaka-Azevedo, A.M. Ontogenetic Study of *Bothrops jararacussu* Venom Composition Reveals Distinct Profiles. *Toxicon* **2020**, *186*, 67–77, doi: 10.1016/j.toxicon.2020.07.030.
47. Antunes, T.C.; Yamashita, K.M.; Barbaro, K.C.; Saiki, M.; Santoro, M.L. Comparative Analysis of Newborn and Adult *Bothrops jararaca* Snake Venoms. *Toxicon* **2010**, *56*, 1443–1458, doi: 10.1016/j.toxicon.2010.08.011.
48. Menezes, M.C.; Furtado, M.F.; Travaglia-Cardoso, S.R.; Camargo, A.C.M.; Serrano, S.M.T. Sex-Based Individual Variation of Snake Venom Proteome among Eighteen *Bothrops jararaca* Siblings. *Toxicon* **2006**, *47*, 304–312, doi:10.1016/j.toxicon.2005.11.007.
49. Chippaux, J.P.; Boche, J.; Courtois, B. Electrophoretic Patterns of the Venoms from a Litter of *Bitis gabonica* Snakes. *Toxicon* **1982**, *20*, 521–522, doi:10.1016/0041-0101(82)90019-8.
50. Williams, V.; White, J.; Schwaner, T.D.; Sparrow, A. Variation in Venom Proteins from Isolated Populations of Tiger Snakes (*Notechis ater niger, N. scutatus*) in South Australia. *Toxicon* **1988**, *26*, 1067–1075, doi:10.1016/0041-0101(88)90205-X.
51. Fry, B.G.; Scheib, H.; van der Weerd, L.; Young, B.; McNaughtan, J.; Ryan Ramjan, S.F.; Vidal, N.; Poelmann, R.E.; Norman, J.A. Evolution of an Arsenal: Structural and Functional Diversification of the Venom System in the Advanced Snakes (*Caenophidia*). *Molecular and Cellular Proteomics* **2008**, *7*, 215–246, doi:10.1074/mcp.M700094-MCP200.
52. Gutiérrez, J.M.; Escalante, T.; Rucavado, A.; Herrera, **C.** Hemorrhage Caused by Snake Venom Metalloproteinases: A Journey of Discovery and Understanding. *Toxins (Basel)* **2016**, *8*, 93, doi:10.3390/toxins8040093.
53. Gutiérrez, J.M.; Rucavado, A.; Escalante, T.; Díaz, C. Hemorrhage Induced by Snake Venom Metalloproteinases: Biochemical and Biophysical Mechanisms Involved in Microvessel Damage. *Toxicon* **2005**, *45*, 997–1011, doi: 10.1016/j.toxicon.2005.02.029.
54. Takeda, S. ADAM and ADAMTS Family Proteins and Snake Venom Metalloproteinases: A Structural Overview. *Toxins (Basel)* **2016**, *8*, doi:10.3390/TOXINS8050155.
55. Gomis-Rüth, F.X. Structural Aspects of the Metzincin Clan of Metalloendopeptidases. *Mol Biotechnol* **2003**, *24*, 157–202, doi:10.1385/MB:24:2:157.
56. Bode, W.; Gomis-Rüth, F.X.; Stöckler, W. Astacins, Serralysins, Snake Venom and Matrix Metalloproteinases Exhibit Identical Zinc-Binding Environments (HEXXHXXGXXH and Met-Turn) and Topologies and Should Be Grouped into a Common Family, the "Metzincins." *FEBS Lett* **1993**, *331*, 134–140, doi:10.1016/0014-5793(93)80312-i.

57. Fox, J.W.; Serrano, S.M.T. Insights into and Speculations about Snake Venom Metalloproteinase (SVMP) Synthesis, Folding and Disulfide Bond Formation and Their Contribution to Venom Complexity. *FEBS J* **2008**, *275*, 3016–3030, doi:10.1111/j.1742-4658.2008.06466. x.
58. Olaoba, O.T.; Karina Dos Santos, P.; Selistre-de-Araujo, H.S.; Ferreira de Souza, D.H. Snake Venom Metalloproteinases (SVMPs): A Structure-Function Update. *Toxicon X* **2020**, *7*, 100052, doi: 10.1016/j.toxcx.2020.100052.
59. Takeda, S.; Takeya, H.; Iwanaga, S. Snake Venom Metalloproteinases: Structure, Function and Relevance to the Mammalian ADAM/ADAMTS Family Proteins. *Biochim Biophys Acta* **2012**, *1824*, 164–176, doi:10.1016/j.bbapap.2011.04.009.
60. Tallant, C.; Marrero, A.; Gomis-Rüth, F.X. Matrix Metalloproteinases: Fold and Function of Their Catalytic Domains. *Biochim Biophys Acta* **2010**, *1803*, 20–28, doi: 10.1016/j.bbamcr.2009.04.003.
61. Coronado, M.A.; de Moraes, F.R.; Ullah, A.; Masood, R.; Santana, V.S.; Mariutti, R.; Brognaro, H.; Georgieva, D.; Murakami, M.T.; Betzel, C.; et al. Three-Dimensional Structures and Mechanisms of Snake Venom Serine Proteinases, Metalloproteinases, and Phospholipase A_2s. In *Venom Genomics and Proteomics*; Gopalakrishnakone, P., Calvete, J.J., Eds.; Toxinology; Springer Netherlands: Dordrecht, **2016**; pp. 239–267 ISBN 978-94-007-6416-3.
62. Escalante, T.; Rucavado, A.; Pinto, A.F.M.; Terra, R.M.S.; Gutiérrez, J.M.; Fox, J.W. Wound Exudate as a Proteomic Window to Reveal Different Mechanisms of Tissue Damage by Snake Venom Toxins. *J Proteome Res* **2009**, *8*, 5120–5131, doi:10.1021/pr900489m.
63. Escalante, T.; Ortiz, N.; Rucavado, A.; Sanchez, E.F.; Richardson, M.; Fox, J.W.; Gutiérrez, J.M. Role of Collagens and Perlecan in Microvascular Stability: Exploring the Mechanism of Capillary Vessel Damage by Snake Venom Metalloproteinases. *PLoS One* **2011**, *6*, e28017, doi: 10.1371/journal.pone.0028017.
64. Moreira, L.; Borkow, G.; Ovadia, M.; Gutiérrez, J.M. Pathological Changes Induced by BaH1, a Hemorrhagic Proteinase Isolated from *Bothrops asper* (Terciopelo) Snake Venom, on Mouse Capillary Blood Vessels. *Toxicon* **1994**, *32*, 976–987, doi:10.1016/0041-0101(94)90376-x.
65. Ohsaka, A. Hemorrhagic, Necrotizing and Edema-Forming Effects of Snake Venoms. In *Handbook of Experimental Pharmacology*; Springer Verlag: Berlin, Germany, **1979**; Vol. 52, Snake, pp. 480–546.
66. Araki, S. Endothelial Cell Toxicity of Vascular Apoptosis-Inducing Proteins from Hemorrhagic Snake Venom. In *Snake Venoms*; Inagaki, H., Vogel, C.-W., Mukherjee, A.K., Rahmy, T.R., Gopalakrishnakone, P., Eds.; Toxinology; Springer Netherlands: Dordrecht, **2017**; pp. 145–159 ISBN 978-94-007-6410-1.
67. Díaz, C.; Valverde, L.; Brenes, O.; Rucavado, A.; Gutiérrez, J.M. Characterization of Events Associated with Apoptosis/Anoikis Induced by Snake Venom Metalloproteinase BaP_1 on Human Endothelial Cells. *J Cell Biochem* **2005**, *94*, 520–528, doi:10.1002/jcb.20322.
68. Wan, S.-G.; Jin, Y.; Lee, W.-H.; Zhang, Y.A. Snake Venom Metalloproteinase That Inhibited Cell Proliferation and Induced Morphological Changes of ECV304 Cells. *Toxicon* **2006**, *47*, 480–489, doi:10.1016/j.toxicon.2006.01.006.

69. Wang, S.H.; Shen, X.C.; Yang, G.Z.; Wu, X.F. CDNA Cloning and Characterization of Agkistin, a New Metalloproteinase from *Agkistrodon halys*. *Biochem Biophys Res Commun* **2003**, *301*, 298–303, doi:10.1016/s0006-291x(02)03001-2.
70. You, W.-K.; Seo, H.-J.; Chung, K.-H.; Kim, D.-S. A Novel Metalloprotease from *Gloydius halys* Venom Induces Endothelial Cell Apoptosis through Its Protease and Disintegrin-like Domains. *J Biochem* **2003**, *134*, 739–749, doi:10.1093/jb/mvg202.
71. Rucavado, A.; Núñez, J.; Gutiérrez, J.M. Blister Formation and Skin Damage Induced by BaP1, a Haemorrhagic Metalloproteinase from the Venom of the Snake *Bothrops asper*. *Int J Exp Pathol* **1998**, *79*, 245–254.
72. Moura-da-Silva, A.M.; Marcinkiewicz, C.; Marcinkiewicz, M.; Niewiarowski, S. Selective Recognition of Alpha2beta1 Integrin by Jararhagin, a Metalloproteinase/Disintegrin from *Bothrops jararaca* Venom. *Thromb Res* **2001**, *102*, 153–159, doi:10.1016/s0049-3848(01)00216-x.
73. Baldo, C.; Jamora, C.; Yamanouye, N.; Zorn, T.M.; Moura-da-Silva, A.M. Mechanisms of Vascular Damage by Hemorrhagic Snake Venom Metalloproteinases: Tissue Distribution and in Situ Hydrolysis. *PLoS Negl Trop Dis* **2010**, *4*, e727, doi: 10.1371/journal.pntd.0000727.
74. Herrera, C.; Escalante, T.; Voisin, M.-B.; Rucavado, A.; Morazán, D.; Macêdo, J.K.A.; Calvete, J.J.; Sanz, L.; Nourshargh, S.; Gutiérrez, J.M.; et al. Tissue Localization and Extracellular Matrix Degradation by PI, PII and PIII Snake Venom Metalloproteinases: Clues on the Mechanisms of Venom-Induced Hemorrhage. *PLoS Negl Trop Dis* **2015**, *9*, e0003731, doi: 10.1371/journal.pntd.0003731.
75. Baramova, E.N.; Shannon, J.D.; Bjarnason, J.B.; Gonias, S.L.; Fox, J.W. Interaction of Hemorrhagic Metalloproteinases with Human Alpha 2-Macroglobulin. *Biochemistry* **1990**, *29*, 1069–1074, doi:10.1021/bi00456a032.
76. Camacho, E.; Villalobos, E.; Sanz, L.; Pérez, A.; Escalante, T.; Lomonte, B.; Calvete, J.J.; Gutiérrez, J.M.; Rucavado, A. Understanding Structural and Functional Aspects of PII Snake Venom Metalloproteinases: Characterization of BlatH1, a Hemorrhagic Dimeric Enzyme from the Venom of Bothriechis Lateralis. *Biochimie* **2014**, *101*, 145–155, doi: 10.1016/j.biochi.2014.01.008.
77. Kamiguti, A.S.; Desmond, H.P.; Theakston, R.D.; Hay, C.R.; Zuzel, M. Ineffectiveness of the Inhibition of the Main Haemorrhagic Metalloproteinase from *Bothrops jararaca* Venom by Its Only Plasma Inhibitor, Alpha 2-Macroglobulin. *Biochim Biophys Acta* **1994**, *1200*, 307–314, doi:10.1016/0304-4165(94)90172-4.
78. Akao, P.K.; Tonoli, C.C.C.; Navarro, M.S.; Cintra, A.C.O.; Neto, J.R.; Arni, R.K.; Murakami, M.T. Structural Studies of BmooMPalpha-I, a Non-Hemorrhagic Metalloproteinase from *Bothrops moojeni* Venom. *Toxicon* **2010**, *55*, 361–368, doi:10.1016/j.toxicon.2009.08.013.
79. Bello, C.A.; Hermogenes, A.L.N.; Magalhaes, A.; Veiga, S.S.; Gremski, L.H.; Richardson, M.; Sanchez, E.F. Isolation and Biochemical Characterization of a Fibrinolytic Proteinase from *Bothrops leucurus* (White-Tailed Jararaca) Snake Venom. *Biochimie* **2006**, *88*, 189–200, doi:10.1016/j.biochi.2005.07.008.

80. Gutiérrez, J.M.; Romero, M.; Núñez, J.; Chaves, F.; Borkow, G.; Ovadia, M. Skeletal Muscle Necrosis and Regeneration after Injection of BaH1, a Hemorrhagic Metalloproteinase Isolated from the Venom of the Snake *Bothrops asper* (Terciopelo). *Exp Mol Pathol* **1995**, *62*, 28–41, doi:10.1006/exmp.1995.1004.
81. Patiño, A.C.; Pereañez, J.A.; Núñez, V.; Benjumea, D.M.; Fernandez, M.; Rucavado, A.; Sanz, L.; Calvete, J.J. Isolation and Biological Characterization of Batx-I, a Weak Hemorrhagic and Fibrinogenolytic PI Metalloproteinase from Colombian *Bothrops atrox* Venom. *Toxicon* **2010**, *56*, 936–943, doi:10.1016/j.toxicon.2010.06.016.
82. Wallnoefer, H.G.; Lingott, T.; Gutiérrez, J.M.; Merfort, I.; Liedl, K.R. Backbone Flexibility Controls the Activity and Specificity of a Protein-Protein Interface: Specificity in Snake Venom Metalloproteases. *J Am Chem Soc* **2010**, *132*, 10330–10337, doi:10.1021/ja909908y.
83. de Souza, R.A.; Díaz, N.; Nagem, R.A.P.; Ferreira, R.S.; Suárez, D. Unraveling the Distinctive Features of Hemorrhagic and Non-Hemorrhagic Snake Venom Metalloproteinases Using Molecular Simulations. *J Comput Aided Mol Des* **2016**, *30*, 69–83, doi:10.1007/s10822-015-9889-5.
84. Preciado, L.M.; Pereañez, J.A.; Singam, E.R.A.; Comer, J. Interactions between Triterpenes and a P-I Type Snake Venom Metalloproteinase: Molecular Simulations and Experiments. *Toxins (Basel)* **2018**, *10*, 1–20, doi:10.3390/toxins10100397.
85. Gutiérrez, J.M.; Escalante, T.; Hernández, R.; Gastaldello, S.; Saravia-Otten, P.; Rucavado, A. Why Is Skeletal Muscle Regeneration Impaired after Myonecrosis Induced by Viperid Snake Venoms? *Toxins (Basel)* **2018**, *10*, E182, doi:10.3390/toxins10050182.
86. Tidball, J.G. Regulation of Muscle Growth and Regeneration by the Immune System. *Nat Rev Immunol* **2017**, *17*, 165–178, doi:10.1038/nri.2016.150.
87. Queiroz, L.S.; Santo Neto, H.; Assakura, M.T.; Reichl, A.P.; Mandelbaum, F.R. Pathological Changes in Muscle Caused by Haemorrhagic and Proteolytic Factors from *Bothrops jararaca* Snake Venom. *Toxicon* **1985**, *23*, 341–345, doi:10.1016/0041-0101(85)90158-8.
88. Homma, M.; Tu, A.T. Morphology of Local Tissue Damage in Experimental Snake Envenomation. *Br J Exp Pathol* **1971**, *52*, 538–542.
89. Jiménez, N.; Escalante, T.; Gutiérrez, J.M.; Rucavado, A. Skin Pathology Induced by Snake Venom Metalloproteinase: Acute Damage, Revascularization, and Re-Epithelization in a Mouse Ear Model. *J Invest Dermatol* **2008**, *128*, 2421–2428, doi:10.1038/jid.2008.118.
90. Macêdo, J.K.A.; Joseph, J.K.; Menon, J.; Escalante, T.; Rucavado, A.; Gutiérrez, J.M.; Fox, J.W. Proteomic Analysis of Human Blister Fluids Following Envenomation by Three Snake Species in India: Differential Markers for Venom Mechanisms of Action. *Toxins (Basel)* **2019**, *11*, E246, doi:10.3390/toxins11050246.
91. Laing, G.D.; Clissa, P.B.; Theakston, R.D.G.; Moura-da-Silva, A.M.; Taylor, M.J. Inflammatory Pathogenesis of Snake Venom Metalloproteinase-Induced Skin Necrosis. *Eur J Immunol* **2003**, *33*, 3458–3463, doi:10.1002/eji.200324475.
92. Fernandes, C.M.; Zamuner, S.R.; Zuliani, J.P.; Rucavado, A.; Gutiérrez, J.M.; Teixeira, C. de F.P. Inflammatory Effects of BaP1 a Metallopro-

teinase Isolated from *Bothrops asper* Snake Venom: Leukocyte Recruitment and Release of Cytokines. *Toxicon* **2006**, *47*, 549–559, doi: 10.1016/j.toxicon.2006.01.009.
93. Teixeira, C. de F.P.; Fernandes, C.M.; Zuliani, J.P.; Zamuner, S.F. Inflammatory Effects of Snake Venom Metalloproteinases. *Mem Inst Oswaldo Cruz* **2005**, *100 Suppl*, 181–184, doi:10.1590/s0074-02762005000900031.
94. Clissa, P.B.; Laing, G.D.; Theakston, R.D.; Mota, I.; Taylor, M.J.; Moura-da-Silva, A.M**.** The Effect of Jararhagin, a Metalloproteinase from *Bothrops jararaca* Venom, on pro-Inflammatory Cytokines Released by Murine Peritoneal Adherent Cells. *Toxicon* **2001**, *39*, 1567–1573, doi:10.1016/s0041-0101(01)00131-3.
95. Schaloske, R.H.; Dennis, E.A. The Phospholipase A_2 Superfamily and Its Group Numbering System. *Biochim Biophys Acta* **2006**, *1761*, 1246–1259, doi: 10.1016/j.bbalip.2006.07.011.
96. Filkin, S.Y.; Lipkin, A. V; Fedorov, A.N**.** Phospholipase Superfamily: Structure, Functions, and Biotechnological Applications. *Biochemistry (Mosc)* **2020**, *85*, S177–S195, doi:10.1134/S0006297920140096.
97. Kini, R.M**.** Excitement Ahead: Structure, Function and Mechanism of Snake Venom Phospholipase A_2 Enzymes. *Toxicon* **2003**, *42*, 827–840, doi: 10.1016/j.toxicon.2003.11.002.
98. Días, E.H.V.; Dos Santos Paschoal, T.; da Silva, A.P.; da Cunha Pereira, D.F.; de Sousa Simamoto, B.B.; Matias, M.S.; Santiago, F.M.; Rosa, J.C.; Soares, A.; Santos-Filho, N.A.; et al. BaltPLA2: A New Phospholipase A2 from *Bothrops alternatus* Snake Venom with Antiplatelet Aggregation Activity. *Protein Pept Lett* **2018**, *25*, 943–952, doi:10.2174/0929866525666181004101622.
99. Nunes, E.; Frihling, B.; Barros, E.; de Oliveira, C.; Verbisck, N.; Flores, T.; de Freitas Júnior, A.; Franco, O.; de Macedo, M.; Migliolo, L.; et al. Antibiofilm Activity of Acidic Phospholipase Isoform Isolated from *Bothrops erythromelas* Snake Venom. *Toxins (Basel)* **2020**, *12*, E606, doi:10.3390/toxins12090606.
100. Jiménez-Charris, E.; Montealegre-Sánchez, L.; Solano-Redondo, L.; Castro-Herrera, F.; Fierro-Pérez, L.; Lomonte, B. Divergent Functional Profiles of Acidic and Basic Phospholipases A_2 in the Venom of the Snake *Porthidium lansbergii lansbergii*. *Toxicon* **2016**, *119*, 289–298, doi: 10.1016/j.toxicon.2016.07.006.
101. Posada Arias, S.; Rey-Suárez, P.; Pereáñez J, A.; Acosta, C.; Rojas, M.; Delazari Dos Santos, L.; Ferreira, R.S.; Núñez, V. Isolation and Functional Characterization of an Acidic Myotoxic Phospholipase A_2 from Colombian *Bothrops asper* Venom. *Toxins (Basel)* **2017**, *9*, E342, doi:10.3390/toxins9110342.
102. Vargas, L.J.; Londoño, M.; Quintana, J.C.; Rua, C.; Segura, C.; Lomonte, B.; Núñez, V. An Acidic Phospholipase A_2 with Antibacterial Activity from *Porthidium nasutum* Snake Venom. *Comp Biochem Physiol B Biochem Mol Biol* **2012**, *161*, 341–347, doi: 10.1016/j.cbpb.2011.12.010.
103. Kini, R.M.; Evans, H.J. A Model to Explain the Pharmacological Effects of Snake Venom Phospholipases A_2. *Toxicon* **1989**, *27*, 613–635, doi:10.1016/0041-0101(89)90013-5.
104. Scott, D. Phospholipase A2: Structure and Catalytic Properties. In

Venom phospholipase A2 enzymes: structure, function and mechanism; Kini, R., Ed.; John Wiley & Sons: Chichester, **1997**; pp. 97–128.
105. Berg, O.G.; Gelb, M.H.; Tsai, M.D.; Jain, M.K. Interfacial Enzymology: The Secreted Phospholipase A_2-Paradigm. *Chem Rev* **2001**, *101*, 2613–2654, doi:10.1021/cr990139w.
106. Maraganore, J.M.; Merutka, G.; Cho, W.; Welches, W.; Kézdy, F.J.; Heinrikson, R.L. A New Class of Phospholipases A_2 with Lysine in Place of Aspartate 49. Functional Consequences for Calcium and Substrate Binding. *J Biol Chem* **1984**, *259*, 13839–13843.
107. Gutiérrez, J.M.; León, G.; Lomonte, B. Pharmacokinetic-Pharmacodynamic Relationships of Immunoglobulin Therapy for Envenomation. *Clin Pharmacokinet* **2003**, *42*, 721–741, doi:10.2165/00003088-200342080-00002.
108. Lambeau, G.; Schmid-Alliana, A.; Lazdunski, M.; Barhanin, J. Identification and Purification of a Very High Affinity Binding Protein for Toxic Phospholipases A_2 in Skeletal Muscle. *Journal of Biological Chemistry* **1990**, *265*, 9526–9532, doi:10.1016/s0021-9258(19)38881-7.
109. Massimino, M.L.; Simonato, M.; Spolaore, B.; Franchin, C.; Arrigoni, G.; Marin, O.; Monturiol-Gross, L.; Fernández, J.; Lomonte, B.; Tonello, F. Cell Surface Nucleolin Interacts with and Internalizes *Bothrops asper* Lys49 Phospholipase A_2 and Mediates Its Toxic Activity. *Sci Rep* **2018**, *8*, 10619, doi:10.1038/s41598-018-28846-4.
110. Fernandes, C.A.H.; Borges, R.J.; Lomonte, B.; Fontes, M.R.M. A Structure-Based Proposal for a Comprehensive Myotoxic Mechanism of Phospholipase A_2-like Proteins from Viperid Snake Venoms. *Biochim Biophys Acta Proteins Proteom* **2014**, *1844*, 2265–2276, doi: 10.1016/j.bbapap.2014.09.015.
111. Gutiérrez, J.M.; Ownby, C.L. Skeletal Muscle Degeneration Induced by Venom Phospholipases A_2: Insights into the Mechanisms of Local and Systemic Myotoxicity. *Toxicon* **2003**, *42*, 915–931, doi: 10.1016/j.toxicon.2003.11.005.
112. Montecucco, C.; Gutiérrez, J.M.; Lomonte, B. Cellular Pathology Induced by Snake Venom Phospholipase A_2 Myotoxins and Neurotoxins: Common Aspects of Their Mechanisms of Action. *Cell Mol Life Sci* **2008**, *65*, 2897–2912, doi:10.1007/s00018-008-8113-3.
113. Otero-Patiño, R. Snake Bites in Colombia. In *Clinical Toxinology: Clinical Toxinology*; Gopalakrishnakone, P., Faiz, S.M.A., Gnanathasan, C.A., Habib, A.G., Fernando, R., Yang, C.-C., Eds.; Springer Netherlands: Dordrecht, **2013**; pp. 1–42 ISBN 978-94-007-6288-6.
114. Sarkar, S.; Sinha, R.; Chaudhury, A.R.; Maduwage, K.; Abeyagunawardena, A.; Bose, N.; Pradhan, S.; Bresolin, N.L.; Garcia, B.A.; McCulloch, M. Snake Bite Associated with Acute Kidney Injury. *Pediatr Nephrol* **2020**, *36*, 3829–3840, doi:10.1007/s00467-020-04911-x.
115. Sitprija, V. Animal Toxins and the Kidney. *Nat Clin Pract Nephrol* **2008**, *4*, 616–627, doi:10.1038/ncpneph0941.
116. Teixeira, C.F.P.; Landucci, E.C.T.; Antunes, E.; Chacur, M.; Cury, Y. Inflammatory Effects of Snake Venom Myotoxic Phospholipases A_2. *Toxicon* **2003**, *42*, 947–962, doi: 10.1016/j.toxicon.2003.11.006.
117. Costa, S.K.P.; Camargo, E.A.; Antunes, E. Inflammatory Action of Secretory Phospholipases A_2 from Snake Venoms. In *Toxins and Drug*

Discovery; Cruz, L.J., Luo, S., Gopalakrishnakone, P., Eds.; Toxinology; Springer Netherlands: Dordrecht, **2017**; pp. 35–52 ISBN 978-94-007-6452-1.
118. Pungerčar, J.; Križaj, I. Understanding the Molecular Mechanism Underlying the Presynaptic Toxicity of Secreted Phospholipases A_2. *Toxicon* **2007**, *50*, 871–892, doi: 10.1016/j.toxicon.2007.07.025.
119. Montecucco, C.; Rossetto, O. How Do Presynaptic PLA2 Neurotoxins Block Nerve Terminals? *Trends Biochem Sci* **2000**, *25*, 266–270, doi:10.1016/s0968-0004(00)01556-5.
120. Tonello, F.; Rigoni, M. Cellular Mechanisms of Action of Snake Phospholipase A_2 Toxins. In *Snake Venoms*; Inagaki, H., Vogel, C.-W., Mukherjee, A.K., Rahmy, T.R., Gopalakrishnakone, P., Eds.; Toxinology; Springer Netherlands: Dordrecht, **2017**; pp. 49–65 ISBN 978-94-007-6410-1.
121. Lambeau, G.; Barhanin, J.; Schweitz, H.; Qar, J.; Lazdunski, M. Identification and Properties of Very High Affinity Brain Membrane-Binding Sites for a Neurotoxic Phospholipase from the Taipan Venom. *Journal of Biological Chemistry* **1989**, *264*, 11503–11510, doi:10.1016/s0021-9258(18)60492-2.
122. Paoli, M.; Rigoni, M.; Koster, G.; Rossetto, O.; Montecucco, C.; Postle, A.D. Mass Spectrometry Analysis of the Phospholipase A_2 Activity of Snake Pre-Synaptic Neurotoxins in Cultured Neurons. *J Neurochem* **2009**, *111*, 737–744, doi:10.1111/j.1471-4159.2009.06365. x.
123. Rigoni, M.; Caccin, P.; Gschmeissner, S.; Koster, G.; Postle, A.D.; Rossetto, O.; Schiavo, G.; Montecucco, C. Equivalent Effects of Snake PLA_2 Neurotoxins and Lysophospholipid-Fatty Acid Mixtures. *Science* **2005**, *310*, 1678–1680, doi:10.1126/science.1120640.
124. Nicotera, P.; Bellomo, G.; Orrenius, S. Calcium-Mediated Mechanisms in Chemically Induced Cell Death. *Annu Rev Pharmacol Toxicol* **1992**, *32*, 449–470, doi:10.1146/annurev.pa.32.040192.002313.
125. Montecucco, C.; Rossetto, O.; Caccin, P.; Rigoni, M.; Carli, L.; Morbiato, L.; Muraro, L.; Paoli, M. Different Mechanisms of Inhibition of Nerve Terminals by Botulinum and Snake Presynaptic Neurotoxins. *Toxicon* **2009**, *54*, 561–564, doi: 10.1016/j.toxicon.2008.12.012.
126. Kovacic, L.; Novinec, M.; Petan, T.; Krizaj, I. Structural Basis of the Significant Calmodulin-Induced Increase in the Enzymatic Activity of Secreted Phospholipases A_2. *Protein Eng Des Sel* **2010**, *23*, 479–487, doi:10.1093/protein/gzq019.
127. Mattiazzi, M.; Sun, Y.; Wolinski, H.; Bavdek, A.; Petan, T.; Anderluh, G.; Kohlwein, S.D.; Drubin, D.G.; Križaj, I.; Petrovič, U. A Neurotoxic Phospholipase A_2 Impairs Yeast Amphiphysin Activity and Reduces Endocytosis. *PLoS One* **2012**, *7*, e40931, doi: 10.1371/journal.pone.0040931.
128. Šribar, J.; Oberčkal, J.; Križaj, I. Understanding the Molecular Mechanism Underlying the Presynaptic Toxicity of Secreted Phospholipases A_2: An Update. *Toxicon* **2014**, *89*, 9–16, doi: 10.1016/j.toxicon.2014.06.019.
129. Kordiš, D.; Križaj, I. Secreted Phospholipases A_2 with β-Neurotoxic Activity. In *Snake Venoms*; Inagaki, H., Vogel, C.-W., Mukherjee, A.K., Rahmy, T.R., Gopalakrishnakone, P., Eds.; Toxinology; Springer Netherlands: Dordrecht, **2017**; pp. 67–86 ISBN 978-94-007-6410-1.
130. Pereañez, J.A.; Núñez, V.; Huancahuire-Vega, S.; Marangoni, S.; Ponce-Soto, L.A. Biochemical and Biological Characterization of a PLA_2 from

Crotoxin Complex of *Crotalus durissus cumanensis*. *Toxicon* **2009**, *53*, 534–542, doi: 10.1016/j.toxicon.2009.01.021.
131. Hendon, R.A.; Fraenkel-Conrat, H. Biological Roles of the Two Components of Crotoxin. *Proc Natl Acad Sci USA* **1971**, *68*, 1560–1563, doi:10.1073/pnas.68.7.1560.
132. Habermann, E.; Breithaupt, H. Mini-Review. The Crotoxin Complexan Example of Biochemical and Pharmacological Protein Complementation. *Toxicon* **1978**, *16*, 19–30, doi:10.1016/0041-0101(78)90056-9.
133. Canziani, G.; Seki, C.; Vidal, J.C. The Mechanism of Inhibition of Phospholipase Activity of Crotoxin B by Crotoxin A. *Toxicon* **1983**, *21*, 663–674, doi:10.1016/0041-0101(83)90272-6.
134. Pereañez, J.A.; Gómez, I.D.; Patiño, A.C**.** Relationship between the Structure and the Enzymatic Activity of Crotoxin Complex and Its Phospholipase A_2 Subunit: An in-Silico Approach. *J Mol Graph Model* **2012**, *35*, 36–42, doi: 10.1016/j.jmgm.2012.01.004.
135. Rey-Suárez, P.; Núñez, V.; Saldarriaga-Cordoba, M.; Lomonte, B. Primary Structures and Partial Toxicological Characterization of Two Phospholipases A_2 from *Micrurus mipartitus* and *Micrurus dumerilii* Coral Snake Venoms. *Biochimie* **2017**, *137*, 88–98, doi: 10.1016/j.biochi.2017.03.008.
136. Kini, R.M. Structure-Function Relationships and Mechanism of Anticoagulant Phospholipase A_2 Enzymes from Snake Venoms. *Toxicon* **2005**, *45*, 1147–1161, doi: 10.1016/j.toxicon.2005.02.018.
137. Verheij, H.M.; Boffa, M.C.; Rothen, C.; Bryckaert, M.C.; Verger, R.; de Haas, G.H. Correlation of Enzymatic Activity and Anticoagulant Properties of Phospholipase A_2. *Eur J Biochem* **1980**, *112*, 25–32, doi:10.1111/j.1432-1033. 1980.tb04982. x.
138. Kini, R.M.; Evans, H.J. Structure-Function Relationships of Phospholipases. The Anticoagulant Region of Phospholipases A_2. *J Biol Chem* **1987**, *262*, 14402–14407.
139. Stefansson, S.; Kini, R.M.; Evans, H.J. The Basic Phospholipase A_2 from *Naja nigricollis* Venom Inhibits the Prothrombinase Complex by a Novel Nonenzymatic Mechanism. *Biochemistry* **1990**, *29*, 7742–7746, doi:10.1021/bi00485a024.
140. Faure, G.; Gowda, V.T.; Maroun, R.C. Characterization of a Human Coagulation Factor Xa-Binding Site on Viperidae Snake Venom Phospholipases A_2 by Affinity Binding Studies and Molecular Bioinformatics. *BMC Struct Biol* **2007**, *7*, 82, doi:10.1186/1472-6807-7-82.
141. Faure, G.; Xu, H.; Saul, F. Anticoagulant Phospholipases A_2 Which Bind to the Specific Soluble Receptor Coagulation Factor Xa. In *Toxins and Hemostasis: From Bench to Bedside*; Kini, R.M., Clemetson, K.J., Markland, F.S., McLane, M.A., Morita, T., Eds.; Springer Netherlands: Dordrecht, **2010**; pp. 201–217 ISBN 978-90-481-9295-3.
142. Saikia, D.; Mukherjee, A.K. Anticoagulant and Membrane Damaging Properties of Snake Venom Phospholipase A2 Enzymes. In *Snake Venoms*; Gopalakrishnakone, P., Inagaki, H., Mukherjee, A.K., Rahmy, T.R., Vogel, C.-W., Eds.; Toxinology; Springer Netherlands: Dordrecht, **2015**; pp. 1–14 ISBN 978-94-007-6648-8.
143. Kini, R.M.; Evans, H.J. Effects of Phospholipase Enzymes on Platelet Aggregation. In *Venom Phospholipase A_2 Enzymes: Structure, Function and Mechanism*; John Wiley: Chichester, England, **1997**; pp. 369–387.

144. Ouyang, C.; Teng, C.M. The Action Mechanism of the Purified Platelet Aggregation Principle of *Trimeresurus mucrosquamatus* Venom. *Thromb Haemost* **1979**, *41*, 475–490.
145. Landucci, E.C.; Condino-Neto, A.; Perez, A.C.; Hyslop, S.; Corrado, A.P.; Novello, J.C.; Marangoni, S.; Oliveira, B.; Antunes, E.; de Nucci, G**.** Crotoxin Induces Aggregation of Human Washed Platelets. *Toxicon* **1994**, *32*, 217–226, doi:10.1016/0041-0101(94)90111-2.
146. Ouyang, C.; Yeh, H.I.; Huang, T.F. A Potent Platelet Aggregation Inhibitor Purified from *Agkistrodon halys* (Mamushi) Snake Venom. *Toxicon* **1983**, *21*, 797–804, doi:10.1016/0041-0101(83)90068-5.
147. Li, Y.S.; Liu, K.F.; Wang, Q.C.; Ran, Y.L.; Tu, G.C. A Platelet Function Inhibitor Purified from *Vipera russelli siamensis* (Smith) Snake Venom. *Toxicon* **1985**, *23*, 895–903, doi:10.1016/0041-0101(85)90381-2.
148. Ouyang, C.; Huang, T.F. Effect of the Purified Phospholipases A_2 from Snake and Bee Venoms on Rabbit Platelet Function. *Toxicon* **1984**, *22*, 705–718, doi:10.1016/0041-0101(84)90154-5.
149. Péterfi, O.; Boda, F.; Szabó, Z.; Ferencz, E.; Bába, L. Hypotensive Snake Venom Components-A Mini-Review. *Molecules* **2019**, *24*, 1–16, doi:10.3390/molecules24152778.
150. Andrião-Escarso, S.H.; Soares, A.M.; Fontes, M.R.M.; Fuly, A.L.; Corrêa, F.M.A.; Rosa, J.C.; Greene, L.J.; Giglio, J.R. Structural and Functional Characterization of an Acidic Platelet Aggregation Inhibitor and Hypotensive Phospholipase A_2 from *Bothrops jararacussu* Snake Venom. *Biochem Pharmacol* **2002**, *64*, 723–732, doi:10.1016/s0006-2952(02)01210-8.
151. Chaisakul, J.; Isbister, G.K.; Tare, M.; Parkington, H.C.; Hodgson, W.C**.** Hypotensive and Vascular Relaxant Effects of Phospholipase A_2 Toxins from Papuan Taipan (*Oxyuranus scutellatus*) Venom. *Eur J Pharmacol* **2014**, *723*, 227–233, doi: 10.1016/j.ejphar.2013.11.028.
152. Silveira, L.B.; Marchi-Salvador, D.P.; Santos-Filho, N.A.; Silva, F.P.; Marcussi, S.; Fuly, A.L.; Nomizo, A.; da Silva, S.L.; Stábeli, R.G.; Arantes, E.C.; et al. Isolation and Expression of a Hypotensive and Anti-Platelet Acidic Phospholipase A_2 from *Bothrops moojeni* Snake Venom. *J Pharm Biomed Anal* **2013**, *73*, 35–43, doi: 10.1016/j.jpba.2012.04.008.
153. Almeida, J.R.; Palacios, A.L. V; Patiño, R.S.P.; Mendes, B.; Teixeira, C.A.S.; Gomes, P.; da Silva, S.L. Harnessing Snake Venom Phospholipases A_2 to Novel Approaches for Overcoming Antibiotic Resistance. *Drug Dev Res* **2019**, *80*, 68–85, doi:10.1002/ddr.21456.
154. Hiu, J.J.; Yap, M.K.K. Cytotoxicity of Snake Venom Enzymatic Toxins: Phospholipase A2 and l-Amino Acid Oxidase. *Biochem Soc Trans* **2020**, *48*, 719–731, doi:10.1042/BST20200110.
155. Asano, Y.; Yasukawa, K**.** Identification and Development of Amino Acid Oxidases. *Curr Opin Chem Biol* **2019**, *49*, 76–83, doi: 10.1016/j.cbpa.2018.10.020.
156. Hossain, G.S.; Li, J.; Shin, H.D.; Du, G.; Liu, L.; Chen, J. L-Amino Acid Oxidases from Microbial Sources: Types, Properties, Functions, and Applications. *Appl Microbiol Biotechnol* **2014**, *98*, 1507–1515, doi:10.1007/s00253-013-5444-2.
157. Paloschi, M. V.; Pontes, A.S.; Soares, A.M.; Zuliani, J.P. An Update on Potential Molecular Mechanisms Underlying the Actions of Snake

Venom L-Amino Acid Oxidases (LAAOs). *Curr Med Chem* **2018**, *25*, 2520–2530, doi:10.2174/0929867324666171109114125.
158. Tan, N.H. L-Amino Acid Oxidases and Lactate Deshydrogenases. In *Enzymes from snake venom*; Bailey, G.S., Ed.; Alaken: Fort Collins, **1998**; pp. 579–598.
159. Ullah, A. Structure–Function Studies and Mechanism of Action of Snake Venom L-Amino Acid Oxidases. *Front Pharmacol* **2020**, *11*, 110, doi:10.3389/fphar.2020.00110.
160. Izidoro, L.F.M.; Sobrinho, J.C.; Mendes, M.M.; Costa, T.R.; Grabner, A.N.; Rodrigues, V.M.; Da Silva, S.L.; Zanchi, F.B.; Zuliani, J.P.; Fernandes, C.F.C.; et al. Snake Venom L-Amino Acid Oxidases: Trends in Pharmacology and Biochemistry. *Biomed Res Int* **2014**, *2014*, doi:10.1155/2014/196754.
161. Moustafa, I.M.; Foster, S.; Lyubimov, A.Y.; Vrielink, A. Crystal Structure of LAAO from *Calloselasma rhodostoma* with an L-Phenylalanine Substrate: Insights into Structure and Mechanism. *J Mol Biol* **2006**, *364*, 991–1002, doi: 10.1016/j.jmb.2006.09.032.
162. Guo, C.; Liu, S.; Yao, Y.; Zhang, Q.; Sun, M.Z. Past Decade Study of Snake Venom L-Amino Acid Oxidase. *Toxicon* **2012**, *60*, 302–311.
163. Liu, J.W.; Chai, M.Q.; Du, X.Y.; Song, J.G.; Zhou, Y.C**.** [Purification and characterization of L-amino acid oxidase from Agkistrodon halys pallas venom]. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* **2002**, *34*, 305–310.
164. Torii, S.; Yamane, K.; Mashima, T.; Haga, N.; Yamamoto, K.; Fox, J.W.; Naito, M.; Tsuruo, T. Molecular Cloning and Functional Analysis of Apoxin I, a Snake Venom- Derived Apoptosis-Inducing Factor with L-Amino Acid Oxidase Activity. *Biochemistry* **2000**, *39*, 3197–3205, doi:10.1021/bi992416z.
165. Stábeli, R.G.; Marcussi, S.; Carlos, G.B.; Pietro, R.C.L.R.; Selistre-De-Araújo, H.S.; Giglio, J.R.; Oliveira, E.B.; Soares, A.M. Platelet Aggregation and Antibacterial Effects of an L-Amino Acid Oxidase Purified from *Bothrops alternatus* Snake Venom. *Bioorg Med Chem* **2004**, *12*, 2881–2886, doi: 10.1016/j.bmc.2004.03.049.
166. Rodrigues, R.S.; da Silva, J.F.; Boldrini França, J.; Fonseca, F.P.P.; Otaviano, A.R.; Henrique Silva, F.; Hamaguchi, A.; Magro, A.J.; Braz, A.S.K.; dos Santos, J.I.; et al. Structural and Functional Properties of Bp-LAAO, a new l-Amino Acid Oxidase Isolated from *Bothrops pauloensis* Snake Venom. *Biochimie* **2009**, *91*, 490–501, doi: 10.1016/j.biochi.2008.12.004.
167. Ciscotto, P.; Machado de Avila, R.A.; Coelho, E.A.F.; Oliveira, J.; Diniz, C.G.; Farías, L.M.; de Carvalho, M.A.R.; Maria, W.S.; Sanchez, E.F.; Borges, A.; et al. Antigenic, Microbicidal and Antiparasitic Properties of an l-Amino Acid Oxidase Isolated from *Bothrops jararaca* Snake Venom. *Toxicon* **2009**, *53*, 330–341, doi: 10.1016/j.toxicon.2008.12.004.
168. Vargas, L.J.; Quintana, J.C.; Pereañez, J.A.; Núñez, V.; Sanz, L.; Calvete, J. Cloning and Characterization of an Antibacterial L-Amino Acid Oxidase from Crotalus *durissus cumanensis* Venom. *Toxicon* **2013**, *64*, 1–11, doi: 10.1016/j.toxicon.2012.11.027.
169. Vargas Muñoz, L.J.; Estrada-Gomez, S.; Núñez, V.; Sanz, L.; Calvete, J.J. Characterization and CDNA Sequence of *Bothriechis schlegelii* L-

Amino Acid Oxidase with Antibacterial Activity. *Int J Biol Macromol* **2014**, *69*, 200–207, doi: 10.1016/j.ijbiomac.2014.05.039.
170. Bedoya-Medina, J.; Mendivil-Perez, M.; Rey-Suarez, P.; Jimenez-Del-Rio, M.; Núñez, V.; Velez-Pardo, C. L-Amino Acid Oxidase Isolated from *Micrurus mipartitus* Snake Venom (MipLAAO) Specifically Induces Apoptosis in Acute Lymphoblastic Leukemia Cells Mostly via Oxidative Stress-Dependent Signaling Mechanism. *Int J Biol Macromol* **2019**, *134*, 1052–1062, doi: 10.1016/j.ijbiomac.2019.05.174.
171. Izidoro, L.F.M.; Ribeiro, M.C.; Souza, G.R.L.; Sant'Ana, C.D.; Hamaguchi, A.; Homsi-Brandeburgo, M.I.; Goulart, L.R.; Beleboni, R.O.; Nomizo, A.; Sampaio, S. V; et al. Biochemical and Functional Characterization of an L-Amino Acid Oxidase Isolated from *Bothrops pirajai* Snake Venom. *Bioorg Med Chem* **2006**, *14*, 7034–7043, doi: 10.1016/j.bmc.2006.06.025.
172. Toyama, M.H.; Toyama, D. de O.; Passero, L.F.D.; Laurenti, M.D.; Corbett, C.E.; Tomokane, T.Y.; Fonseca, F. V; Antunes, E.; Joazeiro, P.P.; Beriam, L.O.S.; et al**.** Isolation of a New L-Amino Acid Oxidase from *Crotalus durissus cascavella* Venom. *Toxicon* **2006**, *47*, 47–57, doi: 10.1016/j.toxicon.2005.09.008.
173. Soares, T.G.; Santos, J.L. Dos; Alvarenga, V.G. de; Santos, J.S.C.; Leclercq, S.Y.; Faria, C.D.; Oliveira, M.A.A.; Bemquerer, M.P.; Sanchez, E.O.F.; de Lima, M.E.; et al. Biochemical and Functional Properties of a New L-Amino Acid Oxidase (LAAO) from *Micrurus lemniscatus* Snake Venom. *Int J Biol Macromol* **2020**, *154*, 1517–1527, doi: 10.1016/j.ijbiomac.2019.11.033.
174. Samel, M.; Vija, H.; Rönnholm, G.; Siigur, J.; Kalkkinen, N.; Siigur, E. Isolation and Characterization of an Apoptotic and Platelet Aggregation Inhibiting L-Amino Acid Oxidase from *Vipera berus berus* (Common Viper) Venom. *Biochim Biophys Acta* **2006**, *1764*, 707–714, doi: 10.1016/j.bbapap.2006.01.021.
175. Suhr, S.M.; Kim, D.S. Comparison of the Apoptotic Pathways Induced by L-Amino Acid Oxidase and Hydrogen Peroxide. *J Biochem* **1999**, *125*, 305–309, doi: 10.1093/oxfordjournals.jbchem.a022287.
176. Ande, S.R.; Kommoju, P.R.; Draxl, S.; Murkovic, M.; Macheroux, P.; Ghisla, S.; Ferrando-May, E. Mechanisms of Cell Death Induction by L-Amino Acid Oxidase, a Major Component of Ophidian Venom. *Apoptosis* **2006**, *11*, 1439–1451, doi:10.1007/s10495-006-7959-9.
177. Souza, D.H.; Eugenio, L.M.; Fletcher, J.E.; Jiang, M.S.; Garratt, R.C.; Oliva, G.; Selistre-de-Araujo, H.S. Isolation and Structural Characterization of a Cytotoxic L-Amino Acid Oxidase from *Agkistrodon contortrix laticinctus* Snake Venom: Preliminary Crystallographic Data. *Arch Biochem Biophys* **1999**, *368*, 285–290, doi:10.1006/abbi.1999.1287.
178. Alves, R.M.; Antonucci, G.A.; Paiva, H.H.; Cintra, A.C.O.; Franco, J.J.; Mendonça-Franqueiro, E.P.; Dorta, D.J.; Giglio, J.R.; Rosa, J.C.; Fuly, A.L.; et al. Evidence of Caspase-Mediated Apoptosis Induced by l-Amino Acid Oxidase Isolated from *Bothrops atrox* Snake Venom. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology* **2008**, *151*, 542–550, doi: 10.1016/j.cbpa.2008.07.007.
179. Zhang, H.; Yang, Q.; Sun, M.; Teng, M.; Niu, L. Hydrogen Peroxide Produced by Two Amino Acid Oxidases Mediates Antibacterial Actions. *J Microbiol* **2004**, *42*, 336–339.

180. Wei, X.-L.; Wei, J.-F.; Li, T.; Qiao, L.-Y.; Liu, Y.-L.; Huang, T.; He, S.-H. Purification, Characterization and Potent Lung Lesion Activity of an L-Amino Acid Oxidase from *Agkistrodon blomhoffii ussurensis* Snake Venom. *Toxicon* **2007**, *50*, 1126–1139, doi: 10.1016/j.toxicon.2007.07.022.
181. Izidoro, L.F.M.; Alves, L.M.; Rodrigues, V.M.; Silva, D.A.O.; Mineo, J.R. Bothrops Pirajai Snake Venom L-Amino Acid Oxidase: In Vitro Effects on Infection of *Toxoplasma gondii* in Human Foreskin Fibroblasts. *Revista Brasileira de Farmacognosia* **2011**, *21*, 477–485, doi:10.1590/S0102-695X2011005000108.
182. Du, X.-Y.; Clemetson, K.J. Snake Venom L-Amino Acid Oxidases. *Toxicon* **2002**, *40*, 659–665, doi:10.1016/s0041-0101(02)00102-2.
183. Belisario, M.A.; Tafuri, S.; Di Domenico, C.; Squillacioti, C.; Della Morte, R.; Lucisano, A.; Staiano, N. H_2O_2 Activity on Platelet Adhesion to Fibrinogen and Protein Tyrosine Phosphorylation. *Biochim Biophys Acta Mol Cell Res* **2000**, *1495*, 183–193, doi:10.1016/S0167-4889(99)00160-3.
184. Pignatelli, P.; Pulcinelli, F.M.; Lenti, L.; Gazzaniga, P.P.; Violi, F. Hydrogen Peroxide Is Involved in Collagen-Induced Platelet Activation. *Blood* **1998**, *91*, 484–490.
185. Bregge-Silva, C.; Nonato, M.C.; de Albuquerque, S.; Ho, P.L.; Junqueira de Azevedo, I.L.M.; Vasconcelos Diniz, M.R.; Lomonte, B.; Rucavado, A.; Díaz, C.; Gutiérrez, J.M.; et al**.** Isolation and Biochemical, Functional and Structural Characterization of a Novel l-Amino Acid Oxidase from *Lachesis muta* Snake Venom. *Toxicon* **2012**, *60*, 1263–1276, doi: 10.1016/j.toxicon.2012.08.008.
186. Izidoro, L.F.M.; Sobrinho, J.C.; Mendes, M.M.; Costa, T.R.; Grabner, A.N.; Rodrigues, V.M.; da Silva, S.L.; Zanchi, F.B.; Zuliani, J.P.; Fernandes, C.F.C.; et al. Snake Venom L-Amino Acid Oxidases: Trends in Pharmacology and Biochemistry. *Biomed Res Int* **2014**, *2014*, 196754, doi:10.1155/2014/196754.
187. Wiezel, G.A.; Rustiguel, J.K.; Morgenstern, D.; Zoccal, K.F.; Faccioli, L.H.; Nonato, M.C.; Ueberheide, B.; Arantes, E.C**.** Insights into the Structure, Function and Stability of Bordonein-L, the First L-Amino Acid Oxidase from *Crotalus durissus terrificus* Snake Venom. *Biochimie* **2019**, *163*, 33–49, doi: 10.1016/j.biochi.2019.05.009.
188. Rey-Suárez, P.; Acosta, C.; Torres, U.; Saldarriaga-Cordoba, M.; Lomonte, B.; Núñez, V. MipLAAO, a New L-Amino Acid Oxidase from the Redtail Coral Snake *Micrurus mipartitus*. *PeerJ* **2018**, *2018*, e4924, doi:10.7717/peerj.4924.
189. Zhang, L.; Wei, L.-J. ACTX-8, a Cytotoxic L-Amino Acid Oxidase Isolated from *Agkistrodon acutus* Snake Venom, Induces Apoptosis in Hela Cervical Cancer Cells. *Life Sci* **2007**, *80*, 1189–1197, doi: 10.1016/j.lfs.2006.12.024.
190. Tan, K.K.; Bay, B.H.; Gopalakrishnakone, P. L-Amino Acid Oxidase from Snake Venom and Its Anticancer Potential. *Toxicon* **2018**, *144*, 7–13.
191. Bhattacharjee, P.; Mitra, J.; Bhattacharyya, D. L-Amino Acid Oxidase from Venoms BT - Toxins and Drug Discovery. In; Cruz, L.J., Luo, S., Gopalakrishnakone, P., Eds.; Springer Netherlands: Dordrecht, **2017**; pp. 295–320 ISBN 978-94-007-6452-1.
192. Huang, T.F.; Holt, J.C.; Lukasiewicz, H.; Niewiarowski, S. Trigramin. A Low Molecular Weight Peptide Inhibiting Fibrinogen Interaction with

Platelet Receptors Expressed on Glycoprotein IIb-IIIa Complex. *J Biol Chem* **1987**, *262*, 16157–16163.
193. Calvete, J.J. The Continuing Saga of Snake Venom Disintegrins. *Toxicon* **2013**, *62*, 40–49, doi: 10.1016/j.toxicon.2012.09.005.
194. Calvete, J.J.; Moreno-Murciano, M.P.; Theakston, R.D.G.; Kisiel, D.G.; Marcinkiewicz, C. Snake Venom Disintegrins: Novel Dimeric Disintegrins and Structural Diversification by Disulphide Bond Engineering. *Biochemical Journal* **2003**, *372*, 725–734, doi:10.1042/BJ20021739.
195. Bilgrami, S.; Tomar, S.; Yadav, S.; Kaur, P.; Kumar, J.; Jabeen, T.; Sharma, S.; Singh, T.P**.** Crystal Structure of Schistatin, a Disintegrin Homodimer from Saw-Scaled Viper (*Echis carinatus*) at 2.5 Å Resolution. *J Mol Biol* **2004**, *341*, 829–837, doi: 10.1016/j.jmb.2004.06.048.
196. Carbajo, R.J.; Sanz, L.; Perez, A.; Calvete, J.J. NMR Structure of Bitistatin – a Missing Piece in the Evolutionary Pathway of Snake Venom Disintegrins. *FEBS J* **2015**, *282*, 341–360, doi:10.1111/FEBS.13138.
197. Arruda Macedo, J.; Fox, J.; Souza Castro, M. Disintegrins from Snake Venoms and Their Applications in Cancer Research and Therapy. *Curr Protein Pept Sci* **2015**, *16*, 532–548, doi:10.2174/1389203716666150515125002.
198. Calvete, J.; Juárez, P.; Sanz, L. Snake Venomics. Strategy and Applications. *Journal of Mass Spectrometry* **2007**, *42*, 1405–1414, doi:10.1002/jms.1242.
199. Jang, Y.J.; Jeon, O.H.; Kim, D.S. Saxatilin, a Snake Venom Disintegrin, Regulates Platelet Activation Associated with Human Vascular Endothelial Cell Migration and Invasion. *J Vasc Res* **2007**, *44*, 129–137, doi:10.1159/000098519.
200. Kuo, Y.J.; Chung, C.H.; Huang, T.F. From Discovery of Snake Venom Disintegrins to A Safer Therapeutic Antithrombotic Agent. *Toxins (Basel)* **2019**, *11*, doi:10.3390/TOXINS11070372.
201. Lazarovici, P.; Marcinkiewicz, C.; Lelkes, P.I. From Snake Venom's Disintegrins and C-Type Lectins to Anti-Platelet Drugs. *Toxins 2019, Vol. 11, Page 303* **2019**, *11*, 303, doi:10.3390/TOXINS11050303.
202. Sánchez, E.E.; Galán, J.A.; Russell, W.K.; Soto, J.G.; Russell, D.H.; Pérez, J.C. Isolation and Characterization of Two Disintegrins Inhibiting ADP-Induced Human Platelet Aggregation from the Venom of *Crotalus scutulatus scutulatus* (Mohave Rattlesnake). *Toxicol Appl Pharmacol* **2006**, *212*, 59–68, doi: 10.1016/j.taap.2005.07.004.
203. Sánchez, E.E.; Rodríguez-Acosta, A.; Palomar, R.; Lucena, S.E.; Bashir, S.; Soto, J.G.; Pérez**,** J.C. Colombistatin: A Disintegrin Isolated from the Venom of the South American Snake (*Bothrops colombiensis*) That Effectively Inhibits Platelet Aggregation and SK-Mel-28 Cell Adhesion. *Arch Toxicol* **2009**, *83*, 271–279, doi:10.1007/s00204-008-0358-y.
204. Gan, Z.R.; Gould, R.J.; Jacobs, J.W.; Friedman, P.A.; Polokoff, M.A. Echistatin. A Potent Platelet Aggregation Inhibitor from the Venom of the Viper, *Echis carinatus*. *Journal of Biological Chemistry* **1988**, *263*, 19827–19832, doi:10.1016/s0021-9258(19)77710-2.
205. Scarborough, R.M.; Rose, J.W.; Hsu, M.A.; Phillips, D.R.; Fried, V.A.; Campbell, A.M.; Nannizzi, L.; Charo, I.F. Barbourin: A GPIIb-IIIa-Specific Integrin Antagonist from the Venom of Sistrurus M. Barbouri. *Journal of Biological Chemistry* **1991**, *266*, 9359–9362, doi:10.1016/s0021-9258(18)92826-7.

206. Selistre-de-Araujo, H.S.; Pontes, C.L.S.; Montenegro, C.F.; Martin, A.C.B.M. Snake Venom Disintegrins and Cell Migration. *Toxins* **2010**, *2*, 2606–2621, doi:10.3390/toxins2112606.
207. Swenson, S.; Ramu, S.; Markland, F. Anti-Angiogenesis and RGD-Containing Snake Venom Disintegrins. *Curr Pharm Des* **2007**, *13*, 2860–2871, doi:10.2174/138161207782023793.
208. Uzair, B.; Atlas, N.; Malik, S.B.; Jamil, N.; Ojuolape, S.T.; Rehman, M.U.; Khan, B.A. Snake Venom as an Effective Tool Against Colorectal Cancer. *Protein Pept Lett* **2018**, *25*, 626–632, doi:10.2174/0929866525666180614112935.
209. Kini, R.M.; Doley, R. Structure, Function and Evolution of Three-Finger Toxins: Mini Proteins with Multiple Targets. *Toxicon* **2010**, *56*, 855–867, doi: 10.1016/j.toxicon.2010.07.010.
210. Rey-Suárez, P.; Floriano, R.S.; Rostelato-Ferreira, S.; Saldarriaga-Cordoba, M.; Núñez, V.; Rodrigues-Simioni, L.; Lomonte, B. Mipartoxin-I, a Novel Three-Finger Toxin, Is the Major Neurotoxic Component in the Venom of the Redtail Coral Snake *Micrurus mipartitus* (Elapidae). *Toxicon* **2012**, *60*, 851–863.
211. Kessler, P.; Marchot, P.; Silva, M.; Servent, D. The Three-Finger Toxin Fold: A Multifunctional Structural Scaffold Able to Modulate Cholinergic Functions. *J Neurochem* **2017**, *142*, 7–18, doi:10.1111/jnc.13975.
212. Aird, S.D.; da Silva, N.J. Chemistry of Coralsnake Venoms. In *Advances in Coralsnake Biology: With an Emphasis on South America*; Eagle Mountain Publishing, L.C., **2021**; pp. 399–484 ISBN 9780972015462.
213. Nastopoulos, V. Structure of Dimeric and Monomeric Erabutoxin a Refined at 1.5 Å Resolution. *Acta Crystallogr D Biol Crystallogr* **1998**, *54*, 964–974, doi:10.1107/S0907444998005125.
214. Scarselli, M.; Spiga, O.; Ciutti, A.; Bernini, A.; Bracci, L.; Lelli, B.; Lozzi, L.; Calamandrei, D.; Maro, D. Di; Klein, S.; et al. NMR Structure of R-Bungarotoxin Free and Bound to a Mimotope of the Nicotinic Receptor. *Methods* **2002**, 1457–1463.
215. Chung, C.; Wu, B.N.; Yang, C.C.; Chang, L.S. Muscarinic Toxin-Like Proteins from Taiwan Banded Krait (*Bungarus multicinctus*) Venom: Purification, Characterization and Gene Organization. *Journal of Biological Chemistry* **2002**, *383*, 1397–1406, doi:10.1023/A:1019760401692.
216. Lukyanova, E.N.; Shenkarev, Z.O.; Shulepko, M.A.; Paramonov, A.S.; Chugunov, A.O.; Janickova, H.; Dolejsi, E.; Dolezal, V.; Utkin, Y.N.; Tsetlin, V.I.; et al. Structural Insight into Specificity of Interactions between Nonconventional Three-Finger Weak Toxin from *Naja kaouthia* (WTX) and Muscarinic Acetylcholine Receptors. *Journal of Biological Chemistry* **2015**, *290*, 23616–23630, doi:10.1074/jbc.M115.656595.
217. Nickitenko, A. V.; Michailov, A.M.; Betzel, C.; Wilson, K.S. Three-Dimensional Structure of Neurotoxin-1 from *Naja naja oxiana* Venom at 1.9 Å Resolution. *FEBS Lett* **1993**, *320*, 111–117, doi:10.1016/0014-5793(93)80073-4.
218. Pawlak, J.; Mackessy, S.P.; Fry, B.G.; Bhatia, M.; Mourier, G.; Fruchart-Gaillard, C.; Servent, D.; Ménez, R.; Stura, E.; Ménez, A.; et al. Denmotoxin, a Three-Finger Toxin from the Colubrid Snake *Boiga dendrophalia* (Mangrove Catsnake) with Bird-Specific Activity. *Journal of Biological Chemistry* **2006**, *281*, 29030–29041, doi:10.1074/jbc.M605850200.

219. Roy, A.; Zhou, X.; Chong, M.Z.; D'Hoedt, D.; Foo, C.S.; Rajagopalan, N.; Nirthanan, S.; Bertrand, D.; Sivaraman, J.; Manjunatha Kini, R. Structural and Functional Characterization of a Novel Homodimeric Three-Finger Neurotoxin from the Venom of *Ophiophaga hannah* (King Cobra). *Journal of Biological Chemistry* **2010**, *285*, 8302–8315, doi:10.1074/jbc.M109.074161.
220. Pawlak, J.; Mackessy, S.P.; Sixberry, N.M.; Stura, E.A.; Le Du, M.H.; Ménez, R.; Foo, C.S.; Ménez, A.; Nirthanan, S.; Kini, R.M. Irditoxin, a Novel Covalently Linked Heterodimeric Three-Finger Toxin with High Taxon-Specific Neurotoxicity. *The FASEB Journal* **2009**, *23*, 534–545, doi: https://doi.org/10.1096/fj.08-113555.
221. Aoki-Shioi, N.; Jobichen, C.; Sivaraman, J.; Kini, R.M. Unusual Quaternary Structure of a Homodimeric Synergistic-Type Toxin from Mamba Snake Venom Defines Its Molecular Evolution. *Biochemical Journal* **2020**, *477*, 3951–3962, doi:10.1042/BCJ20200529.
222. Anadón, A.; Martínez-Larrañaga, M.R.; Valerio, L.G. Onchidal and Fasciculins. In *Handbook of Toxicology of Chemical Warfare Agents: Second Edition*; Elsevier Inc., **2015**; pp. 411–420 ISBN 9780128001592.
223. Utkin, Y. Last Decade Update for Three-Finger Toxins: Newly Emerging Structures and Biological Activities. *World J Biol Chem* **2019**, *10*, 17–27.
224. Kleiz-Ferreira, J.M.; Cirauqui, N.; Trajano, E.A.; Almeida, M. da S.; Zingali, R.B. Three-Finger Toxins from Brazilian Coral Snakes: From Molecular Framework to Insights in Biological Function. *Toxins (Basel)* **2021**, *13*, 1–19, doi:10.3390/toxins13050328.
225. Castillo-Beltrán, M.C.; Hurtado-Gómez, J.P.; Corredor-Espinel, V.; Ruiz-Gómez, F.J. A Polyvalent Coral Snake Antivenom with Broad Neutralization Capacity. *PLoS Negl Trop Dis* **2018**, *13*, 1–14, doi: 10.1371/journal.pntd.0007250.
226. Rey-Suárez, P.; Saldarriaga, M.; Torres, U.; Marin-villa, M.; Lomonte, B.; Núñez, V. Novel three-finger toxins from *Micrurus dumerilii* and *Micrurus mipartitus* coral snake venoms: Phylogenetic relationships and characterization of Clarkitoxin-I-Mdum. *Toxicon* **2019**, *170*, 85–93.
227. Lomonte, B.; Sasa, M.; Rey-Suárez, P.; Bryan, W.; Gutiérrez, J.M. Venom of the Coral Snake Micrurus Clarki: Proteomic Profile, Toxicity, Immunological Cross-Neutralization, and Characterization of a Three-Finger Toxin. *Toxins (Basel)* **2016**, *8*, doi:10.3390/toxins8050138.
228. Bertoni, M.; Kiefer, F.; Biasini, M.; Bordoli, L.; Schwede, T. Modeling Protein Quaternary Structure of Homo- and Hetero-Oligomers beyond Binary Interactions by Homology. *Sci Rep* **2017**, *7*, 10480, doi:10.1038/s41598-017-09654-8.
229. Utkin, Y.N. Three-Finger Toxins, a Deadly Weapon of Elapid Venom - Milestones of Discovery. *Toxicon* **2013**, *62*, 50–55, doi: 10.1016/j.toxicon.2012.09.007.
230. Kini, R.M.; Koh, C.Y. Snake Venom Three-Finger Toxins and Their Potential in Drug Development Targeting Cardiovascular Diseases. *Biochem Pharmacol* **2020**, *181*, 114105, doi: 10.1016/j.bcp.2020.114105.
231. Zaqueo, K.D.; Kayano, A.M.; Domingos, T.F.S.; Moura, L.A.; Fuly, A.L.; da Silva, S.L.; Acosta, G.; Oliveira, E.; Albericio, F.; Zanchi, F.B.; et al. BbrzSP-32, the First Serine Protease Isolated from Bothrops Brazili

Venom: Purification and Characterization. *Comp Biochem Physiol A Mol Integr Physiol* **2016**, *195*, 15–25, doi: 10.1016/j.cbpa.2016.01.021.
232. Roldán-Padrón, O.; Castro-Guillén, J.; García-Arredondo, J.; Cruz-Pérez, M.; Díaz-Peña, L.; Saldaña, C.; Blanco-Labra, A.; García-Gasca, T. Snake Venom Hemotoxic Enzymes : Biochemical Comparison between *Crotalus* Species from Central Mexico. *Molecules* **2019**, *24*, 1–16.
233. Latinović, Z.; Leonardi, A.; Koh, C.Y.; Kini, R.M.; Bakija, A.T.; Pungerčar, J.; Križaj, I. The Procoagulant Snake Venom Serine Protease Potentially Having a Dual, Blood Coagulation Factor v and X-Activating Activity. *Toxins* **2020**, *12*, 1–15, doi:10.3390/toxins12060358.
234. Stefanelli, V.L.; Barker, T.H. The Evolution of Fibrin-Specific Targeting Strategies. *J Mater Chem B* **2015**, *3*, 1177–1186, doi:10.1039/c4tb01769b.
235. Mackessy, S. Venom Composition in Rattlesnakes: Trends and Biological Significance. In *The Biology of Rattlesnakes*; Hayes, W.K., Beaman, K.R., Cardwell, M.D., Bush, S.P., Eds.; Loma Linda University Press: Loma Linda, CA, **2008**; pp. 495–510.
236. Angulo, Y.; Lomonte, B. Biochemistry and Toxicology of Toxins Purified from the Venom of the Snake *Bothrops asper*. *Toxicon* **2009**, *54*, 949–957.
237. Gutiérrez, J.M.; Calvete, J.; Habib, A.; Harrison, R.; Williams, D.; Warrell, D. Snakebite Envenoming. *Nat Rev Dis Primers* **2017**, *3*, 1–20.
238. Serrano, S.M.T.; Maroun, R.C. Snake Venom Serine Proteinases: Sequence Homology vs. Substrate Specificity, a Paradox to Be Solved. *Toxicon* **2005**, *45*, 1115–1132, doi: 10.1016/j.toxicon.2005.02.020.
239. Serrano, S.M.T. The Long Road of Research on Snake Venom Serine Proteinases. *Toxicon* **2013**, *62*, 19–26, doi: 10.1016/j.toxicon.2012.09.003.
240. Yonamine, C.M.; Kondo, M.Y.; Nering, M.B.; Gouvêa, I.E.; Okamoto, D.; Andrade, D.; Alberto da Silva, J.A.; Prieto da Silva, Á.R.; Yamane, T.; Juliano, M.A.; et al. Enzyme Specificity and Effects of Gyroxin, a Serine Protease from the Venom of the South American Rattlesnake *Crotalus durissus terrificus*, on Protease-Activated Receptors. *Toxicon* **2014**, *79*, 64–71.
241. Carvalho, D.D.; Marangoni, S.; Oliveira, B.; Novello, J.C. Isolation and Characterization of a New Lectin From the Venom of the Snake *Bothrops jararacussu. Biochem Mol Biol Int* **1998**, *44*, 933–938.
242. Clemetson, K.; Morita, T.; Manjunatha Kini, R. Classification and Nomenclature of Snake Venom C-Type Lectins and Related Proteins. *Toxicon* **2009**, *54*, 83.
243. Sartim, M.A.; Sampaio, S. V Snake Venom Galactoside-Binding Lectins: A Structural and Functional Overview. *Journal of Venomous Animals and Toxins including Tropical Diseases* **2015**, *21*, 1–11, doi:10.1186/s40409-015-0038-3.
244. Arlinghaus, F.T.; Eble, J.A. C-Type Lectin-like Proteins from Snake Venoms. *Toxicon* **2012**, *60*, 512–519.
245. Vonk, F.J.; Jackson, K.; Doley, R.; Madaras, F.; Mirtschin, P.J.; Vidal, N. Snake Venom: From Fieldwork to the Clinic: Recent Insights into Snake Biology, Together with New Technology Allowing High-Throughput Screening of Venom, Bring New Hope for Drug Discovery. *BioEssays* **2011**, *33*, 269–279.
246. Chakrabarty, D.; Sarkar, A. Cytotoxic Effects of Snake Venoms. **2017**, *111*, 1–7.

247. Thakur, R.; Mukherjee, A.K**.** Pathophysiological Significance and Therapeutic Applications of Snake Venom Protease Inhibitors. *Toxicon* **2017**, *131*, 37–47.
248. Girish, K.S.; Jagadeesha, D.K.; Rajeev, K.B.; Kemparaju, K. Snake Venom Hyaluronidase: An Evidence for Isoforms and Extracellular Matrix Degradation. *Mol Cell Biochem* **2002**, *240*, 105–110, doi:10.1023/A:1020651607164.
249. Girish, K.S.; Shashidharamurthy, R.; Nagaraju, S.; Gowda, T. V.; Kemparaju, K. Isolation and Characterization of Hyaluronidase a "Spreading Factor" from Indian Cobra (*Naja naja*) Venom. *Biochimie* **2004**, *86*, 193–202, doi: 10.1016/j.biochi.2004.02.004.
250. Jiang, D.; Liang, J.; Noble, P.W. Hyaluronan as an Immune Regulator in Human Diseases. *Physiol Rev* **2011**, *91*, 221–264, doi:10.1152/physrev.00052.2009.
251. Noble, P.W. Hyaluronan and Its Catabolic Products in Tissue Injury and Repair. *Matrix Biology* **2002**, *21*, 25–29, doi:10.1016/S0945-053X(01)00184-6.
252. Ohno, S.; Im, H.J.; Knudson, C.B.; Knudson, W. Hyaluronan Oligosaccharides Induce Matrix Metalloproteinase 13 via Transcriptional Activation of NFκB and P38 MAP Kinase in Articular Chondrocytes. *Journal of Biological Chemistry* **2006**, *281*, 17952–17960, doi:10.1074/jbc.M602750200.
253. Frobert, Y.; Créminon, C.; Cousin, X.; Rémy, M.H.; Chatel, J.M.; Bon, S.; Bon, C.; Grassi, J. Acetylcholinesterases from *Elapidae* Snake Venoms: Biochemical, Immunological and Enzymatic Characterization. *Biochimica et Biophysica Acta - Protein Structure and Molecular Enzymology* **1997**, *1339*, 253–267, doi:10.1016/S0167-4838(97)00009-5.
254. Karlsson, E.; Mbugua, P.M.; Rodriguez-Ithurralde, D. Fasciculins, Anticholinesterase Toxins from the Venom of the Green Mamba Dendroaspis Angusticeps. *J Physiol (Paris)* **1984**, *79*, 232–240.
255. Bin Asad, M.H.H.; Iqbal, M.; Akram, M.R.; Khawaja, N.R.; Muneer, S.; Shabbir, M.Z.; Khan, M.S.; Murtaza, G.; Hussain, I. 5′-Nucleotidases of *Naja naja* Karachiensis Snake Venom: Their Determination, Toxicities and Remedial Approach by Natural Inhibitors (Medicinal Plants). *Acta Poloniae Pharmaceutica - Drug Research* **2016**, *73*, 667–673.
256. Ouyang, C.; Huang, T.F. Inhibition of Platelet Aggregation by 5′-Nucleotidase Purified from *Trimeresurus gramineus* Snake Venom. *Toxicon* **1983**, *21*, 491–501, doi:10.1016/0041-0101(83)90127-7.
257. Ouyang, C.; Huang, T.F. Platelet Aggregation Inhibitors from *Agkistrodon acutus* Snake Venom. *Toxicon* **1986**, *24*, 1099–1106, doi:10.1016/0041-0101(86)90136-4.
258. Trummal, K.; Samel, M.; Aaspõllu, A.; Tõnismägi, K.; Titma, T.; Subbi, J.; Siigur, J.; Siigur, E. 5′-Nucleotidase from *Vipera lebetina* Venom. *Toxicon* **2015**, *93*, 155–163, doi: 10.1016/j.toxicon.2014.11.234.
259. Aird, S.D. Ophidian Envenomation Strategies and the Role of Purines. *Toxicon* **2002**, *40*, 335–393, doi:10.1016/S0041-0101(01)00232-X.
260. Aloulou, A.; Rahier, R.; Arhab, Y.; Noiriel, A.; Abousalham, A. Phospholipases: An Overview. In *Methods in Molecular Biology*; Methods Mol Biol, **2018**; Vol. 1835, pp. 69–105.
261. Jiménez-Charris, E.; Montealegre-Sanchez, L.; Solano-Redondo, L.; Mora-Obando, D.; Camacho, E.; Castro-Herrera, F.; Fierro-Pérez, L.;

Lomonte, B. Proteomic and Functional Analyses of the Venom of *Porthidium lansbergii lansbergii* (Lansberg's Hognose Viper) from the Atlantic Department of Colombia. *J Proteomics* **2015**, *114*, 287–299, doi: 10.1016/j.jprot.2014.11.016.

262. Mora-Obando, D.; Salazar-Valenzuela, D.; Pla, D.; Lomonte, B.; Guerrero-Vargas, J.A.; Ayerbe, S.; Gibbs, H.L.; Calvete, J.J. Venom Variation in Bothrops Asper Lineages from North-Western South America. *J Proteomics* **2020**, *229*, doi: 10.1016/j.jprot.2020.103945.
263. Pereañez, J.A.; Preciado, L.M.; Fernández, J.; Camacho, E.; Lomonte, B.; Castro, F.; Cañas, C.A.; Galvis, C.; Castaño, S. Snake Venomics, Experimental Toxic Activities and Clinical Characteristics of Human Envenomation by *Bothrocophias myersi* (Serpentes: Viperidae) from Colombia. *J Proteomics* **2020**, *220*, doi: 10.1016/j.jprot.2020.103758.
264. Rey-Suárez, P.; Núñez, V.; Fernández, J.; Lomonte, B. Integrative Characterization of the Venom of the Coral Snake *Micrurus dumerilii* (Elapidae) from Colombia: Proteome, Toxicity, and Cross-Neutralization by Antivenom. *J Proteomics* **2016**, *136*, 262–273, doi: 10.1016/j.jprot.2016.02.006.
265. Bernheimer, A.W.; Linder, R.; Weinstein, S.A.; Kim, K.S. Isolation and Characterization of a Phospholipase B from Venom of Collett's Snake, *Pseudechis colleti*. *Toxicon* **1987**, *25*, 547–554, doi:10.1016/0041-0101(87)90290-x.
266. Yamazaki, Y.; Koike, H.; Sugiyama, Y.; Motoyoshi, K.; Wada, T.; Hishinuma, S.; Mita, M.; Morita, T. Cloning and Characterization of Novel Snake Venom Proteins That Block Smooth Muscle Contraction. *Eur J Biochem* **2002**, *269*, 2708–2715, doi:10.1046/j.1432-1033.2002.02940. x.
267. Brown, R.L.; Lynch, L.L.; Haley, T.L.; Arsanjani, R. Pseudechetoxin Binds to the Pore Turret of Cyclic Nucleotide-Gated Ion Channels. *Journal of General Physiology* **2003**, *122*, 749–760, doi:10.1085/jgp.200308823.
268. Lodovicho, M.E.; Costa, T.R.; Bernardes, C.P.; Menaldo, D.L.; Zoccal, K.F.; Carone, S.E.; Rosa, J.C.; Pucca, M.B.; Cerni, F.A.; Arantes, E.C.; et al. Investigating Possible Biological Targets of Bj-CRP, the First Cysteine-Rich Secretory Protein (CRISP) Isolated from *Bothrops jararaca* Snake Venom. *Toxicol Lett* **2017**, *265*, 156–169, doi: 10.1016/j.toxlet.2016.12.003.
269. Kostiza, T.; Meier, J. Nerve Growth Factors from Snake Venoms: Chemical Properties, Mode of Action and Biological Significance. *Toxicon* **1996**, *34*, 787–806, doi:10.1016/0041-0101(96)00023-2.
270. Yamazaki, Y.; Matsunaga, Y.; Tokunaga, Y.; Obayashi, S.; Saito, M.; Morita, T. Snake Venom Vascular Endothelial Growth Factors (VEGF-Fs) Exclusively Vary Their Structures and Functions among Species. *Journal of Biological Chemistry* **2009**, *284*, 9885–9891, doi:10.1074/jbc.M809071200.
271. Osipov, A. V.; Terpinskaya, T.I.; Kryukova, E. V.; Ulaschik, V.S.; Paulovets, L. V.; Petrova, E.A.; Blagun, E. V.; Starkov, V.G.; Utkin, Y.N. Nerve Growth Factor from Cobra Venom Inhibits the Growth of Ehrlich Tumor in Mice. *Toxins (Basel)* **2014**, *6*, 784–795, doi:10.3390/toxins6030784.
272. Koh, D.C.I.; Armugam, A.; Jeyaseelan, K. Sputa Nerve Growth Factor Forms a Preferable Substitute to Mouse 7S-β Nerve Growth Factor. *Biochemical Journal* **2004**, *383*, 149–158, doi:10.1042/BJ20040569.

273. Takahashi, H.; Hattori, S.; Iwamatsu, A.; Takizawa, H.; Shibuya, M. A Novel Snake Venom Vascular Endothelial Growth Factor (VEGF) Predominantly Induces Vascular Permeability through Preferential Signaling via VEGF Receptor-1. *Journal of Biological Chemistry* **2004**, *279*, 46304–46314, doi:10.1074/jbc.M403687200.
274. Flight, S.M.; Johnson, L.A.; Du, Q.S.; Warner, R.L.; Trabi, M.; Gaffney, P.J.; Lavin, M.F.; De Jersey, J.; Masci, P.P. Textilinin-1, an Alternative Anti-Bleeding Agent to Aprotinin: Importance of Plasmin Inhibition in Controlling Blood Loss. *Br J Haematol* **2009**, *145*, 207–211, doi:10.1111 /j.1365-2141.2009.07605. x.
275. Masci, P.P.; Whitaker, A.N.; Sparrow, L.G.; De Jersey, J.; Winzor, D.J.; Watters, D.J.; Lavin, M.F.; Gaffney, P.J. Textilinins from *Pseudonaja textilis textilis*. Characterization of Two Plasmin Inhibitors That Reduce Bleeding in an Animal Model. *Blood Coagulation and Fibrinolysis* **2000**, *11*, 385–393, doi:10.1097/00001721-200006000-00011.
276. Morjen, M.; Kallech-ziri, O.; Bazaa, A.; Othman, H.; Mabrouk, K.; Zouari-kessentini, R.; Sanz, L.; Calvete, J.J.; Srairi-Abid, N.; El Ayeb, M.; et al. PIVL, a New Serine Protease Inhibitor from *Macrovipera lebetina* Transmediterranea Venom, Impairs Motility of Human Glioblastoma Cells. *Matrix Biology* **2013**, *32*, 52–62, doi: 10.1016/j.matbio.2012.11.015.
277. Fernández, J.; Gutiérrez, J.M.; Calvete, J.J.; Sanz, L.; Lomonte, B. Characterization of a Novel Snake Venom Component: Kazal-Type Inhibitor-like Protein from the Arboreal Pitviper *Bothriechis schlegelii*. *Biochimie* **2016**, *125*, 83–90, doi: 10.1016/j.biochi.2016.03.004.
278. Wagstaff, S.C.; Favreau, P.; Cheneval, O.; Laing, G.D.; Wilkinson, M.C.; Miller, R.L.; Stöcklin, R.; Harrison, R.A. Molecular Characterisation of Endogenous Snake Venom Metalloproteinase Inhibitors. *Biochem Biophys Res Commun* **2008**, *365*, 650–656, doi: 10.1016/j.bbrc.2007.11.027.
279. Ullah, A.; Ullah, K.; Ali, H.; Betzel, C.; Rehman, S.U. The Sequence and a Three-Dimensional Structural Analysis Reveal Substrate Specificity among Snake Venom Phosphodiesterases. *Toxins (Basel)* **2019**, *11*, doi:10.3390/toxins11110625.
280. Uzair, B.; Khan, B.A.; Sharif, N.; Shabbir, F.; Menaa, F. Phosphodiesterases (PDEs) from Snake Venoms: Therapeutic Applications. *Protein Pept Lett* **2018**, *25*, 612–618, doi:10.2174/092986652566180628160616.
281. Gao, J.F.; Qu, Y.F.; Zhang, X.Q.; He, Y.; Ji, X. Neonate-to-Adult Transition of Snake Venomics in the Short-Tailed Pit Viper, *Gloydius brevicaudus*. *J Proteomics* **2013**, *84*, 148–157, doi: 10.1016/j.jprot.2013.04.003.
282. Yuh, F.P.; Wong, P.T.H.; Kumar, P.P.; Hodgson, W.C.; Kini, R.M. Ohanin, a Novel Protein from King Cobra Venom, Induces Hypolocomotion and Hyperalgesia in Mice. *Journal of Biological Chemistry* **2005**, *280*, 13137–13147, doi:10.1074/jbc.M414137200.
283. Vejayan, J.; Khoon, T.L.; Ibrahim, H. Comparative Analysis of the Venom Proteome of Four Important Malaysian Snake Species. *Journal of Venomous Animals and Toxins Including Tropical Diseases* **2014**, *20*, doi:10.1186/1678-9199-20-6.
284. Tan, C.H.; Tan, K.Y.; Tan, N.H. A Protein Decomplexation Strategy in Snake Venom Proteomics. *Methods Mol Biol* **2019**, *1871*, 83–92, doi:10.1007/978-1-4939-8814-3_5.

285. Rey-Suárez, P.; Núñez, V.; Gutiérrez, J.M.; Lomonte, B. Proteomic and Biological Characterization of the Venom of the Redtail Coral Snake, *Micrurus mipartitus* (Elapidae), from Colombia and Costa Rica. *J Proteomics* **2011**, *75*, 655–667, doi: 10.1016/J.JPROT.2011.09.003.
286. Lomonte, B.; Rey-Suárez, P.; Fernández, J.; Sasa, M.; Pla, D.; Vargas, N.; Bénard-Valle, M.; Sanz, L.; Corrêa-Netto, C.; Núñez, V.; et al. Venoms of *Micrurus* Coral Snakes: Evolutionary Trends in Compositional Patterns Emerging from Proteomic Analyses. *Toxicon* **2016**, *122*, 7–25, doi: 10.1016/J.TOXICON.2016.09.008.
287. Fox, J.W.; Serrano, S.M.T. Exploring Snake Venom Proteomes: Multifaceted Analyses for Complex Toxin Mixtures. *Proteomics* **2008**, *8*, 909–920, doi:10.1002/PMIC.200700777.
288. Ghezellou, P.; Garikapati, V.; Kazemi, S.M.; Strupat, K.; Ghassempour, A.; Spengler, B. A Perspective View of Top-down Proteomics in Snake Venom Research. *Rapid Communications in Mass Spectrometry* **2019**, *33*, 20–27, doi:10.1002/rcm.8255.
289. Alape-Girón, A.; Sanz, L.; Escolano, J.; Flores-Díaz, M.; Madrigal, M.; Sasa, M.; Calvete, J.J. Snake Venomics of the Lancehead Pitviper *Bothrops asper*: Geographic, Individual, and Ontogenetic Variations. *J Proteome Res* **2008**, *7*, 3556–3571, doi:10.1021/PR800332P.
290. Núñez, V.; Cid, P.; Sanz, L.; de La Torre, P.; Angulo, Y.; Lomonte, B.; Gutiérrez, J.M.; Calvete, J.J. Snake Venomics and Antivenomics of *Bothrops atrox* Venoms from Colombia and the Amazon Regions of Brazil, Perú and Ecuador Suggest the Occurrence of Geographic Variation of Venom Phenotype by a Trend towards Paedomorphism. *J Proteomics* **2009**, *73*, 57–78, doi: 10.1016/j.jprot.2009.07.013.
291. Jiménez-Charris, E.; Montealegre-Sanchez, L.; Solano-Redondo, L.; Mora-Obando, D.; Camacho, E.; Castro-Herrera, F.; Fierro-Pérez, L.; Lomonte, B. Proteomic and Functional Analyses of the Venom of *Porthidium lansbergii lansbergii* (Lansberg's Hognose Viper) from the Atlantic Department of Colombia. *J Proteomics* **2015**, *114*, 287–299, doi: 10.1016/j.jprot.2014.11.016.
292. Salazar-Valenzuela, D.; Mora-Obando, D.; Fernández, M.L.; Loaiza-Lange, A.; Gibbs, H.L.; Lomonte, B. Proteomic and Toxicological Profiling of the Venom of *Bothrocophias campbelli*, a Pitviper Species from Ecuador and Colombia. *Toxicon* **2014**, *90*, 15–25, doi:10.1016/j.toxicon.2014.07.012.
293. Quintana-Castillo, J.C.; Johana vargas, L.; Segura, C.; Estrada-Gómez, S.; Bueno-Sánchez, J.C.; Alarcón, J.C. Characterization of the Venom of C. d. Cumanesis of Colombia: Proteomic Analysis and Antivenomic Study. *Toxins (Basel)* **2018**, *10*, doi:10.3390/toxins10020085.
294. Madrigal, M.; Sanz, L.; Flores-Díaz, M.; Sasa, M.; Núñez, V.; Alape-Girón, A.; Calvete, J.J. Snake Venomics across Genus *Lachesis*. Ontogenetic Changes in the Venom Composition of *Lachesis stenophrys* and Comparative Proteomics of the Venoms of Adult *Lachesis melanocephala* and *Lachesis acrochorda*. *J Proteomics* **2012**, *77*, 280–297, doi: 10.1016/j.jprot.2012.09.003.
295. Céspedes, N.; Castro, F.; Jiménez, E.; Montealegre, L.; Castellanos, A.; Cañas, C.; Arévalo-Herrera, M.; Herrera, S. Biochemical Comparison of Venoms from Young Colombian *Crotalus durissus cumanensis*

and Their Parents. *Journal of Venomous Animals and Toxins including Tropical Diseases* **2010**, *16*, 268–284.
296. Culma, M.F.; Pereañez, J.A.; Rangel, V.Ń.; Lomonte, B. Snake Venomics of *Bothrops punctatus,* a Semiarboreal Pitviper Species from Antioquia, Colombia. *PeerJ* **2014**, *2*, 1–16, doi:10.7717/PEERJ.246.
297. Lomonte, B.; Pla, D.; Sasa, M.; Tsai, W.C.; Solórzano, A.; Ureña-Díaz, J.M.; Fernández-Montes, M.L.; Mora-Obando, D.; Sanz, L.; Gutiérrez, J.M.; et al. Two Color Morphs of the Pelagic Yellow-Bellied Sea Snake, *Pelamis platurus*, from Different Locations of Costa Rica: Snake Venomics, Toxicity, and Neutralization by Antivenom. *J Proteomics* **2014**, *103*, 137–152, doi: 10.1016/j.jprot.2014.03.034.
298. Lazarovici, P.; Marcinkiewicz, C.; Lelkes, P.I. From Snake Venom's Disintegrins and C-Type Lectins to Anti-Platelet Drugs. *Toxins (Basel)* **2019**, *11*, 1–15, doi:10.3390/toxins11050303.
299. Karapetian, H. Reptilase Time (RT). *Methods Mol Biol* **2013**, *992*, 273–277, doi:10.1007/978-1-62703-339-8_20.
300. Rodríguez-Vargas, A.; Franco-Vásquez, A.; Bolivar-Barbosa, J.; Vega, N.; Reyes-Montaño, E.; Arreguín-Espinosa, R.; Carbajal-Saucedo, A.; Angarita-Sierra, T.; Ruíz-Gómez, F. Unveiling the Venom composition of the Coral Venom Snakes *Micrurus helleri, M. medemi, and M. sangilensis. Toxins* **2023**, *15*, doi:10.3390/toxins15110622.
301. Garrido Garrido, M.B.; Herráez, A. *Guía de Jmol*. Available online: https://biomodel.uah.es/Jmol/jmolguia/otrasopciones.html (accessed on 12/04/**2023**).
302. González Mañas, J.M. *Curso de Biomoléculas*. Universidad del País Vasco. Available online: https://www.ehu.eus/biomoleculas/index.htm (accessed on 12/04/**2023**).
303. Protein Data Bank (PDB). Available online: https://www.rcsb.org/ (accessed on 12/04/**2023**).
304. Blender. Available online: https://www.blender.org/ (accessed on 12/04/**2024**).
305. Jmol: An Open-Source Java Viewer for Chemical Structures in 3D. Available online: http://jmol.sourceforge.net/ (accessed on 12/04/**2023**).
306. Sketchfab. Website for Visualizing and Sharing 3D Content Online. Available online: https://sketchfab.com/ (accessed on 12/04/**2023**).



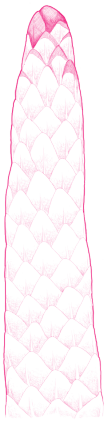
Chapter 6

Snakebite therapies in Colombia: antivenoms as pivotal responses

Ariadna Rodriguez-Vargas, Jaime Andres Pereañez, Maria Carlina Castillo-Beltran, Camila Figueredo-Salinas, Adrian Marcelo Franco-Vasquez, Teddy Angarita-Sierra

Abstract: Since the time of Dr. Albert Calmette (1890s), antivenoms derived from hyperimmunized animals, such as sheep or horses, have been the main treatment for snakebite envenomation. However, there have been significant advancements in pharmaceutical presentations and molecular antivenom formats, which have optimized their kinetic and pharmacodynamic performance, prevented or reversed clinical effects, and reduced adverse effects after use. Currently, research is focused on the neutralization of specific protein components with antivenoms. Additionally, complementary immunorecognition techniques are being explored to improve the development of antivenoms, making them more efficient, effective, and cost-effective. In this chapter, we present a brief historical review of antivenom production at the Colombian National Health Institute, as well as a detailed account of the regulatory and legal framework that governs and addresses the production of antivenoms in Colombia. Furthermore, we explore complementary immunorecognition techniques aimed at enhancing the development of antivenoms to make them more efficient, effective, and cost-effective. Finally, we highlight that the high variability of snake venoms has motivated scientific research aimed at improving our understanding of snakebite events and their treatment, as well as implementing better management tools.

Keywords: Snakebite treatment, antivenoms, antisera, antibodies.



Citation: Rodriguez-Vargas A; Pereañez A; Castillo-Beltran MC; Figueredo-Salinas C; Franco-Vasquez, AM; Angarita- Sierra T. Chapter 6. Snakebite therapies in Colombia: antivenoms as pivotal response. In Book: *Bites, venoms, and venomous snakes of Colombia*; Angarita-Sierra, T., Ruiz-Gomez, FJ, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 275–302.
doi: 10.33610/135454jvioed

Copyright: © 2024by the authors. Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0) license (http://creativecommons.org/licenses/by/4.0/).

Illustrations by:
Oscar A. Ramirez Ruiz

1. Snakebite therapies in Colombia

1.1 What is an antivenom?

Although there is no consensus on the exact definition of an antivenom, it is generally thought to be a drug or treatment that counteracts the harmful effects attributed to a venom or disease [1]. Humans have developed specific antidotes to defend themselves against these substances, including the group of antivenoms, also known as antisera. Purified antibodies (immunoglobulin-based therapies) have historically played a fundamental role in deactivating toxins from specific venoms [2–4].

According to the Colombian regulatory framework for obtaining sanitary registrations described in Decree 386 of 2018 [10], antivenoms are defined as "purified fractions of immunoglobulins or immunoglobulin fragments from plasma obtained from animals immunized with a venom or a mixture of venoms." Antivenoms are prepared by hyperimmunizing animals such as sheep or horses and injecting them with small doses of venom to produce antibodies that deactivate harmful toxins. The antibodies are collected by drawing blood from the animal [3–5]. After the antibodies are isolated, the antivenoms are produced and used to counteract the envenoming caused by snakebites. An antivenom is composed of equine-origin antibodies, which correspond to a purified fraction of hyperimmune sera. This is the only way to save the lives of envenomed victims [5,6,7]. The treatment consists of administering these antibodies that are responsible for neutralizing the venom's toxins.

Although this antivenom is an essential drug for treating snakebite accidents, especially in tropical and subtropical countries [8], its distribution is unfortunately inadequate. Only a few countries in tropical latitudes meet the adequate quality standards to manufacture these substances. In addition, some countries lack proper regulation and control over their production, and this greatly impedes assessment and intervention in the control of the quality and effectiveness of these antidotes [9].

1.2 A brief history of antivenom production in the Colombian National Health Institute

The National Institute of Health (INS) is a governmental scientific and technical entity that has been formally producing polyvalent antivenom serum since the 1970s (see Chapter 7). This serum is developed from hyperimmune plasmas obtained through standardized immunization protocols using venoms from medically important snake species across different ecoregions of Colombia [11].

According to INS production records, the first extractions of equine-derived hyperimmune plasma for antivenom production date back to the early 1970s. Hyperimmune anti-rabies plasma production was also initiated during this period. In February 1972, the INS registered the production of Bothropic and Crotalic hyperimmune plasmas, along with initial trials for antivenom production. At that time, challenges arose due to limited

experience in the handling of snake venoms, immunization protocols, titration techniques, and production procedures. The first batch consisted of approximately 150 vials, but production was interrupted due to these difficulties (see Chapter 7).

Two years later, the physicians Augusto Corredor, Miguel Guzman, and Ernesto Barbosa from the INS attended the Tropical Medicine Congress in Medellin. MD. Roger Bolaños, renowned for his extensive experience in antivenom production and snakebite management, also attended the event. The INS physicians contacted MD. Bolaños through the Pan American Health Organization (PAHO) to hire him as a consultant for the INS serum laboratory. MD. Bolaños trained the production team, including Juan Manuel Renjifo, Guiomar Caicedo de Pardo, and Carlos Caceres, in antivenom production (see Chapter 7). They successfully produced an experimental batch of hyperimmune plasma from horses immunized with *Bothrops asper* venom, which was approved for its neutralizing capacity. This early success facilitated the development of new production protocols and immunization schemes, solidifying the formal establishment of antivenom production in Colombia.

Later, Juan Manuel Renjifo and Guiomar Caicedo de Pardo received further training at the Clodomiro Picado Institute, where they gained expertise in serpentarium management, immunization protocols, and antivenom production processes. As a result of this training, the INS manufactured its first official batch of polyvalent antivenom serum (Batch No. 4), with official production starting on June 23, 1975. Currently, the INS employs a variety of venoms (see Chapter 8). The previously produced batches No. 1, No. 2, and No. 3 were used to standardize production and quality control for Bothropic-Crotalic antivenom (AV B/C) production.

In 1975, a total of 4,020 vials of antivenom serum were produced, including 2,800 vials of polyvalent antivenom serum (SAP) and 1,220 vials of monovalent antivenom serum (SAM). From 1978 to 1990, the INS produced a total of 120,472 SAP vials and 20,334 SAM vials. SAP production peaked in the 1980s, reaching 14,811 vials in a single year.

In 1993, Law 100 of 1993, through Article 245, established the National Institute for Food and Drug Surveillance (INVIMA Spanish acronym). Its primary objective is to implement health surveillance policies and ensure the quality control of medications, biological products, food, beverages, cosmetics, medical-surgical and dental devices, homeopathic and natural products, biotechnology-derived products, and household cleaning and hygiene products. In 1994, Decree 1290 defined INVIMA's responsibilities, including enforcing policies formulated by the Colombian Ministry of Health for health surveillance and quality control of the products described in Article 245 of Law 100 of 1993, as well as other relevant regulations and recommendations from the Reviewing Commission mentioned in Article 9.

In 1995, Decree 677 partially regulated the registration and licensing regime, quality control, and health surveillance for pharmaceuticals,

cosmetics, pharmaceutical preparations derived from natural resources, cleaning and hygiene products, and other household products. Additional provisions were also issued on the subject. Article 2 defines a drug as "*a pharmaceutical preparation obtained from active ingredients with or without auxiliary substances, presented in a pharmaceutical form, and used for the prevention, relief, diagnosis, treatment, cure, or rehabilitation of disease*." Containers, labels, and packaging are considered integral parts of the drug, as they ensure its quality, stability, and appropriate use. This classification includes serums or antivenoms.

In 2002, the INS Serum Group developed, produced, and delivered 182 vials of pan-African polyspecific serum to the WHO Collaborating Center for Antivenom Control (WHO CCCA) at the Liverpool School of Tropical Medicine, demonstrating its capability to manufacture antivenom for the African continent. During this period, the INS faced intermittent production issues, prompting the Ministry of Health to issue Resolution 122 of 2002 and declare a health emergency to ensure the supply of biologics in the country, including vaccines and antivenoms. In response, the Ministry requested the necessary resources from the PAHO/WHO Revolving Fund to reactivate antivenom production, in compliance with paragraph b) of Article 96 of Decree 677 of 1995.

In 2004, snakebite envenomations were classified as mandatory notifiable events, leading to the systematic reporting of cases in Colombia's Epidemiological Surveillance System (SIVIGILA Spanish acronym). Resolution 2934 (2004) declared a health emergency due to antivenom shortages for six months. However, this emergency declaration was extended until December 31, 2010, as the conditions necessitating it remained unresolved. This extension followed Resolution 2672 (2010) and amendments via Resolutions 613 and 5078 (2005), Resolution 2325 (2006), Resolution 2198 (2007), Resolution 2413 (2008), and Resolution 2206 (2009).

From 1991 to 2011, SAP production reached a total of 133,869 vials, while SAM production reached 19,898 vials, peaking at 16,206 SAP vials in 1998 (Figure 1). In 2005, the final production of SAM was recorded, with a total of 1,662 vials. In 2014, the INS increased its production capacity, successfully releasing approximately 3,500 vials and establishing a standard batch size of 9,500±500 SAP vials. Additionally, in 2017, the INS implemented technological upgrades to enhance and sustain production capacity, ensuring the continued availability of SAP nationwide.

In the early 2010s, the INS aimed to improve antivenom production and expand coverage of medically significant snake species. To achieve this, it developed the polyvalent coral antivenom (PAA Spanish acronym). The initial batch of antivenom, identified as 15AMP01, was released by INVIMA on October 4, 2016. This milestone in Colombian antivenom production marked the development of a polyvalent therapeutic treatment for envenomation caused by coralsnake bites (genus *Micrurus*), covering the four most medically important species: *Micrurus dumerilii*, *M. isozonus*, *M. surinamensis* (triads), and *M. mipartitus* (bicolor), as well as *M. helleri,*

M. medemi, M. sangilensis, and *M. obscurus* by cross-neutralization [12]. Since 2016, the INS has maintained continuous PAA production, exceeding 2,000 vials per year. Additionally, it actively monitors patients receiving this antivenom as a therapy to neutralize envenomation caused by *Micrurus* snakes (Figure 1).

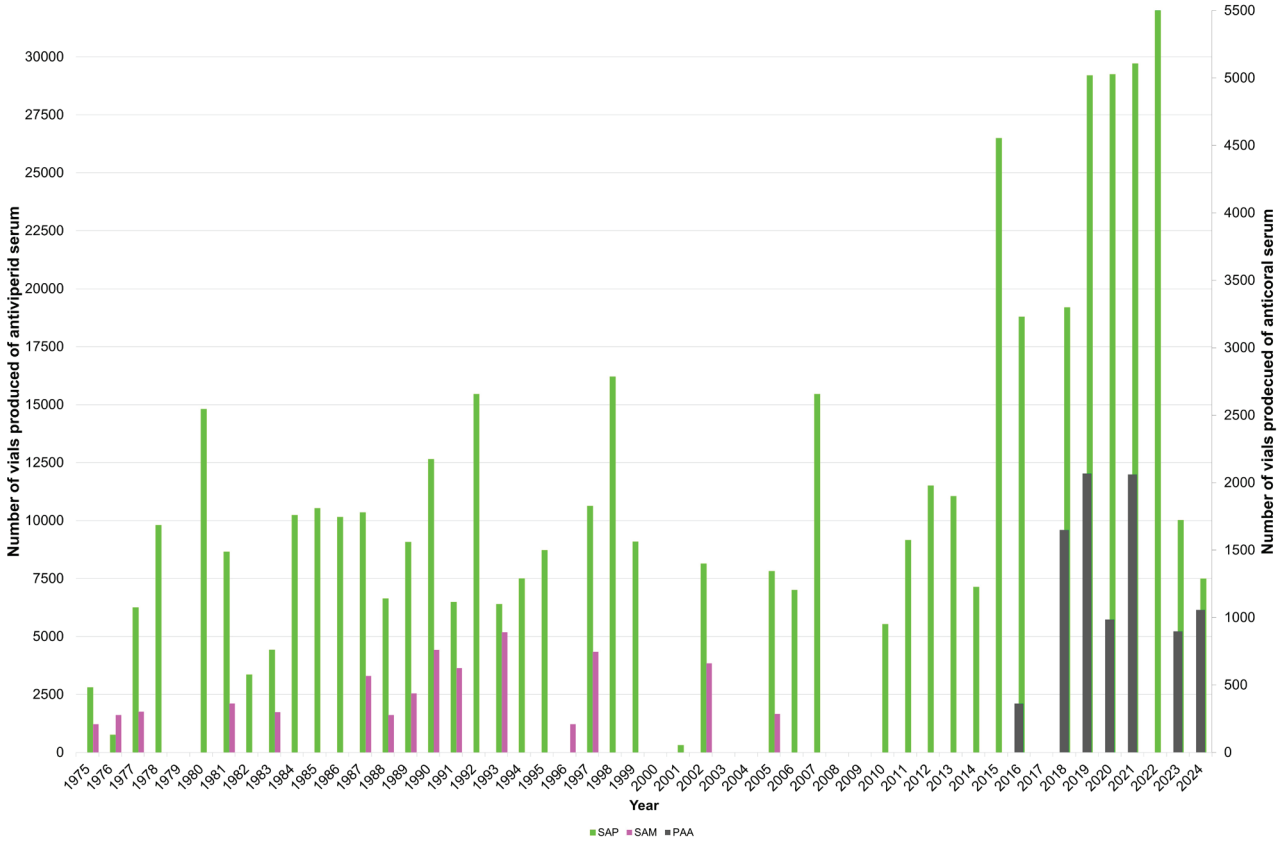


Figure 1. Historical antivenom production at the Colombian National Health Institute.

Despite the enormous efforts made by the INS to ensure the production of antivenoms, Resolutions 1300 and 1301 of April 14, 2014, declared a health emergency to prevent SAP shortages. In the same year, Decree 1375 of July 22 established the sanitary requirements for the manufacture and importation of antivenom and antilonomic sera (used as therapy for envenoming caused by caterpillars of moths of the genus *Lonomia*) during the declaration of a national public health emergency. Subsequently, Resolution 1209 of April 21, 2017, extended the health emergency declared by Resolutions 1300, 1301, and 1302 of 2014, which had already been extended by Resolutions 1241 of 2015, and 1478 of 2016, for twelve months.

Finally, in December 2017, the INS production plant was certified for good manufacturing practices, and the production of SAP and PAA was authorized. Additionally, INVIMA authorized the INS to manufacture pilot batches of lachetic, scorpionic, and lonomic antivenoms. Due to the scarcity of lonomic antivenom (ALP Spanish acronym), whose sole producer was the Butantan Institute in Brazil, the INS developed ALP and packaged a total of 1,663 vials between December 2017 and October 2018.

From 2012 to the present, the INS has produced a total of 182,333 vials of SAP and 7,127 vials of PAA. The maximum production per type of antivenom was achieved in 2022 with 49,931 vials of SAP and in 2019 with 2,068 vials of PAA; overall antivenom production was highest in 2022, totaling 50,525 vials, of which 594 corresponded to antilonomic antivenom (Figure 2). The plant's maximum production capacity was estimated at approximately 100,000 vials, with the qualities of SAP, which has the highest neutralization capacity among all available antivenoms in Central and South America [13].

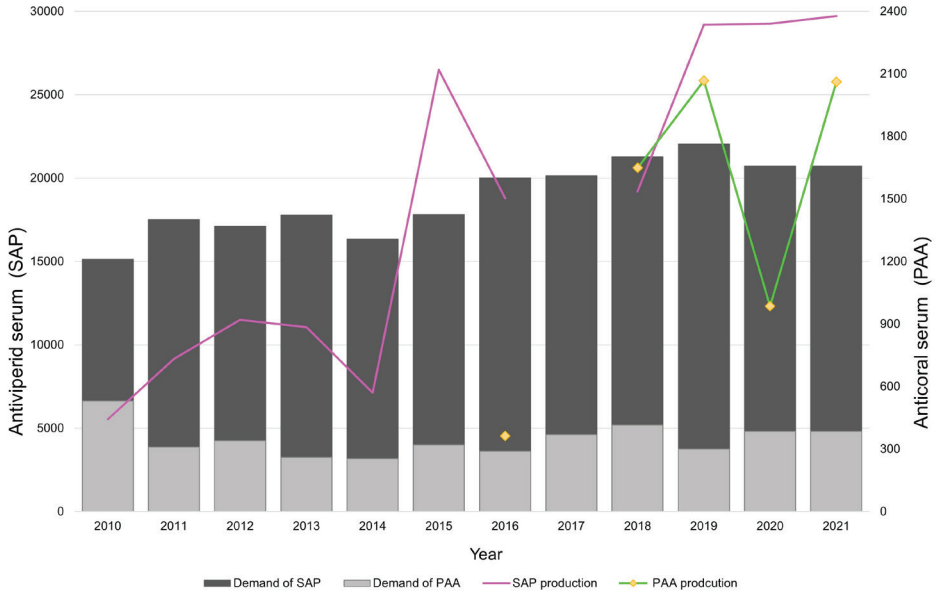


Figure 2. Antivenom production under the Good Manufacturing Practices certificate. SAP= polyvalent antiviperid serum;PAA= polyvalent anticoral antivenom.

The efficacy and quality of SAP produced by the INS have been optimized since the early 2000s by incorporating new medically important snake venoms into immunization protocols, covering all of the country's ecoregions. This enhancement increased its versatility and ensured that antivenoms retained the same antigen responsible for triggering the equine immune response. Since 2011, the INS has expanded the representativity of snake venoms from various species and biogeographical regions (see Chapters 7 and 8). This effort has resulted in a robust venom bank, which enhances the availability of venoms used for antivenom production and research. The INS maintains a stability study program for antivenom production, periodically conducting quality control tests to verify venom lethality (LD_{50}) and evaluating the median effective dose (ED_{50}) of all produced antivenoms to ensure their long-term stability.

The production of INS antivenoms complies with the applicable regulatory framework. Since 2010, antivenoms have been manufactured in a new hyperimmune serum plant, which holds a Good Manufacturing Practices (GMP) certification. This certification enables the INS to produce polyvalent snake antivenom (SAP), polyvalent coral snake antivenom (PAA), and polyvalent lonomic antivenom (ALP).

The polyvalent snake antivenom has held INVIMA sanitary registration number 2012M-0013350 since 2012, granted through Resolution 2012019512 (July 13, 2012), and this was renewed under number 2019M-0013350-R1 by Resolution 2019034649 (August 12, 2019). Notably, the INS manages the entire production chain, from snake collection and venom extraction to characterization and final product development, ensuring compliance with all quality standards authorized by INVIMA.

The INS guarantees antivenom production by adhering to manufacturing and quality control protocols. Compliance with all established quality specifications and parameters is verified at each stage of the production chain. Additionally, quality control includes a stability study program and active pharmacovigilance. Each manufactured batch is certified by INVIMA before commercialization (Table 1).

Table 1. Production of antivenoms carried out at the Instituto Nacional de Salud de Colombia between 1975 and 2024.

Historical production	**Antivenom production**		
Period	**SAP Vials**	**SAM Vials**	**PAA Vials**
1975 to 1990	120,472	20,334	0
1991 to 2011	133,869	19,898	0
2012 to 2024	249,771	0	9,082

SAP: Polyvalent Antiviperid serum, PAA: Polyvalent Anticoral Antivenom, SAM: Monovalent Antiviperid serum.

To estimate the required number of vials of polyvalent antivenom serum (SAP) and polyvalent coral antivenom (PAA) produced by the INS, the minimum toxicity neutralization capacity is considered to be 7 mg/mL for *Bothrops* spp. venom, 1 mg/mL for *Crotalus durissus* venom, and 0.2 mg/mL for *M. dumerilii, M. isozonus*, *M. surinamensis*, and *M. mipartitus* venoms [14]. The number of vials was determined based on the criteria established by the Ministry of Health and Social Security in the "*Guide for the Management of Toxicological Emergencies*" and considering the number of snakebite incidents reported in Colombia between 2010 and 2021. Figure 2 illustrates the production of SAP and PAA during the 2010–2021 period, demonstrating that the INS's production capacity is sufficient to meet national demand.

1.3 Regulation of antivenom production

The INVIMA is a governmental entity in Colombia tasked with protecting the health of individuals and the population as a whole. INVIMA regulates and monitors the consumption of food, the use of pharmaceuticals, medical devices, and other products requiring sanitary oversight.

According to INVIMA's regulations for medicines and biological products, antivenoms are classified as biological products [15]. INVIMA systematically monitors biological products to ensure their quality and safety standards. The National Pharmacovigilance Program under INVI-

MA aims to oversee the safety, efficacy, and quality of medicines during their commercialization phase after obtaining marketing authorization from INVIMA. These activities complement the control and monitoring of products throughout the production chain, minimizing health risks and impacts [11].

INVIMA authorization is required for the commercialization, importation, or manufacturing of antivenoms. This permit is granted only after compliance with all requirements established under current sanitary regulations, as validated by INVIMA [11]. The key requirements include standardization and validation of production processes and testing methods, pharmacological evaluation, manufacturing certification, sanitary registration (RS), and compliance with stability standards for the marketed antivenoms. In Colombia, biological products such as antivenoms cannot be marketed without meeting all these requirements. However, the Ministry of Health and Social Protection is responsible for ensuring the health and safety of the population. Therefore, it must take the necessary measures to prevent and control any contingencies that may affect public health, such as a potential shortage of antivenoms. As previously mentioned, in exceptional circumstances, the Ministry has declared a national health emergency on multiple occasions due to antivenom shortages.

Colombia has limited regulations governing antivenom production. Decree 821 of 2017 established the "*Emergency Technical Regulation for the Obtaining of Antivenom Sanitary Registration*" and adopted the "*Good Manufacturing Practices (GMP) Guide*" for their production. However, this decree was partially repealed by Decree 386 of 2018, which stipulates that a "*scaled drawing of label drafts and packaging designs must be created, including: product name, manufacturer name, batch number, pharmaceutical form, labeled volume, specificity (neutralized venom including the common names of the animals against which the product is effective), neutralizing potency, storage conditions (including those for the reconstituted product, if applicable), description of the reconstitution process, route of administration, recommended dosage, contraindications, warnings, and expiration date*." The technical annex of the decree corresponds to the "*Good Manufacturing Practices (GMP) Guide for Antivenom Production*".

Decree 386 of 2018 outlines the procedure for obtaining, renewing, or modifying the sanitary registration of antivenoms and includes measures to ensure their availability. Countries such as Mexico and Brazil have their own pharmacopeias, which contain specific monographs for each type of antivenom. These monographs define the quality parameters that different antivenoms must meet for production and/or commercialization within each country. In Mexico, this document is known as the "United Mexican States Pharmacopeia," issued by the Ministry of Health, while in Brazil, it is the "Brazilian Pharmacopeia," published by the National Health Surveillance Agency (ANVISA Portugues acronym).

In these countries, compliance with the monographs is mandatory, and they specify the minimum neutralizing capacity required for each

type of antivenom and the venomous species it is effective against. However, Colombia lacks reference sera or venoms, and there is no pharmacological regulation establishing the minimum neutralizing capacity that antivenoms must meet or against which venoms this capacity should be tested. This legal, technical, and regulatory gap must be addressed to enhance the procedures for the sanitary registration of antivenom. Establishing such standards would ensure that all antivenoms available in the Colombian market meet the minimum neutralizing capacity required for envenomations caused by medically significant species in the country.

2. Antivenom development

Traditional antivenom production methods involve inducing antibody production in a host through serial inoculations of snake venom, taking into account its median lethal dose (LD_{50}) [16,17,50,59,63]. The horse is the most commonly used animal for immunoglobulin production due to its large blood volume, resilience, and capacity to generate antibodies against venom [18–20]. A review of studies indicates that direct inoculation of raw venom is not well tolerated by or lethal to the host [16,17,50,59,63]. Therefore, adjuvants must be used during venom administration to form antigen deposits, ensuring a controlled release that preserves the immunogenicity of purified toxoids while effectively stimulating the immune response [21].

The immunization schedule varies depending on the LD_{50} of the venom [22], the species used for immunoglobulin production, and the protein purification technique employed [23]. It is essential to periodically analyze antibody titers at the end of the immunization cycle to assess the production of the desired immunoglobulins [5,24]. Once the target antibody titer is achieved, the horses undergo production bleeding, during which approximately eight liters of blood can be collected from each animal over a period of three to four days, depending on its physiological condition. The plasma is subsequently separated by centrifugation and precipitated using ammonium sulfate. Red blood cells and albumin are reinfused into the animal via plasmapheresis to mitigate the risk of anemia [25,26]. Historically, the production and commercialization of antivenoms in pharmaceutical form have evolved through four generations [27]. The methodology is described by Pope [28,29], which serves as the foundation for antivenom production (Figure 3).

First-generation antivenoms consist of unpurified serum derived from the blood of animals hyperimmunized with venoms [30,31]. In contrast, second-generation antivenoms are composed of purified whole immunoglobulins obtained through precipitation techniques using ammonium sulfate or caprylic acid [32]. The composition of second-generation antivenoms primarily includes whole immunoglobulin G (IgG) molecules, with an approximate molecular weight of 150 kDa. It is important to note that other protein fractions, such as albumin, may also be present in these preparations [33,34].



Figure 3. Immunization procedure and immunoglobulin production. (A) Extraction of snake venom. (B–C) Inoculation of snake venom combined with adjuvants into a horse as an animal model for immunoglobulin production. (D) Catheterization of the jugular vein for blood collection. (E) Blood storage following catheterization. (F) Cell sedimentation after 12 hours of storage. (G) Mechanical separation of plasma. (H) Collection of 8 liters of hyperimmune plasma following separation. (I) Bags containing hyperimmune plasma (the raw material for antivenom production) and reconstituted cell packs in saline solution for reinfusion into the horses. (J) Plasma in quarantine, designated for processing at the manufacturing facility. Photos by: (A) Javier Crespo; (B)–(J) German Diaz.

Second-generation antivenoms are formulated with purified IgG and devoid of serum proteins. They contain trace amounts of medium and high molecular weight proteins, typically ranging between 1% and 5% [35–37]. Commercially, first- and second-generation antivenoms are referred to as sera, whereas those composed of F(ab')2 or Fab fragments are classified as fabotherapeutics [38].

Third-generation antivenoms are derived from immunoglobulin fractions of approximately 100 kDa, produced by digesting whole IgG plasma proteins with pepsin, an aspartic protease. This process cleaves the F(ab')2 fragment from the heavy chains and separates the Fc fragment

from the immunoglobulins. The F(ab')2 fragment is subsequently purified through a second ammonium sulfate precipitation step [31]. These F(ab')2 fragments inhibit the interaction between the venom's active site and its receptor, thereby neutralizing the venom's effects and facilitating its elimination [39]. The molecular size is reduced as a result of Fc digestion via proteolytic cleavage, which hydrolyzes over 50% of the IgG constant regions [40]. The efficiency of this process can be influenced by the type of enzyme used and the thermocoagulation of IgG [23,26]. Additional methods for lipoprotein removal and F(ab')2 purification include ion-exchange chromatography and caprylic acid precipitation, which are occasionally employed in the purification of F(ab')2 [25].

Fourth-generation antivenoms are produced by digesting immunoglobulins with papain at neutral pH [41,42]. However, cleavage of the antibodies at the hinge region and disruption of disulfide bonds require incubation with a reducing agent, such as mercaptoethylamine, under mild conditions [43]. This results in the generation of two Fab fragments and one Fc fragment [44]. The Fc fragment can be removed from the IgG molecule through chromatographic methods [45], or by pepsin treatment, yielding purified monovalent Fab fragments [43,44]. The reduction of disulfide bonds linking the F(ab) fragments results in individual structures with intact recognition sites. Each fragment has an approximate molecular weight of 50 kDa, which enhances their clearance from the patient's body [37].

Depending on the enzymatic digestion process or the recombinant DNA technique employed, various antibody conformations can be generated to suit specific purposes [46,47]. Following antivenom production, the initial immunogenicity assessment is conducted using gel electrophoresis or western blot techniques [48,49]. An antivenom with positive immunogenicity is one that reacts with all protein fractions of the venom [50–54]. Additionally, the microbiological quality and efficacy of the product must be evaluated using the median effective dose (ED_{50}) to determine its neutralizing capacity [55–57]. After rigorous safety and efficacy evaluations, the antivenom is packaged in vials, either in liquid or lyophilized form [25,58,59].

Antivenoms are classified as monospecific or polyspecific, depending on whether the immunization process utilizes venom from a single snake species or from multiple snake species, respectively. In both cases, the venoms are sourced from multiple populations of medically important snake species native to the region or country for which the antivenom is designed [33,60].

The production of monospecific antivenoms has largely been discontinued worldwide. However, some manufacturers continue to produce monovalent antivenoms. For instance, in Peru, the National Center for Biological Products manufactures two types of monospecific sera for snakebite treatment [7]. The injectable anticrotalic serum is used to treat envenomations caused by *Crotalus durissus* (South American rattlesnake), while the antilachesic serum is employed for envenomations caused by *Lachesis muta* (Amazonian bushmaster) [61]. In contrast, most manufac-

turers produce polyspecific antivenoms that neutralize the venom of viperids or elapids, such as the antiviperid and anticoral antivenoms produced in Colombia by the INS [62,63].

Experimental approaches have been proposed to develop novel antivenoms using diverse antibody formats, with the aim of creating more efficient and effective pharmaceutical treatments [64,65]. These approaches include not only the enzymatic digestion of immunoglobulins, as previously described, but also other innovative methods. For example: (1) generating monoclonal or recombinant antibodies targeting specific venom components [66]; (2) utilizing synthetic epitope chains for immunization [67]; and (3) producing recombinant antigens based on the consensus of specific toxins that are poor immunogens but can enhance existing antivenoms or lead to the development of new antivenoms with exclusive specificity [68].

Currently, international biotechnological advancements have achieved promising results in both *in vivo* and *in vitro* experiments [69,70], However, they still need to be evaluated in clinical studies. In recent research performed in Colombia, Romero-Giraldo et al. [71], cloned and expressed the most abundant phospholipase A2 (PLA_2) from the venom of *Micrurus dumerilii* and generated IgGs against the recombinant toxin. These antibodies successfully neutralized the myotoxic activities produced by PLA_2 as well as those induced by the whole venom. While these results are promising, clinical trials are still required to validate their efficacy and efficiency.

In Colombia, the use of these modern technologies for improving antivenoms as therapeutic treatments for snake envenomations remains in its nascent stages. Nevertheless, we urge researchers to explore these avenues to advance the development of next-generation antivenoms.

3. Preclinical evaluation of antivenoms

For a biological medicinal product intended for human use, such as antivenoms, to meet quality requirements, it must undergo rigorous evaluation to demonstrate its neutralizing capacity against venom toxins, as well as its effectiveness in controlling or reversing the clinical manifestations caused by envenomation. The World Health Organization (WHO) proposes methodologies for evaluating such medications. This is because the parenteral administration of antivenoms derived from animal immunoglobulins is the cornerstone of treating snakebite envenomations, which constitute a serious public health issue in tropical regions worldwide [72].

To ensure the efficacy and safety of antivenoms for human use, it is necessary to evaluate their neutralizing capacity and safety at the preclinical level. This is due to the significant variation in venom composition and the types of venoms used for immunization (see Chapters 2, 3, and 5), as well as the heterogeneity in clinical efficacy [73]. The preclinical evaluation of snake antivenoms provides valuable information for selecting appropriate antivenoms for use in various countries around the world

[74,75]. The WHO recommends that the essential preclinical assays to determine the neutralizing capacity of antivenoms against venom-induced lethality are the median effective dose of the antivenom (ED_{50}) and the median lethal dose (LD_{50}) of the venom, respectively. However, since venoms induce other toxic activities that can also be assessed, Figure 4 outlines the most relevant steps for determining the preclinical efficacy of an antivenom during its production process.

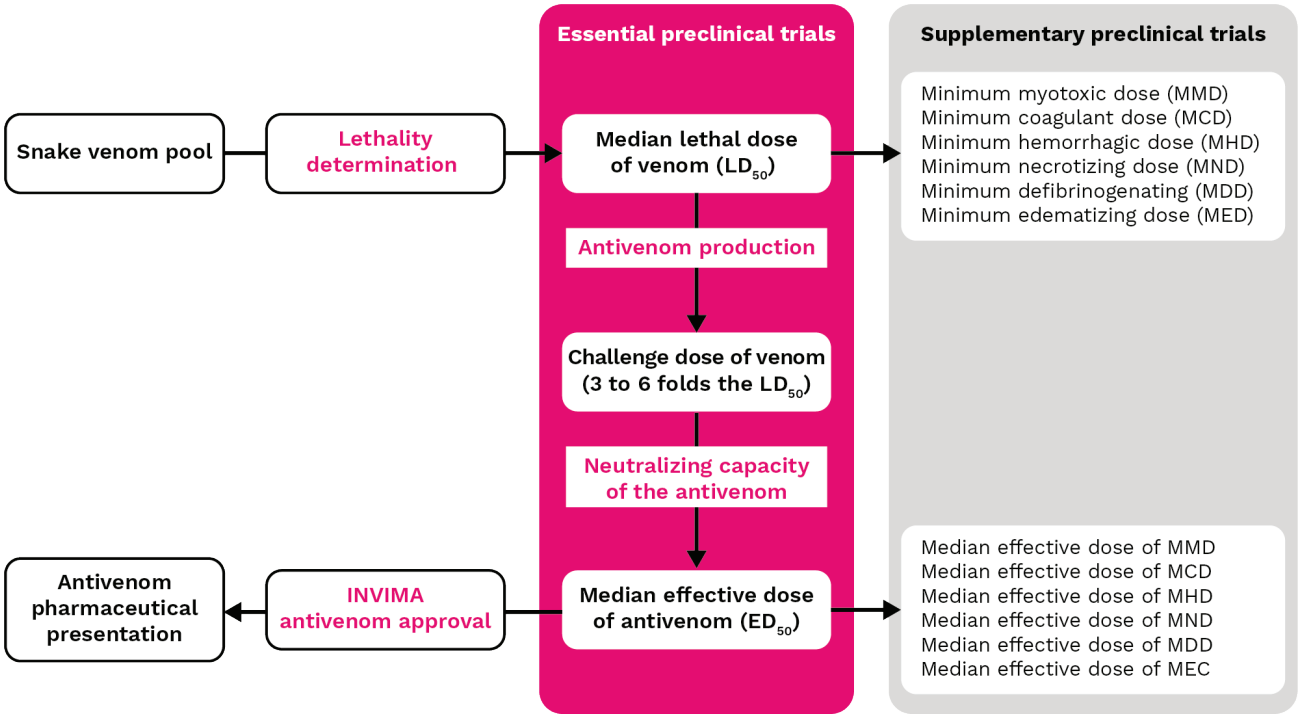


Figure 4. Preclinical evaluation process of antivenoms: Essential and supplementary tests.

3.1 Determination of the biological activities of venoms

Determination of the median lethal dose

The median lethal dose (LD_{50}) of a venom is defined as the quantity of venom required to cause death in 50% of the test animals, typically mice. The LD_{50} is determined by preparing a series of venom dilutions in saline solution and administering them intraperitoneally (i.p., maximum 0.5 mL) or intravenously (i.v., maximum 0.2 mL) to groups of 4-6 mice. According to WHO guidelines [76], a sample size of five mice (of the same strain and within a defined weight range, usually 18-22 g) is recommended. Mortality is recorded at 24 hours (for intravenous injections) or 48 hours (for intraperitoneal injections). Control groups receive saline solution. The LD_{50} is calculated using statistical methods such as the Spearman-Karber method, Probit analysis, or other non-parametric approaches [77–80].

Determination of the median effective dose of the antivenom

The median effective dose (ED_{50}) of an antivenom is defined as the amount of antivenom, or the venom/antivenom ratio, that ensures the survival of

50% of mice injected with a mixture of antivenom and a lethal dose of venom [76]. To perform this assay, a challenge dose of venom is first selected, typically equivalent to 3-6 times the LD_{50} of the venom. This dose is then mixed with varying volumes of antivenom and adjusted to a constant final volume with saline solution. The mixtures are incubated for 30 minutes at 37 °C, after which aliquots are injected into mice via the selected route (i.v. or i.p.). Survival rates are recorded based on the prepared dilutions. Two control groups are included: one injected with saline solution (negative control) and another with venom alone (positive control). The ED_{50} is calculated using the same statistical methods as for the LD_{50} and is commonly expressed as the amount of venom (in mg) neutralized per milliliter of antivenom.

In addition to these essential preclinical evaluations, which assess the ability of antivenoms to neutralize lethal effects, supplementary assays may be conducted to evaluate their efficacy against other toxic activities of venoms. These assays are optional and depend on the specific venom used, the laboratory, and the regulatory requirements of the country. For example, under Colombian regulations (Decree 386 of 2018), specific preclinical studies are required to demonstrate the neutralizing capacity of antivenoms against the target venom. The INS has developed standardized and validated biological tests to support these evaluations, including assays for myotoxicity, hemorrhage, dermal necrosis, coagulant activity, and edematizing activity, among others (Figure 4).

Determination of the minimum hemorrhagic dose and the median effective antivenom dose

The minimum hemorrhagic dose (MHD) is defined as the smallest quantity of venom that induces a hemorrhagic lesion with a diameter of 10 mm. To determine the MHD, various doses of venom are prepared in saline solution (final injection volume: 50 µL) and injected intradermally into the shaved ventral skin of mice. After 2-3 hours, the mice are ethically euthanized, and the skin is dissected to measure the diameter of the hemorrhagic lesion. A sample size of five mice per dose is recommended, with a control group receiving saline solution.

For the neutralization assay, a challenge dose of 1-5 MHD is mixed with varying doses of antivenom. The mixtures are incubated for 30 minutes at 37°C, after which 50 µL aliquots are injected intradermally. The mice are euthanized 2-3 hours later, and the skin is dissected to measure the lesion diameter. The median effective dose for MHD (MHD_{50}) is defined as the volume of antivenom (in µL) or the venom/antivenom ratio that reduces the hemorrhagic lesion diameter by 50% compared to the control group injected with venom and saline [76].

Determination of minimum necrotizing dose and the median effective antivenom dose

The minimum necrotizing dose (MND) is defined as the smallest amount of venom that, when injected intradermally, induces necrotic lesions of 5 mm in diameter three days post-injection. The methodology is similar to

that used for the MHD, except that the skin is examined three days after venom administration.

For the neutralization assay, a dose of 1-2 MND is mixed with varying doses of antivenom. The mixtures are incubated for 30 minutes at 37°C, and 50 µL aliquots are injected intradermally. Three days later, the necrotic lesion diameter is measured. The median effective dose for MND (MND_{50}) is defined as the volume of antivenom (in µL) or the venom/antivenom ratio that reduces the necrotic lesion diameter by 50% compared to the control group [76].

Determination of minimum coagulant dose and the effective antivenom dose

The minimum coagulant dose (MCD) can be determined using either a bovine fibrinogen solution (2.0 g/L; MCD-F) or standard citrated human plasma (MCD-P). The MCD is defined as the smallest amount of venom that coagulates the fibrinogen solution or plasma within 60 seconds at 37°C. To measure the MCD, 50 µL of venom (ranging from 240 to 0.5 mg/L) is added to 0.2 mL of fibrinogen or plasma in glass coagulation tubes at 37°C. The coagulation time is recorded within 60 seconds.

For the neutralization assay, a dose of MCD-P or MCD-F is mixed with varying doses of antivenom. The mixtures are incubated for 30 minutes at 37°C, and the coagulation time is recorded. The effective dose ($MCD\text{-}F_{100}$ or $MCD\text{-}P_{100}$) is defined as the minimum volume of antivenom or the venom/antivenom ratio required to completely prevent coagulation within 30 minutes [76].

Determination of minimum myotoxic dose and the median effective antivenom dose

The minimum myotoxic dose (MMD) is defined as the amount of venom that increases serum or plasma creatine kinase (CK) activity to four times the level observed in control animals injected with saline solution. To determine the MMD, 50 µL of venom is injected into the right gastrocnemius muscle. After 3 hours, blood samples are collected via tail incision, and CK activity is measured using commercial diagnostic kits.

For the neutralization assay, a dose of 3 MMD is mixed with varying doses of antivenom. The mixtures are incubated for 30 minutes at 37°C and injected into the right gastrocnemius muscle. After another 3 hours, blood samples are collected, and CK activity is measured. The median effective dose for MMD (MMD_{50}) is defined as the volume of antivenom (in µL) or the venom/antivenom ratio that reduces CK activity by 50% compared to the control group [76].

3.2 Neutralization assays

The evaluation of antivenom effectiveness is crucial for the treatment of snakebite envenomation. The WHO [76] specifies that each batch of antivenom must be tested to determine its capacity to neutralize the

lethal effects of relevant venoms, using a testing method approved by the national regulatory authority of the country where the antivenom is manufactured [81]. Research on antivenoms aims to improve the treatment of snakebite envenomation through lethality assays. As previously described, these assays include the LD_{50}, which quantifies venom lethality, and the ED_{50}, which estimates the *in vivo* neutralizing potential of an antivenom [81,82].

However, several *in vitro* techniques have been developed to quantify venom lethality and the neutralizing capacity of corresponding antivenoms. These include immunochemical assays that evaluate the immunological interaction between antivenoms and venoms [57,82,83]. Immunochemical methods, such as enzyme-linked immunosorbent assay (ELISA), can be combined with electrophoresis techniques like western blot and antivenomics studies to provide specific detection. Proteomic techniques can also be employed to identify venom proteins and their epitopes recognized by antivenoms [86], These methods allow for the quantification of antibody-antigen recognition and reduce the need for animal experimentation [83,87,88].

ELISA assays

The principle of this technique is based on the binding of soluble antigens or antibodies to an insoluble solid phase, such as the wells of a microtiter plate, which preserves the immunoreactivity of the components [59]. An antigen-antibody complex is detected using a specific enzyme-conjugated antibody, followed by a washing step. A substrate specific to the enzyme is then added, and the amount of hydrolysis (color change measured visually or spectrophotometrically) is proportional to the amount of antigen present in the test sample [87,88]. The ELISA assay is highly sensitive, capable of detecting protein levels as low as 1 to 400 ng/mL. The specificity of the assay depends on the quality of the reagents used [82,89].

Preliminary studies have shown that the ELISA system can serve as an in vitro alternative to the ED_{50} assay in mice, and there is a significant correlation between *in vivo* and *in vitro* ED_{50} values [90]. For example, the ED_{50} of 38 different batches of antivenom was estimated for four medically important snake venoms in Africa and compared with the *in vivo* ED_{50} [90]. ELISA plates were coated with the same venoms used for the reference curves. A significant correlation ($p < 0.001$) was observed between *in vitro* and *in vivo* methods when the ED_{50} results of the antisera were related to their optical densities [87].

To serve as a positive control, reference antivenoms (those with an established ED_{50} in mice) must be tested on each ELISA plate and maintained as sterile aliquots at 4°C. Generally, opacities develop before potency is lost. Lyophilized and frozen antivenoms have demonstrated long-term stability. However, standardizing and distributing reference antivenoms for each venom is impractical, which represents a significant limitation [82].

Western Blot

The term "western blot" (WB) was first coined by Dr. W. Neal Burnette [91,92]. He observed that electrophoresis facilitated the transfer of proteins from SDS-PAGE gels to nitrocellulose paper membranes and that this method performed better than chemically modified paper [92].

The western blot process begins with the electrophoretic separation of a mixture of antigens using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). This method relies on the electrotransfer of venom proteins to a nitrocellulose membrane, which is then treated with two antibodies. One of these antibodies is conjugated to an enzyme that enables the visualization of the target components.

The components bound to the nitrocellulose are accessible for interaction with antibodies or other molecules [59]. The first antibody is the antivenom, which binds to the venom proteins, while the second antibody is conjugated to an enzyme that degrades a substrate, producing a colorimetric signal that indicates the components recognized by the antivenom [59]. This technique cannot detect the recognition of structural epitopes that may be relevant, as the venom proteins in the gel are denatured. The ELISA technique is more suitable for this purpose. However, the advantage of western blot lies in its use of SDS-PAGE gels, which can detect most protein bands in the venom and provide information on the molecular weight of the recognized antigens [84,93].

3.3 Antivenomics

Variability in venom composition across different geographic populations, developmental stages, and individuals of the same species highlights the critical need for comprehensive proteomic, toxicological, and immunological studies both within and between populations (see Chapters 2, 3, and 5). These studies are essential for formulating an optimal venom mixture and developing effective antivenoms capable of neutralizing snakebites caused by any member of the target species [94].

Antivenomic assays represent a powerful tool for evaluating the ability of antivenoms to recognize specific venom components, which are often identified through mass spectrometry [52,95]. This approach utilizes a proteomics-based platform, such as antivenomic immunoreactivity analysis (Figure 5), to provide a quantitative assessment of therapeutic antibody molecules and toxin-binding molecules present in an antivenom [96]. This analysis complements *in vivo* and *in vitro* venom neutralization assays and has the potential to replace traditional immunological methods, such as ELISA and western blot, in some applications [97].

First-generation antivenomics

First-generation antivenomic techniques rely on the immunoprecipitation of antivenom-bound toxins. In this method, the whole venom is incubated with the antivenom, followed by the addition of a secondary antibody, such as horse anti-IgG [86,98]. The resulting immunoprecipitated antigen-

antibody complexes contain toxins that are recognized with sufficient affinity by the antivenom antibodies. The non-immunoprecipitated toxins, which remain in the supernatant, represent venom components that are either not recognized by the antivenom or elicited the production of antibodies with very low affinity [99]. To identify these components, the reverse-phase chromatograms of the non-immunoprecipitated fraction are compared with the venom profile previously characterized through venomics [99].

Second-generation antivenomics

There are antivenom assessments based on F(ab')2 and Fab, which cannot be evaluated by immunoprecipitation [98]. An affinity chromatography column is filled with Sepharose, and a coupling buffer is added (Figure 5). After an incubation period, the molecules of IgG, F(ab')2, Fab', or Fab of an antivenom will covalently bind [100]. Next, the matrix is incubated with fixed amounts of venom, the fractions containing the unretained molecules that were not retained and those containing the proteins that were retained in the column due to their affinity to the antivenom molecules are analyzed separately by reverse phase chromatography [100]. An affinity chromatography approach is used to compare the retained and unretained fractions of the complete venom and quantify the degree to which an antivenom recognizes individual venom proteins [97,101].

The protein fraction not bound to the immobilized antibody matrix "i" is estimated using Equation 1 [101]:

$$\%NRi = 100 - \left(\left[\frac{Ri}{Ri + NRi}\right] \times 100\right) \quad (1)$$

Equition 1. The chromatographic peak area of protein "i" in the immune retention and eluded fraction of the affinity column is presented by "Ri". This value is used to determine the "antivenomic capacity".

A %NRi value of ≥ 25% indicates a good result in *in vivo* neutralization tests [8].

Third-generation antivenomics

Third-generation antivenomics is based on the same operational principle as the second generation, which involves affinity chromatography on an immobilized antivenom matrix (Figure 5). However, instead of using a single venom concentration incubated on the antivenom matrix, a series of identical affinity columns are used. Each column is incubated with increasing amounts of venom. This modification enables determining the maximum binding capacity of each toxin in the venom to the immobilized antivenom [95,102].

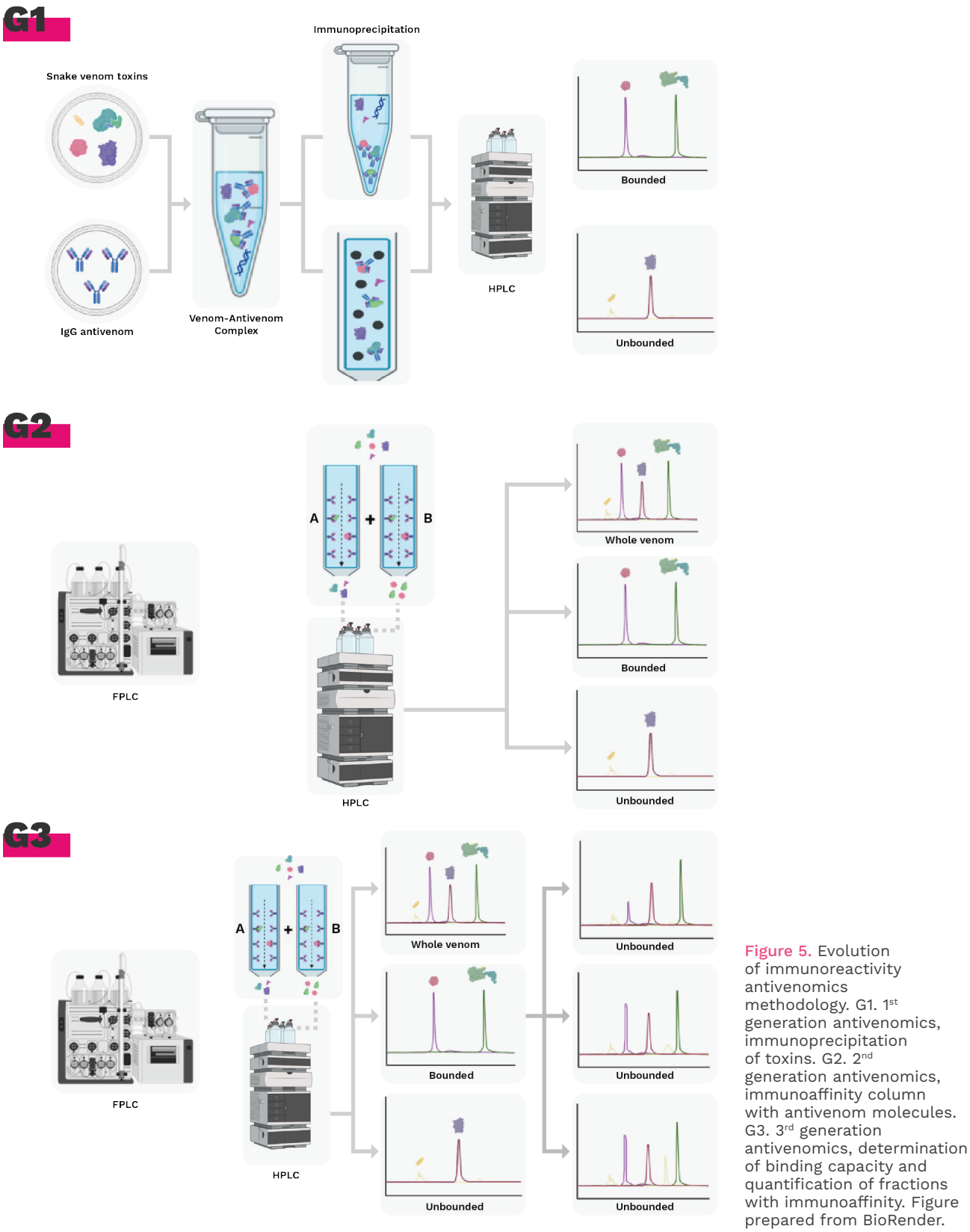


Figure 5. Evolution of immunoreactivity antivenomics methodology. G1. 1st generation antivenomics, immunoprecipitation of toxins. G2. 2nd generation antivenomics, immunoaffinity column with antivenom molecules. G3. 3rd generation antivenomics, determination of binding capacity and quantification of fractions with immunoaffinity. Figure prepared from BioRender.

However, antivenomics can only propose potential reasons for observed cross-reactivity, like identical subfamilies of crucial toxins in two venoms, one of which is employed in antivenom manufacturing. Antivenomics cannot elucidate cross-reactivity at the epitope level [98].

Despite this, the antivenoms are the only scientifically validated therapy for envenoming by snakebites. One urgent aspect that requires attention in institutions that produce antivenoms is the implementation of Good Manufacturing Practices (GMPs) to ensure the sustainability of production projects, the availability and accessibility of safe and effective antivenoms; as well as the implementation and testing of new technologies such as nano-body production (Figure 5).

4. Challenges and actions in snakebite treatment

Unlike other public health threats, snakebite envenomation is not susceptible to eradication due to the inevitable coexistence between humans and snakes, particularly in tropical regions, and the increase in encounters with these reptiles as a consequence of the expansion of human territories. Despite this reality, efforts to develop therapeutic alternatives for the management of ophidism are ongoing, and the optimization and modernization of antivenom production processes merit increased attention.

The progressive implementation of animal welfare regulations in some regions of the world poses significant challenges, including potential decreases in the number of countries with the capacity to produce antivenoms on a large scale. Consequently, clinical trial protocols using animal models need to be reviewed and updated to evaluate the cost/benefit ratio of current methods. The development and validation of alternative platforms, such as *in vitro* and *in silico* assays, designed to generate robust, reproducible, and clinically relevant results are needed. These tools will not only facilitate the transition to a more ethical paradigm in antivenom research and production but also ensure the sustainability of antivenom manufacture and universal access to this strategic biological input for public health.

Although antivenoms remain the “gold standard” therapy for the treatment of snakebite envenomation, there is a need for the production of antivenoms to be oriented towards the generation of broadly specific, affordable, safe, and effective antivenoms. This would contribute to greater homogeneity of antivenoms in all regions, reducing the cost of production and stabilizing the market for these biomolecules. The availability of good immunogens, appropriate production hosts, improved antibody purification methods, antivenom testing, including the use of alternative animal models, *in vitro* and *in silico* assays, and the establishment of appropriate distribution chains and systems to ensure that antivenoms are available in all locations, especially those closest and most prone to snakebite, are needed.

References

1. Dalefield, R. Antidotes. In *Veterinary Toxicology for Australia and New Zealand*; Elsevier, **2017**; pp. 33–39 ISBN 978-0-12-420227-6.
2. The Smithsonian Institution Antivenom Available online: https://www.si.edu/spotlight/antibody-initiative/antivenom (accessed on 1 May **2022**).
3. The University of Melbourne What Is Antivenom? Available online: https://biomedicalsciences.unimelb.edu.au/departments/department-of-biochemistry-and-pharmacology/engage/avru/discover/what-is-antivenom (accessed on 1 May **2022**).
4. Espino-Solis, G.P.; Riaño-Umbarila, L.; Becerril, B.; Possani, L.D. Antidotes against Venomous Animals: State of the Art and Prospectives. *J Proteomics* **2009**, *72*, 183–199, doi:10.1016/j.jprot.2009.01.020.
5. Waghmare, A.B.; Salvi, N.C.; Deopurkar, R.L.; Shenoy, P.A.; Sonpetkar, J.M. Evaluation of Health Status of Horses Immunized with Snake Venom and Montanide Adjuvants, IMS 3012 (Nanoparticle), ISA 206 and ISA 35 (Emulsion Based) during Polyvalent Snake Antivenom Production: Hematological and Biochemical Assessment. *Toxicon* **2014**, *82*, 83–92, doi:10.1016/j.toxicon.2014.02.012.
6. Bogado, F.; Núñez, S.; Mussart, N.B.; Leiva, L.; Acosta, O.C. Cambios Clínicos, Hemáticos y Coagulativos Consecuentes al Aumento de Anticuerpos En Equinos Productores de Suero Antiofídico. *Revista Veterinaria* **2013**, *24*, 3–9, doi:10.30972/vet.2411141.
7. Fan, H.W.; Vigilato, M.A.N.; Pompei, J.C.A.; Gutiérrez, J.M. Situation of Public Laboratories Manufacturing Antivenoms in Latin America. *Revista Panamericana de Salud Publica/Pan American Journal of Public Health* **2019**, *43*, doi:10.26633/RPSP.2019.92.
8. World Health Organization *Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins*; **2017**.
9. WHO Issues New Recommendation on Antivenom for Snakebites Available online: https://www.who.int/news/item/19-08-2018-who-issues-new-recommendation-on-antivenom-for-snakebites (consultado 1 May **2022**).
10. Ministerio de Salud y Protección, C. *Decreto 386 de 2018*; Ministerio de Salud y Protección social: República de Colombia, **2018**; pp. 1–9.
11. INVIMA Sistema de Tramites En Línea - Consultas Públicas disponible en línea: http://consultaregistro.invima.gov.co/Consultas/consultas/consreg_encabcum.jsp (consultado el 5 mayo **2022**).
12. Castillo-Beltrán, M.C.; Hurtado-Gómez, J.P.; Corredor-Espinel, V.; Ruiz-Gómez, F.J. A Polyvalent Coral Snake Antivenom with Broad Neutralization Capacity. *PLoS Negl Trop Dis* **2018**, *13*, 1–14, doi:10.1371/journal.pntd.0007250.
13. Gutiérrez, J.M.; Fan, H.W.; Silvera, C.L.M.; Angulo, Y. Stability, Distribution and Use of Antivenoms for Snakebite Envenomation in Latin America: Report of a Workshop. *Toxicon* **2009**, *53*, 625–630, doi:10.1016/J.TOXICON.2009.01.020.
14. Minsalud *Guía Para El Manejo de Emergencias Toxicológicas*; **2017**; ISBN 978-958-5401-33-4.
15. Oficina de laboratorios y control de calidad del INVIMA *Guía de Liberación de Lotes de Vacunas, Hemoderivados y Sueros de Origen Animal (Suero Antiofídico)*; https://www.invima.gov.co/sites/default/files/el-

instituto/laboratorio/Guia%20Liberacion%20de%20Lote%20LPB%20 2021-03-26.pdf (consultado el 1 May **2022**).

16. Jiménez-Charris, E.; Montealegre-Sánchez, L.; Solano-Redondo, L.; Castro-Herrera, F.; Fierro-Pérez, L.; Lomonte, B. Divergent Functional Profiles of Acidic and Basic Phospholipases A_2 in the Venom of the Snake *Porthidium lansbergii lansbergii*. *Toxicon* **2016**, *119*, 289–298, doi:10.1016/j.toxicon.2016.07.006.17.
17. Posada Arias, S.; Rey-Suárez, P.; Pereáñez J, A.; Acosta, C.; Rojas, M.; Delazari Dos Santos, L.; Ferreira, R.S.; Núñez, V. Isolation and Functional Characterization of an Acidic Myotoxic Phospholipase A_2 from Colombian *Bothrops asper* Venom. *Toxins* **2017**, *9*, E342, doi:10.3390/ toxins9110342.
18. Fernandes, C.M.; Zamuner, S.R.; Zuliani, J.P.; Rucavado, A.; Gutiérrez, J.M.; Teixeira, C. de F.P. Inflammatory Effects of BaP1 a Metalloproteinase Isolated from *Bothrops asper* Snake Venom: Leukocyte Recruitment and Release of Cytokines. *Toxicon* **2006**, *47*, 549–559, doi:10.1016/j.toxicon.2006.01.009.
19. Clissa, P.B.; Laing, G.D.; Theakston, R.D.; Mota, I.; Taylor, M.J.; Moura-da-Silva, A.M. The Effect of Jararhagin, a Metalloproteinase from *Bothrops jararaca* Venom, on pro-Inflammatory Cytokines Released by Murine Peritoneal Adherent Cells. *Toxicon* **2001**, *39*, 1567–1573, doi:10.1016/s0041-0101(01)00131-3.
20. Vargas, L.J.; Londoño, M.; Quintana, J.C.; Rua, C.; Segura, C.; Lomonte, B.; Núñez, V. An Acidic Phospholipase A_2 with Antibacterial Activity from *Porthidium nasutum* Snake Venom. *Comp Biochem Physiol B Biochem Mol Biol* **2012**, *161*, 341–347, doi:10.1016/j.cbpb.2011.12.010.
21. Kini, R.M.; Evans, H.J. A Model to Explain the Pharmacological Effects of Snake Venom Phospholipases A_2. *Toxicon* **1989**, *27*, 613–635, doi:10.1016/0041-0101(89)90013-5.
22. Sanjuán, J.; Vargas, J.; Ortiz, F.; Gonzalez-Herrera, L.; Watanabe-Minto, B.; Granja-Salcedo, Y. Determinación de la DL_{50} del Veneno de serpientes adultas de la especie *Bothrops atrox* en ratones albinos. *Momentos de Ciencia* **2015**, *9*, 147–152.
23. Segura, Á.; Herrera, M.; Villalta, M.; Vargas, M.; Gutiérrez, J.M.; León, G. Assessment of Snake Antivenom Purity by Comparing Physicochemical and Immunochemical Methods. *Biologicals* **2013**, *41*, 93–97, doi:10.1016/j.biologicals.2012.11.001.
24. Vázquez, H.; Olvera, F.; Alagón, A.; Sevcik, C. Production of Anti-Horse Antibodies Induced by IgG, F(Ab')2 and Fab Applied Repeatedly to Rabbits. Effect on Antivenom Pharmacokinetics. *Toxicon* **2013**, *76*, 362–369, doi:10.1016/j.toxicon.2013.09.004.
25. Chippaux, J.P. Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins. *Biol Aujourdhui* **2010**, *204*, 87–91, doi:10.1051/jbio/2009043.
26. De Roodt; Rafael, A.; Eduardo, G.; Jorge, A.; Malbrán, I.N.P.B.A.N.L.I.S.C.G.; Vélez, A.; Aires, C.D.B.; Adolfo, C.O.S.; Roodt, R. De; Litwin, S.; et al. Comparación Entre Dos Métodos de Producción Para La Elaboración de Antivenenos Ofídicos. *Acta Toxicol. Argent* **2010**, *18*, 10–20.
27. Amazonas, D.R.; Portes-Junior, J.A.; Nishiyama-Jr, M.Y.; Nicolau, C.A.; Chalkidis, H.M.; Mourão, R.H.V.; Grazziotin, F.G.; Rokyta, D.R.; Gibbs,

H.L.; Valente, R.H.; et al. Molecular Mechanisms Underlying Intraspecific Variation in Snake Venom. *J Proteomics* **2018**, *181*, 60–72, doi:10.1016/j.jprot.2018.03.032.
28. Pope, C.G. The Action of Proteolytic Enzymes on the Antitoxins and Proteins in Immune Sera: I. *Brit. J. exp. Path* **1939**, *20*, 132–149.
29. Pope, C.G. The Action of Proteolytic Enzymes on the Antitoxins and Proteins in Immune Sera: II. *Br J Exp Pathol* **1939**, *20*, 201–212.
30. Rojas, G.; Jiménez, J.; Gutiérrez, J. Caprylic Acid Fractionation of Hyperimmune Horse Plasma: Description of a Simple Procedure for Antivenom Production. *Toxicon* **1994**, *32*, 351–363, doi:10.1016/0041-0101(94)90087-6.
31. Simsiriwong, P.; Eursakun, S.; Ratanabanangkoon, K. A Study on the Use of Caprylic Acid and Ammonium Sulfate in Combination for the Fractionation of Equine Antivenom F(Ab')2. *Biologicals* **2012**, *40*, 338–344, doi:10.1016/j.biologicals.2012.05.002.
32. Calvete, J.J.; Sanz, L.; Angulo, Y.; Lomonte, B.; Gutiérrez, J.M. Venoms, Venomics, Antivenomics. *FEBS Lett* **2009**, *583*, 1736–1743, doi:10.1016/j.febslet.2009.03.029.
33. Valenta, J. *Venomous Snakes-Envenoming Therapy*; Nova Science, **2010**; ISBN 6312317269.
34. Casewell, N.R.; Jackson, T.N.W.; Laustsen, A.H.; Sunagar, K. Causes and Consequences of Snake Venom Variation. *Trends Pharmacol Sci* **2020**, *41*, 570–581.
35. Casewell, N.R.; Wagstaff, S.C.; Wüster, W.; Cook, D.A.N.; Bolton, F.M.S.; King, S.I.; Pla, D.; Sanz, L.; Calvete, J.J.; Harrison, R.A. Medically Important Differences in Snake Venom Composition Are Dictated by Distinct Postgenomic Mechanisms. *Proc Natl Acad Sci U S A* **2014**, *111*, 9205–9210, doi:10.1073/pnas.1405484111.
36. Engmark, M.; Lomonte, B.; Gutiérrez, J.M.; Laustsen, A.H.; De Masi, F.; Andersen, M.R.; Lund, O. Cross-Recognition of a Pit Viper (Crotalinae) Polyspecific Antivenom Explored through High-Density Peptide Microarray Epitope Mapping. *PLoS Negl Trop Dis* **2017**, *11*, e0005768, doi:10.1371/journal.pntd.0005768.
37. Chippaux, J.P.; Goyffon, M. Venoms, Antivenoms and Immunotherapy. *Toxicon* **1998**, *36*, 823–846, doi:10.1016/S0041-0101(97)00160-8.
38. Mackessy, S. *Handbook of Venoms and Toxins of Reptiles*; Mackessy, S., Ed.; CRC Press, **2016**; ISBN 9780429186394.
39. Vázquez, H.; Chávez-Haro, A.; García-Ubbelohde, W.; Mancilla-Nava, R.; Paniagua-Solís, J.; Alagón, A.; Sevcik, C. Pharmacokinetics of a F(Ab')2 Scorpion Antivenom in Healthy Human Volunteers. *Toxicon* **2005**, *46*, 797–805, doi:10.1016/j.toxicon.2005.08.010.
40. Andrew, S.M.; Titus, J.A. Purification of Immunoglobulin G. In *Current Protocols in Cell Biology*; **2001**; Chapter 2: Unit 2.7. doi: 10.1002/0471142735.im0207s21
41. Virtual Amrita lab Fragmentation of IgG Using Papain Available online: https://vlab.amrita.edu/?sub=3&brch=70&sim=1349&cnt=1 (Consultado el 3 May **2022**).
42. Ismail, M.; Abd-Elsalam, M.A. Pharmacokinetics of 125I-Labelled IgG, F(Ab')2 and Fab Fractions of Scorpion and Snake Antivenins: Merits and Potential for Therapeutic Use. *Toxicon* **1998**, *36*, 1523–1528, doi:10.1016/S0041-0101(98)00144-5.

43. Stowers, R.S.; Callihan, J.; Bryers, J. Optimal Conditions for F(Ab) 2 Antibody Fragment Production from Mouse IgG2a. *Undergraduate Research in Bioengineering* **2008**, 16–20.
44. Otero-Patiño, R.; Segura, Á.; Herrera, M.; Angulo, Y.; León, G.; Gutiérrez, J.M.; Barona, J.; Estrada, S.; Pereañez, A.; Quintana, J.C.; et al. Comparative Study of the Efficacy and Safety of Two Polyvalent, Caprylic Acid Fractionated [IgG and F(Ab')2] Antivenoms, in *Bothrops asper* Bites In Colombia. *Toxicon* **2012**, *59*, 344–355, doi:10.1016/j.toxicon.2011.11.017.
45. Otero, R.; León, G.; Gutiérrez, J.M.; Rojas, G.; Toro, M.F.; Barona, J.; Rodríguez, V.; Díaz, A.; Núñez, V.; Quintana, J.C.; et al. Efficacy and Safety of Two Whole IgG Polyvalent Antivenoms, Refined by Caprylic Acid Fractionation with or without β-Propiolactone, in the Treatment of *Bothrops asper* Bites in Colombia. *Trans R Soc Trop Med Hyg* **2006**, *100*, 1173–1182, doi:10.1016/j.trstmh.2006.01.006.
46. Mendes, T.M.; Oliveira, D.; Figueiredo, L.F.M.; Machado-de-Avila, R.A.; Duarte, C.G.; Dias-Lopes, C.; Guimarães, G.; Felicori, L.; Minozzo, J.C.; Chávez-Olortegui, C. Generation and Characterization of a Recombinant Chimeric Protein (RCpLi) Consisting of B-Cell Epitopes of a Dermonecrotic Protein from *Loxosceles intermedia* Spider Venom. *Vaccine* **2013**, *31*, 2749–2755, doi:10.1016/j.vaccine.2013.03.048.
47. Gutiérrez, J.M.; León, G.; Burnouf, T. Antivenoms for the Treatment of Snakebite Envenomings: The Road Ahead. *Biologicals* **2011**, *39*, 129–142, doi:10.1016/j.biologicals.2011.02.005.
48. Rucavado, A.; Núñez, J.; Gutiérrez, J.M. Blister Formation and Skin Damage Induced by BaP1, a Haemorrhagic Metalloproteinase from the Venom of the Snake *Bothrops asper*. *Int J Exp Pathol* **1998**, *79*, 245–254.
49. Queiroz, L.S.; Santo Neto, H.; Assakura, M.T.; Reichl, A.P.; Mandelbaum, F.R. Pathological Changes in Muscle Caused by Haemorrhagic and Proteolytic Factors from *Bothrops jararaca* Snake Venom. *Toxicon* **1985**, *23*, 341–345, doi:10.1016/0041-0101(85)90158-8.
50. Krifi, M.N.; El Ayeb, M.; Dellagi, K. The improvement and standardization of antivenom production in developing countries: comparing antivenom quality, therapeutical efficiency, and cost. *Journal of Venomous Animals and Toxins* **1999**, *5*, 128–141, doi:10.1590/s0104-79301999000200002.
51. Seifert, S.A.; Boyer, L. V. Recurrence Phenomena after Immunoglobulin Therapy for Snake Envenomations: Part 1. Pharmacokinetics and Pharmacodynamics of Immunoglobulin Antivenoms and Related Antibodies. *Ann Emerg Med* **2001**, *37*, 189–195, doi:10.1067/mem.2001.113135.
52. Núñez, V.; Cid, P.; Sanz, L.; De La Torre, P.; Angulo, Y.; Lomonte, B.; Gutiérrez, J.M.; Calvete, J.J. Snake Venomics and Antivenomics of *Bothrops atrox* Venoms from Colombia and the Amazon Regions of Brazil, Perú and Ecuador Suggest the Occurrence of Geographic Variation of Venom Phenotype by a Trend towards Paedomorphism. *J Proteomics* **2009**, *73*, 57–78, doi:10.1016/j.jprot.2009.07.013.
53. Gutiérrez, J.M.; Lomonte, B.; León, G.; Alape-Girón, A.; Flores-Díaz, M.; Sanz, L.; Angulo, Y.; Calvete, J.J. Snake Venomics and Antivenomics: Proteomic Tools in the Design and Control of Antivenoms for the Treatment of Snakebite Envenoming. *J Proteomics* **2009**, *72*, 165–182, doi:10.1016/j.jprot.2009.01.008.

54. Gutiérrez, J.M.; León, G.; Lomonte, B. Pharmacokinetic-Pharmacodynamic Relationships of Immunoglobulin Therapy for Envenomation. *Clin Pharmacokinet* **2003**, *42*, 721–741, doi:10.2165/00003088-200342080-00002.
55. Casewell, N.R.; Cook, D.A.N.; Wagstaff, S.C.; Nasidi, A.; Durfa, N.; Wüster, W.; Harrison, R.A. Pre-Clinical Assays Predict Pan-African Echis Viper Efficacy for a Species-Specific Antivenom. *PLoS Negl Trop Dis* **2010**, *4*, e851, doi:10.1371/journal.pntd.0000851.
56. Fernandes, I.; Assumpção, G.G.; Silveira, C.R.F.; Faquim-Mauro, E.L.; Tanjoni, I.; Carmona, A.K.; Alves, M.F.M.; Takehara, H.A.; Rucavado, A.; Ramos, O.H.P.; et al. Immunochemical and Biological Characterization of Monoclonal Antibodies against BaP1, a Metalloproteinase from *Bothrops asper* Snake Venom. *Toxicon* **2010**, *56*, 1059–1065, doi:10.1016/j.toxicon.2010.07.014.
57. Maria, W.S.; Cambuy, M.O.; Costa, J.O.; Velarde, D.T.; Chávez-Olórtegui, C. Neutralizing Potency of Horse Antibothropic Antivenom. Correlation between *in vivo* and *in vitro* Methods. *Toxicon* **1998**, *36*, 1433–1439, doi:10.1016/S0041-0101(98)00077-4.
58. Kapp, R.W. *Clarke's Analysis of Drugs and Poisons*, 3rd ed.; Moffat, A.C., Osselton, M.D., Widdop, B., Eds.; Pharmaceutical Press: London, UK, 2004; Vol. I, pp. 1–480; Vol. II, pp. 1–1176; ISBN 0-853-69473-7. *Int. J. Toxicol.* **2006**, *25*, 81–82.
59. Lomonte, B. Manual de Métodos Inmunológicos. *Métodos Inmunológicos* **2007**, 138.
60. World Health Organization *Guidelines for the Clinical Management of Snake Bites in the South-East Asia Region*; New-Delhi, **2005**.
61. Instituto Nacional de Salud de Perú Sueros Antiponzoñosos disponible en línea: https://web.ins.gob.pe/es/productos-biologicos/productos (consultado el 1 mayo **2022**).
62. Scanes, C.G. Animals and Human Disease: Zoonosis, Vectors, Food-Borne Diseases, and Allergies. In *Animals and Human Society*; Academic Press, **2017**; pp. 331–354 ISBN 9780128052471.
63. Sarmiento, K.; Rodríguez, A.; Quevedo-Buitrago, W.; Torres, I.; Ríos, C.; Ruiz, L.; Salazar, J.; Hidalgo-Martínez, P.; Diez, H. Comparación de La Eficacia, La Seguridad y La Farmacocinética de Los Antivenenos Antiofídicos: Revisión de Literatura. *Universitas Médica* **2019**, *61*, doi:10.11144/javeriana.umed61-1.anti.
64. Isbister, G.K. Antivenom Efficacy or Effectiveness: The Australian Experience. *Toxicology* **2010**, *268*, 148–154.
65. Laustsen, A.H.; María Gutiérrez, J.; Knudsen, C.; Johansen, K.H.; Bermúdez-Méndez, E.; Cerni, F.A.; Jürgensen, J.A.; Ledsgaard, L.; Martos-Esteban, A.; Øhlenschlæger, M.; et al. Pros and Cons of Different Therapeutic Antibody Formats for Recombinant Antivenom Development. *Toxicon* **2018**, *146*, doi:10.1016/j.toxicon.2018.03.004.
66. Espino-Solis, G.P.; Riaño-Umbarila, L.; Becerril, B.; Possani, L.D. Antidotes against Venomous Animals: State of the Art and Prospectives. *J Proteomics* **2009**, *72*, 183–199, doi:10.1016/j.jprot.2009.01.020.
67. Laustsen, A.; Solà, M.; Jappe, E.C.; Oscoz, S.; Lauridsen, L.P.; Engmark, M. Biotechnological Trends in Spider and Scorpion Antivenom Development. *Toxins* **2016**, *8*, doi:10.3390/toxins8080226.

68. de la Rosa, G.; Olvera, F.; Archundia, I.G.; Lomonte, B.; Alagón, A.; Corzo, G. Horse Immunization with Short-Chain Consensus α-Neurotoxin Generates Antibodies against Broad Spectrum of Elapid Venomous Species. *Nat Commun* **2019**, *10*, 1–8, doi:10.1038/s41467-019-11639-2.
69. Kadkhodazadeh, M.; Rajabibazl, M.; Motedayen, M.; Shahidi, S.; Veisi Malekshahi, Z.; Rahimpour, A.; Yarahmadi, M. Isolation of Polyclonal Single-Chain Fragment Variable (ScFv) Antibodies Against Venomous Snakes of Iran and Evaluation of Their Capability in Neutralizing the Venom. *Iran J Pharm Res* **2020**, *19*, 288–296, doi:10.22037/ijpr.2019.14400.12358.
70. Kazemi-Lomedasht, F.; Yamabhai, M.; Sabatier, J.-M.; Behdani, M.; Zareinejad, M.R.; Shahbazzadeh, D. Development of a Human ScFv Antibody Targeting the Lethal Iranian Cobra (*Naja oxiana*) Snake Venom. *Toxicon* **2019**, *171*, 78–85, doi:10.1016/j.toxicon.2019.10.006.
71. Romero-Giraldo, L.E.; Pulido, S.; Berrío, M.A.; Flórez, M.F.; Rey-Suárez, P.; Nuñez, V.; Pereañez, J.A. Heterologous Expression and Immunogenic Potential of the Most Abundant Phospholipase A_2 from Coral Snake *Micrurus dumerilii* to Develop Antivenoms. *Toxins* **2022**, *14*, doi:10.3390/TOXINS14120825.
72. Gutiérrez, J.M.; Calvete, J.J.; Habib, A.G.; Harrison, R.A.; Williams, D.J.; Warrell, D.A. Snakebite Envenoming. *Nat Rev Dis Primers* **2017**, *3*, 17063, doi:10.1038/nrdp.2017.63.
73. Resiere, D.; Arias, A.S.; Villalta, M.; Rucavado, A.; Brouste, Y.; Cabié, A.; Névière, R.; Césaire, R.; Kallel, H.; Mégarbane, B.; et al. Preclinical Evaluation of the Neutralizing Ability of a Monospecific Antivenom for the Treatment of Envenomings by *Bothrops lanceolatus* in Martinique. *Toxicon* **2018**, *148*, 50–55, doi:10.1016/j.toxicon.2018.04.010.
74. Theakston, R.D.G.; Reid, H.A. Development of Simple Standard Assay Procedures for the Characterization of Snake Venoms. *Bull World Health Organ* **1983**, *61*, 949–956.
75. WHO/SEARO: Guidelines for the clinical management of snake bites in the Southeast Asian region *The Southeast Asian Journal of Tropical Medicine and Public Health*; **1999**.
76. World Health Organization: Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins. *World Health Organization* **2010**, *204*, 87–91.
77. World Health Organization: The Biological Potency Assay for Antivenom Preparations. *Expert Committee on Biological Standardization* **1990**.
78. World Health Organization: Progress in the Characterization of Venoms and Standardization of Antivenoms. *World Health Organization Offset Publication* **1981**, 1–44.
79. Meier, J.; Theakston, R.D.G. Approximate LD_{50} Determinations of Snake Venoms Using Eight to Ten Experimental Animals. *Toxicon* **1986**, *24*, 395–401, doi:10.1016/0041-0101(86)90199-6.
80. Ramakrishnan, M.A. Determination of 50% Endpoint Titer Using a Simple Formula. *World J Virol* **2016**, *5*, 85, doi:10.5501/WJV.V5.I2.85.
81. World Health Organization: *Requirements for Biological Substances No. 21, WHO Technical Report Series No. 463, Annex 1. WHO Expert Committee on Biological Standardisation, 23rd Report*; **1971**.

82. Sells, P.G. Animal Experimentation in Snake Venom Research and *in vitro* Alternatives. *Toxicon* **2003**, *42*, 115–133, doi:10.1016/S0041-0101(03)00125-9.
83. Engvall, E.; Perlmann, P. Enzyme-Linked Immunosorbent Assay (ELISA) Quantitative Assay of Immunoglobulin G. *Immunochemistry* **1971**, *8*, 871–874, doi:10.1016/0019-2791(71)90454-X.
84. Mahmood, T.; Yang, P.C. Western Blot: Technique, Theory, and Trouble Shooting. *N Am J Med Sci* **2012**, *4*, 429–434, doi:10.4103/1947-2714.100998.
85. Gavini, K.; Parameshwaran, K. Western Blot. StatPearls [Internet]; StatPearls Publishing: Treasure Island, FL, USA, 2025. Available online: https://www.ncbi.nlm.nih.gov/books/NBK542290/ (Consultado el 1 noviembre **2023**).
86. Lomonte, B.; Escolano, J.; Fernández, J.; Sanz, L.; Angulo, Y.; Gutiérrez, J.M.; Calvete, J.J. Snake Venomics and Antivenomics of the Arboreal Neotropical Pitvipers *Bothriechis lateralis* and *Bothriechis schlegelii*. *J Proteome Res* **2008**, *7*, 2445–2457, doi:10.1021/pr8000139.
87. Theakston, R.D.G.; Jane Lloyd-Jones, M.; Reid, H.A. Micro-elisa for detecting and assaying snake venom and venom-antibody. *The Lancet* **1977**, *310*, 639–641, doi:10.1016/S0140-6736(77)92502-8.
88. Theakston, R.D.G. The Application of Immunoassay Techniques, Including Enzyme-Linked Immunosorbent Assay (ELISA), to Snake Venom Research. *Toxicon* **1983**, *21*, 341–352, doi:10.1016/0041-0101(83)90090-9.
89. Voller, A.; Bartlett, A.; Bidwell, D.E. Enzyme Immunoassays with Special Reference to ELISA Techniqies. *J Clin Pathol* **1978**, *31*, 507–520, doi:10.1136/jcp.31.6.507.
90. Theakston, R.D.G.; Reid, H.A. Enzyme-Linked Immunosorbent Assay (ELISA) in Assessing Antivenom Potency. *Toxicon* **1979**, *17*, 511–515, doi:10.1016/0041-0101(79)90284-8.
91. Burnette, W.N. "Western Blotting": Electrophoretic Transfer of Proteins from Sodium Dodecyl Sulfate-Polyacrylamide Gels to Unmodified Nitrocellulose and Radiographic Detection with Antibody and Radioiodinated Protein A. *Anal Biochem* **1981**, *112*, 195–203, doi:10.1016/0003-2697(81)90281-5.
92. Sawyer, A. What Is Western Blotting? The Story of the Western Blot - BioTechniques Available online: https://www.biotechniques.com/biochemistry/south-north-east-and-west-ern-the-story-of-how-the-western-blot-came-into-being/ (Consulatodo el 4 May **2022**).
93. Isbister, G.K. Antivenom Efficacy or Effectiveness: The Australian Experience. *Toxicology* **2010**, *268*, 148–154.
94. Calvete, J.J.; Lomonte, B.; Sanz, L.; Pérez, A.; Rodríguez, Y.; Gutiérrez, J.M.; Pla, D. Venómica y antivenómica: herramientas proteómicas para hacer frente a la patología desatendida del envenenamiento ofídico. In *Estrategias y Avances en el Estudio de Toxinas de Interés para la Biomedicina*; Calvete, J.J., Ed.; Instituto de Biomedicina de Valencia: Valencia, España, **2015**; pp. 79–97.
95. Calvete, J.J.; Rodríguez, Y.; Quesada-Bernat, S.; Pla, D. Toxin-Resolved Antivenomics-Guided Assessment of the Immunorecognition Landscape of Antivenoms. *Toxicon* **2018**, *148*, 107–122, doi:10.1016/j.toxicon.2018.04.015.

96. Gutiérrez, J.M.; Solano, G.; Pla, D.; Herrera, M.; Segura, Á.; Vargas, M.; Villalta, M.; Sánchez, A.; Sanz, L.; Lomonte, B.; et al. Preclinical Evaluation of the Efficacy of Antivenoms for Snakebite Envenoming: State-of-the-Art and Challenges Ahead. *Toxins* **2017**, *9*, 163, doi:10.3390/toxins9050163.
97. Pla, D.; Gutiérrez, J.M.; Calvete, J.J. Second Generation Snake Antivenomics: Comparing Immunoaffinity and Immunodepletion Protocols. *Toxicon* **2012**, *60*, 688–699, doi:10.1016/j.toxicon.2012.04.342.
98. Ledsgaard, L.; Jenkins, T.P.; Davidsen, K.; Krause, K.E.; Martos-Esteban, A.; Engmark, M.; Andersen, M.R.; Lund, O.; Laustsen, A.H. Antibody Cross-Reactivity in Antivenom Research. *Toxins* **2018**, 10:393. doi: 10.3390/toxins10100393.
99. Calvete, J.J. Proteomic Tools against the Neglected Pathology of Snake Bite Envenoming. *Expert Rev Proteomics* **2011**, *8*, 739–758.
100. Calvete, J.J.; Lomonte, B.; Sanz, L.; Pérez, A.; Rodríguez, Y.; Gutiérrez, J.M.; Pla, D. Venómica y Antivenómica: Herramientas Proteómicas para Hacer Frente a la Patología Desatendida del Envenenamiento Ofídico. In *Estrategias y Avances en el Estudio de Toxinas de Interés para la Biomedicina*; Calvete, J.J., Ed.; Instituto de Biomedicina de Valencia: Valencia, España, **2015**; pp. 79–97; ISBN 978-607-525-046-5.
101. Gutiérrez, J.M.; Lomonte, B.; Sanz, L.; Calvete, J.; Pla, D. Immunological Profile of Antivenoms: Preclinical Analysis of the Efficacy of a Polyspecific Antivenom through Antivenomics and Neutralization Assays. *J Proteomics* **2014**, 340–350, doi:http://dx.doi.org/10.1016/j.jprot.2014.02.021.
102. Pla, D.; Rodríguez, Y.; Calvete, J.J. Third Generation Antivenomics: Pushing the Limits of the *in vitro* Preclinical Assessment of Antivenoms. *Toxins* **2017**, *9*, doi:10.3390/toxins9050158.

Chapter 7

Venoms, love, and hate

Amira Abultaif Kadamani

Abstract: This chapter presents the history of the production of antivenom serum in Colombia by public health entities. The records show that this process began in the 1930s in the so-called Samper Martinez National Institute of Hygiene, the foundation of today's National Institute of Health. An exhaustive review of official and unofficial documents and interviews with some of the main protagonists involved in the production of these biologics was carried out. As a result, a historical contextualization of the conception and scientific study of venoms and antivenoms is presented, as well as a timeline that explains the most significant events in this process, which was not carried out continuously, but had interruptions throughout different decades due to various circumstances.

Keywords: Venoms, antivenom serum, National Institute of Health, antivenoms, polyvalent serum, monovalent serum, snakes.



Citation: Abultaif Kadamani, A. Chapter 7: Venoms, Love, and Hate. In Book: *Bites, venoms, and venomous snakes of Colombia*; Angarita-Sierra, T., Ruiz-Gomez, F.J, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 303-348. doi: 10.33610/691703ctwwtl

Copyright: © 2024 by the authors. Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0) license (http://creativecommons.org/licenses/by/4.0/).

Illustrations by:
Oscar A. Ramirez Ruiz

1. Introduction

"Gilgamesh, thou camest hither, sorrowing and striving, What shall I deliver thee, that thou mayest return to thy land? I will reveal, O Gilgamesh, a hidden thing, and [a secret of the gods] I will tell thee: This plant, like the cambron [tree in Ramanceae family, gen. Lyceum]. Its thorns will prick you [char your hands] like the rose. If your hands obtain the plant, [you will find new life]".

The eyes of this warrior opened wide, and he dived into the depths of the sea to take some branches, determined to eat them once he reached the wall of Uruk and with the illusion of becoming young forever. But something unexpected happened...

"Gilgamesh saw a well whose water was cool. He went down to bathe in the water. A snake sniffed the fragrance of the plant; it came out [of the water] and snatched the plant. As it retreated it shed its skin. At this Gilgamesh sat down and wept, tears streaming down his face. [He grasped the hand] of Urshanabi, the ferryman: "For whom, Urshanabi, did my hands labor? For whom is the blood of my heart spent? I obtained no mercy for myself" [1].

And so, fleeting and elusive, a serpent stole the dream of immortality from Gilgamesh, the Sumerian hero of Uruk whose exploits, written on stone tablets in cuneiform script, constitute the first epic of mankind.

This is not the only episode in which the yearning for transcendence is shattered by a sinuous creature. It is well known the grief that this reptile bears in Genesis, where it is the first and only animal mentioned in that biblical story that, among others, exalts its cunning and ability to hide.

Although for the ancient Israelites it was a symbol of the renewal of the cycle of life - this is why its image was used in some healing amulets; for Christians it was the animal of original sin, and that stigma has marked much of its odious reputation in Western culture, to the point of being labeled a demon.

But as everything in nature is perfectly circular and contingent - after all, a mirror of life - in snakes there is not only a curse, but also a blessing: poison and antidote in the same being that harbors the hope of salvation and that is why it stands unscathed on the staff of Asklepios, the Greek god of medicine and, later, on that of Aesculapius, its Roman version (see Chapter 10). From Rome also comes the name of its venom, venenum, conceived then as that "magic potion to make oneself loved or to awaken sexual desire in oneself or in others" [2]. And why is it called this? Because it honors Venus, the deity of love.

Ophidians, so cylindrical, have been a symbol of privilege throughout the centuries, being associated with renewal, fertility, longevity, prudence, wisdom, strength, and immortality. Hence their presence as logo-symbols in multiple health entities, from academia - in medical or dental schools

- to associations of professionals dedicated to this branch of knowledge and research, as well as multilateral organizations, such as the World Health Organization (see Chapter 10).

And it is the goodness of these reptiles that concerns us here: the science behind antiophidic serums. These substances, capable of neutralizing the devastating effects of serpentine venoms, are made using the very toxins in their venoms. It looks like magic, but it is more than that: in this case, the act of illusionism does not contradict the natural facts but corroborates them with proven clarity.

The origin of how serums began to change the course of history for a snakebite victim is attributed to the French microbiologist and infectologist Albert Calmette, a very disciplined, brilliant, and structured disciple of Louis Pasteur. As recounted in a publication of the Royal Society for the Advancement of Natural Science in London in the late 19th century, Calmette was sent to Saigon - today Ho Chi Minh City - to establish the headquarters of the nascent Pasteur Institute on the Indochinese peninsula. Overwhelmed by the number of people who died when attacked by cobras, he decided to conduct an experiment: to apply small and repeated doses of cobra venom to poultry and rabbits, waiting for them to acquire resistance to it. He was inspired by what other researchers had done with the bacillus causing diphtheria and tetanus, which, when inoculated in small amounts in different animals, generated neutralizing substances in them [3].

And, eureka, Calmette's hypothesis was proven and supported by experiments carried out simultaneously by other scientists of the time, particularly Henry Sewall with rattlesnakes (*Crotalus*) and Gabriel Bertrand, Cesaire Phisalix and his wife Marie with the common European viperid, *Vipera berus* [4]. Thus, from different shores, but with a common focus, serotherapy was born in 1894 as a mechanism for approaching ophidian accidents. With the drive and resources of the Pasteur Institute, at the end of that year Calmette went a step further by successfully demonstrating how such a serum could be created: by inoculating venom into horses, vigorous animals capable of developing sufficient antibodies in their blood to eventually be used in humans as a remedy against snake venom [3].

When, on the other side of the Atlantic, the Brazilian physician Vital Brazil (Figure 1) learned of Calmette's work, he did not hesitate to accept these findings and apply them in his native Brazil, a fertile land of ophidians of multiple types. As described in the review Pesquisa de la Fundação de Apoio à Pesquisa da Fundação à Pesquisa Cientifica do Estado de São Paulo (FAPESP) [5], Brazil tested the serum developed by the Pasteur Institute in France, but to his surprise it had no effect on guinea pigs attacked by the so-called jararacas (*Bothrops jararaca*) or rattlesnakes of this part of the world (*Crotalus durissus*). These were "the species causing the greatest number of incidences in the state of Sao Paulo", where he directed the Instituto Serumtherapico do Estado de São Paulo.



Figure 1. Ten thousand cruceiro banknote with the portrait of Dr Vital Brazil, Vital Brazil Mineiro da Campanha. (Taken from the book Traumas, ponzoñas y venenos de origen animal en Colombia by Hugo Sotomayor, Fundación Universitaria de Ciencias de la Salud, 2017, pg. 57).

The reason for this ineffectiveness? The French serum was produced from the venom of the Indian cobra or spectacled cobra (*Naja naja*), and everything indicated that this antidote could not be universal. In other words, "to specific venom, specific serum", as Brazil enshrined it, and this finding reinforced a very valuable principle for immunology: antigenic specificity, according to which the immune system of a human being responds differently to each toxin or antigen [5].

At the beginning of the 20th century, the famous Brazilian scientist devoted himself to the production of antivenom sera - among other biological products - from venoms obtained from regional species. But not completely satisfied, this shrewd researcher decided to make a combination of venoms to obtain from them a serum that would be useful in multiple cases of envenoming [6], thinking that it would be very complex for any victim to notice what type of snake was the cause of his suffering. Thus, at the end of 1901, the Instituto Serumtherapico do Estado de São Paulo - which would later be renamed Instituto Butantan - developed the polyvalent antivenom serum, prepared with the venoms of the most challenging specimens for public health in the South American giant: the jararaca and the rattlesnake [7].

This creation was the spearhead for other institutions in other latitudes to generate similar products, based on local venoms. This was the case

of the Clodomiro Picado Institute, another reference from America to the world. This Costa Rican institution, attached to the Faculty of Microbiology of the University of Costa Rica, was formally founded in 1970. However, the first batches of antivenom on Costa Rican soil were produced in 1967 by a group of enthusiasts of the process, who structured the Antiophidic Serum Program, which was under the initial administration of the Ministry of Health [8]. The breeding ground that allowed this desire to materialize had been cooked decades earlier and was led by the prestigious scientist Clodomiro Picado (Figure 2) who, although born in Nicaragua, grew up and was educated in Costa Rica, the country where his parents were born.



Figure 2. 20 colones banknote with the image of Clodomiro Picado (Costa Rica 1964-1970). (Taken from the book Traumas, ponzoñas y venenos de origen animal en Colombia by Hugo Sotomayor, Fundación Universitaria de Ciencias de la Salud, 2017, Pag. 58).

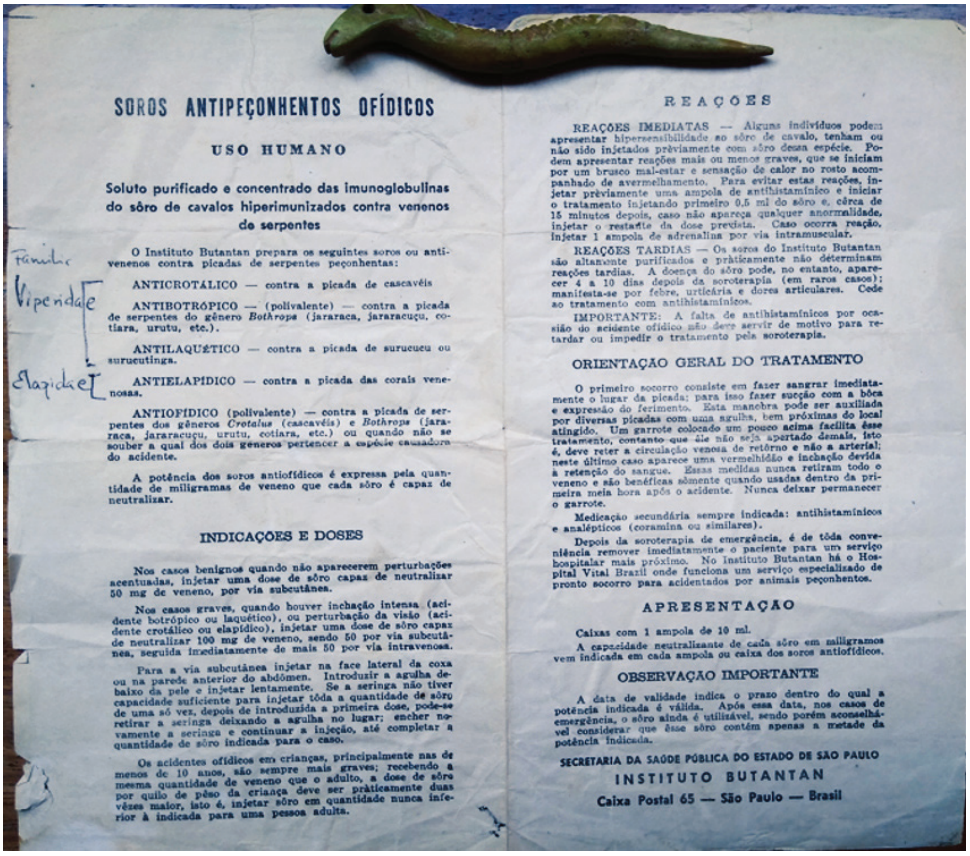
Trained in different disciplines, including pharmacy, zoology, microbiology, immunology, and chemistry, Picado began to explore the universe of snakes while working in the clinical analysis laboratory of the San Juan de Dios Hospital in Costa Rica, where he received many snakebite victims, especially peasants. He got in touch with the Butantan Institute of Brazil, to establish a collaborative relationship and began an intense research and study of snakes and their venoms. At the same time, he paved the way for the elaboration and approval of Law # 13 of 1926, known as "The Law of Defense against Ophians" that was a pioneering legislation in the continent because, among other reasons, it stipulated that antivenom serum should be free of charge for the most disadvantaged people [9].

Decades later, the founder of the Clodomiro Picado Institute was Roger Bolaños, the Costa Rican microbiologist and chemist who instructed officials of the Colombian National Health Institute in the development of

antivenom serums. His training and guidance were fundamental for the creation of Colombian antivenoms, a process that was formalized and strengthened in 1974.

2. As the crow flies

Before Colombia produced its own antivenoms on a regular basis, antivenoms produced in Brazil and Costa Rica were used. Brazilian antivenoms were the most widely used (Figure 3). However, for many communities, this was not and is not the way - or at least not the only way - to deal with snakebite. As part of the ancestral wisdom of indigenous and black ethnic groups, there are multiple mechanisms to deal with snakebites; and today, in some areas, they are preserved as a cultural inheritance [10].



SOROS ANTIPEÇONHENTOS OFÍDICOS

USO HUMANO

Soluto purificado e concentrado das imunoglobulinas do sôro de cavalos hiperimunizados contra venenos de serpentes

O Instituto Butantan prepara os seguintes soros ou antivenenos contra picadas de serpentes peçonhentas:

ANTICROTÁLICO — contra a picada de cascavéis

ANTIBOTRÓPICO — (polivalente) — contra a picada de serpentes do gênero *Bothrops* (jararaca, jararacuçu, cotiara, urutu, etc.).

ANTILAQUÉTICO — contra a picada de surucucu ou surucutinga.

ANTIELAPÍDICO — contra a picada das corais venenosas.

ANTIOFÍDICO (polivalente) — contra a picada de serpentes dos gêneros *Crotalus* (cascavéis) e *Bothrops* (jararaca, jararacuçu, urutu, cotiara, etc.) ou quando não se souber a qual dos dois gêneros pertencer a espécie causadora do acidente.

A potência dos soros antiofídicos é expressa pela quantidade de miligramas de veneno que cada sôro é capaz de neutralizar.

INDICAÇÕES E DOSES

Nos casos benignos quando não aparecerem perturbações acentuadas, injetar uma dose de sôro capaz de neutralizar 50 mg de veneno, por via subcutânea.

Nos casos graves, quando houver inchação intensa (acidente botrópico ou laquético), ou perturbação da visão (acidente crotálico ou elapídico), injetar uma dose de sôro capaz de neutralizar 100 mg de veneno, sendo 50 por via subcutânea, seguida imediatamente de mais 50 por via intravenosa.

Para a via subcutânea injetar na face lateral da coxa ou na parede anterior do abdômen. Introduzir a agulha debaixo da pele e injetar lentamente. Se a seringa não tiver capacidade suficiente para injetar tôda a quantidade de sôro de uma só vez, depois de introduzida a primeira dose, pode-se retirar a seringa deixando a agulha no lugar; encher novamente a seringa e continuar a injeção, até completar a quantidade de sôro indicada para o caso.

Os acidentes ofídicos em crianças, principalmente nas de menos de 10 anos, são sempre mais graves; recebendo a mesma quantidade de veneno que o adulto, a dose de sôro por quilo de pêso da criança deve ser praticamente duas vêzes maior, isto é, injetar sôro em quantidade nunca inferior à indicada para uma pessoa adulta.

REAÇÕES

REAÇÕES IMEDIATAS — Alguns indivíduos podem apresentar hipersensibilidade ao sôro de cavalo, tenham ou não sido injetados prèviamente com sôro desta espécie. Podem apresentar reações mais ou menos graves, que se iniciam por um brusco mal-estar e sensação de calor no rosto acompanhado de avermelhamento. Para evitar estas reações, injetar prèviamente uma ampola de antihistamínico e iniciar o tratamento injetando primeiro 0,5 ml do sôro e, cêrca de 15 minutos depois, caso não apareça qualquer anormalidade, injetar o restante da dose prevista. Caso ocorra reação, injetar 1 ampola de adrenalina por via intramuscular.

REAÇÕES TARDIAS — Os soros do Instituto Butantan são altamente purificados e praticamente não determinam reações tardias. A doença do sôro pode, no entanto, aparecer 4 a 10 dias depois da soroterapia (em raros casos); manifesta-se por febre, urticária e dores articulares. Cede ao tratamento com antihistamínicos.

IMPORTANTE: A falta de antihistamínicos por ocasião do acidente ofídico não deve servir de motivo para retardar ou impedir o tratamento pela soroterapia.

ORIENTAÇÃO GERAL DO TRATAMENTO

O primeiro socorro consiste em fazer sangrar imediatamente o lugar da picada; para isso fazer sucção com a bôca e expressão do ferimento. Esta mancha pode ser auxiliada por diversas picadas com uma agulha, bem próximas do local atingido. Um garrote colocado um pouco acima facilita êsse tratamento, contanto que êle não seja apertado demais, isto é, deve reter a circulação venosa de retôrno e não a arterial; neste último caso aparece uma vermelhidão e inchação devida à retenção do sangue. Essas medidas nunca retiram todo o veneno e são benéficas sòmente quando usadas dentro da primeira meia hora após o acidente. Nunca deixar permanecer o garrote.

Medicação secundária sempre indicada: antihistamínicos e analépticos (coramina ou similares).

Depois da soroterapia de emergência, é de tôda conveniência remover imediatamente o paciente para um serviço hospitalar mais próximo. No Instituto Butantan há o Hospital Vital Brazil onde funciona um serviço especializado de pronto socorro para acidentados por animais peçonhentos.

APRESENTAÇÃO

Caixas com 1 ampola de 10 ml.

A capacidade neutralizante de cada sôro em miligramos vem indicada em cada ampola ou caixa dos soros antiofídicos.

OBSERVAÇÃO IMPORTANTE

A data de validade indica o prazo dentro do qual a potência indicada é válida. Após essa data, nos casos de emergência, o sôro ainda é utilizável, sendo porém aconselhável considerar que êsse sôro contém apenas a metade da potência indicada.

SECRETARIA DA SAÚDE PÚBLICA DO ESTADO DE SÃO PAULO
INSTITUTO BUTANTAN
Caixa Postal 65 — São Paulo — Brasil

Figure 3. Photo of the instructions for use of the antivenom serum produced by the Butantan Institute of Brazil. This original copy was used in 1974 in Puerto Asís, Putumayo, by MD Hugo Sotomayor, during his rural stay in this municipality in southern Colombia, in order to complete his medical studies at the National University. Courtesy: Collection of Hugo Sotomayor.

Did a snake condom ever exist? Yes, in the 18th century. This was the name given to the bejuco del guaco, the most popular plant a little more than 200 years ago that attributed the incredible power to cure the sufferer of a venemous snakebite and to preserve oneself from its venom - that is, to protect oneself from the effects of the venom by obtaining immunity by frequently drinking its juice. The philosopher, lawyer, and acolyte of the Botanical Expedition Pedro Fermin de Vargas was the one hastened to underline its effectiveness, after venturing to verify it. In 1791 he published the *Estudio sobre el Guaco contra el Veneno de las Culebras* [11], in which he describes the antiophidic properties of the genus *Mikania* (mainly *Mikania laevigata* and *M. glomerata*, which are commonly called guaco).

In the year 1788, this illustrious neo Granadian, who was in Mariquita, arranged the worthy mission of experimental medicine for that purpose:

"We destined this operation for May 30: After summoning on the afternoon of the previous day, a black man from a local hacienda (...) who was known as the most skilled in those dangerous experiences. The black brought with him a venomous snake, known there by the name of talla equis, because of the white spots on its back that are somewhat similar to the letter X. On the appointed day the negro took the snake into his hands and made several movements in front of the snake without it becoming restless or biting him. I judged that the negro had previously removed its fangs, or that the snake was of a nonvenomous species. However, the man made the snake open its mouth; and I saw it had all its fangs. Everybody assured me that it was one of the most venomous snakes around, so I had no doubt of the efficacy of the preservative. Consequently, I determined to do the same thing myself, and so subjected myself to the same practice that the blacks do for their cures, to obtain the fearful satisfaction of handling the snakes.

The operation that was performed on me was as follows: The negro squeezed the juice of some leaves of the guaco herb into a glass and made me drink two spoonfuls of it. He then inoculated my skin, making six incisions: One on each foot, another on the index finger and thumb of each hand, and the last two on both sides of my chest. When these small wounds bled, a little of the guaco juice was poured over the wounds, and they were rubbed with the same leaf. After this preparation the sugeto (sic) is reputed to be truly cured and in a state to catch any snake without danger. I executé (sic) it (sic) immediately.

That day not only did I initiate myself into these mysteries, but also several other people tried it who were at Mr. Mutis' house. (...) To satisfy myself in an unquestionable way of the efficacy of the Guaco herb, I took the snake into my own hands; the snake was a little restless; but the reptile did not seem to want to bite. Once I lost my fear, I handled the snake twice more in the presence of Mr. Mutis (...) and in the presence of many other people who were there for this novel experience".

The illustrious man narrates that, in view of the good offices attributed to the plant, others dared to repeat the audacious experiment, among them, the painter Francisco Matis. Matis was wounded in his right hand during the snake-handling maneuver. However, the episode did not escalate because the black rubbed the herb on Matis´ body to stop any evil consequences.

And so, in Vargas' opinion, the myth, now converted into truth, of the benefits of the guaco that owes its name, according to his study, to a bird of the same name (probably *Herpetotheres cachinnans*, a known snake eater and named as guaco in Colombia) that consumes this herb when chasing and hunting snakes in warm zones. It is said that it was the Afrodescendants of the Choco who noticed the behavior of these birds that used the peculiar bejuco for two purposes: either to repel the venomous

effects they could incur if they were bitten during the hunt or to lull their potential prey to sleep and achieve their surrender in combat.

The explanation for this protection is that the repugnant odor emanating from the body of the eater alienates the snakes so that they do not bite, or it may weaken their venom. It is not clear, however, "if the vapor of the herb works by force of the annoyance it causes (...), as a true *anguicide* (sic), or by virtue of a pleasant sensation that alienates them (sic) and makes them forget their natural ferocity". For the healers, what is a fact is that its extract must be consumed constantly - at least for five or six days every month - and that it serves as an antidote against all types of snakes.

Who would not refrain from sharing his views on the matter either was another exceptional naturalist: Jorge Tadeo Lozano. He, as part of his scientific studies published in 1808 the book Sobre las serpientes [12], offers notes on the natural history of these reptiles in the New Kingdom of Granada and remedies for dealing with their bites. His entry into this universe could not be less accented:

"Among the various and enjoyable themes in zoology, there is perhaps no other more beautiful and important than herpetology or the study of reptiles; and among the themes of herpetology, the theme of snakes stands out. For in this animal order, which the ordinary people do not think about except with the greatest fright and horror, the naturalist admires the diversity that nature has evolved, portents upon portents, bringing together the most contradictory things in appearance. Here is where one sees a body, apparently inert and deprived of motor limbs, which enjoys the speed of lightning, and with equal ease runs over the surface of the earth, crosses the widest lakes and mighty rivers, and rises to the top of the loftiest trees. It is here that, under the appearance of a helpless animal that seems to be at the mercy of its enemies, the gigantic buio (güio or boa) is found that, with no other weapons than its enormous length and strength, fights against our most daring and largest animals [...], and not only defeats them, but also defeats them with its own strength and swallows them whole for its sustenance. Here is where in several species of the genus Coluber (Family Colubridae) and in all those of the Crotalus (Crotalurus = rattlesnakes), is hidden the venomous dart, whose wound is mortal although so small, and which by the place it occupies in the mouth is called a fang, and because it is movable and retractable and by its figure can be compared to the nail of a cat; here it is that, without proper organs for mastication, the most active digestive virtue is found; here the oviparous and viviparous generation are seen as confused and mixed; and finally, here it is that art recognizes its impotence to imitate the brilliant fugitive shades that adorn snakes and that somehow diminish the horror that their sight inspires with the consideration of the fatal effects caused by the venom of those species that are provided with such a deadly and terrible weapon".

With great literary flair, this marquis, who was part of the Royal Botanical Expedition of Santafe de Bogota and was in charge of the zoological part of the expedition, described and classified some of the most surprising characteristics of these reptiles. He also made an x-ray of the physi-

ological reactions of those bitten by snakes of different species and their way of experiencing it, according to the testimonies of victims of bites or of those who assisted them. He also offered his conjectures on the subject, among them being that humidity and warm weather worsen the situation by intensifying the active power of the venom and that this not only serves to make the snakes defend themselves or attack and eat, but also them to digest the prey they engulf.

Bandaging a little above the bitten area, burying the affected limb in a hole in the ground and remaining there for 7 or 8 hours or sucking the venom with the mouth and then spitting it out (a maneuver performed by healers who chew some herbs as a counter to avoid their own venoming) are some of the primary mechanisms described in the book to deal with an ophidian bite. The final and definitive lunge proposed is the amputation of the affected part (a practice carried out by the *afrodecendientes bozales*, emphasizing the illustrious Santa Fe native) after a prior analysis of the severity with which the "deadly liquor" acts.

To these mechanical remedies, whose essential purpose is to limit as much as possible the circulation of the venom in the bloodstream and to alleviate somewhat the burden for the victim, are usually added other non-medical therapeutic tactics such as the administration of substances with a chemical effect on the venom, the blood, or another part of the body (see Chapter 9). The aim of these is to "disorganize" the venom, neutralize its activity, slow its dispersion through the body, replenish "the irritability of the nervous system" - which is indicated as the main one affected -, combat inflammation and/or resist tissue putrefaction. The remedies can be one of the above, some, or all of the above. Today we know that all these practices act against the survival of the patient, generating serious injuries, worsening their condition or, in the worst case, causing their death (see Chapter 9).

In the palette of Creole options, he mentions, not without first criticizing the capriciousness and ignorance with which charlatan healers offer therapeutic miracles: the use of oil, aguardiente, cane with gunpowder and a host of plants such as *Polygala senega* (a plant that he says is effective against rattlesnake venom), native to North America [13], the tobacco leaf, and the popular guaco, to which not only curative but also preventive properties are attributed after drinking it in juice or cooked, as Pedro Fermin de Vargas explained in his writing. "Notwithstanding this assertion which, they say, is founded on repeated observations, and notwithstanding that it is assured that there are people cured who handle snakes with impunity, I know for a fact that a black woman who was bitten by a taya in the Bajamon hacienda and had guaco applied inside and out in very considerable portions, and in spite of the decanted virtues of this specific remedy, died miserably thirty hours after that misfortune happened to her", affirmed the marquis who was versed in chemistry due to his studies at the Royal Laboratory of Spain.

Although Jorge Tadeo Lozano praised the work of Abbé Fontana in his studies on the venom of European vipers and his experimentation with

caustic potash as a powerful antidote, he recognized the difficulty of accepting this as a universal remedy, given the great ignorance about the nature of the venoms and their variability according to the origin of the animal, age, sex, and other conditions that could affect their venom (see Chapter 5). In this way he glimpsed the principle of "a specific venom, a specific serum", postulated almost a century later by Vital Brazil.

He recommended the red cinchona (*Cinchona oblongifolia*) and pointed out that it could be used to counteract the effects caused by snake venom since, according to the "observations of the immortal Mutis, it possesses in supreme degree an antiputrid virtue that is a powerful astringent and febrifuge tonic". He clarified, however, that this was only his conjecture and that as such, it must be validated by experiment.

Finally, he offered some guidelines for those who want to study these animals: Although they have earned the hatred of mortals, they are honorable, on the one hand, because they fulfill a function in the ecosystem where they live - such as containing the proliferation of certain pests - and on the other hand, because they attack man only if they have been provoked, voluntarily or involuntarily.

Decades later, other texts on ophiology, toxicology, clinical effects, and case studies of victims were published in the country. As part of these writings, the publications of the physician Andres Posada Arango, his counterparts Manuel Uribe Angel and Evaristo Garcia, the monks Niceforo Maria, Apolinar Maria and Daniel Julian Gonzalez, the herpetologist Federico Medem, and the physicians Rodrigo Angel, Rafael Otero, and Santiago Ayerbe stand out. These last three physicians were trained in toxicology (the science that studies toxins, which are produced by living organisms such as microorganisms, plants, and small vertebrates, as opposed to toxins that are synthetic substances harmful to living organisms), and they were the precursors of two of the most robust academic programs of study and research on the subject in the country: The Ophiology/Scorpiology Program of the Faculty of Medicine of the University of Antioquia and the Herpetological and Toxicological Research Group of the University of Cauca.

3. Between ancestral knowledge, science and living memories

The above are just a few examples of how different populations during centuries have dealt with snakebites or their possible occurrences in Colombia. Although some practices are preserved, because they are part of the idiosyncrasy of the communities, many have welcomed with great receptivity the options that science has developed in the last decades, mainly the antiophidic sera, but also the scientific research on the effectiveness of plant extracts.

In fact, this is one of the lines of study of the Ophiology/Scorpiology Program of the University of Antioquia that in the 2000s initiated sev-

eral ethnobotanical studies with plants used by healers in Antioquia and Choco (one of them was presented at the XV International Congress of Tropical Medicine and Malaria, held in Cartagena in 2000 [14]). In this study a total of 101 plant species were identified, of which 74 were tested for neutralization of the venom of the talla x snake (*Bothrops asper*); and of these, seven proved to be the most promising by completely inhibiting the lethal effects in mice [15].

So-called "traditional medicine" is still deeply rooted in indigenous and Afro-Colombian populations, especially in remote and warm rural areas, where most snake accidents occur. Officially, around 4,700 cases of snakebite are reported annually (see Chapter 9), with a mortality rate of 1%. According to the analysis of clinical statistics in Colombia, of the total number of people bitten by venomous snakes in the last decade, 56% presented a mild degree of envenomation, 36% showed moderate symptoms, and the remaining 8% were severe (see Chapter 9).

It is worth clarifying that not all accidents in which a snake bites a person involve envenomation; first, because in many cases snakes are not venomous or because they have inefficient bite mechanisms or have venoms of very low toxicity for humans (see Chapters 5 and 9); and second, because around 60% of the bites of species that are venomous are warning signs [10], i.e., they are done only to dissuade predators or scare off threats, not to inject venom.

Although in 2009 the WHO included snake envenomation in the list of neglected tropical diseases, sometime later it was removed from that classification, but in 2017 it was reincorporated [15,16]). In Colombia there has been great interest in remedying this situation through three different mechanisms: (1) local production of antivenoms - a process for which there are records since the 1930s [17]); (2) training of health personnel in the clinical care of snakebite accidents, provided by health authorities and physicians passionate about the subject who have been trained and have extended their knowledge; and (3) research and study of the species present in our country, the second most megadiverse on the planet.

Unfortunately, not all nations have the capacity to produce antidotes to treat snake envenomation. Colombia has this capacity, and today its antiophidic serums stand out in the American continent for their quality. Thanks to our biological richness, the antivenoms produced by the National Institute of Health accept the venoms of various snake species from the different Colombian regions, and with them serums are formulated that, by cross-reaction, neutralize the effect of the venoms of most of the snakes of medical interest in the country (see Chapter 6).

The following is an account of this entire snake odyssey, documented and told by the protagonists at each stage. In this timeline, constituted of course by *Bothrops asper* -the cause of the greatest number of envenomings in the country- the most relevant facts of this history of venoms: love and hate are referenced. Welcome!

3.1. Timeline

-1916-: The national government, presided by Jose Vicente Concha, buys the Hacienda Santo Domingo, where decades later the Armero Serpentarium will operate.

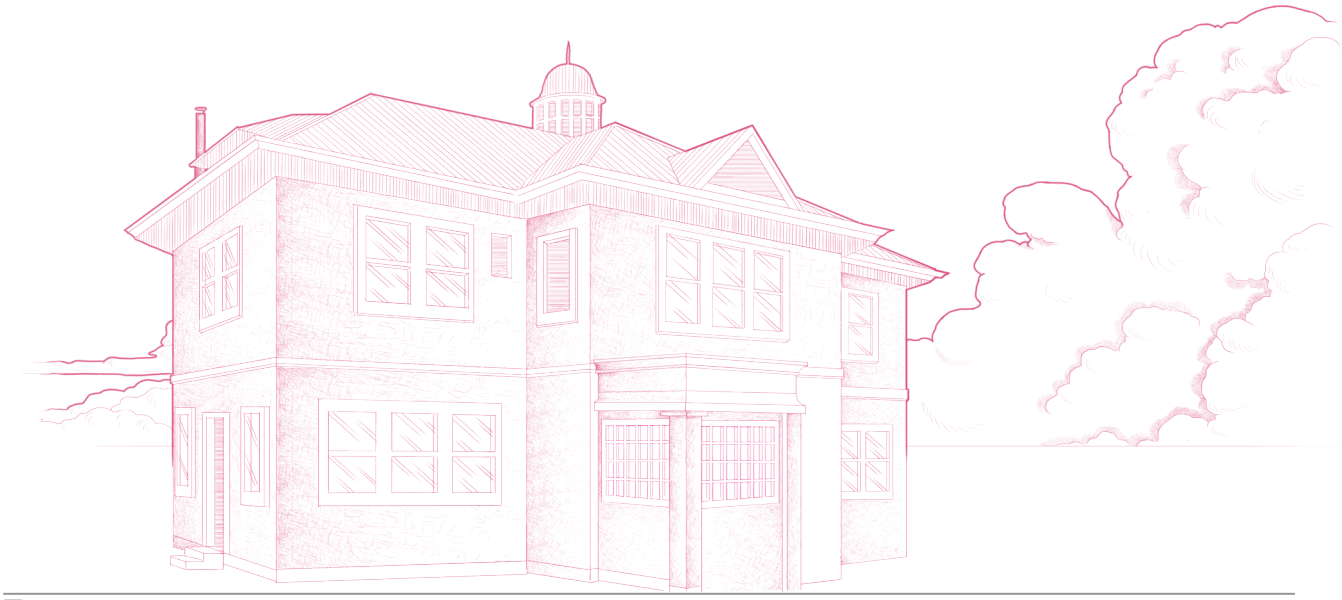
Detail: According to the deed of sale, this hacienda belonged to the jurisdiction of the municipality of San Lorenzo, which years later would be called Armero in honor of the independence hero Jose Leon Armero. This property, of 1,141 hectares located in the department of Tolima, was acquired for a value of 40.00 legal gold pesos.

-1925-: Law 15 of 1925 is enacted in which the Colombian Congress authorizes the government to purchase the Samper-Martinez Laboratory as a private entity capable of meeting public health needs, among them, the production of antiophidic serum.

Detail: Article 69 of this law states: "This Laboratory will preferably prepare serums, vaccines, and other biological products to combat the infectious diseases prevalent in Colombia: rabies, snake bite, and epizootics such as anthrax, symptomatic anthrax, etc. All these products will be sold at principal and cost, except for those destined to combat infectious diseases of the poorer classes, and for epidemics, cases in which these products will be supplied free of charge. To the biological products of this Laboratory that are exported, the commercial price will be fixed".

-1937-: The Colombian government issues Decree 1578 of 1937, which in its article 78 allocates 15,000 pesos for the construction, equipping, and operation of a serpentarium to produce antiophidic serum.

-1937-: MD. Bernardo Samper Sordo, founder of the Samper-Martinez Hygiene Institute (Figure 4), the foundation of the later National Health Institute, proceeded with a vigorous impulse for the eventual production of sera and, thus, strengthened the Armero Serpentarium. The development of polyvalent antiophidic serum was established.



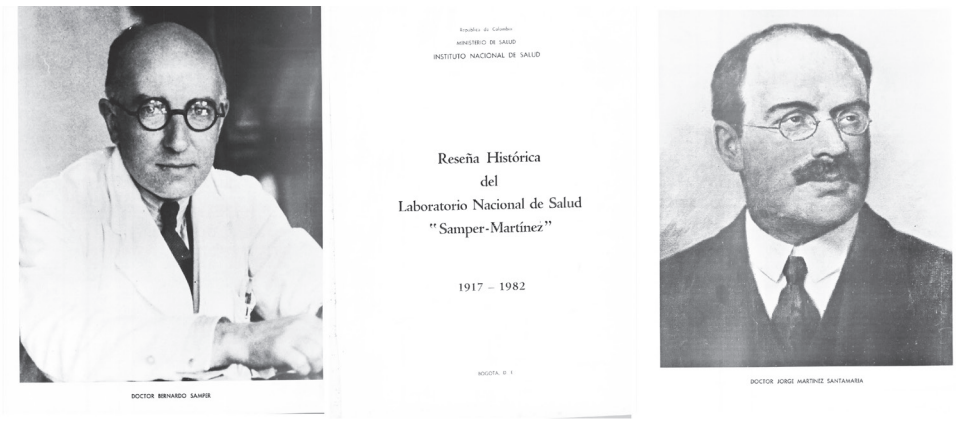
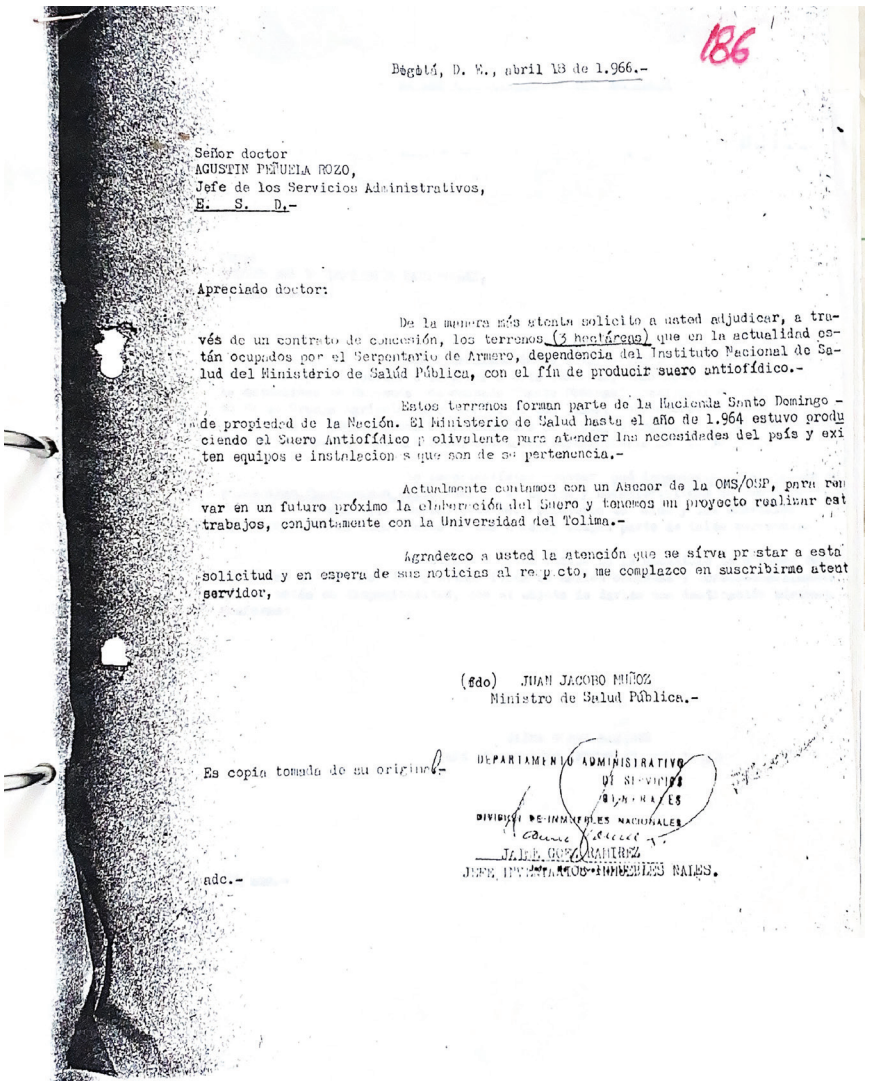


Figure 4. Photos from the book Reseña Histórica del Laboratorio Nacional de Salud Samper Martínez, 1917-1982. (Right) MD. Bernardo Samper. (Left) MD. Jorge Martinez Santamaria.

-1966-: The Minister of Health at the time, Juan Jacobo Muñoz, requested the Directorate of the Administrative Services to formally allocate a three-hectare plot of land, occupied by the Armero Serpentarium, to produce antiophidic serum (Figure 5). This document establishes that there was production of antiophidic serum until 1964.



186

Bogotá, D. E., abril 13 de 1.966.-

Señor doctor
AGUSTIN PEÑUELA ROZO,
Jefe de los Servicios Administrativos,
E. S. D.-

Apreciado doctor:

De la manera más atenta solicito a usted adjudicar, a través de un contrato de concesión, los terrenos (3 hectáreas) que en la actualidad están ocupados por el Serpentario de Armero, dependencia del Instituto Nacional de Salud del Ministerio de Salúd Pública, con el fín de producir suero antiofídico.-

Estos terrenos forman parte de la Hacienda Santo Domingo - de propiedad de la Nación. El Ministerio de Salud hasta el año de 1.964 estuvo produciendo el Suero Antiofídico y olivalente para atender las necesidades del país y existen equipos e instalaciones que son de su pertenencia.-

Actualmente contamos con un Asesor de la OMS/OSP, para reanudar en un futuro próximo la elaboración del Suero y tenemos un proyecto realizar estos trabajos, conjuntamente con la Universidad del Tolima.-

Agradezco a usted la atención que se sirva prestar a esta solicitud y en espera de sus noticias al respecto, me complazco en suscribirme atento servidor,

(Edo) JUAN JACOBO MUÑOZ
Ministro de Salud Pública.-

Es copia tomada de su original.-

DEPARTAMENTO ADMINISTRATIVO DE SERVICIOS GENERALES
DIVISION DE INMUEBLES NACIONALES
JEFE INVENTARIOS INMUEBLES NALES.

adc.-

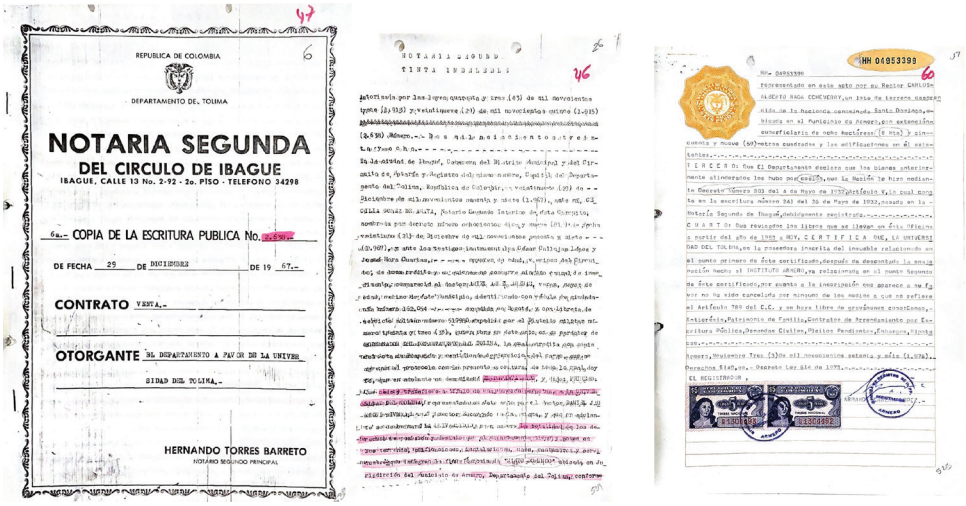
Figure 5. Letter in the dossier of documents on the serpentarium held by the INS.

-1967-: The Congress of the Republic enacted Law 39 of October 9, 1967, by means of which the nation ceded to the Department of Tolima a piece of land that would eventually be transferred in gratuitous bailment to the National Institute of Health to be used for the Armero Serpentarium.

Detail: The official publication of the act of promulgation of Law 39 of 1967 dated October 9 of that year states: the nation cedes to the "Department of Tolima a 350-hectare lot of land that was part of the former Hacienda Santo Domingo, located in the municipality of Armero, which was reserved in favor of the nation for the Army Remount." In the same document, the assignment to the Department of "the rights that may correspond to the Nation in buildings and installations constructed for the Armero Experimental Farm on the land ceded by the former to the Department, which was also part of the former Hacienda Santo Domingo" was also made.

Almost three months later, on December 29 the Department of Tolima granted to the University of Tolima the property rights and ownership of 200 hectares of the land, with the commitment to use this property for "exclusively educational, research, and extension functions carried out by the University", as stated in public deed # 2638 of 1967, Second Notary of Ibague (Figure 6).

In 1969, the University ceded, in turn, by way of perpetual alienation, a lot of eight hectares and 59 meters from the Hacienda Santo Domingo to the so-called Armero Institute, designated as an official educational establishment with legal status in 1966 and governed by Carlos Alberto Rada Echeverry.


REPUBLICA DE COLOMBIA

DEPARTAMENTO DE TOLIMA

NOTARIA SEGUNDA

DEL CIRCULO DE IBAGUE

IBAGUE, CALLE 13 No. 2-92 - 2o. PISO - TELEFONO 34298

COPIA DE LA ESCRITURA PUBLICA No.

DE FECHA 29 DE DICIEMBRE DE 19 67.-

CONTRATO VENTA.-

OTORGANTE EL DEPARTAMENTO A FAVOR DE LA UNIVERSIDAD DEL TOLIMA.-

HERNANDO TORRES BARRETO

NOTARIO SEGUNDO PRINCIPAL

Figure 6. Photos of the perpetual alienation title, a lot of eight hectares and 59 meters that is detached from Hacienda Santo Domingo.

-1969-: The Armero Serpentarium was remodeled and included the construction of a laboratory for regional epidemiological research -with parasitology, entomology and arbovirus sections- and a mouse biotherium for snake feeding.

-1972-: In the Biological Products section of the then National Institute for Special Health Programs (INPES, later National Health Institute-INS), therapeutic sera were developed, including antiophidic sera; and laboratory tests (electrophoresis) were carried out to determine the potency of venoms and antivenoms. A batch of 150 vials was created, but months later production was suspended due to the lack of experience and knowledge in handling venoms, immunization protocols, and titration and production techniques. The need for international training arose.

-1974-: A tropical medical congress was held in Medellin with renowned international guests, among them, Roger Bolaños, Costa Rican microbiologist and chemist who was a speaker at a conference on ophiology in Costa Rica.

Testimony of Juan Manuel Renjifo, biologist, former coordinator of the Serum Group of the National Health Institute

"That congress was vital because it brought to the table a very important reunion: that of Miguel Guzman and Ernesto Barbosa, INS researchers, with Roger Bolaños; all three were fellow students of the doctorate in microbiology at Tulane University, United States. The Congress was also attended by the Institute's director of parasitology, Augusto Corredor, who at the end of the event and upon his return to Bogota, began formal arrangements with the Clodomiro Picado Institute in Costa Rica to summon Professor Bolaños - who was also a consultant for the Pan American Health Organization - as an advisor for our Serum Laboratory.

I was just a recent biology graduate from the Universidad Javeriana who did his undergraduate thesis on snakes. Several people from the Institute helped me in my development and even allowed me to go several times to the Armero serpentarium to learn how to handle snakes. Since I was a little boy, I was interested in snakes because my father always had animals in the house, including snakes (Figure 7). He, Santiago Renjifo Salcedo, was a health doctor, based in Cali, and traveled through many parts of the country, mainly doing malarial studies. He was Minister of Health between 1963 and 1965.



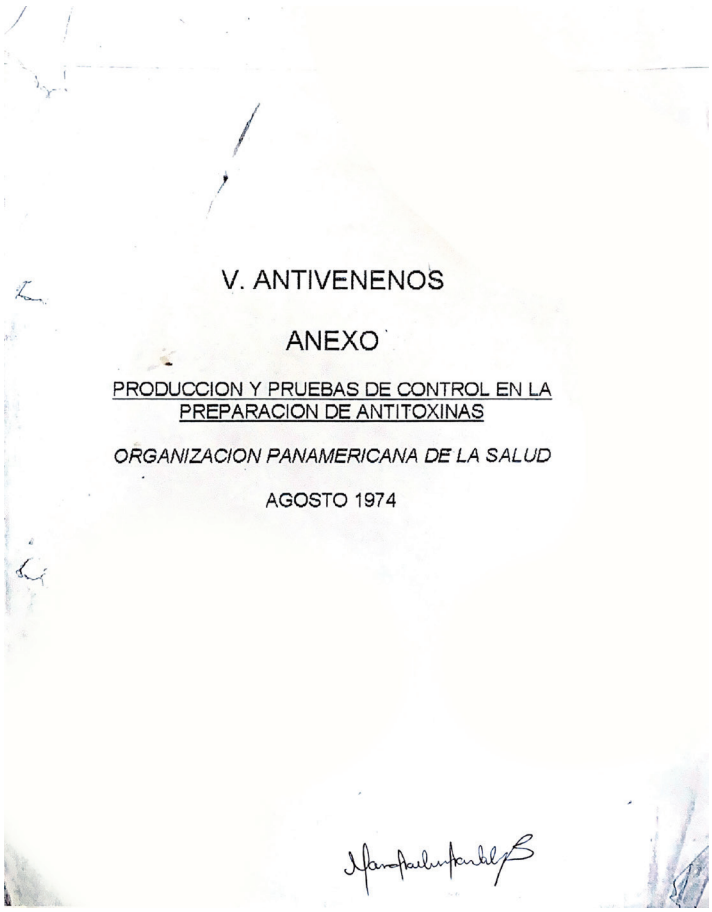


Figure 7. Juan Manuel Renjifo, eight years old, looking closely at a boa constrictor (*Boa constrictor*) wandering around his house. Courtesy: Juan Manuel Renjifo's private archive.

Many knowledgeable people warned me that working with these reptiles was very risky and thinking about developing sera was very complicated because attempts to date had been unsuccessful. However, I insisted for one fundamental reason: for me there is no better incentive than to be told no. As they say, if "I ask God and they don't give it to me, then that's my way".

I joined the Institute as a research assistant, and since I did well with the handling of the snakes of Armero, the directors proposed that I take charge of the production of antiophidic serums. And so, in the blink of an eye, I ended up being the coordinator of the Serum Group. Although I knew something about snakes, I had no idea about chemistry or immunology, so the first person I thought of to join the team was Guiomar Caicedo, who at that time was working in the DPT (Diphtheria, Pertussis and Tetanus) vaccine development laboratory. When I told her of my intentions, she cried with emotion".

-1974-: Roger Bolaños advised the Serum Group of the INS, given his experience and deep knowledge in the creation of antivenoms in Costa Rica (Figure 8), which he founded and was the first director of the Clodomiro Picado Institute, a world reference in the field.


V. ANTIVENENOS

ANEXO

PRODUCCION Y PRUEBAS DE CONTROL EN LA PREPARACION DE ANTITOXINAS

ORGANIZACION PANAMERICANA DE LA SALUD

AGOSTO 1974

Figure 8. Photo of the report made after the consultancy of Roger Bolaños, in 1974.

Testimony of Juan Manuel Renjifo, biologist, former coordinator of the Serum Group of the National Institute of Health

"PhD Bolaños' guidance proved crucial (Figure 8). It was Guiomar Caicedo who explained to him what the Institute had done so far in the production of antiophidic sera and he, very quickly, understood the scenario and trained us on what we should do and what we should not do. He toured the laboratory, checked the supplies, went to Hacienda Galindo to see the horses and to understand how the process of injecting venom and extracting blood was being done, among other things.

What we did was to dilute the venom with Freund's oil: we took a syringe with venom diluted in saline solution and another one with the oily substance and, little by little, we introduced both compounds with multiple steps and in both directions, inside the horse. A very homogeneous mixture with the consistency of toothpaste had to be achieved, which, after being injected, remained encapsulated inside the horse, allowing the progressive and slow release of the venom and the constant stimulation of the immune system.

But with Bolaños' instructions, what we did was to take sodium alginate and mix it with the liquid (dilution of venom in saline solution). Once we had a kind of gelatin, we added calcium chloride to create a softer

toothpaste that releases venom not in 20 days but in 10, with a maximum release after five days. This not only shortened production times, but also allowed us to have a better immune response.

Bolaño also instructed us in the Spearman Karber technique (statistical calculation tool) to determine the lethal doses and effective or neutralizing doses and, in this way, to determine with certainty and very quickly the immune response of the horses.

During the month that Bolaños was with us at the Institute, we produced a complete batch. Then we had a second phase of training in Costa Rica, where we traveled with Guiomar to learn about the work being done at the Clodomiro Picado Institute. Upon our return, we began full production".

-1975-: The INS produces batch 4 of polyvalent antivenom for the country marked the definitive and standardized restart of the production of antiophidic serum in Colombia (Figure 9).

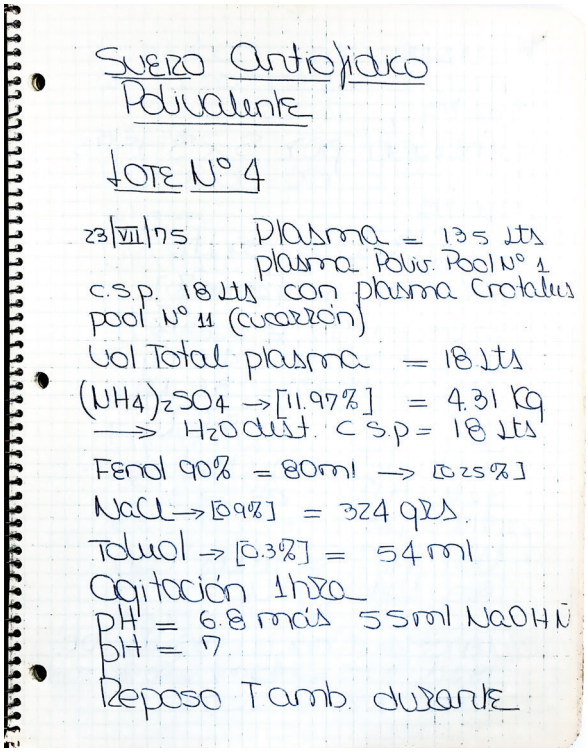

Suero Antiofídico
Polivalente
Lote N° 4
23/VII/75 Plasma = 135 Lts
plasma Poliv. Pool N° 1
c.s.p. 18 Lts con plasma Crotalus
pool N° 11 (Cucarrón)
Vol Total plasma = 18 Lts
$(NH_4)_2SO_4$ → [11.97%] = 4.31 Kg
→ H_2O dest. c.s.p = 18 Lts
Fenol 90% = 80ml → [0.25%]
NaCl → [0.9%] = 324 grs.
Toluol → [0.3%] = 54 ml
Agitación 1hra
pH = 6.8 más 55ml NaOH N
pH = 7
Reposo Temp. durante

Figure 9. Image of the document recording its production. This photo shows the record made in the handwriting of bacteriologist Guiomar Caicedo in the notebook of the time of the characteristics of the plasma of Cucarrón, as one of the horses used in the production of antiophidic serum was called.

Testimony of Francisco Ruiz, current leader of the INS Serpentarium

"By 1975, our Institute had developed two types of serum: the monovalent, which neutralizes the toxic effects of the venoms of the *Bothrops* group (tayas or mapanas), and the polyvalent, which counteracts the toxicity of the venoms of the *Bothrops* and *Crotalus* genera (rattlesnakes), the most representative in terms of ophiology in Colombia. Venom is a functional trait in snakes that has evolved according to the prey from the habitats where they live, and in this way snake venom becomes specific, accord-

ing to the area where they are located. In work done by the INS, we have found that the venom of *Bothrops asper* (the species that causes most accidents in our country and is distributed in the Andean, Pacific and Caribbean regions), has slightly different degrees of toxicity in the so-called lethal dose 50 (LD_{50}), which measures the amount of a toxic substance necessary to cause the death of 50% of a set of test animals.

Additionally, there are marked differences in the other biological tests that determine the effects that cause bleeding and inflammation. Several explanations for this are included in the scientific literature, including the type of diet or prey. Moreover, a very illustrative case of this phenomenon is that of the venom of rattlesnakes that inhabit the southern United States and Central America, which cause inflammation, coagulation alterations, necrosis and, in the case of some specific species, even paralysis (neurotoxicity). In contrast, the venom of South American rattlesnakes has evolved effects that are mainly neurotoxic and occasionally also myotoxic (hemorrhage).

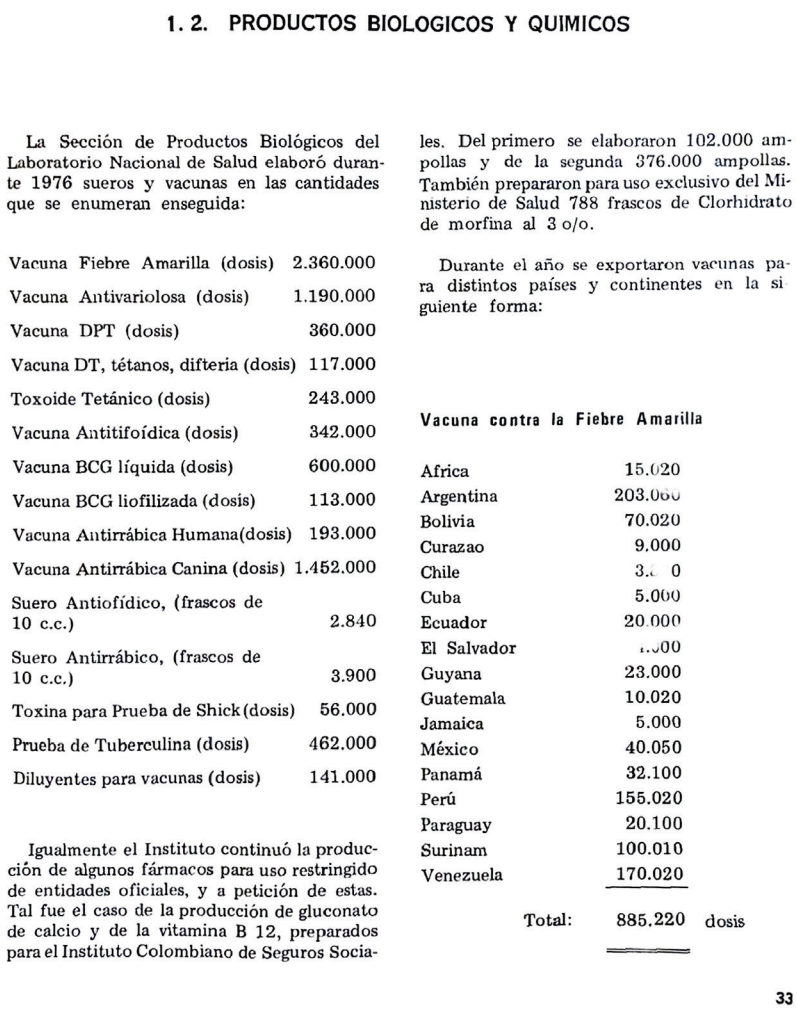
It is, therefore, extremely important that the venoms used as immunogens for serum production are regional or local. From the beginning, our serum group understood that it was necessary to meet the criterion of polyvalence, and in the production, development, and optimization of antivenom manufacturing, the mixtures of venoms used as immunogens were readjusted. Thus, today we use venoms of viperids (tallas, mapanas, cuatronarices, among others) from the five Colombian ecoregions. Thus, the INS antiophidic sera - the antiviperid and anticoral - have a greater neutralizing capacity than those commercialized in Colombia. Hence, one of the most valuable contributions of the Institute to the country is to enhance the serums by expanding their capacity to respond to an ophidian accident caused by a snake in any corner of the national territory".

***-1975-*:** The INS acquired the 70-hectare property called Hacienda Galindo y Ocho Rios, where horses were initially concentrated for the production of antiophidic serum (in addition to sheep and cattle for the production of culture media and vaccines); and, decades later, the Institute's serpentarium was also relocated (Figure 10).



Figure 10. Hacienda Galindo y Ocho Ríos, 70 hectares, where horses were initially concentrated to produce antiophidic serum.

-1976-: First detected record of exportation of antiophidic serum. That year there was a production of 2840 vials of 10cc, of which 170 were exported to Ecuador (Figure 11).



1. 2. PRODUCTOS BIOLOGICOS Y QUIMICOS

La Sección de Productos Biológicos del Laboratorio Nacional de Salud elaboró durante 1976 sueros y vacunas en las cantidades que se enumeran enseguida:

Vacuna Fiebre Amarilla (dosis)	2.360.000
Vacuna Antivariolosa (dosis)	1.190.000
Vacuna DPT (dosis)	360.000
Vacuna DT, tétanos, difteria (dosis)	117.000
Toxoide Tetánico (dosis)	243.000
Vacuna Antitifoídica (dosis)	342.000
Vacuna BCG líquida (dosis)	600.000
Vacuna BCG liofilizada (dosis)	113.000
Vacuna Antirrábica Humana(dosis)	193.000
Vacuna Antirrábica Canina (dosis)	1.452.000
Suero Antiofídico, (frascos de 10 c.c.)	2.840
Suero Antirrábico, (frascos de 10 c.c.)	3.900
Toxina para Prueba de Shick (dosis)	56.000
Prueba de Tuberculina (dosis)	462.000
Diluyentes para vacunas (dosis)	141.000

Igualmente el Instituto continuó la producción de algunos fármacos para uso restringido de entidades oficiales, y a petición de estas. Tal fue el caso de la producción de gluconato de calcio y de la vitamina B 12, preparados para el Instituto Colombiano de Seguros Sociales. Del primero se elaboraron 102.000 ampollas y de la segunda 376.000 ampollas. También prepararon para uso exclusivo del Ministerio de Salud 788 frascos de Clorhidrato de morfina al 3 o/o.

Durante el año se exportaron vacunas para distintos países y continentes en la siguiente forma:

Vacuna contra la Fiebre Amarilla

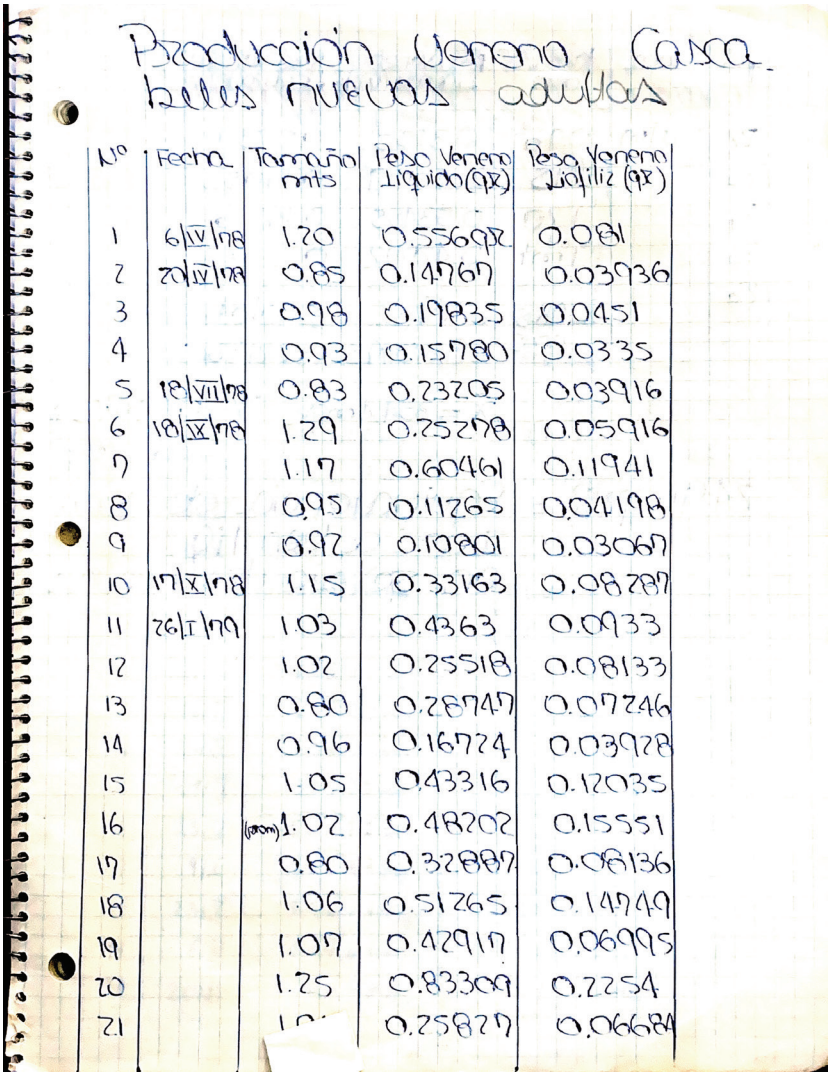
Africa	15.020
Argentina	203.060
Bolivia	70.020
Curazao	9.000
Chile	3.[illegible]0
Cuba	5.000
Ecuador	20.000
El Salvador	[illegible]00
Guyana	23.000
Guatemala	10.020
Jamaica	5.000
México	40.050
Panamá	32.100
Perú	155.020
Paraguay	20.100
Surinam	100.010
Venezuela	170.020
Total:	885.220 dosis

33

Figure 11. INS activity report in 1976.

-1978-: Lyophilization of venoms obtained from different species begins.

Detail: Since the end of the 1970s, venom extracted from snakes began to be lyophilized to favor its conservation, transport, and maintenance. This procedure consists of extracting the water from the venom by sublimation, that is, by freezing it and then evaporating it, subjecting it to a vacuum; in this way it passes from the solid to the gaseous state without passing through the liquid and, as a result, a powder is obtained that remains stable for long periods of time. Before 1978, the venoms were dried, i.e., the water was extracted from them by applying heat. "The venom is composed mainly of proteins with enzymatic action, i.e., they have the capacity to destroy other proteins or to destroy themselves. If left at room temperature between 12 and 37 degrees Celsius, it begins to denature and becomes, as Juan Manuel Renjifo used to say, panela water," explains Francisco Ruiz, leader of the Serpentarium (Figure 12).



Producción Veneno Cauca
bella nuevas adultas

Nº	Fecha	Tamaño mts	Peso Veneno Liquido (gr)	Peso Veneno Liofiliz (gr)
1	6/IV/78	1.20	0.55695	0.081
2	20/IV/78	0.85	0.14767	0.03936
3		0.98	0.19835	0.0451
4		0.93	0.15780	0.0335
5	18/VII/78	0.83	0.23205	0.03916
6	18/IX/78	1.29	0.25278	0.05916
7		1.17	0.60461	0.11941
8		0.95	0.11265	0.04198
9		0.92	0.10801	0.03067
10	17/X/78	1.15	0.33163	0.08787
11	26/I/79	1.03	0.4363	0.0933
12		1.02	0.25518	0.08133
13		0.80	0.28747	0.07746
14		0.96	0.16774	0.03978
15		1.05	0.43316	0.12035
16		1.02	0.48202	0.15551
17		0.80	0.32887	0.06136
18		1.06	0.51265	0.14749
19		1.07	0.42917	0.06995
20		1.25	0.83309	0.2254
21			0.25827	0.06684

Figure 12. Photo of the production notebook of that year showing a column for recording the freeze-dried venom, in the handwriting of Guiomar Caicedo.

-1981-: Report and analysis of a case of an ophidian accident in Barranquilla.

Testimony of Santiago Ayerbe, pediatrician and toxinologist, founder of the Herpetological and Toxinological Research Group and the Biomedical Research Center of the University of Cauca

"Since I was a resident studying my specialization in pediatrics, I have studied the subject with a feverish passion and that has allowed me to help save many lives from a distance. I have a particular anecdote from 1981. At that time, I was at the Central Military Hospital in Bogota. One day the Departmental Hospital of Barranquilla called the National Health Institute requesting antiophidic anticoral serum. Then Juan Manuel Renjifo (director of the Serum Group at that time) contacted me and said: "Santiago, they are asking us for an anticoral serum from the Departmental Hospital of Barranquilla for a bite, but I have my doubts that it was due to a coral because although there is neurotoxicity, the patient is intubated and everything, the bite was in the back. Please, find out what it is like".

I spoke, then, not to the doctor but to the head nurse of the hospital's ICU:

-Good afternoon nurse, this is Dr. Santiago Ayerbe speaking. I was contacted by the National Health Institute about a case of an ophidian accident. Please tell me why they require anti-coral serum.

-Good afternoon, because the patient is paralyzed it is a coral venom.

-I was just informed that the bite is on the back.

-Yes, it is on the back.

-Do you happen to know how far apart the fangs are?

-Wait a minute..., it's about three to four centimeters.

-Well, for starters, there is no coral of that size on the Caribbean Coast; the only one that could have a bite three centimeters between fangs is in the Amazon. But as I know that you have a zoo in Barranquilla, run by Roberto Tinoco, the only possibility is that this person would have gone to the zoo and there they had such a snake. But it would be an exotic and very rare bite. And I don't understand why he was bitten on the back either. Did this patient come from the zoo?"

-No. He's a 12-year-old boy.

-Okay, so that's not a coral bite but a rattlesnake bite. And there are more details: a rattlesnake doesn't bite on the back unless the patient was crawling on the ground, like crawling under a fence or something, and didn't notice the animal, and as soon as it got close, it bit him. And you know what? I think that animal would have had its rattles ripped off because otherwise the boy would have heard it...unless he's deaf and that's why he didn't hear the warning sound. To me that is the cause and there is not the slightest doubt in my mind that it is a bite from a South American rattlesnake, whose venom is neurotoxic. Please, give him the antiophidic serum, because Dr. Renjifo has just informed me that the hospital has antiophidic bothropic and antichrotalic serum, and that is the one you have to give him. Give him at least six bottles.

-But doctor, the doctor here has been investigating, and the venom of the rattlesnake produces hemorrhage and swelling, but not paralysis.

-The doctor is researching in Harrison's book, from the United States, and in the part of snakebite accidents he talks about the venoming of rattlesnakes, but from North American, not South American snakes. So, in that context he is wrong. This is a neurotoxic South American rattlesnake, so please, apply the serum as I indicated

-Well doctor, I'll let you know, thank you very much.

The next day, at 8 o'clock in the morning, I phoned the hospital. I asked to speak to the doctor, but they told me that he was currently on rounds reviewing cases, so I asked to speak to the nurse I had spoken to.

-What happened, tell me how did it go with the patient?

-How did it seem to you? the doctor said no, that it was not a rattlesnake, that it was a coral and that he needed the anti-coral serum

-So *sumercé (Colombian idiom to say sir or madam in a respectful way)*, with all the respect I have for you, it's not your fault. But listen to what I am going to tell you: I am in the Central Military Hospital of Bogota, I am going to try to arrange a plane to bring this patient here because they are going to kill him there. And if something happens to this patient, believe me, there is going to be a big problem. The situation is that big. The doctor is about to commit murder out of arrogance and ignorance. Tell him that and if he wants to talk to me, he can call me.

-Well doctor, thank you very much, I will.

-At eight o'clock in the evening I will call. The nurse was delivering the shift, about to leave, and she said to me:

-Oh doctor, thank goodness you called!

-Tell me, what happened?

-Well, I told the doctor what you said, he got scared and sent me for the serum. They gave him 10 vials.

-And what happened?

-Well, right away the patient started to open his eyes and to move. He was fighting with the tube and we extubated him.

-Ahh, so he recovered?

-Yes, he recovered.

-Well, tell me something, did you ask him how big the coral was?

-No, how do you think, doctor, it was like you said. The boy said it was a rattlesnake whose rattles had been torn off, and that's why he didn't hear it when he was going under the fence. I think we are going to keep the patient under observation for one more day and then he will be discharged.

-Well, I am very grateful to you because you listened to me and convinced the doctor.

This is one of the most dramatic cases I have had and it is part of the several episodes in which I have supported the Institute to solve situations derived from ophidian accidents.

When I was young, I wanted to study biology, but in Popayan there was no such career and the closest thing was medicine, so I started studying to become a doctor. In my fourth semester I saw the first case of an ophidian accident at the San Jose University Hospital. I was terrified by what I saw in that patient, who arrived with very advanced venoming. Nobody knew what to do or how to treat the case; they said we had to use the antiophidic serum available at the hospital, which was from the Butantan Institute, but they did not know how to use it, not even if it should be applied intramuscularly, intravenously, or subcutaneously. And when a professor was consulted, he only managed to say that he should look in his medical books because he really did not have much experience. A little more than 12 hours later the patient died. At that moment I understood my parents' concern when they warned me as a child to stay away from snakes.

Sometime later, I began to specialize in pediatrics. It was 1977 and one day, while I was doing my hematology rotation, the head of the department, MD. Alejandro Vasquez Godoy, a Chilean, told me that I had to do a research project during the four months of my rotation. We were discussing what I was going to work on when a colleague from internal medicine who had already been in the department for 15 days came in and the professor asked him about his work on ophidic accidents. He answered: "I am working on it, professor, I think I have something for next week". When I heard that, all the lights went on, and from that moment on my life began to take a 180-degree turn. It caught my attention and I dared to ask:

-Doctor, are you going to do a paper on ophidian accidents?

-Yes, Santiago. I am Chilean and in Chile there are no venomous snakes. When I studied hematology in the United States, I learned something about that because there are venous snakes there, but I am totally unaware of what is happening here, and I want us to start researching on that subject.

-Is it possible for my partner and I to work on it?

-Why, does it attract your attention, do you like the subject?"

-Doctor, I don't like it. It fascinates me.

-And what do you know about snakes?

-Nothing, absolutely nothing; but I, if you authorize me, very soon I will be talking to you about snakes, and in another language. Then he called his colleague and asked him if he could admit me to his work, to which he immediately answered yes. I thanked the doctor, and when I talked to my colleague, he told me that he had not really done anything because he was not interested; he thought the subject was a brick. I simply told him not

to worry, that we were going to do the job. I told him what I had in mind about how to carry out the study, and he was fine with that. I immediately went to the hospital archive and asked the coordinator, who was a friend of mine, to help me get the medical records of ophidian accidents for the last five years. At that time there was nothing systematized, but the truth is that in a matter of an hour she gave me a folder with 46 medical records.

I picked up those documents and went to the hematology laboratory. When Dr. Vasquez Godoy saw me he asked me what I had in my hands, and I told him. Suddenly, he opened his eyes and told me: "Hey Santiago, but you are going fast, aren't you?" And I simply told him: "No doctor, there is a lot to do here".

From that moment on, with my partner we began to read history by history. We made a big 'sheet' of office-size gridded sheets that we taped together and began to make a chart with different variables. I went to the Cauca Museum of Natural History to look at books and some magazines on ophiology, and there my passion for the study of the subject began to develop until I was professionally trained as a toxicologist in Brazil.

Thanks to God I have been able to help many people who consult me from many parts of the country, at any time, and for me it is a great satisfaction to be able to analyze each case and give clinical guidance (Figure 13)".



Figure 13. Left: Santiago Ayerbe searching for snakes in the Vaupes jungle with Hollman Miller (white skeleton and blue pants) and two guides from the community of Trinidad Tiquie, November 30, 2006. Right: Santiago Ayerbe handling a *Bothriechis schlegelii* species complex (eyelash viper).

***-1985-*:** A provisional serpentarium is set up at the INS headquarters in Bogota, due to the difficulties in accessing and maintaining the Armero Serpentarium after the natural disaster that affected this municipality in Tolima (Figure 14).

Detail: The images of the destruction caused by the mud avalanche after the melting of the Nevado del Ruiz, which erupted in November 1985, remain in the memory of all Colombians. But there is one fact that passed by: the implications that this natural disaster caused in the Bernardo Samper Serpentarium in Armero, known as the Serpentarium; the largest and most diverse in Colombia remained intact because the layer of mud at that point did not exceed half a meter. But the animals housed there began to starve to death because the staff in charge of it had to

evacuate the area as soon as they could. The Institute was left without its primary source of obtaining venoms to produce antiophidic serum.

"A month after the tragedy, the removal of the survivors began: most of the snakes and a group of *Aotus* spp monkeys used for malaria investigation," recounts the INS book Vigilantes de la Salud (Health Watchers). Where did they end up? On El Dorado Avenue in Bogota. There, at the main headquarters of the National Health Institute, a space was adapted for the sinuous creatures, to the horror of many and the delight of those who had the noble task of creating antidotes against their venom.

Although it was a provisional place, since it did not have the ideal conditions of temperature and humidity for their stay, decades passed before they were moved, in 2011, to an installation of their own: the Hacienda Galindo y Ocho Rios, in the municipality of Bojaca, Cundinamarca (see Chapter 8).



Figure 14. Photo of the provisional serpentarium located in the Bogota facilities of the INS. Originally, the snakes were housed in wooden boxes with sliding doors. Later, they were placed in aluminum boxes with acrylic lids (left), a type of shelter that was too cold for the snakes; for this reason, it was later decided to place them in plastic boxes (right) that give them greater comfort and allow for superior asepsis of their habitat in captivity.

Testimony of Juan Manuel Renjifo, former coordinator of the Serum Group of the National Health Institute

"When the avalanche happened, we went there by helicopter. Since I joined the Institute in 1974, I was in charge of the Armero Serpentarium, since that was our collection center for snakes from that region of the country. From the air I noticed that nothing had happened to the serpentarium. The mud reached only to where some of the mice were, and they had high cages; the rest, nothing.

Weeks later we moved the snakes to Bogota, and eventually I managed to keep them in separate boxes and to have air conditioning in the room where they were housed. In Armero my intention was to keep the animals in individual cages, but it was not possible because the serpentarium was built in the image and likeness of the Butantan Institute in Brazil: they were covered with transparent domes as a tourist showcase, where the snakes lived with each other. The problem with having them like that was that no sanitary control was possible; the sick ones were mixed with the healthy ones. One of our biggest headaches was the ticks they passed among them.

In the Armero Serpentarium we had specimens from San Juan de Rioseco, Lerida, Mariquita, Guayabal and all the surrounding area. But since it

was also crucial to have snakes from other regions of the country - to have a variety of venoms and, therefore, antivenoms - I personally took on this task. I could not call someone on the phone and say "send me snakes"; if I did that, the answer was obvious: "you are crazy, come and catch them yourself". That's why I traveled and got to know the entire country, and in one of those trips I even found a cobra that a man had in Manizales.

I remember one time Rodrigo Angel (a physician from Antioquia with vast knowledge of ophiology in Colombia), Roger Bolaños (the founder of the Clodomiro Picado Institute of Costa Rica) and I went with him. We captured them and put them in triplex wooden boxes with sliding doors that I asked Silvio, the carpenter, to make.

Testimony of Santiago Ayerbe, pediatrician and toxicologist, founder of the Herpetological and Toxicological Research Group of the Biomedical Research Center of the University of Cauca

"I met Juan Manuel Renjifo in 1980 and he became my teacher and guide in snake research. Together we began a series of field trips to build collections. I learned a lot in biological terms about these animals. Then I, from Cauca, began to send him the snakes that I collected by air to Bogota to contribute to the production of antiophidic serums, whose neutralization capacity for the patients of this region was marvelous, because in the manufacture of these antivenoms came from local specimens. The shipments were made by Intercontinental de Aviacion, which did not charge us for the shipment, but the boxes had to be delivered two hours before the passengers arrived to avoid panic reactions. There was only one daily flight from Popayan.

Juan Manuel was a great teacher for me in ophiology, a subject for which I felt an unusual attraction from childhood. My interest in herpetology comes from those early years, particularly since 1957, when I saw a photograph of a Tyrannosaurus rex that my father had brought me from the American Museum of Natural History. In the process of researching lizards, I came across snakes; and I was struck by the fact that these animals, despite not having limbs, sometimes moved faster than the lizards themselves. I took some snakes home and that caused problems since my parents told me that they represented a great risk, and that I should dedicate myself to studying birds, which I did. However, deep inside me, that taste for snakes remained intact.

I always had some degree of difficulty learning. During my career there were medical subjects that were very difficult for me to understand, and I didn´t have a very good memory, but everything I read about ophiology I absorbed like a sponge. This was impressive, anything about snakes I understood with unique ease, as if all my life that knowledge had been waiting for me".

Testimony of Francisco Ruiz, leader of the serpentarium

"The first time I went to the Serpentarium I had an unexpected reaction: the snakes didn´t scare me, I didn´t feel panic; actually, I thought they were beautiful... their scales, their mouth, their length... The only thing I

did was take notes. At that time, 1993, I was working in the Immunogenetics Laboratory of the INS Research Subdirection with primates of the genus Aotus. But one day Juan Manuel Renjifo came to my office, smoking and scratching his head, to tell me:

-Pacho, brother, my rattlesnakes are dying.

-I know about monkeys, but nothing about snakes.

-But you're a veterinarian, how could you not know!

-Well, no, I don't know.

At that moment I had just taken blood samples from several primates; I looked at his worried face and said to him:

-"Well, I'll gladly go and take a look. I have no idea about snakes, but I could consult someone.

-Great, little brother!

He always called me "manito"(buddy). Then he took me to the Instituto's serpentarium, a space that had been provisionally adapted in 1985 due to the Armero tragedy, but it continued working there until December 2011, that is, for 26 years. There were two rooms: a large one and another one like a small basement, where there was also a small bathroom. I had never seen a snake, I am from Bogota, a pure city dweller. And since I knew nothing, I consulted the person I thought who did know: Jorge Gardeazabal from the Cali Zoo, where I had done an unforgettable internship after finishing my veterinary career at the National University, and where I found my own thing, wild animals, because I never felt comfortable with domestic animal medicine. Jorge is a wonderful, brilliant person, a great teacher and, at the time, the veterinarian who knew the most about wildlife management and medicine in the country.

I called him, described to him the snake scenario at the Institute and he gave me clear and precise instructions: 'Remember population medicine, that is what must be applied now, so separate the animals that are in good condition and leave them downstairs, in separate boxes, inside separate containers and with separate handling tongs; and classify the sick animals into less serious, more serious, and moribund. Let's take advantage of the fact that you are at the Institute, so take some swabs as samples and send them to microbiology. In the meantime, let's start with one of those broad-spectrum antibiotics.

Well, I tightened my underpants, and Juan Manuel told the snake technician to help me; he was a great man, and it is really to him that I owe my apprenticeship in snake handling. His name is Arquimedes Gonzales, a veteran paisa (how persons native from Antioquia department are called.), very judicious and a hard worker, a lover like no other of snakes,

to the point that he distinguished each one, he knew their character, he knew perfectly which one was the escapist, the aggressive, the relaxed and how they should be handled. With his help and the guidance of Jorge Gardeazabal we began to treat them medically. Effectively we managed to save a group of snakes, others had died. And that was the beginning of my career as a snake catcher.

I must confess in all honesty: it was a great challenge for me to learn everything about snakes, i.e., species, distributions, and, of course, their correct handling. In the serpentarium, the ventilation system and the humidifier were deficient and had recurrent failures, so I had to spray water in the two rooms where they were located, in the morning and in the afternoon, to guarantee humidity. I often went on Saturdays to perform this task; and sometimes, I took out snakes and practiced how to hold them, by myself, and at my own risk. Fortunately, nothing ever happened to me.

Shortly afterwards, in 1994, Carlos Caceres, the veterinarian in charge of the horses used in the serum production, retired. For two years his work was taken over by other veterinarians, but eventually they also left their positions, so Juan Manuel sought me out again, in 1996, to help him. He made me a proposal.

-Pacho, you did well with the snakes. You know how they are kept and how they are milked. I want a professional who manages the process in an integral way, that is, the venoms, the snakes and the horses, and not the way we are managing it now, which is that we prepare the venoms to inject the horses, we put them in a refrigerator, the veterinarian takes them, injects the horses, after a month and a half he brings me the horse blood, we do the titration test, we tell him which horses developed the desired immunological response, and we ask him to take blood from those animals.

This is not practical, and we have communication problems with the biotherium; to avoid this, we must approach the process in an integrated manner.

-Oh, is that so?

-Yes, you do it; you can do it, that's why you are a veterinarian. If you already have your hands in snakes, I am sure that handling horses will not be difficult... Besides, I am going to tell you that the primate project is going to end, and you are going to be out of a job.

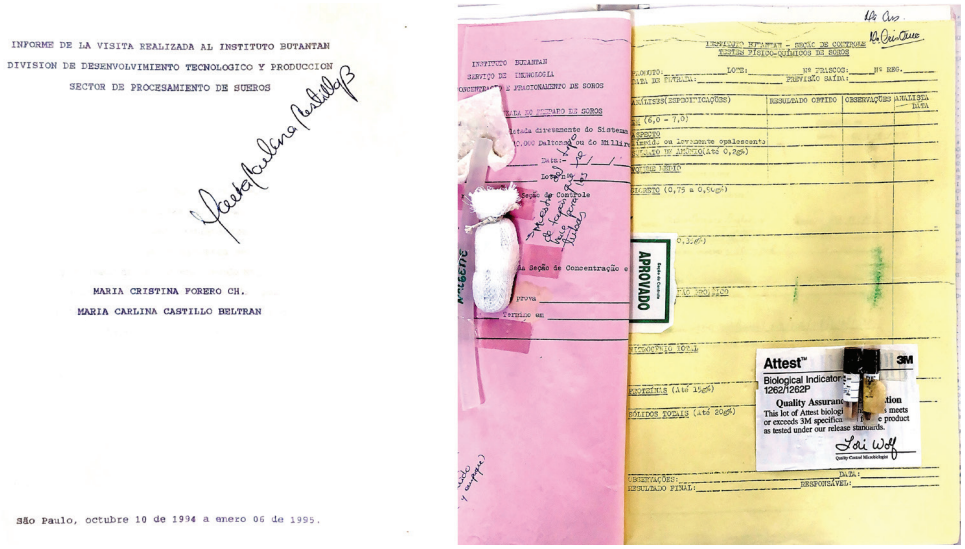
He told me all this, and I remained thoughtful. I didn't want to be left without something to do, and although I was beginning to have a passion for snakes, I had no idea about equine management because, so far, I had concentrated only on cytogenetics with nocturnal monkeys. However, I decided to accept the challenge and was endorsed by Moises Wasserman, the director at the time; so I arrived at the Hacienda Galindo y Ocho Rios, which is accessed through the Madrid Air Base until reach-

ing the Cortes trail, in the jurisdiction of Bojaca. There was no time to join the previous veterinarian, so I received my training with the horses from the technician, Evangelista Salazar, another great human being who had learned quite well about immunizations, bleeding, and other phases inherent to the process. When the Institute bought the farm in 1975 he was the administrator, and he decided to stay working with the Institute until he retired.

I confessed to him that I knew very little about equines in general and less about hyperimmune plasma production in particular. But he, kind as always, told me: 'Don't worry doctor, I will support you in whatever you need'. The first thing he did was to show me the medical histories of the horses, and I read them judiciously. The first two years involved a lot of reading and gradually getting involved in the process, under Salazar´s guidance. His expertise in handling the horses was overwhelming. He had arms as strong as Mike Tyson's and unbelievable strength.

With Juan Manuel Renjifo, Jaime Ramirez, and Maria Cristina Forero I was learning the basics of the work with the snakes: venom collection, handling, and control, biological tests, and inoculum preparation; the work at Hacienda Galindo with the horses involved injecting venom in a controlled manner so that they develop an immune response, performing the bloodletting, and obtaining the hyperimmune plasma – all of this I owe to Salazar. Looking back, it has been a very challenging job and one that we are very proud of."

-***1994-*:** INS officials travel to the Butantan Institute in Brazil to receive training in the production of antiophidic serum.


INFORME DE LA VISITA REALIZADA AL INSTITUTO BUTANTAN
DIVISION DE DESENVOLVIMIENTO TECNOLOGICO Y PRODUCCION
SECTOR DE PROCESAMIENTO DE SUEROS

MARIA CRISTINA FORERO CH.
MARIA CARLINA CASTILLO BELTRAN

São Paulo, octubre 10 de 1994 a enero 06 de 1995.

APROVADO

Attest™ 3M
Biological Indicator
1262/1262P
Quality Assurance

Figure 15. INS officials travel to the Butantan Institute in Brazil. (Left) Copy of the report prepared by INS Serum Group officials. (Right) Samples brought from Brazil to be used as a model for local production.

Testimony of Maria Carlina Castillo, coordinator of the Production and Technological Development Group of the Biologicals Production Division of the INS

"This training in production and quality control for the production of hyperimmune sera gave us a more realistic and very broad view of the degree of demand for production traceability and quality control, in compliance with good manufacturing practices. It was three months in which we had the opportunity to follow all the stages and steps involved in the production and control of hyperimmune sera.

I was 27 years old when we traveled, and it was the first time I heard only Portuguese. The first day I understood absolutely nothing, and of course at that time there were no technological tools that we know today and that are available, such as translators. In fact, the emotion I felt when I received a message in Spanish is unforgettable: it was a fax that my family had sent me with a warm greeting. Little by little, I picked up the rhythm of the language and later on I ended up with fairly fluent Portuguese (Figure 15).

At the time, Jose Roberto Marcelino was in charge of production at the Butantan Institute, and over the years we have continued to talk to each other. We met again in 2010 and 2013; he has always been a very kind person and available to support and address any concerns".

-***1998-*:** The INS obtains the sanitary registration for 10 years for the manufacture and commercialization of antiophidic serums, from the Direction of Surveillance and Control, Division of Biochemical Products of the Ministry of Health (Figure 16).


INSTITUTO NACIONAL DE SALUD

EL DIRECTOR DEL INSTITUTO NACIONAL DE SALUD

CERTIFICA

MOISES WASSERMAN LERNER
Director General

REPUBLICA DE COLOMBIA
MINISTERIO DE SALUD
DIRECCION DE VIGILANCIA Y CONTROL
DIVISION DE VIGILANCIA DE PRODUCTOS BIOQUIMICOS

Número: 001450 R-1 Vence:
Resolución Número: 1945 Fecha: 15 JUL 1998

FABRICAR Y VENDER.

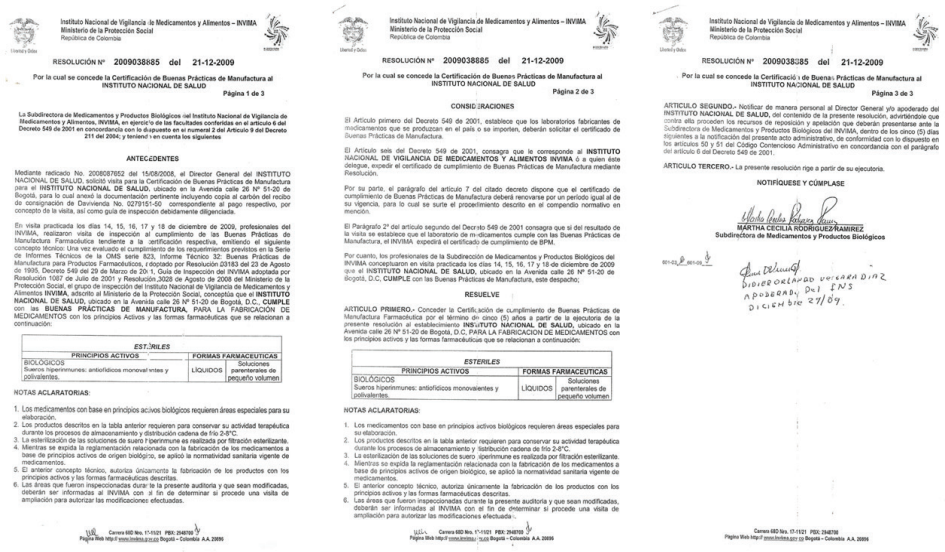
FORMULA MEDICA

EXPEDIENTE: 32.209

Figure 16. Photo of the documents certifying sanitary registration for 10 years to manufacture and commercialize antiophidic serums.

-***2002-*:** The INS publishes the book *Accidente por animales venenosos*, edited by the toxinologist Daniel Pineda, who describes in detail what happens in ophidian accidents and reviews the literature on venoms, types of snakes and their behaviors, treatments, and preventative mechanisms.

-***2009-***: The INS receives from the National Institute of Food and Drug Surveillance (INVIMA) a certificate for five years of Good Pharmaceutical Manufacturing Practices (GMP) of hyperimmune serums (including antiophidic sera; Figure 17).



Instituto Nacional de Vigilancia de Medicamentos y Alimentos – INVIMA
Ministerio de la Protección Social
República de Colombia

RESOLUCIÓN Nº 2009038885 del 21-12-2009

Por la cual se concede la Certificación de Buenas Prácticas de Manufactura al INSTITUTO NACIONAL DE SALUD

Página 1 de 3

La Subdirectora de Medicamentos y Productos Biológicos del Instituto Nacional de Vigilancia de Medicamentos y Alimentos, INVIMA, en ejercicio de las facultades conferidas en el artículo 6 del Decreto 549 de 2001 en concordancia con lo dispuesto en el numeral 2 del Artículo 9 del Decreto 211 del 2004, y teniendo en cuenta los siguientes

ANTECEDENTES

Mediante radicado No. 2008087652 del 15/08/2008, el Director General del INSTITUTO NACIONAL DE SALUD, solicitó visita para la Certificación de Buenas Prácticas de Manufactura para el INSTITUTO NACIONAL DE SALUD, ubicado en la Avenida calle 26 Nº 51-20 de Bogotá, para lo cual anexó la documentación pertinente incluyendo copia al carbón del recibo de consignación de Davivienda No. 0279151-50 correspondiente al pago respectivo, por concepto de la visita, así como guía de inspección debidamente diligenciada.

En visita practicada los días 14, 15, 16, 17 y 18 de diciembre de 2009, profesionales del INVIMA, realizaron visita de inspección al cumplimiento de las Buenas Prácticas de Manufactura Farmacéutica tendiente a la certificación respectiva, emitiendo el siguiente concepto técnico: Una vez evaluado el cumplimiento de los requerimientos previstos en la Serie de Informes Técnicos de la OMS serie 823, Informe Técnico 32: Buenas Prácticas de Manufactura para Productos Farmacéuticos, y acogido por Resolución 03183 del 23 de Agosto de 1995, Decreto 549 del 29 de Marzo de 20-1, Guía de Inspección del INVIMA adoptada por Resolución 1087 de Julio de 2001 y Resolución 3028 de Agosto de 2008 del Ministerio de la Protección Social, el grupo de inspección del Instituto Nacional de Vigilancia de Medicamentos y Alimentos INVIMA, adscrito al Ministerio de la Protección Social, concepta que el INSTITUTO NACIONAL DE SALUD, ubicado en la Avenida calle 26 Nº 51-20 de Bogotá, D.C., CUMPLE con las BUENAS PRÁCTICAS DE MANUFACTURA, PARA LA FABRICACIÓN DE MEDICAMENTOS con los principios Activos y las formas farmacéuticas que se relacionan a continuación:

ESTERILES

PRINCIPIOS ACTIVOS	FORMAS FARMACEUTICAS	
BIOLÓGICOS Sueros hiperinmunes: antiofídicos monovalentes y polivalentes.	LÍQUIDOS	Soluciones parenterales de pequeño volumen

NOTAS ACLARATORIAS:

1. Los medicamentos con base en principios activos biológicos requieren áreas especiales para su elaboración.
2. Los productos descritos en la tabla anterior requieren para conservar su actividad terapéutica durante los procesos de almacenamiento y distribución cadena de frío 2-8°C.
3. La esterilización de las soluciones de suero hiperinmune es realizada por filtración esterilizante.
4. Mientras se expida la reglamentación relacionada con la fabricación de los medicamentos a base de principios activos de origen biológico, se aplicó la normatividad sanitaria vigente de medicamentos.
5. El anterior concepto técnico, autoriza únicamente la fabricación de los productos con los principios activos y las formas farmacéuticas descritas.
6. Las áreas que fueron inspeccionadas durante la presente auditoría y que sean modificadas, deberán ser informadas al INVIMA con el fin de determinar si procede una visita de ampliación para autorizar las modificaciones efectuadas.

Carrera 68D No. 17-11/21 PBX: 2948700
Página Web http:// www.invima.gov.co Bogotá – Colombia A.A. 28996

Instituto Nacional de Vigilancia de Medicamentos y Alimentos – INVIMA
Ministerio de la Protección Social
República de Colombia

RESOLUCIÓN Nº 2009038885 del 21-12-2009

Por la cual se concede la Certificación de Buenas Prácticas de Manufactura al INSTITUTO NACIONAL DE SALUD

Página 2 de 3

CONSIDERACIONES

El Artículo primero del Decreto 549 de 2001, establece que los laboratorios fabricantes de medicamentos que se produzcan en el país o se importen, deberán solicitar el certificado de Buenas Prácticas de Manufactura.

El Artículo seis del Decreto 549 de 2001, consagra que le corresponde al INSTITUTO NACIONAL DE VIGILANCIA DE MEDICAMENTOS Y ALIMENTOS INVIMA ó a quien éste delegue, expedir el certificado de cumplimiento de Buenas Prácticas de Manufactura mediante Resolución.

Por su parte, el parágrafo del artículo 7 del citado decreto dispone que el certificado de cumplimiento de Buenas Prácticas de Manufactura deberá renovarse por un período igual al de su vigencia, para lo cual se surte el procedimiento descrito en el compendio normativo en mención.

El Parágrafo 2º del artículo segundo del Decreto 549 de 2001 consagra que si del resultado de la visita se establece que el laboratorio de m-dicamentos cumple con las Buenas Prácticas de Manufactura, el INVIMA expedirá el certificado de cumplimiento de BPM.

Por cuanto, los profesionales de la Subdirección de Medicamentos y Productos Biológicos del INVIMA conceptuaron en visita practicada los días 14, 15, 16, 17 y 18 de diciembre de 2009 que el INSTITUTO NACIONAL DE SALUD, ubicado en la Avenida calle 26 Nº 51-20 de Bogotá, D.C. CUMPLE con las Buenas Prácticas de Manufactura, este despacho;

RESUELVE

ARTICULO PRIMERO.- Conceder la Certificación de cumplimiento de Buenas Prácticas de Manufactura Farmacéutica por el término de cinco (5) años a partir de la ejecutoria de la presente resolución al establecimiento INSTITUTO NACIONAL DE SALUD, ubicado en la Avenida calle 26 Nº 51-20 de Bogotá, D.C. PARA LA FABRICACION DE MEDICAMENTOS con los principios activos y las formas farmacéuticas que se relacionan a continuación:

ESTERILES

PRINCIPIOS ACTIVOS	FORMAS FARMACEUTICAS	
BIOLÓGICOS Sueros hiperinmunes: antiofídicos monovalentes y polivalentes.	LÍQUIDOS	Soluciones parenterales de pequeño volumen

NOTAS ACLARATORIAS:

1. Los medicamentos con base en principios activos biológicos requieren áreas especiales para su elaboración.
2. Los productos descritos en la tabla anterior requieren para conservar su actividad terapéutica durante los procesos de almacenamiento y distribución cadena de frío 2-8°C.
3. La esterilización de las soluciones de suero hiperinmune es realizada por filtración esterilizante.
4. Mientras se expida la reglamentación relacionada con la fabricación de los medicamentos a base de principios activos de origen biológico, se aplicó la normatividad sanitaria vigente de medicamentos.
5. El anterior concepto técnico, autoriza únicamente la fabricación de los productos con los principios activos y las formas farmacéuticas descritas.
6. Las áreas que fueron inspeccionadas durante la presente auditoría y que sean modificadas, deberán ser informadas al INVIMA con el fin de determinar si procede una visita de ampliación para autorizar las modificaciones efectuadas.

Carrera 68D No. 17-11/21 PBX: 2948700
Página Web http:// www.invima.gov.co Bogotá – Colombia A.A. 28996

Instituto Nacional de Vigilancia de Medicamentos y Alimentos – INVIMA
Ministerio de la Protección Social
República de Colombia

RESOLUCIÓN Nº 2009038885 del 21-12-2009

Por la cual se concede la Certificación de Buenas Prácticas de Manufactura al INSTITUTO NACIONAL DE SALUD

Página 3 de 3

ARTICULO SEGUNDO.- Notificar de manera personal al Director General y/o apoderado del INSTITUTO NACIONAL DE SALUD, del contenido de la presente resolución, advirtiéndole que contra ella procederán los recursos de reposición y apelación que deberán presentarse ante la Subdirectora de Medicamentos y Productos Biológicos del INVIMA, dentro de los cinco (5) días siguientes a la notificación del presente acto administrativo, de conformidad con lo dispuesto en los artículos 50 y 51 del Código Contencioso Administrativo en concordancia con el parágrafo del artículo 6 del Decreto 549 de 2001.

ARTICULO TERCERO.- La presente resolución rige a partir de su ejecutoria.

NOTIFÍQUESE Y CÚMPLASE

MARTHA CECILIA RODRIGUEZ RAMIREZ
Subdirectora de Medicamentos y Productos Biológicos

DIDIER ORLANDO VIVERAS DIAZ
APODERADO DEL INS
DICIEMBRE 29/09

Carrera 68D No. 17-11/21 PBX: 2948700
Página Web http:// www.invima.gov.co Bogotá – Colombia A.A. 28996

Figure 17. Photo of the resolution granting the five-year Good Pharmaceutical Manufacturing Practices certification.

-2010-: The Serum Group implements negative control of equine plasma or serum in the testing scheme to determine the efficacy of antibodies produced by horses.

Detail: In the production of antiophidic serum, it is essential to perform biological tests of lethal dose fifty (LD_{50}) that determines the toxicity of the venoms, and the effective dose fifty (ED_{50}), intended to assess the neutralizing capacity of the serum or plasma of a horse against the control venoms, so as to know whether or not the animal can be an effective donor.

For this purpose, varying amounts of saline solution and equine plasma are mixed with the same dose of venom in several test tubes to create serial dilutions. They are then incubated at 37 degrees Celsius for the antigen-antibody (horse venom-plasma) reaction to occur. Subsequently, these mixtures are taken to the biotherium to inoculate mice.

Five mice are inoculated intraperitoneally from each tube, and each is injected with the same amount of substance. Five other mice serve as positive controls, i.e., they are injected with a combination of venom and saline solution and will therefore inevitably die. Since all the tubes of the serial dilutions have the same amount of venom, this means that the mice that survive do so because the horse's serum has developed the necessary antibodies to neutralize the venom. In this way it is possible to determine the level of titers (antibodies) that a horse is able to produce, and this power is translated into the amount of venom that neutralizes one milliliter of equine plasma.

Since 2010, negative controls have also been implemented, i.e., one group of mice is inoculated with a saline solution and another group with plasma. This verifies that these two substances are harmless for the mice and that the operator performs the intraperitoneal injection correctly, so that these animals should not die (Figure 18).

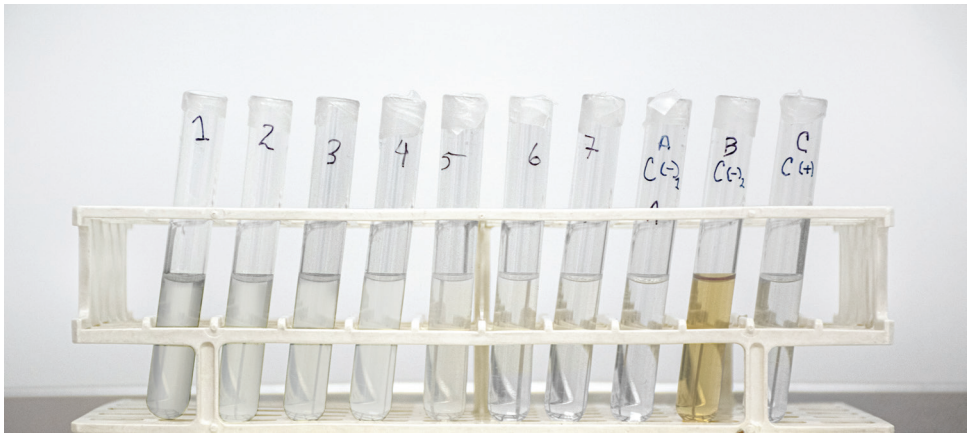


Figure 18. The test is performed in test tubes with volumes of 6 mL, each. Tubes 1 to 7 are serial dilutions with varying amounts of saline and horse serum and a fixed amount of venom. This together forms a suspension that looks milky in tube 1 and gradually decreases until tube 7 due to the reaction between the venom and the immunoglobulins (IgG) of the horse plasma so that the higher the IgG, the milkier it looks. Tube "A" has only saline and tube "B" has only horse serum, and both are the negative controls. Meanwhile, tube "C" has only venom, and is a positive control.

-2011-: The new and definitive home of all INS snakes is inaugurated: the Serpentarium at Hacienda Galindo y Ocho Rios. (Figure 20).



Figure 19. Photo of the transfer of the snakes from INS headquarters in Bogota (left) to Hacienda Galindo and Ocho Rios, in Bojaca (right).

Testimony of Monica Sarmiento, veterinarian, and leader of the INS Serpentarium

"The serpentarium was conceived as a laboratory, because this is where the inputs for the manufacture of serum come from. Therefore, everything is designed to maintain the aseptic conditions necessary for a biological product. Each snake lives inside a plastic box that is subjected to a rigorous process of cleaning and disinfection when it needs to be changed, for example, after receiving its physiological needs. Inside their environment, a container with water is kept for the snakes´ consumption and, in some boxes, a thermohygrometer is placed to constantly check temperature and humidity. Although in the wild many of the animals would be hidden or mimicked in natural substrates such as leaf litter, trunks, bushes, and trees, here in captivity we do not use these elements because it would be very complicated to disinfect them and change them for new ones.



Figura 20. Panoramic view of the Serpentarium facilities at Hacienda Galindo (left). Horses destined for serum production being examined by Francisco Ruiz (right).

As replacement for more natural elements, we use a substrate that we already had standardized, a mixture of imported sterile poplar or maple chips with vermiculite, which is mixed with water to increase the relative humidity in the enclosures to reach 80% or more humidity. For the elapids we implement a deeper layer of the same substrate. This conditioning allows the coral snakes to hide and literally 'swim' within this environment, which ensures that they can hide from predators, minimizing stress and achieving greater comfort. If we did not create these conditions these species would have problems with molt retention, and they would not eat and would easily become sick and die. That is why we have made all these adaptations.

For the group of mapanas, tayas or *Bothrops* in general, we use the same substrate, and for those that are arboreal, we provide them with branches to serve as perches. We have found that these adjustments have been very important because their stay in captivity has improved in time and animal welfare (see Chapter 8).

In the case of the rattlesnakes, since they do not have so many qualms about being exposed and are from dry habitats, the environments we build for them do not have vermiculite. Likewise, they are housed in the warmest part of the serpentarium that maintains an average temperature of 26 degrees Celsius.

Each time an animal enters the collection, it is quarantined and observed for three months. During this period, it is kept separate from the general population for evaluation whether it is sick or shows signs and symptoms of any disease, whether or not it is receiving food, whether it is moulting correctly, among other situations. At the end of this trimester, it is milked. If at the end of that time it shows any condition that does not compromise its life but does not evolve, it remains in quarantine. In the meantime, if there is a snake that is already part of the collection, but showing lesions -usually skin lesions-, we quarantine it until its health condition is restored (see Chapter 8).

Except for feeding days, venom milking, medical check-ups, or enclosure changes, the serpentarium is kept in the dark or in very dim light. However, every morning there is an inspection tour and recording of any news.

These are wild animals of solitary habits that are not accustomed to being handled, restrained with hooks, tongs, or hands, lifted, stretched, and having their glands squeezed to milk them. For them it is uncomfortable and highly stressful, but it is a discomfort that passes after a few hours, when they return to their primary confinement, where they can remain coiled and calm. The success of the technique consists of manipulating them as little as possible and in the shortest time possible so that these interventions do not result in illness or death. Having snakes as long-lived as the ones we have today (for example, an eight and one-half-year-old coral in captivity), we can confirm that we have greatly refined the process of keeping and maintaining the specimens under our care (see Chapter 8).

Before joining the Institute, I had no idea about snakes because I was dedicated to the veterinary medical management of horses, but learning about snakes has been incredible, very challenging, and not without risks. In fact, only four months after I started working in the serpentarium I was bitten on my left index finger by a *Bothrops asper* from the Choco department. It happened on January 27, 2014.

It was a day of venom collection; I grabbed the snake, lifted it out of its box, and it bit me. It was very fast; I didn't see what happened. Obviously, I let go of the snake and my partner Carlos took care of organizing it and securing it in its box. Francisco washed my finger and massaged it so that some blood would come out. We immediately notified the ARL (notified the work risk insurance company), got the car and the antivenom that we have ready in the serpentarium and left in a hurry for the Hospital San Rafael de Facatativa; this is our medical center of reference in case of an accident in the serpentarium. We arrived after about 45 minutes and went straight to the emergency room. There, the doctor who attended me had little knowledge of snakebite, so we called MD. Santiago Ayerbe. Over the phone, he guided the doctor on how to handle my case. Fortunately, the doctor who attended me was very kind and receptive to Dr. Ayerbe's indications.

Fortunately, the snake only bit me with one fang. At the hospital there was still a small hemorrhage from the finger, just a trickle of blood. However, the whole hand was already swollen up to the wrist. They took tests and determined that I was not clotting, so they began to administer the Institute's serum; the treatment was with 10 complete ampoules. That day they left me in intensive care as a preventive measure because I was anticoagulated. Six hours later I was examined again, and I was already clotting, but the inflammation of the hand continued, so they ordered an X-ray. The orthopedist determined that there was bone involvement, specifically of the phalanx of the finger, so he was going to schedule surgery to debride that part. However, after a second call to Dr. Ayerbe, we followed his advice not to operate at that time and so he did not authorize the procedure.

The next day I was taken down to recuperate and completed a week in the hospital, while I finished the antibiotic treatment. I was discharged,

and at home I had serum sickness, which is a late and expected reaction to antivenom. Fortunately, I had no complications. A few days later, all the skin on my finger fell off, and there was a scab at the point of the bite that eventually fell off. In an evaluation by ARL they determined that I had to have a small plastic surgery to correct the defect on my finger. This they did a month later, and after about 45 days I had a small scar; but I was able to return to work with snakes. I passed the test!

Testimony of Carlos Castro, animal technician at the INS serpentarium

"I have been working here at the farm for 18 years. When I started, the serpentarium functioned within the Institute, at the CAN headquarters in Bogota, and I worked exclusively on the farm in the production phase with the horses: that is, in the inoculation of venom, the titration tests to determine how effective the antibodies generated by the horse are in neutralizing the venom, the bleeding of the horses, the re-infusion of red blood cells, along with other work (Figure 21).



Figure 21. Carlos Castro in the current facilities of the Serpentarium at Hacienda Galindo ready to start the milking day (venom extraction).

At the beginning of 2011, when they thought of bringing the serpentarium to Hacienda Galindo, they took me to see the one at the Institute's headquarters in the CAN. I was very wary of snakes, I could not conceive of the idea of holding one in my hands, but I was very curious. So, I watched very carefully all the procedures, the handling, and the risks involved. I went twice to the Institute´s headquarters and then, at the end of that year, they finally moved the serpentarium to the Hacienda.

Mr. Francisco would come to milk the snakes and I would not go there, not for anything. But, little by little, he got me involved and taught me the whole process until I learned how to handle the snakes, and today I do the milking.

At the beginning, to catch the snakes, we immobilized them with a hook just above the head and inside the enclosures. This caused them to bite, their mouths filled with the substrate in which they live and so milk-

ing was not so effective. Sometimes, as a defense mechanism, they would expel the venom before we were able to put their mouth inside the vial, so it would end up on the floor or inside the box. To avoid this waste, I modified the technique that Mr. Francisco taught me, and for some years now what we have been doing is to restrain an animal with a clamp and hold it by the head, keeping it closed so that it does not prematurely release the venom.

With the snakes you have to be very calm and feel very safe at all times. When we are doing this work, everyone in the team is very connected; many times, we don't need to talk to say 'I'm going to open the box', 'you have to take it', 'I'm going to release it'... we are so synchronized that sometimes just a look is enough.

From the moment we handle them, anything can happen to them: from a very slight external skin wound, to a fracture of the vertebrae, to a fang injury when they bite the vial. But we are very careful, and this rarely happened. It is not for nothing that we have snakes that are several years old. Sometimes, for example, the animals hurt their gums a little during extraction, or as they are constantly changing teeth (it depends on their size and how much they are eating), many times when the new tooth pushes the old one and the old one is already loose, we have to remove it to prevent it from getting stuck. After milking them, we clean them with an antiseptic; and, in some cases, we apply antibiotic cream (see Chapter 8).

For hygiene and protection of the snakes we use latex gloves that we change between snakes; the only consideration is that they fit tightly so as not to lose grip and sensitivity. But with the small snakes, like the corals, we have refined the technique a lot. The person who handles the small snakes manipulates them 'clean-handed', that is we do not use hooks, tweezers or gloves in order to preserve greater motor skills and sensitivity and minimize the risk of mistreating them. This also gives greater security to the handler. Fortunately, to date we have not had an accident with the corals, which demonstrates the wisdom of our technique.

I live here at the Hacienda with my family, who are already very accustomed to the entire process of producing antiophidic serum. In fact, my daughters, ages 8 and 17, already know quite a bit about snakes".

-***2016-*:** The INS, the National University of Colombia and the Ministry of Environment and Sustainable Development publish the document of the National Program for the Conservation of Snakes in Colombia (see Chapter 1), in which they address general and diagnostic aspects of the country's snakes, establishing the species in the different ecogeographic regions and project how the program for their conservation should be (Figure 22).



Figure 22. Covers of the National Program for the Conservation of Snakes in Colombia and the guide for the differentiation of venomous snakes in Colombia.

2016-: INS creates the first polyvalent anticoral serum. With the manufacture of 363 vials begins the uninterrupted production of this antiophidic serum in the country.

Testimony of Francisco Ruiz, leader of the Serpentarium

"The production of anti-coral serum had always been a myth, like everything with snakes, largely because corals are much more difficult to handle from their capture to their handling, including their captivity. At this point in my life, I have collected about 300 snakes in the field, and of those, only two have been corals. There are 30 or so different species and they are terribly toxic; if you don't have the serum next to you, it's deadly.

Developing this antivenom was one of our major objectives, so we started by doing two exercises: the first was to ask many biologists and herpetologists which is the most representative coral in terms of ophidian accidents in the country. For this species we have to redouble our efforts to make the serum. The second was to investigate among medical specialists in the field, which is the snake of this type that generates more accidents according to the patients they receive. For this information we went to two experts: Santiago Ayerbe, who is from Cauca, and Rafael Otero, from San Gil (Santander), although all his professional life was carried out at the University of Antioquia.

After talking to many people, we concluded that there were four species of corals that cause the most accidents: two from the trans-Andean region (the Andean, Atlantic and Pacific zones), that is, everything in the western part of the country, where most of the Colombian population is distributed, and two from the eastern part of the country, the cis-Andean region (Orinoco and Amazon). Of the former, the two most representative snakes are the *Micrurus dumerilii* and the *Micrurus mipartitus* -the famous chili bell pepper tail-, and of the latter are the *Micrurus isozonus* and the *Micrurus surinamensis*. The latter is the water coral, and

because of its size and the toxicity of its venom it is considered one of the deadliest in Colombia.

So, with the venoms that Juan Manuel Renjifo had left in our bank of venoms, added to those collected by our group in recent years, we began to think with Maria Carlina Castillo how we were going to mix them and, what was more critical, how much we were going to inoculate each horse.

After scratching our heads, writing to the Brazilians, who obviously had their recipe and are jealous of it, and the same with the Costa Ricans, we came up with a number according to our calculations. It took us a week to figure out the formulation. We injected the horses with venom, and I was at the farm, from 6 am, for 23 days straight, observing the process. We did three venom injections, drew blood, went to titer and we were like little kids in secrecy: we locked ourselves in the lab and did the tests until EUREKA! All four horses gave titers for the venoms we injected into them. We figured it out! That's how, by the beginning of 2016, we made the first batch with 363 vials of polyvalent anticoral serum from the National Institute of Health (Figure 23).



Figure 23. Second batch of polyvalent anticoral serum produced by INS.

We were filled with excitement and fear because, although all the preclinical tests performed were reliable, we did not know what the reaction would be for people. I was very anxious with the first patient who was given our antiophidic serum. We followed his case very closely and had direct communication with him; fortunately, he responded very well. I also saw the second patient; and, in addition, I accompanied the treating toxicologists at the Hospital San Jose de la FUCS, where he had been rereferred from La Mesa, Cundinamarca, due to the bite of a chili bell pepper tail coral (*Micrurus mipartitus*). In 48 hours, the patient had an impressive change after the application of our anticoral serum, and this proved its

enormous efficacy. Since then, every year we receive messages from general practitioners, emergency physicians, and toxicologists sharing with us their successful experiences in the treatment of ophidian accidents due to elapid bites. We made some adjustments and now we are in the final phase of registration with INVIMA".

Testimony of Rafael Otero, pediatrician and toxinologist, founder of the Ophidism and Scorpionism Project in Antioquia and Choco, School of Medicine, University of Antioquia
"Coral venoms are slow-acting, and by slow I mean two hours after the bite for the clinical picture to set in when different symptoms usually appear: the eyelids droop, swallowing and breathing become difficult, there is weakness in the extremities; and, finally, respiratory arrest occurs, which can happen from 6 to 12 hours, depending on whether it is a child or an adult. For this reason, treatment should ideally be applied during the first two hours, as this is the best way to obtain the best results.

The anticoral antivenoms that have been produced in Colombia since 2016 are the result of experimentation in Brazil or Costa Rica with similar antivenoms against Micrurus, the genus of venomous snakes called corals. And these domestically produced sera have nothing to envy to foreign ones in terms of neutralizing efficacy. The INS polyvalent antivenom fills a great void that existed in Colombia due to coral accidents. It is very powerful and covers several species".

Testimony of Santiago Ayerbe, pediatrician and toxicologist, founder of the Herpetological and Toxicological Research Group of the Biomedical Research Center of the University of Cauca
"Coral venom is a very difficult venom to obtain because they are animals with very small fangs, one millimeter or less. Only the largest ones have fangs of two millimeters; and that is why, in general, milking them is extremely difficult. Of course they are potent venoms, but the amount obtained is minimal and the host, which is the horse, needs enough venom to be able to recognize it and produce antibodies. Otherwise, one can hardly have a neutralizing effect. That is the problem with corals, and it is not only here, but in all America.

In Brazil and Costa Rica there are recognition problems, that is, when doing in vitro tests, the venom is recognized, but the antibody is not produced. The anti-coral serum that did prove to have an adequate or recognized neutralizing capacity was that of the venom of the coralillo (*Micrurus dumerilii*), a coral that here in Colombia has four subspecies and occupies the second place in coral ophidian accidents, since the first place is held by the rabo de aji (*Micrurus mipartitus*), with 4 or 5 subspecies that will soon be elevated to species.

The Institute produces the most potent anticoral antivenom in the Americas, but it is used only for the species of the northern part of South America, i.e., Colombia, Ecuador, Peru, Venezuela, and some of Brazil, since the venoms used in its manufacture are from those regions. It is

also used for Panama and Costa Rica because the *Micrurus multifasciatus*, whose venom is almost identical to that of the rabo de aji, reaches there. This serum is the one with the greatest neutralization capacity".

***-2016-*:** Forty years after it was produced, a laboratory study was carried out on a sample of the antiophidic serum of lot 12, manufactured in October 1976, the oldest of its own manufacture kept by the INS.

Testimony of Francisco Ruiz, leader of the Serpentarium
"This is the oldest preserved vial of monovalent antiophidic serum produced by the National Institute of Health (Figure 24). It is lot 12, manufactured in 1976, when the serum group was headed by Juan Manuel Renjifo.

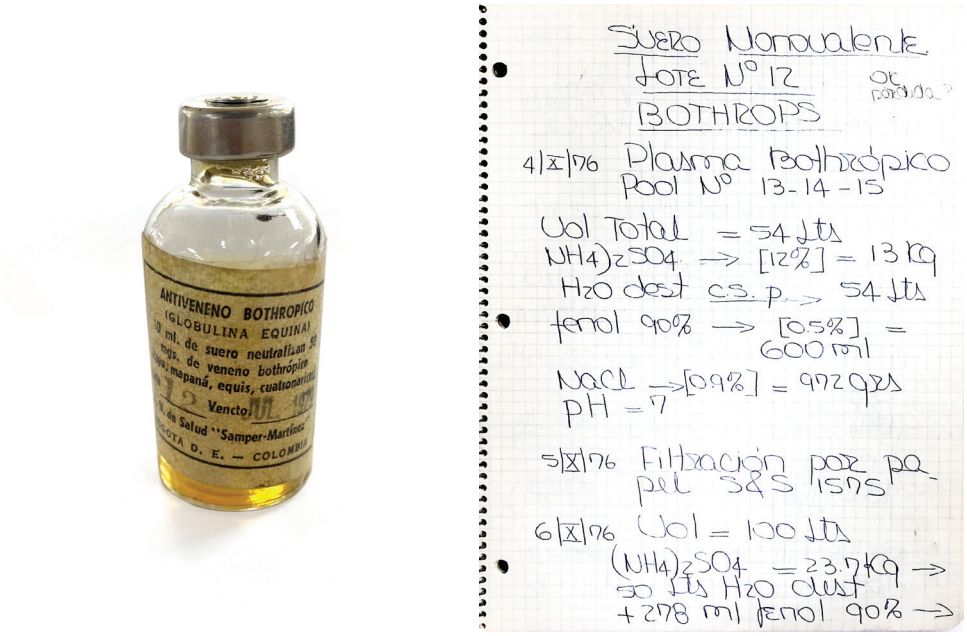
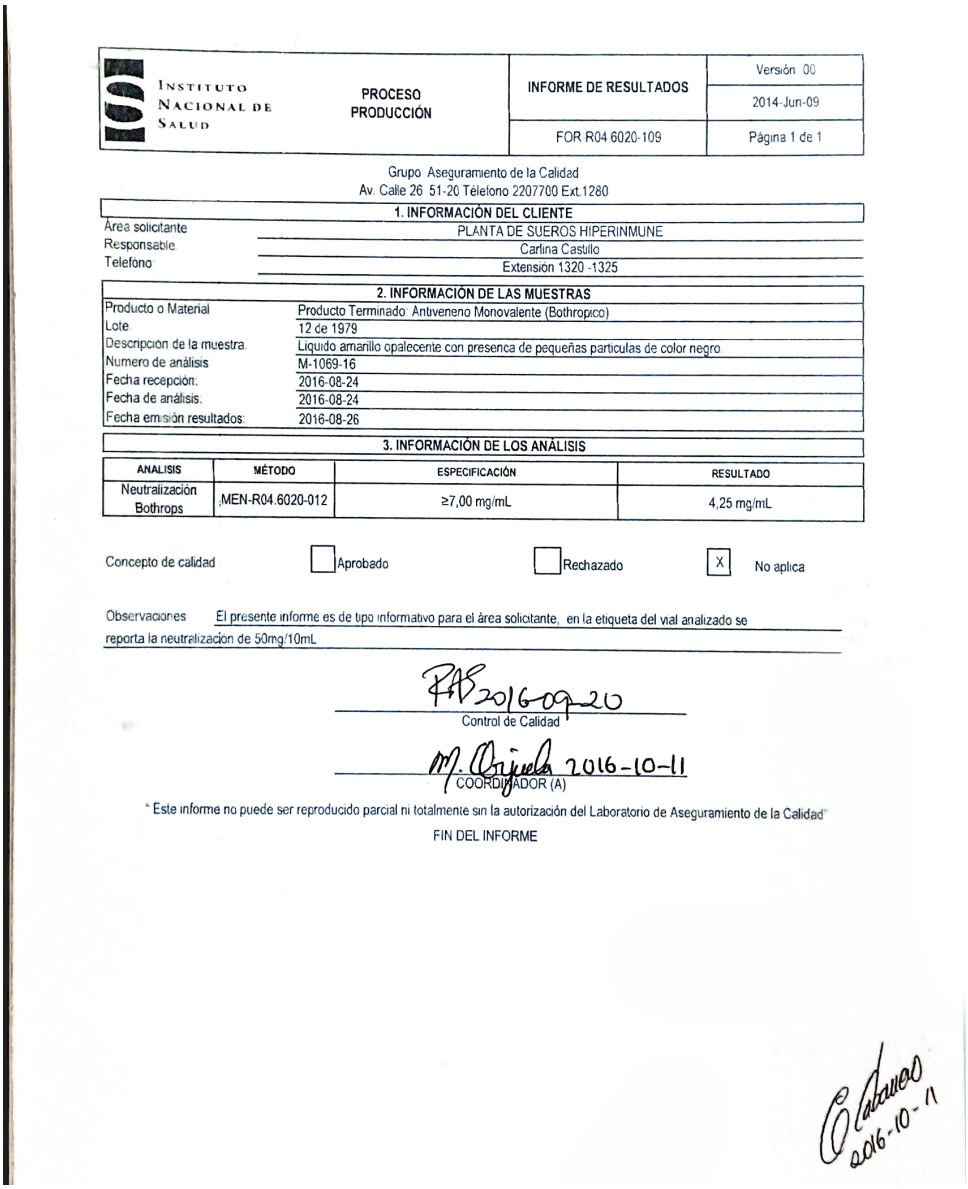


Figure 24. (Left) Polyvalent antiophidic serum produced in the 1970s, corresponding to lot 12. (Right) Photo of the 1976 production notebook showing the lot of the vial containing the antivenom, in the handwriting of Guiomar Caicedo.

This serum was recovered thanks to the fact that one day, towards the end of 2009, a doctor came to the Institute's facilities in the CAN to give it to me personally two vials. According to what he told me, his father used to buy the serums at the Institute and kept them frozen to better preserve them, and when he died they were found inside the refrigerator of his farm. He thought that, instead of throwing them away, the Institute would be interested in having them. He brought us two vials. We tried one and after so many years the serum still neutralized. Juan Manuel used to use a phrase that I will never forget: 'the serum is very grateful'. And indeed, this bottle shows it".

Testimony of Maria Carlina Castillo, coordinator of the Production and Technological Development Group of the Biologicals Production Division of the INS
"One of the vials received by the INS in 2016, with testing optimized to the maximum, was subjected to electrophoresis analysis, protein quantification, and the most critical test—the neutralization titer or potency. The result was conclusive: the serum still maintained its titers 40 years later. I have always

said that the stability of the sera that INS produces is very high because not everybody produces with the same stringency, with the same neutralizing capacity or with the same compliance with the regulatory framework. The INS do not commercialize any batch of antivenom if it does not have the certificate of approval from INVIMA as the regulatory body (Figure 25)".



INSTITUTO NACIONAL DE SALUD

PROCESO PRODUCCIÓN	INFORME DE RESULTADOS	Versión 00
		2014-Jun-09
	FOR R04 6020-109	Página 1 de 1

Grupo Aseguramiento de la Calidad
Av. Calle 26 51-20 Teléfono 2207700 Ext.1280

1. INFORMACIÓN DEL CLIENTE

Área solicitante	PLANTA DE SUEROS HIPERINMUNE
Responsable	Carlina Castillo
Telefono	Extension 1320 -1325

2. INFORMACIÓN DE LAS MUESTRAS

Producto o Material	Producto Terminado: Antiveneno Monovalente (Bothropico)
Lote	12 de 1979
Descripción de la muestra	Liquido amarillo opalecente con presenca de pequeñas particulas de color negro.
Numero de análisis	M-1069-16
Fecha recepción:	2016-08-24
Fecha de análisis:	2016-08-24
Fecha emisión resultados:	2016-08-26

3. INFORMACIÓN DE LOS ANÁLISIS

ANALISIS	MÉTODO	ESPECIFICACIÓN	RESULTADO
Neutralización Bothrops	MEN-R04.6020-012	≥7,00 mg/mL	4,25 mg/mL

Concepto de calidad: [ ] Aprobado [ ] Rechazado [X] No aplica

Observaciones: El presente informe es de tipo informativo para el área solicitante, en la etiqueta del vial analizado se reporta la neutralización de 50mg/10mL

2016-09-20
Control de Calidad

2016-10-11
COORDINADOR (A)

" Este informe no puede ser reproducido parcial ni totalmente sin la autorización del Laboratorio de Aseguramiento de la Calidad"

FIN DEL INFORME

2016-10-11

Figure 25. Photo of the result of the neutralization titer test performed on lot 12. Although this document states that this result is from lot 12 of 1979, in reality this year corresponds to the expiration date of the vial. Its production was in 1976.

-***2017-***: For the second time, INS receives from the Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA) the certificate for 18 months of Good Pharmaceutical Manufacturing Practices (GMP) of the polyvalent antiophidic serum and polyvalent anticoral antivenom.

-***2020-***: The U.S. Armed Forces publish a global management guide in case their members suffer ophidian accidents, and in it they determine the use of the antivenoms created by INS as first-line treatment, in South and Central America.

Detail: The guide for the evaluation and treatment of people who have suffered ophidian venoming hosted by the U.S. Armed Forces establishes, in each region of the world, the protocol to be followed. According to this document, to counteract neurotoxic venoms in South America, the polyvalent anticoral anti-venom produced by the National Institute of Health of Colombia constitutes the first line of action recommended to deal with a case of any severity (page 60 and 64[18]).

-***2021-***: Once again, the Instituto Nacional de Vigilancia de Medicamentos y Ali-mentos (INVIMA) certifies INS in Good Pharmaceutical Manufacturing Practices (GMP) for the manufacture of polyvalent antiophidic serum and polyvalent anticoral antivenom.

-***2021-***: The Institute is conducting lyophilization tests on polyvalent antiophidic serum (Figure 26).



Figura 26. Lyophilization of antiophidic serum. (A) Antiophidic serum before lyophilization. (B) Antiophidic serum in lyophilization chamber. (C) Lyophilized antiophidic serum.

Testimony of Maria Carlina Castillo, coordinator of the Production and Technological Development Group of the Biologicals Production Division of the INS

"Currently, we are doing lyophilization tests, and we are almost ready to make our pilot batches. The challenge is to maintain the same neutralizing capacity, that is, 7 milligrams of antivenom per milliliter of venom. We are planning to manufacture a lyophilized fraction to satisfy a small market of people who want it in that presentation, but we will keep our liquid sera, as all our biological hyperimmune products are today.

With respect to antivenoms, I can say that our serum, if kept at 37 degrees Celsius day and night or frozen, starts to lower its titer (potency) after about six months. The first thing that is affected is its appearance, because it becomes milky, but it does not lose its neutralizing capacity. We also have evidence that at about 20 degrees in the shade, the product is maintained for the entire shelf life, which is three years in the case of the polyvalent bothropic antiophidic serum, according to the authorization of the current sanitary registration.

The Institute has a program for the study of stability, and for this purpose different batches are kept for the entire shelf life. Initially, all of them were tested at 0, 1, 2, 3, 6, 9, 18, 24, 36, and 48 months after manufacture. Nowadays, we are spreading them out a little further - we perform them at 0, 3, 6, 12, 18, 24, 36, and 48 months - in order to confirm the overall characteristics of the product. Sterility, safety, protein, phenol, chloride, and other tests are performed to ensure that the same conditions of efficacy and safety are maintained.

4. Looking to the future

Lyophilization is the most recent chapter in the history of the production and optimization of antiophidic sera, a process that has been around for more than a century and which emerged in Colombia in the 1930s.

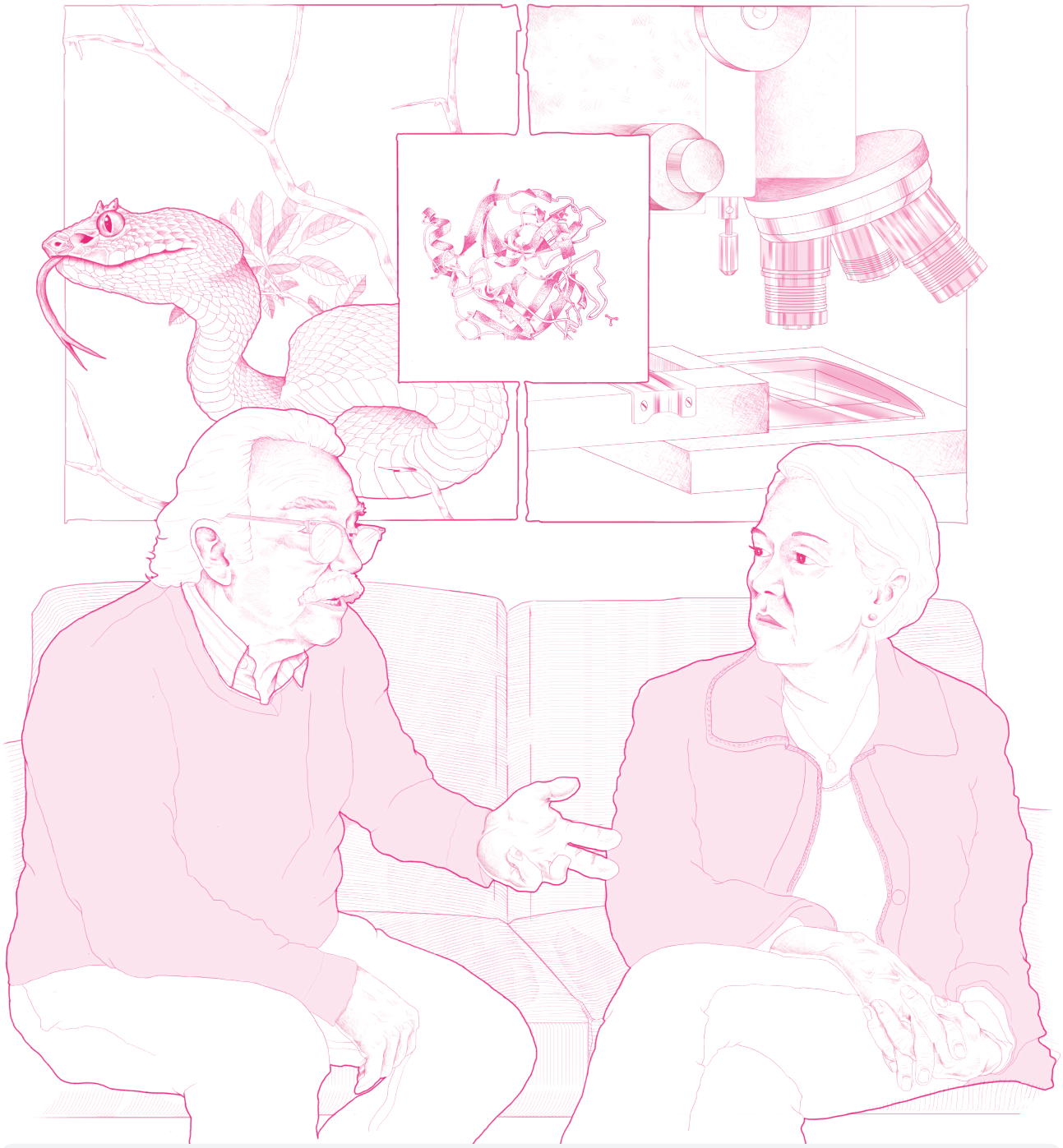
There are technologies that will undoubtedly evolve the development of anti-venoms and allow the use of different methodologies, such as bacteriophages, transcriptomics, or recombinant venoms (see Chapter 6). However, they will take a long time to analyze, evaluate and implement because of their complexity and cost. Not surprisingly, Brazil, Costa Rica, Mexico, England, and Japan, to name but a few countries, are leading the way in the study and development of sera, following in the footsteps of the French infectologist Albert Calmette and other visionary scientists of the late nineteenth and early twentieth centuries.

As a result, the National Institute of Health has strengthened, especially in the last decade, the technical equipment and infrastructure necessary to manufacture safe and effective biological serums against envenomation by the bite of various snake species. Today it has a production capacity that overcome 50,000 vials per year; however, this could easily be doubled if required, to fulfill the mission that snakebite accidents are no longer a neglected tropical disease, neither in Colombia nor in the world.

References

1. Anonymous *La leyenda de Gilgamesh*; Instituto Latinoamericano de la comunicación educativa ILCE: Ciudad de México. http://biblioteca-digital.ilce.edu.mx/Colecciones/ObrasClasicas/_docs/Gilgamesh.pdf
2. Soca, R. La palabra del día Available online: https://www.elcastellano.org/envios/2021-12-02-000000?utm_campaign=LaPalabraDelDía (accessed on Dec 3, **2021**).
3. León C.J.M. Charles Albert Calmette, 1863-1933. *Biogr. Mem. fellows R. Soc.* **1934**, *1*, 1–12, doi:https://doi.org/10.1098/rsbm.1934.0015.
4. Bochner, R. Paths to the discovery of antivenom serotherapy in France. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2016**, *22*, 1–7, doi:10.1186/s40409-016-0074-7.
5. Nassif, M.I. El vital Brazil con Z Available online: https://revistapesquisa.fapesp.br/es/el-vital-brazil-con-z/ (accessed on Dec 3, **2022**).
6. Hawgood, B.J. Pioneers of anti-venomous serotherapy: Dr Vital Brazil (1865-1950). *Toxicon* **1992**, *30*, 573–579.
7. Bochner, R.; Struchiner, C.J. Epidemiologia dos acidentes ofídicos nos últimos 100 anos no Brasil: uma revisão. *Cad. Saude Publica* **2003**, *19*, 07–16, doi:10.1590/s0102-311x2003000100002.
8. Anónimo. Historia del Instituto Clodomiro Picado Available online: https://www.icp.ucr.ac.cr/index.php/es/sobre-el-icp/historia-del-instituto-clodomiro-picado (accessed on Nov 10, **2021**).
9. Congreso Constitucional de la República de Costa Rica *Ley de defensa contra el Ofidismo*; Costa Rica, **1926**; p. 8.
10. Otero, R.; Núñez, V.; Barona, J.; Fonnegra, R.; Jiménez, S..; Osorio, R..; Saldarriaga, M.; Díaz, A. Snakebites and ethnobotany in the northwest region of Colombia Part III: Neutralization of the haemorrhagic effect of Bothrops atrox venom. *J. Ethnopharmacol.* **2000**, *73*, 233–241, doi:10.1016/S0378-8741(00)00243-9.
11. Vargas, P.F. *Pensamientos políticos y memoria sobre la población del Nuevo Reino de Granada*; Biblbioteca Popular Colombiana: Bogotá D.C, **1944**.
12. Lozano, J.T. No Title. *Sem. del Nuevo Reino Granada* **1808**, 117–182.
13. Welborn, J.L. Plant of the Week Available online: https://www.fs.usda.gov/wildflowers/plant-of-the-week/polygala_senega.shtml (accessed on Dec 20, **2021**).
14. Núñez, V.; Otero, R.; Barona, J.; Saldarriaga, M.; Osorio, R.G.; Fonnegra, R.; Jiménez, S.L.; Díaz, A.; Quintana, J.C. Neutralization of the edema-forming, defibrinating and coagulant effects of Bothrops asper venom by extracts of plants used by healers in Colombia. *Brazilian J. Med. Biol. Res. = Rev. Bras. Pesqui. medicas e Biol.* **2004**, *37*, 969–977, doi:10.1590/s0100-879x2004000700005.
15. Pereañez, J.A.; Preciado, L.M.; Romero, L.E. Toxinology in colombia: Contributions of ophidism/scorpionism program and other research groups. *Vitae* **2019**, *26*, 120–134, doi:10.17533/udea.vitae.v26n3a01.
16. The Lancet Snake-bite envenoming: a priority neglected tropical disease. *Lancet* **2017**, *390*, 2, doi:10.1016/S0140-6736(17)31751-8.
17. Ministerio de Salud-Instituto Nacional de Salud *Reseña Histórica del Laboratorio Nacional de Salud "Samper-Martínez" 1917-1982*; Instituto Nacional de Salud: Bogota, **1982**.

18. Benjamin, J.M.; Hall, M.A.J.A.; Collazo, M.; Grisham, C.P.T.J.; Jarema, D.; Dye, S.S.G.C.; Lee, S.S.G.J.; Abo, B.; Loos, S.F.C.P.; Remley, M.S.G.M.; et al. Clinical practice guideline(JTS CPG) Global Snake envenomation management (CPG ID : 81). *Res. Gates* **2020**.



Among the multiple interviews conducted with experts, witnesses, and protagonists of the history of antivenom production in Colombia, this illustration was inspired by the 2021 meeting we had with Juan Manuel Renjifo and Guiomar Caicedo to recall their experiences. This gathering took place in a municipality near Bogota, specifically at Juan Manuel Renjifo's farm, who had not seen his colleague and research partner Guiomar Caicedo in over 30 years.

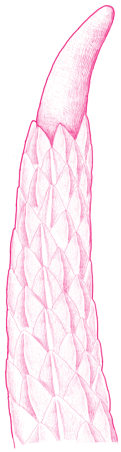
Chapter 8

Venomous snakes in captivity: A history of learned lessons

Teddy Angarita Sierra, Juan Jose Torres-Ramirez, Carlos Antonio Castro-Sandoval, Monica Sarmiento-Perez, Francisco Javier Ruiz-Gomez

Abstract: The key to establishing a large-scale program for the production of snake antivenom is to maintain alive the largest number of medically important snakes in an intensive captive environment. However, captivity is associated with stressors that can provoke chronic, steady physiological and behavioral changes in snakes, causing responses from non-adaptive levels of glucocorticoid secretion that lead to impaired welfare of the captive snakes in a serpentarium. Understanding the pathologies and their impacts on the general population of the serpentarium, as well as learning the best practices to maintain them in captivity, are the main goals for achieving a program for antivenom production. Nevertheless, gaining understanding and learning from lessons is a long trail in which empirical approaches, common sense, and the experience of elder researchers are the best guide. This chapter summarizes and analyzes the historical data available from 1990 to 2021 from the captive snakes employed for antivenom production housed in the serpentarium of the Colombian National Health Institute. Additionally, we provide a brief history of learned lessons about the venomous snakes housed in the serpentarium, as well as the most interesting information about them.

Keywords: Captivity, serpentarium, antivenom, husbandry, handling, feeding, diet, housing Viperidae, Elapidae.



Citation: Angarita-Sierra T; Torres-Ramirez JJ.; Castro-Sandoval CA.; Sarmiento-Perez M.; Ruiz-Gomez FJ. Chapter 8. Venomous snakes in captivity: A history of learned lessons. In: *Bites, venoms, and venomous snakes of Colombia*; Angarita-Sierra, T., Ruiz-Gomez, F.J, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 349–384.
doi: 10.33610/733564phubgm

Copyright: © 2024 by the authors. Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0) license (http://creativecommons.org/licenses/by/4.0/).

Illustrations by:
Oscar A. Ramirez Ruiz

1. The serpentarium jewels

Despite the widespread idea that snakes are common animals in the wild and rural areas, the truth is that they are elusive, cryptic, and uncommon reptiles, even more so in the tropics. Finding snakes in tropical ecosystems is normally a hard task. Most snakes live in secretive, isolated, and difficult-to-access habitats (*e.g.*, hyperhumid Chocoan rainforest, Amazonian rainforest). Because of armed conflict in Colombia, some parts of the country have been historically inaccessible or highly risky for researchers [1,2].

Achieving a robust sampling of tropical snakes is always a major challenge, and even more if the goal is to capture venomous species to establish a large-scale snake captivity program for antivenom production. Historically, experienced researchers have employed intensive survey designs in defined areas in tropical habitats, demonstrating that even devoting a tremendous sampling effort is not sufficient to achieve a completely representative sample of the local snake community [3–5].

So, the acquisition of live snakes for antivenom production, attempting to establish a geographically representative sampling that covers all snakes that are medically important, demands challenges such as: (1) building a strong collaborative partner network that includes researchers, governmental, and non-governmental allies; (2) investing much effort performing intensive snake sampling; and (3) being patient and having a high tolerance for frustration due the elusive and cryptic nature of snakes. This is why all snakes for antivenom production collected across the decades and housed in the Colombian National Health Institute (INS, Spanish acronym) are jewels that deserve to be extremely well-cared for and carefully maintained. This chapter summarizes the history of learned lessons about the venomous snakes housed in the serpentarium of the INS, as well as the most remarkable information about these serpentarium jewels.

1.1. Taxonomic richness and snake representativeness

Historically, the serpentarium of the INS has housed at least 608 snake specimens, according to the clinical records available from 1990 to 2021. However, this number is far from representative of the true history of the serpentarium because during the period 1965-1989 when it was located at the municipality of Armero (Tolima department), around 1,000 snake specimens were kept (*com pers. Juan Manuel Renjifo*, see Chapter 7). However, records of these specimens are scarce because standardized clinical records were not established until the year 2000. (Table 1).

During the early stage of the serpentarium in Armero, all housed snakes were represented by three species: *Crotalus durissus* (cascabel)*, Bothrops asper* (lancehead pitviper), and *B. atrox* (mapanare). After the relocation of Armero's serpentarium (see later) over time, the snake abundance, and species richness of the serpentarium grew gradually, adding occasionally pit vipers like *Bothriechis schlegelii* (eyelash pit viper), *Lachesis muta* (Amazon bushmaster), but maintaining as core species for antivenom production *Crotalus durissus, Bothrops asper*, and *B. atrox*. From 2012 to

the present, a noticeable increase in abundance and species richness of medically important Colombian snakes was achieved (Table 1), incorporating for the first time as permanent species in the collection coralsnakes (*Micrurus* spp.) in the snake production population. These achievements strengthened national antivenom production and allowed the establishment of a large-scale intensive snake captivity program for coralsnake antivenom production [6].

Table 1. Number of venomous snakes housed historically, the serpentarium of the National Health Institute of Colombia.

Period	Family Elapidae (Coral snakes)	Family Viperidae (Pitviper snakes)	Total specimens
1965-1989	0	~1,000	~1,000
1990-2011	0	49	49
2012-2021	181	378	559
Total	**181**	**427**	**608**

Across the last decade, the serpentarium has maintained seven of the eight genera of snakes medically important in the country: pitvipers (*Bothriechis*, *Bothrocophias*, *Bothrops*, *Crotalus*, *Porthidium* and *Lachesis*), and several species of *Micrurus*. Only specimens of *Hydrophis platurus* (Yellow-bellied Sea snake), represented by the single sea snake inhabiting the Colombian Pacific coast has been absent in the entire history of the serpentarium (Figure 1).

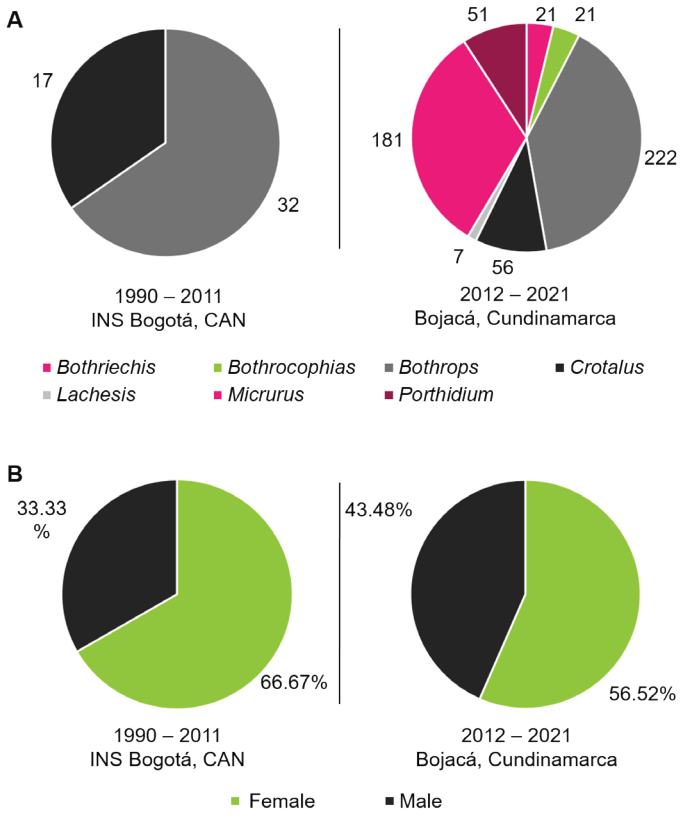


Figure 1. Number of snakes by genus and percentages by sex of snakes housed in the serpentarium of the National Health Institute of Colombia. (A) left, number of snakes by genus in the period 1990 - 2011, (A) right, number of snakes by genus in the period 2012 - 2021. (B) left, percentage by sex of snakes during the period 2012 - 2011, (B) right, percentage by sex of snakes during the period 2012 - 2021.

Interestingly, when the sex ratio of the entire serpentarium snake population was historical reviewed, a sex-bias emerged regardless of year or assessed historical period; the percentage of females was higher than males (Figure 1). Most of the snakes housed in the serpentarium came from occasional encounters or active snake searches.

Thus, there are numerous explanations for the biased sex ratios observed, including the following: sampling bias, skewed primary sex ratios, differential mortality, differential immigration and emigration, and differential maturity of the sexes between and within species [7]. Due to a dearth of knowledge of natural history of most of the Colombian populations of medically important snakes, as well as a lack of knowledge about the knowledge related to human-snake conflict, we cannot explain the reasons for this outcome.

The huge efforts invested by the Colombian government, led by the INS gathering specimens of medically important snakes during the last decade to enhance the capacity of production and neutralization of their own polyvalent snake antivenoms, has had a tremendous impact on the species richness and snake representativeness housed in the serpentarium. Before 2011, the accumulative species richness was less than four species, but from 2012 to 2021 the accumulative species richness showed rapid growth, reaching a total 25 snake species. These species represented 48% of the total snakes that are medically important in the country, and they cover 100% of the main venomous snake species that cause envenomation in Colombia (Figure 2).

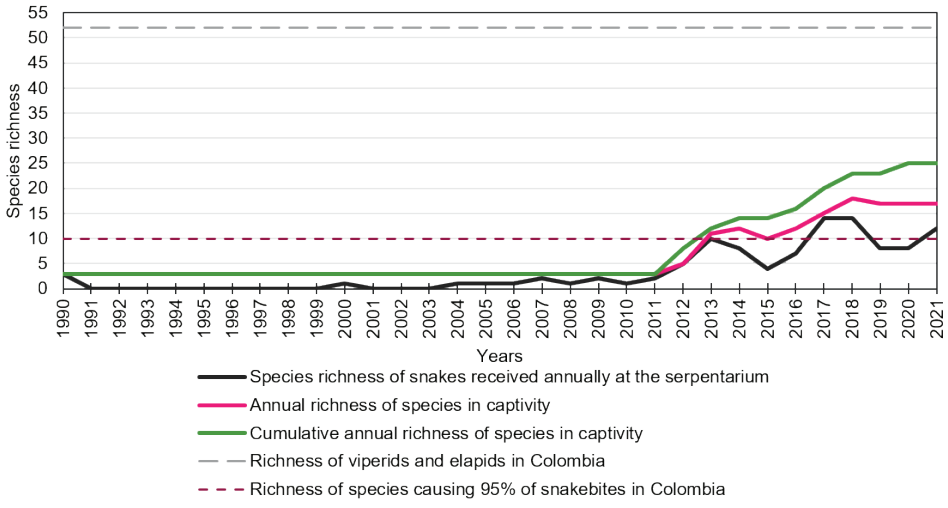


Figure 2. Annual species richness of viperids and elapids housed historically in the serpentarium of the National Institute of Health of Colombia.

Likewise, these efforts have resulted in having the highest number of specimens and species of snakes housed in the serpentarium by geographical and political units during the period 2012-2021 (Figure 3). Therefore, the bank of venoms used for antivenom production and research was strengthened, increasing the number of venoms per snake species, snake species population, and ecoregion. Particularly, in the last decade the political unit's representativeness grew from 12 to 23 departments, from three to five ecoregions, and from an average of 3.9 to 22.6 snakes per departments (Figure 3).

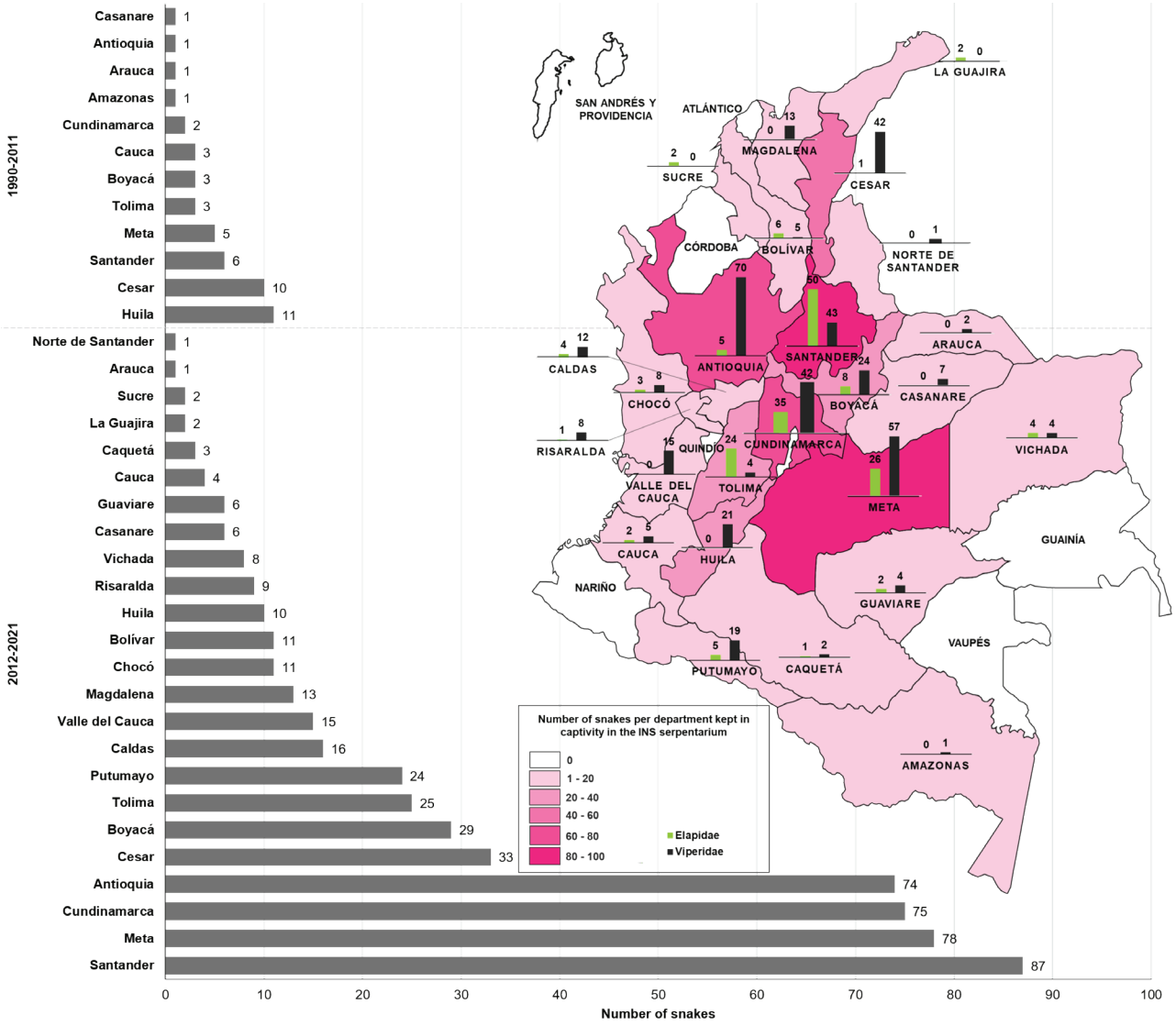


Figure 3. Historical geographical representation of snakes in captivity in the serpentarium of the National Institute of Health.

Despite these huge efforts, there is an important dearth of snakes represented, as well as a low number of specimens per species in ecoregions such as the Amazon, Pacific and Caribbean (Upper La Guajira department). These shortfalls occur because of the expenses involved in carrying out searches for snakes in isolated, unvisited, or difficult-to-access ecosystems found in these ecoregions, like, for example, the xerophytic dry forest (upper La Guajira department), hyperhumid Chocoan rainforest, northern lowland Amazonian rainforest, the highlands of the Pacific, and the eastern slopes of the Andes mountains that encompass the moist montane forests and cloudy montane forests.

Also, when environmental authorities or partner organizations have venomous snake to donate to the snake production population, there are serious constraints as to how these specimens can be delivered from their origin to the serpentarium because shipping and courier companies

in Colombia are unable to handle this type of cargo. Additionally, the Colombian armed conflict has constrained free movement in wild and rural areas, thus making it risky to obtain snakes for antivenom production in several municipalities of the country [2].

Nevertheless, the recent history of the serpentarium showed that snake acquisition in the last decade have noticeably strengthened the production and neutralization capacity of antivenoms, showing an increasing trend for overcoming the shortage of the past (see Chapter 6) and consolidating a robust snake captivity program for antivenom production.

2. Surviving in the lockdown

One of the most challenging goals of any serpentarium is live maintenance of the snakes as long as possible. However, establishing a large-scale intensive snake captivity program for antivenom production is substantially different from a program for exhibition or for biological studies. In captive environments several strict and standardized animal health protocols must be implemented that try to extend as long as possible the lifespan of snakes used for antivenom production [8]. Snake survival in captivity is associated with several factors such as the initial conditions of the snake when it arrives at the serpentarium, the captive environment, the snake's developmental stage, sex, body condition, health condition, region of origin, feeding frequency, and laboratory handling protocols [8].

Previous experiences worldwide have shown that despite careful standardization and a suitable combination of environmental variables (e.g., temperature, humidity, light, feeding frequency, physical space), as well as high sanitary conditions and veterinary care, there are no guarantees that snakes will adapt to captivity [8–13]. About 90% of wild-caught reptiles die in the first year of captivity because of physical trauma prior to capture or because their keepers cannot meet their complex dietary and habitat needs [14].

Maladaptation syndrome or inability of the snake to adapt to a captive environment due to pathological effects of stress is the main source of mortality among serpentarium worldwide [8,12,13]. Captivity is associated with stressors that can provoke chronic, steady physiological and behavioral changes in snakes, causing responses to non-adaptive levels of glucocorticoid secretion that chronically disturb normal activities, leading to the impaired welfare of the snakes [13]. Thus, understanding the factors that can affect a snake's captive survival is paramount for achieving successful implementation of a large-scale intensive snake captivity program for antivenom production [8].

Currently in Colombia, there is only one study that explores in a qualitative way the maintenance conditions and the factors affecting the captive survival of the snakes employed for antivenom production [11]. Thus, captive survival patterns in Colombian snake populations remain unknown. Based on the accumulative clinical records of snakes housed in the INS serpen-

tarium during the period 2010-2021, this section provides a broad outlook of the intrinsic and extrinsic factors that could affect snake's captive survival of the main medically important species of the country. Additionally, a summary of the main clinical findings, pathologies, and natural history remarks observed in venomous snake species in captivity in Colombia are described.

2.1. Survival rates

The moment a snake dies is one of the most regrettable events in a serpentarium for antivenom production. So, understanding how the survival rates of the captive snake population vary, as well as which intrinsic or extrinsic variables are correlated with the snake's survival time is an imperative quest. Through INS serpentarium history, there are three stages with different maintenance schemes. During the period 1965-1989 the snakes were kept in a semi-intensive (a serpentarium system that uses a single enclosure for entire snake population, providing some type of housing or shelters, but lacking the isolated individual enclosures and standardized sanitary protocols) serpentarium in the municipality of Armero that emulated the traditional snake maintenance scheme carried out by the Brazilian Butantan Institute during the first half of the twentieth century [15].

There are few documented records of snake survival rates during this period, but according to staff around this time, snake searches in the wild were frequent because of low snake survival rates (*com pers. Juan Manuel Renjifo*, see Chapter 7); and they equaled similar survival trends observed in the Butantan Institute during the semi-intensive scheme in which snakes' mortality percentages were about 92% - 98% yearly [15].

After the Armero tragedy of 1985 when an enormous avalanche of lahar buried the town due to that mountain's eruption and subsequent melting glaciers that created the flow of lahar from Nevado del Ruiz, during 1990-2011 the INS serpentarium was relocated to Bogota (See Chapter 7). Nevertheless, over time, the facilities in Bogotá had technical and operational constraints, as well as shortfalls for establishing a suitable large-scale intensive snake captivity program for antivenom production. Thus, since 2012 the INS serpentarium was relocated to the Hacienda Galindo in the municipality of Bojacá, Cundinamarca.

Since clinical records have been available since 1990, survival rates of captive snakes in Bogota and Bojaca, as well as snakes translocated from Bogotá to the Bojacá serpentarium have been recorded for assessment. However, since the captive snake population housed in the Bogotá facility during the period 1990-2011 were composed of specimens of *Crotalus durissus, Bothrops asper*, and *B. atrox*, survival rate comparisons between the INS facilities only considered three species.

Survival rates were significantly different between INS serpentarium facilities as well as in translocated snakes (X^2= 31.1, df= 2, P <0.0001). The historical decision to relocate the serpentarium from Bogotá to Bojacá clearly shows that it was a success, representing early victory for es-

tablishing suitable large-scale antivenom production. Indeed, the median lifespan of the captive snake population was increased in 260 days, allowing at least four to six milking of venoms per specimen. Interestingly, snakes translocated from Bogota to Bojaca exhibited a great increase in lifespan with a median survival time of 3870 days (Figure 4). Most of the translocated specimens of *Crotalus durissus* (South American rattlesnake) indicated that Colombian populations of this species, had a low and steady decay exhibiting high survival rate once the adaptation period to captivity was exceeded (see below).

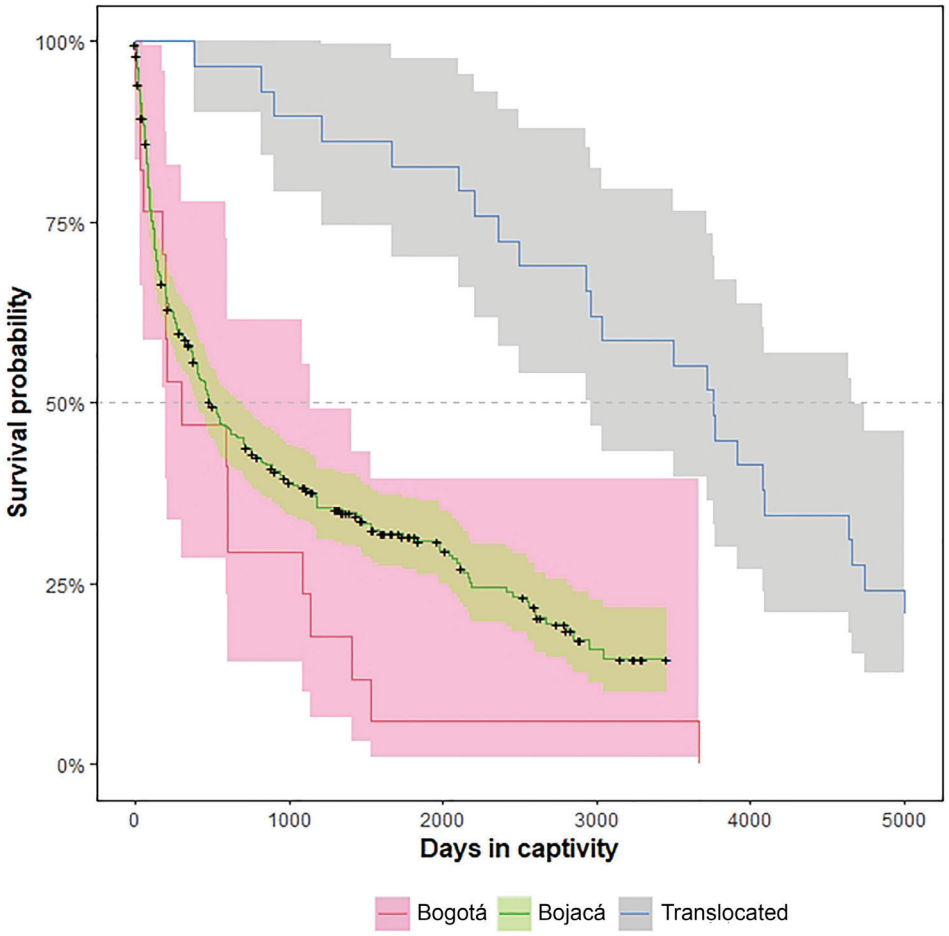


Figure 4. Median survival time of the snakes housed in the INS serpentarium facilities of Bogota, Bojaca, and snakes translocated between facilities.

Over the last decade, survival rates of the captive snake populations from the INS serpentarium exhibited different rates according to their origin, family, genus, and species. In general, the median survival time that represents the time in which 50% of the captivity snake population has died, was 580 days (Figure 5A). This means, that during the last decade, every 1.6 years a population turnover occurred. Differences in the median survival time in the captive snakes according to their ecoregional origins were also observed (X^2= 30.9, df= 5, P <0.0001). These significant differences were expected due the complex Colombian geography and topography that clearly configures ecoregions with their specific biotas and climates [16]. Moreover, climatological and biophysical changes have

impacts on the overall performance of wild and captive snake populations, particularly for the survival rates [13,17].

Species from highly humid environments like the Pacific, Amazon, and Andean regions are prone to show maladaptation syndrome that results in smaller median survival times than snake species from dry or moderately humid environments like the Caribbean, Orinoquia, and Inter-Andean valleys (Figure 5B). Maladaptation syndrome or physiological stress occurs mainly because the environmental conditions of the serpentarium are homogenous for all species despite their region of origin (temperature: 26±0.2 °C; humidity relative (Hr): 65±5), with minor changes across the serpentarium room (vertical variation ~ 3.52°C/ 5.65 Hr; horizontal variation ~ 1.52°C/ 2.65 Hr).

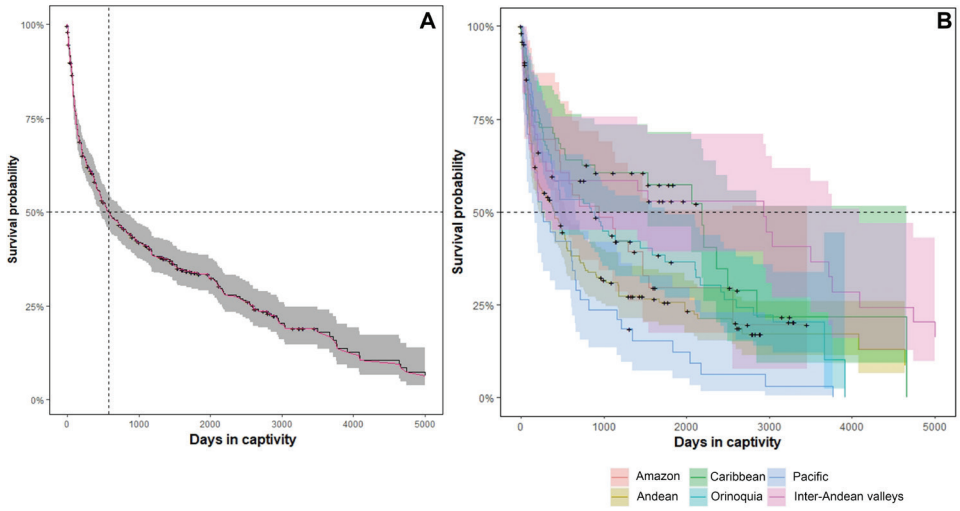


Figure 5. Median survival time of the snakes housed in the serpentarium of the Instituto Nacional de Salud during the period 2010-2021. (A) Median survival time of the entire snake population. (B) Median survival time of the entire snake population by ecoregion.

Therefore, this can cause significant stress on the snakes from highly humid environments, reducing the survival probability. Particularly, some pathologies observed are associated with maladaptation syndrome or physiological stress on the snakes from highly humid environments such as dysecdysis (abnormal pattern of skin shedding), mycosis (fungal infection), dermatitis (skin irritation), and dehydration [18,19]. About 50% of the snakes housed in the INS serpentarium, irrespective of their regional origin, have a median survival time of over 1 year and an average of 2.96 years for the entire snake population, indicating that the snakes in captivity for antivenom production during the last decade have been maintained under good captive conditions (Table 2).

Table 2. Median survival time and lifespan observed during the period 2010-2021 in captive snakes for antivenom production housed at INS serpentarium.

Groups	N	Median survival time (days)	LCI (95%)	UCI (95%)	Lifespan in captivity (years)
Ecoregions					
Amazon	33	963	0.34	0.69	2.83 (0.01-9.61)
Andes	177	398	0.40	0.60	2.11 (0.01-14.45)
Caribbean	73	2,198	0.39	0.70	2.75 (0-12.94)
Orinoquia	86	851	0.39	0.60	2.98 (0.01-10.87)
Pacific	38	260	0.36	0.69	1.85 (0.01-10.48)
Inter-Andean valleys	42	2,930	0.30	0.60	4.83 (0.01-15.5)
Families					
Elapidae	153	192	0.42	0.59	1.44 (0.01-8.01)
Viperidae	308	1,136	0.45	0.56	3.25 (0-15.55)
Viperid genera					
Bothriechis	12	*	*	*	3.28 (0.36-4.94)
Bothrocophias	8	*	*	*	1.09 (0.02-4.62)
Bothrops	190	1,174	0.45	0.60	3.17 (0.01-14.03)
Crotalus	54	3,499	0.36	0.70	5.21 (0-15.55)
Lachesis	6	*	*	*	0.75 (0.01-2.93)
Porthidium	38	198	0.45	0.70	1.70 (0.01-7.12)
Viperid species					
Bothriechis schlegelii	12	*	*	*	3.24 (0.36-4.87)
Bothrocophias tulitoi	8	*	*	*	1.07 (0.02-4.56)
Bothrops asper	100	*			2.64 (0.01-10.33)
Bothrops atrox	85	2,103	0.40		3.77 (0.02-13.84)
Bothrops bilineatus	1	*	*	*	4.21
Bothrops oligobalius	1	*	*	*	3.76
Bothrops punctatus	1	*	*	*	0.18
Bothrops venezuelensis	2	*	*	*	0.52 (0.24-8.0)
Crotalus durissus	54	3,499	0.36	0.70	5.21 (0-15.55)
Lachesis acrochorda	3	*	*	*	0.51 (0.02-1.40)
Lachesis muta	3	*	*	*	0.98 (0.01-2.88)
Porthidium lansbergii	38	198	0.45	0.70	1.70 (0.01-7.12)
Micrurus species					
M. camilae	1	*	*	*	0.55
M. dissoleucus	4	*	*	*	2.41 (0.18-4.91)
M. dumerilii	70	199	0.40	0.65	1.69 (0.01-7.77)
M. filiformis	1	*	*	*	0.17

M. helleri	9	*	*	*	1.66 (0.06-3.18)
M. hemprichii	2	*	*	*	0.90 (0.10-1.70)
M. cf lemniscatus	4	*	*	*	4.08 (3.53-4.38)
M. medemi	11	*	*	*	1.30 (0.09-2.70)
M. mipartitus	35	103	0.41	0.75	0.72 (0.01-4.71)
M. multiscutatus	1	*	*	*	0.01
M. nattereri	1	*	*	*	0.38
M. sangilensis	4	*	*	*	2.37 (0.12-7.90)
M. obscurus	1	*	*	*	0.04
M. surinamensis	9	*	*	*	0.65 (0.24-1.28)

N: Sample size. LCI. Lower confidence interval. UCI: Upper confidence interval. *: Data excluded due the small sample size.

Differences of the median survival time between elapid and viperid snakes are notable (X^2= 36.6, df= 1, P <0.0001), showing that elapid species exhibit lower median survival times than viperid species (Table 2). Thus, coral snakes are more prone to show maladaptation syndromes than do viperids (Figure 6A). Comparatively, elapid species require more specialized care and environmental conditions than viperids for factors such as types of substrates, natural prey, feeding techniques, milking schedules, and handling techniques [9–11,20–24]. In addition, the lack of environmental complexity or enriched environments have been proposed as sources of stressors that directly impact the survival rates of snakes in captivity [9].

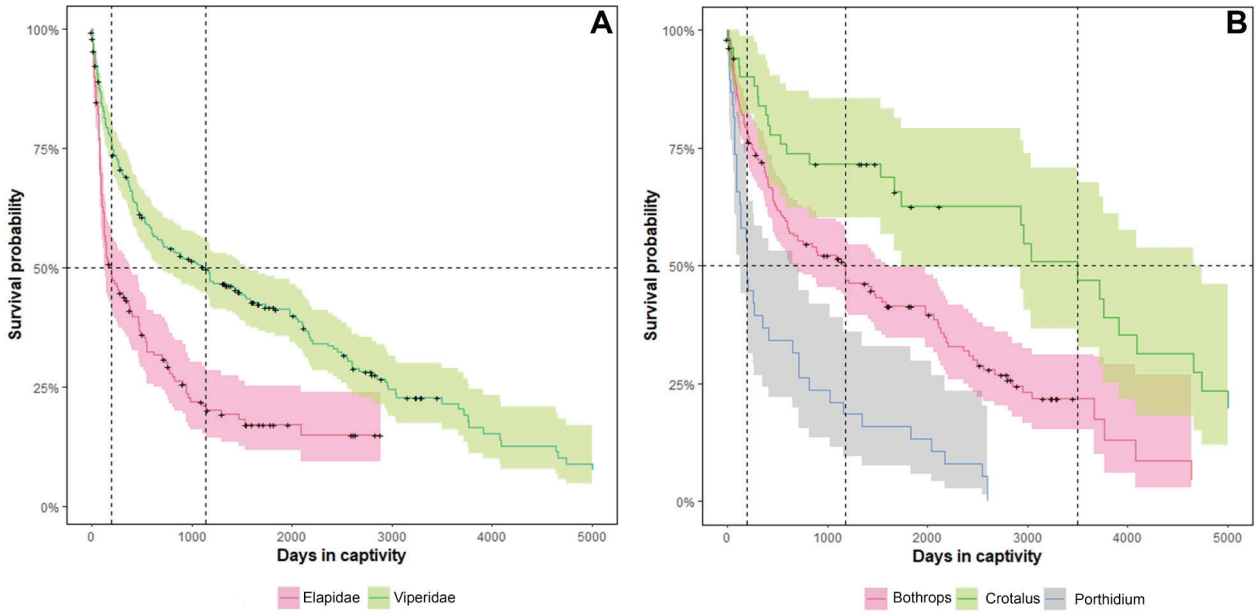


Figure 6. Median survival times. (A) Comparison between the two main families of medically important snakes housed in the serpentarium of the Instituto Nacional de Salud during the period 2010-2021. (B) Comparisons between viperid genera housed in the serpentarium of the Instituto Nacional de Salud during the period 2010-2021.

Nevertheless, median survival time observed in *Micrurus* species housed in the INS serpentarium overcome those reported for other serpentarium for antivenom production [23,25], indicating that the environmental enrichment done provides proper welfare for these snakes (see below). Additionally, no significant differences in the median survival time were observed between *Micrurus* species (X^2= 3.0, df= 1, P =0.08). Lifespan profiles were very similar between *Micrurus dumerilii* and *M. mipartitus*, in which median survival time differed by 91 days (Figure 7A).

In contrast, Colombian pitviper species seem to have a higher adaptive capacity to captive environments, but they do show significant differences between genera (X^2= 47.8, df= 2, P <0.0001). *Crotalus durissus* exhibit the longest lifespan and median survival time, followed by *Bothrops* and *Porthidium* species (Figure 6B). Likewise, significant differences between pitviper species are also observed (X^2= 54.2, df= 3, P <0.0001), exhibiting a distinctive lifespan profile, as well as a median survival time by species (Figure 6B). *Crotalus durissus* shows the highest median survival time (3,499 days), followed by *Bothrops atrox* (2,103 days), *B. asper* (891 days), and *Porthidium lansbergii* (198 days).

In fact, *Porthidium* and *Lachesis* species (bushmasters) show similar lifespans in captivity as observed in *Micrurus* species, indicating that these pitvipers also require specialized care and environmental conditions. They are more likely to show the most extreme maladaptation signs or physiological stress among all viperids species housed in INS serpentarium (Table 2). Interestingly, both *genera* represent body size extremes seen across all the viperid species that inhabit South America; the *Porthidium* species was the shortest and the two *Lachesis* species were the longest and the heaviest, suggesting that body size plays a key role in the ability of snakes to survive in captive environments.

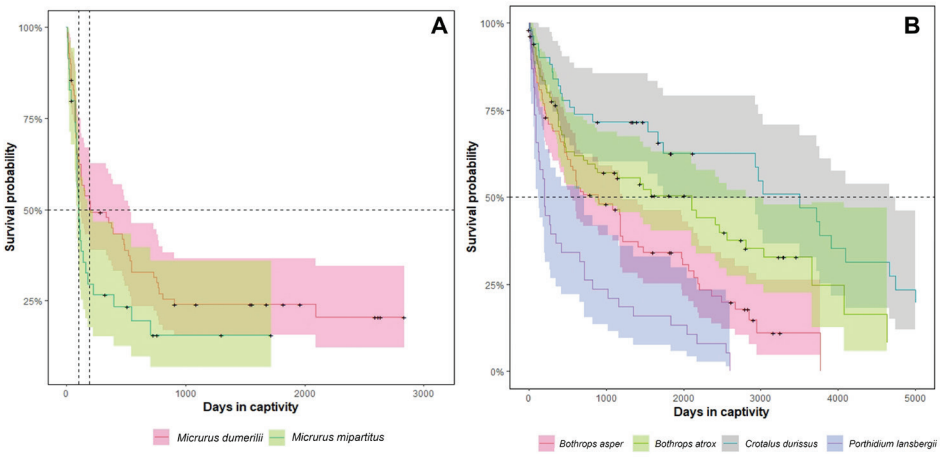


Figure 7. Median survival time. (A) Comparison between the two main medically important elapid species housed in the serpentarium of the Instituto Nacional de Salud during the period 2010-2021. (B) Comparisons between the main medically important viperid species housed in the serpentarium of the Instituto Nacional de Salud during the period 2010-2021.

There are few previously documented data assessing snake survival of venomous species maintained in captivity for antivenom production in Central and South American. Nevertheless, the lifespan profile and median survival time by observed species has close similarities to previ-

ous studies done in Costa Rica, although there are clear differences. For example, Sasa et al. [23] in the serpentarium of the Instituto Clodomiro Picado (ICP) from Costa Rica, stated four statistically distinct survival profiles that closely resemble the survival curves depicted by the captive snake population of the INS serpentarium. Thus, following the Sasa et al. [23] proposal, the statistically distinct survival profiles observed in the INS serpentarium match the following categories (Figure 7B):

- *High survival* —. Snake species with over 70% survival during the first 24 months (720 days) and less than 40% of deaths occurring after the 60th (1800 days) month in captivity. This profile was exhibited only by *Crotalus durissus*.
- *Steady decay* —. Snake species in which less than 50% of the deaths occur during the first 24 months in captivity, and a gradual reduction of survival follows. This profile was shown only by *Bothrops asper* and *B. atrox*.
- *Rapid decline* —. Snake species in which close to 40% survive during the first 24 months (720 days) in captivity but mortality rate decreases after 48 months (1440 days). No species followed this profile.
- *Severe decline*—. Snake species in which close to 30% survival occurs within the first 24 months (720 days) and a steady decrease observed afterward. This profile was exhibited by *Micrurus dumerilii, M. mipartitus,* and *Lachesis acrochorda*, and *Porthidium lansbergii*.

Likewise, in the ICP snake population reported by Sasa et al. [23] the median survival time of *Porthidium nasutum* and *Lachesis stenophrys* (the closest relative of *Porthidium lansbergii*, *Lachesis acrochorda, and L. muta*, respectively) showed a rapid decline in the survival curves, as well as the shortest median survival times, similar to that observed in captive populations of their Colombian congeners. In addition, both Costa Rican species also represent the body size extremes [23,26].

In the case of *Bothrops asper*, the single species shared by both serpentarium, the median survival time seen had marked differences, at 2.3 times longer in the Colombian rather than the Costa Rican captive snake population. In addition, both *Bothrops asper* captive populations showed distinct survival patterns, in which the Colombian population exhibited a steady decay of its survival profile while Costa Rican population showed a rapid decline in the survival profile.

Differences observed between ICP and INS captive snake populations of *Bothrops asper* can be caused by intrinsic and extrinsic factors related to captive conditions or natural history traits of each species, as well as population variability. For example, previous survival studies of coral snakes in captivity have found significant associations to the initial body condition during admission in the serpentarium, as well as to the types of substrates, and feeding schemes during captivity [21,22]. In Colombia, captive populations of the coral snake *Micrurus mipartitus* (redtail coral snake) have shown that weight and body condition at admission play a significant role in the lifespan observed [27].

Table 3. Cox proportional hazards models. The table depicts the P-values of each model, indicating the statistical significance of each covariate. Snout ventral length (SVL), weight, synergetic pathologies, ectoparasites, dysecdysis, and dehydration were included as intrinsic snake covariates during admission in the INS serpentarium. Synergetic pathologies illustrate the cases when a snake exhibited two or more pathologies at the same time (e.g., ectoparasites + dysecdysis)

Analysis categories	Groups	N	SVL	Weight	Synergetic pathologies	Ectoparasites	Dysecdysis	Dehydration
Regions	Amazon	28	0.387	0.0351	0.192	0.224	0.044	0.022
	Andean	143	<0.001	0.0926	0.0049	0.445	0.425	0.135
	Caribbean	64	0.52	0.99	0.273	0.531	0.187	<0.0001
	Orinoquia	67	0.033	0.0111	0.968	0.796	NA	NA
	Pacific	4	0.047	0.025	0.277	0.107	NA	0.909
	Inter-Andean valleys	31	0.13	0.097	NA	NA	NA	NA
Families	Elapidae	101	0.0068	0.736	<0.0001	0.423	0.0167	0.916
	Viperidae	209	0.245	0.265	0.429	0.203	0.06	0.0028
Viperid species	*Bothrops asper*	70	0.701	0.867	0.703	<0.0001	0.131	0.0085
	Bothrops atrox	36	0.0147	0.0054	<0.0001	<0.0001	0.0001	1
	Crotalus durissus	20	0.884	0.976	0.15	NA	0.99	1
Micrurus species	*M. dumerilii*	66	0.0092	0.931	0.0011	0.381	0.019	0.861
	M. mipartitus	31	0.265	0.69	0.171	NA	0.63	NA

P-value color scale 

NA: No data available. P-value color scale illustrating the statistical significance of the local differences between Cox proportional hazards models and intrinsic snake covariates.

We focused on the intrinsic factors of the conditions of snakes at admission in the INS serpentarium, such as body size, weight, and the presence/absence of pathologies, ectoparasites, dysecdysis, and dehydration. Cox proportional hazard models (CPH) indicate that the main pathologies observed during the last decade in the INS serpentarium can act alone or synergistically. In general, survival of captive snakes showed significant correlation with the intrinsic covariates assessed (Table 3). Body size (SVL) was the most frequent explanatory variable and was significant in 46% of the snake groups assessed, followed by dysecdysis (38%), weight, synergetic pathologies, dehydration (31% each one), and ectoparasites (15%). These results were expected since body size and weight loss are common risk factors in captive animals [28].

Dehydration showed a marked correlation according to the ecoregional origin of the snake or its taxonomic family. It indicates that when this sign appears, it alone has a significant effect on the probability of survival of viperid snakes from Amazonian and Caribbean ecoregions. In contrast, when dehydration appears together with synergetic pathologies, the cluster of factors shows a significant correlation of snake species from the Andean region, particularly for *Micrurus dumerilii*.

This finding is interesting because, as above, this same snake population is prone to show a maladaptation syndrome exhibiting smaller median survival times, suggesting that synergistic interactions could modulate or reduce snake lifespans in the serpentarium. Synergistic interactions of two or more pathologies are an important issue during snake maintenance in captivity because they can generate a greater deleterious response than when the pathologies act alone. Unfortunately, there is a dearth of information about this topic in literature, thus the understanding and the state of knowledge of synergistic pathological interactions in South American tropical snake in captivity is still extremely fragmentary.

Survival of captive snakes showed a significant correlation with dysecdysis, particularly with snakes of both viperid and elapid species from the Amazonian ecoregion. Unlike synergistic pathological interactions, this pathology is well- known and is considered as an important risk factor in captive reptiles because of multifactorial causes, such as: (1) inadequate captive management due to unsuitable humidity or temperature conditions; (2) scars from old injuries, surgery, or burns; (3) parasitic infections and systemic disease; and (4) underlying disease causing dehydration or impaired movement (e.g., fused vertebrae) [29].

Nevertheless, each snake group that we assessed exhibited a unique combination of explanatory variables with few commonalities among the CPH models. This suggests that future studies must be performed focusing on explored variables such as origin, locality/ecoregion, cortisol level, endoparasites, *etc*. For example, medically important snake species from the Orinoquia and Amazonian regions share body size and weight as explanatory variables. This would be expected because both regions

encompassed the historical distribution of *Lachesis muta,* a species that represents an extreme body size among South American pitvipers.

3. Understanding the deaths of snakes in captivity

Since 2010, all deceased snake in captivity have been examined using a standardized protocol in the INS serpentarium to understand the causes of death. Results of necropsies have been documented and entered into each specimen's clinical record. The compilation of these records year by year provides the basis of information allowing us to understand the pathologies and their impacts on the general population in the serpentarium. Understanding the causes of death of snake in captive conditions provides an important input in the improvement of management and tenure techniques in a large-scale snake captivity program for antivenom production [23,30].

The following descriptions and analyses were based on 264 necropsies done by veterinarians from 2010 to Jan of 2021 in deceased captive snakes encompassing 99 elapids (*M. camilae, M. dissoleucus, M. dumerilii, M. filiformis, M. helleri, M. hemprichii, M. medemi, M. mipartitus, M. nattereri, M. obscurus*, and *M. surinamensis*) and 165 viperids (*Bothriechis schlegelii*, *Bothrocophias tulitoi*, *Bothrops asper, B. atrox, B. bilineatus, B. venezuelensis*, *Crotalus durissus*, *Lachesis acrochorda*, *L. muta*, and *Porthidium lansbergii*; Table 4). Mostly, necropsy records came from the INS serpentarium since 2012 from the Hacienda Galindo in the municipality of Bojacá, Cundinamarca (see Chapter 7).

Digestive and respiratory system abnormalities were the main findings. They represented 43.3% of the total *postmortem* findings, followed by an absence of abnormalities (Figure 8). Digestive abnormalities in snakes are the main cause for infections. However, due the lack of established diagnostic tests, references for most reptile species and correct interpretation of the clinical signs are challenging [31,32]. Research efforts of diagnostic techniques, particularly imaging (e.g., contrast radiography and ultrasonography) of gastrointestinal tract, have resulted in clinical advancements for practicing veterinarians [32].

Nevertheless, due to limited technical facilities in serpentariums dedicated to antivenom production, as well as the dearth of reptile veterinarians available in Colombia that could to provide an accurate diagnosis, the therapy of digestive system disorders in captive snakes is difficult [31]. Therefore, in both pet veterinary clinics and laboratory animal production units like the INS serpentarium, digestive diseases are common as a cause of death; but this cause mostly cannot be detected until postmortem examination. Currently, there are no feasible factors or enclosure conditions that can be linked to a snake's death nor any digestive system abnormalities that have been observed in the INS serpentarium. This finding requires further research.

Respiratory diseases in snakes have multifactorial origins, including bacterial, viral parasitic, fungal, stress, cage spacing, genetics, physi-

ologic ecdysis, etc. [33]. Respiratory diseases are common in captive snakes since enclosure design and space directly impact the health of the snake. These factors affect the normal behavior and mobility of snakes, as well as the additional captive conditions like sanitation and humidity or physiological conditions like thermoregulation [28]. An ideal enclosure should provide a dry, hot area; a warm, humid area; a cool, dry area; and a cool, damp area [28]. However, as mentioned earlier, due to the limited space available in the INS serpentarium, all enclosures share the same environmental conditions and are homogeneous, lacking a diverse thermal offering. Therefore, prolonged exposition to invariant thermal and humidity conditions in the captive environment could explain the abnormalities observed. However, continued research efforts must be done to assess this hypothesis.

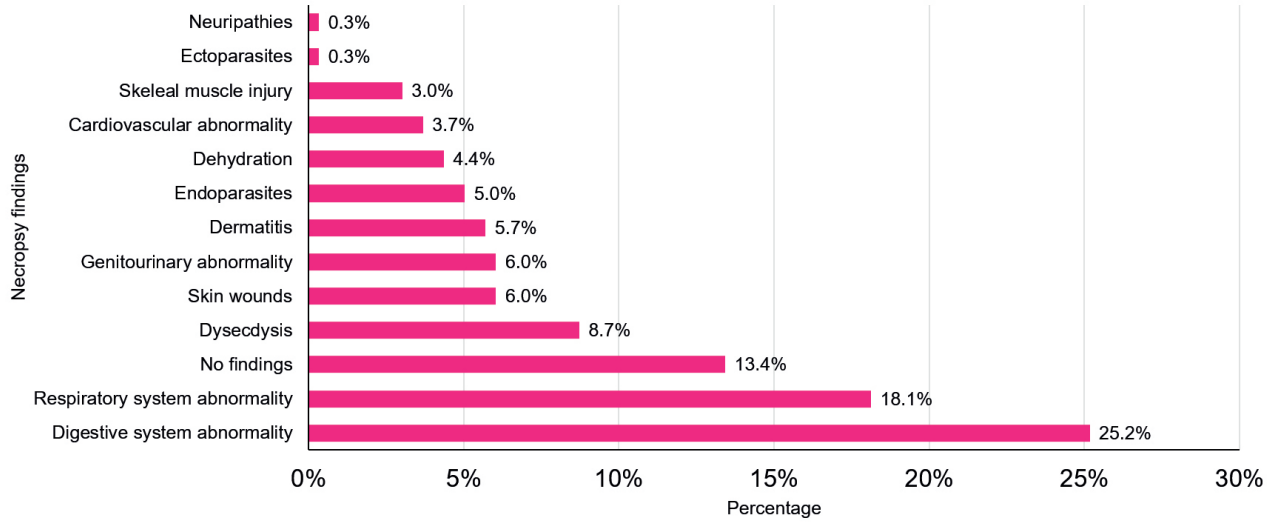


Figure 8. Main postmortem findings in medically important snake species housed in the INS serpentarium during 2010-2021.



Table 4. Main causes of death in medically important snake species housed in the INS serpentarium during 2010-2021.

Taxon	N	BBCA	Iatrogenic	Accidental death	Maladaptation syndrome	Unknown cause
Elapidae	99	14 (14.14%)	5 (5.05%)	6 (6.06%)	36 (36.36%)	38 (38.38%)
M. camilae	1					1 (1.01%)
M. dissoleucus	2		1 (1.01%)		1 (1.01%)	
M. dumerilii	46	9 (9.09%)	2 (2.02%)		15 (15.15%)	20 (20.2%)
M. filiformis	1		1 (1.01%)			
M. helleri	7	1 (1.01%)			3 (3.03%)	3 (3.03%)
M. hemprichii	2			1 (1.01%)		1 (1.01%)
M. medemi	8			1 (1.01%)		7 (7.07%)
M. mipartitus	24	3 (3.03%)		4 (4.04%)	13 (13.13%)	4 (4.04%)
M. nattereri	1		1 (1.01%)			
M. obscurus	1				1 (1.01%)	
M. surinamensis	6	1 (1.01%)			3 (3.03%)	2 (2.02%)
Viperidae	165	14 (8.48%)			49 (29.69%)	102 (61.82%)
Bothriechis						
B. schlegelii	4				1 (0.61%)	3 (1.82%)
Bothrocophias						
B. tulitoi	2	1 (0.61%)			1 (0.61%)	
Bothrops	110	6 (3.64%)			30 (18.18%)	74 (44.85%)
B. asper	66	4 (2.42%)			20 (12.12%)	42 (25.45%)
B. atrox	42	2 (1.21%)			10 (6.06%)	30 (18.18%)
B. bilineatus	1					1 (0.61%)
B. venezuelensis	1					1 (0.61%)
Crotalus						
C. durissus	15	2 (1.21%)				13 (7.88%)
Lachesis	4	2 (1.21%)				2 (1.21%)
L. acrochorda	1	1 (0.61%)				
L. muta	3	1 (0.61%)				2 (1.21%)
Porthidium						
P. lansbergii	30	3 (1.82%)			17 (10.3%)	10 (6.06%)
Total	**264**	**28 (10.61%)**	**5 (1.89%)**	**6 (2.27%)**	**85 (32.2%)**	**140 (53.03%)**

N= sample size. BBCA= Bad body condition before admission.

Knowledge of the diseases that snakes contract and the microbial agents they carry is essential for their management in captivity [34,35]. But there are few scientific studies that focus on the diseases of South American tropical snakes used for antivenom production. Neverthe-

less, the necropsy reports from the last decade of the INS serpentarium showed close similarities to previous observations in captive snakes or zoological exhibitions of specimens [13,18,29,30].

For example, the five most common causes of death in captive snakes in order of importance are (Table 4) as follow:

- *Unknown causes.*— Defined as the impossibility of establishing a medical cause of death from necropsy findings. This cause groups the largest proportion of snake deaths in all previous studies [13,18,29], representing 53% of deaths in the INS serpentarium with a notable prevalence in viperid species.
- *Maladaptation syndrome.*— Prevalence of 32.2% observed in captive snakes in the INS serpentarium, exhibiting an apparent association with body size and ecoregional origin, with significantly high frequency in Micrurus species.
- *Poor body condition.*— Deaths resulting from a deficient body condition before admission. The observed frequency of cases in the INS serpentarium was 10.61%. This cause is highly relevant during the first 150 days of captivity for all species but presents a high frequency in Micrurus species.
- *Accidental death.*— Deaths resulting from an accidental event in captivity. This cause had a low prevalence (2.27%), with mechanical asphyxia produced by attempts to escape from the enclosure being the main accident.
- *Iatrogeny.*— Deaths related to illness or injury caused during medical examination or treatment. This cause had the lowest frequency (1.89%) among observed causes of death in the INS serpentarium. However, when it occurred, coral snakes were the most prone to death from medical procedures.

4. Learned lessons

4.1 Lesson one: Feeding and nutrition

One of the main concerns for maintaining captive snakes for antivenom production is providing suitable feeding and nutrition; the goal is to prolong a healthy life for the animal to the extent possible. Feeding constitutes one of the most important and decisive activities for the maintenance and survival of snakes in captivity. Most captive snakes prefer live prey or prey that has been freshly killed, although they also accept dead prey [36]. The latter option has been commonly adopted by snake keepers in zoos or educational biological exhibitions, but dead prey is not compatible with good manufacturing practices in a program for antivenom production.

To achieve international production standards, special procedures must be designed, considering the following aspects: (1) A suitable selection of the feeding technique according to the diet type for each snake species should be chosen; (2) The establishment of a feeding schedule accord-

ing to the needs of the snake including the following criteria: health status, body size, body mass index, developmental stage (neonate, juvenile, adult); and (3) Feeding procedures performed by an experienced team of snake keepers.

Most feeding and nutritional problems arise from improper care or handling, combined with poor feeding management [37]. This lesson summarizes the main conclusions gained from solving feeding and nutritional problems that arise in the INS serpentarium housing tropical snakes that seeks to minimize deaths and diseases caused by malnutrition.

Voluntary feeding

As a primary directive, snake keepers must maintain and promote as long as possible the voluntary feeding stimuli exhibited by the snakes when they entered the serpentarium from wild environments. Steady voluntary feeding stimuli avoids the occurrence of a maladaptation syndrome. For success, feeding procedures must be performed during the day in a warm enclosure with temperatures ranging between 26° to 30°C and providing live prey. Both elapids and viperids tend to feed using this approach; however, technical steps differ. The technical approach employed to feed viperids snakes is as follows (Figure 9):

1. The snake keeper must remove the snake from its enclosure and put it in a box for weighing; this allows a determination of the prey to be given. This procedure should be done previously to the following steps.
2. The snake keeper selects a rodent according to the snake's weight (~ 10% of the snake's weight).
3. The rodent is held by a long tweezer and offered to the snake using the lid of the enclosure as an additional security barrier. Displaying gentle movements at 10 to 20 cm from the head of the snake, the snake keeper seeks to stimulate the snake to bite or attack the prey. The distance at which prey is offered to snakes varies according to the body size of the snake; we recommend displaying gentle movements when offering the prey at distance not more than 30% of the snake's body length.
4. Once the snake bites or attacks the prey, the prey is released from the tweezer and the enclosure is closed. If the snake refuses to bite the prey it is withdrawn, and an offering of prey is put off until the second round of feeding. The prey must be removed to avoid psychological stress or wounds caused by the prey. Attack by the prey can induce a severe, potentially life-threatening infection. The prey is never released without confirmation of an effective bite by the snake and the prey is dead.
5. In the case that more than one prey is needed to reach 10% of the required feeding weight, the snake keeper must wait for a second round of feeding, checking that the snake has completely swallowed the first rodent; then the additional prey is offered. In the event of a delay in the consumption of the first prey, the snake keeper performs euthanasia on the second prey, and it is left inside the enclosure. Ingestion of the prey by the snake must be confirmed, otherwise it must be removed.

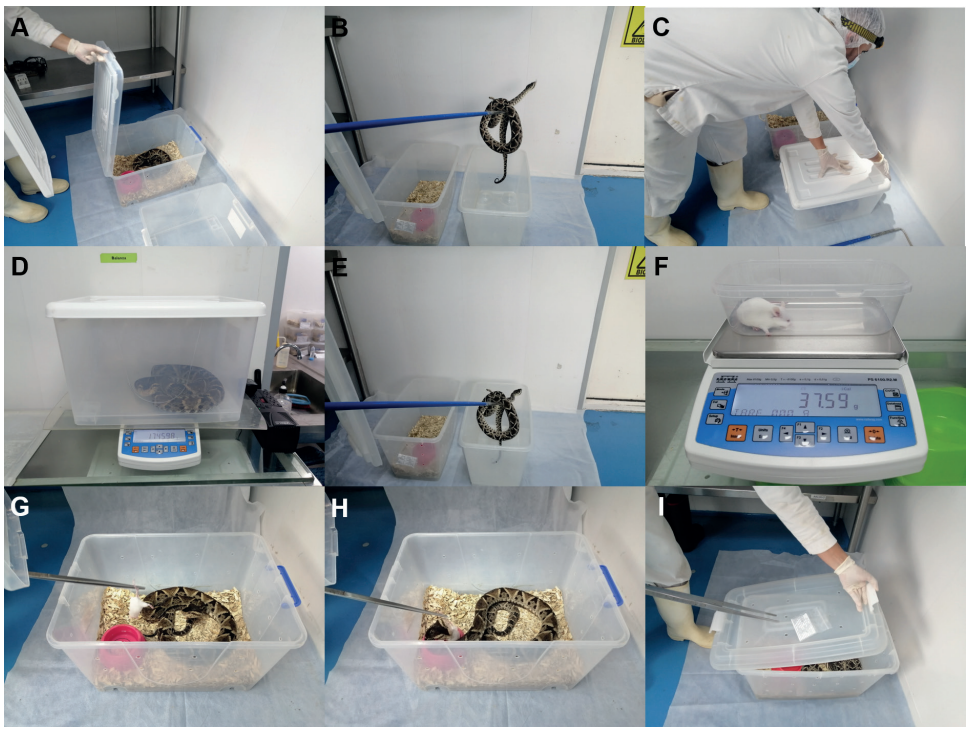


Figure 9. Weighing and feeding viperids. (A) Opening the snake enclosure using the lid as protective barrier. (B) Using a snake-hook, shifting the snake to an empty box for weighing. (C) Closing the box in which the snake will be weighed using the same lid of the snake enclosure. (D) Weighing the snake on a scale. (E) Returning the snake to its enclosure and closing the box. (F) Weighing the rodent on a scale. (G) The mouse is offered to the snake, holding the prey by a long tweezer, and displaying gentle movements. (H) The snake bite or attack on the prey; the prey is released from the tweezer. (I) The enclosure is closed.

The INS has an animal house facility independent of the serpentarium, used to produce certified rodents raised in controlled conditions, providing a suitable diet free of pathogens for viperid snakes in captivity (Table 5). Thus, the INS serpentarium always has broad dietary offerings varying in size and weight as food for the snakes according to their needs.

Table 5. Rodents raised in controlled conditions used feed viperid snakes in the INS serpentarium.

Type	Strain	Strain code	Fur color
Mice	Balb/c	028	Albine
Mice	Cd1(ICR)	022	Albine
Rat	Wistar	003	Albine
Guinea pig	Hartley	051	Albine
Gerbil	Mongolian	243	Agouti
Hamster	Syrian	249	Golden

Unlike viperids, feeding coralsnakes with live animals has enormous constraints and technical issues that reduce the possibility of steady voluntary feeding stimuli. First, due to the fact that coralsnakes are ground and litter dwellers (semi-fossorials), their diet consists mainly of other snakes, limbless lizards, caecilians, swamp eels, and knife fish [38]. In practice, the collection, maintenance or raising in captivity of this kind of prey is very difficult and unsustainable. So, feeding coral snake with live animals in the context of international production standards for snake antivenom production is not feasible or it is highly expensive. Second, due

to such prey being collected form the wild, it is not possible to guarantee they are free of pathogens; and the prey would be very difficult to sanitize. Third, to provide a continuous feeding schedule according to snake needs based on prey collected from the wild is difficult to achieve.

So, in practice feeding coral snakes with live animals is an occasional and opportunistic activity. The object is the promotion of voluntary feeding and avoiding direct handling that has deleterious effects on the snakes' health if force feeding is performed. At the INS serpentarium voluntary feeding with live animals is occasional. Voluntary feeding has been performed successfully in some individuals of *Micrurus dumerilii, M. mipartitus, M. helleri, M. sangilensis* and *M. hemprichi*, fed with live prey to snakes of the genera *Ninia, Atractus, Oxyrhopus* and *Stenorrhina* and limbless lizards of the genus *Bachia*. The technical approach employed to feed elapids is as follows:

1. The snake keeper must remove the snake from its enclosure and put it in a box for weighing that allows the determination of the prey to be administered. This procedure should be done previously to the following steps.
2. The snake keeper selects a prey according to the snake's length and weight (less than 50% of the snake's length and ~ 10% of the snake's weight).
3. The prey is released live into the enclosure and afterwards it is closed. The snake keeper must make the rounds verifying that the snake has attacked and ingested the prey. If it does not ingest the prey, the prey must be removed. In this way, the coral snake is stimulated to forage by using sight, smell, and taste.
4. The consumption of the prey must be confirmed within a period of two days, otherwise the prey must be removed.

Force feeding

Force feeding refers to the provision of mechanically forced food. This procedure is practiced by snake keepers when snakes refuse prey repeatedly for more than four months, due to the prevalence of some medical condition or when operative constraints when feeding with live animals is not feasible. This procedure can be performed in two ways. First, using a tweezer, the entire prey is introducing manually into the digestive tract of the snake, avoiding any kind of restrictive tool to hold the snake (e.g., restraining tube). The prey must be selected according to snake's length and weight as described above. Second, by introducing an orogastric probe into the digestive tract of the snake and gently injecting a nutritive formula. Following, both techniques are described.

Force feeding using a whole prey.— According to the accumulated experience of the INS serpentarium, this technique is mostly used in viperids rather than in elapids because the latter are prone to be highly stressed by handling during feeding procedure. Most viperids endure handling without major difficulties during force feeding. The technical approach employed to feed snakes using a whole prey is as follows:

1. The snake keeper must remove the snake from its enclosure and put it in a cloth bag for weighing. This allows determination of the prey to be administered. The procedure must be done between five to ten days before feeding.
2. The snake keeper selects a prey according to the snake's weight (~ 10% of the snake's weight).
3. The euthanized prey is moistened with water for lubrication.
4. When catching and gently handling the snake to be fed, it is recommended that the following steps be conducted by two snake keepers. The first snake keeper is charged with capturing and holding the snake, the second keeper is in charge of performing the feeding. Avoid the use of any kind of restrictive tool to hold the snake during feeding procedure (e.g., restraining tube).
5. A long tweezer (at least 20 cm) is used to hold the pre-lubricated prey with the tips of the tweezer.
6. Insert the pre-lubricated prey (headfirst) into the mouth of the snake.
7. With gentle and slow pushes, the pre-lubricated prey is pushed into the digestive tract of the snake, passing the head till a distance equivalent to the snake's head-length is reached. Afterwards, release the prey from the tweezer.
8. With the free hand, the snake keeper executing the feeding technique, gently massaged the ventral surface of the snake, slowly displacing the prey till reaching approximately the end of the first third of the snake's body.
9. Releasing the snake into its enclosure. Verify the acceptance of the prey for 24 hours. In case of regurgitating the prey, the prey must be discarded.

Force-feeding using a nutritive formula.— This technique represents one of the greatest successes of the INS serpentarium in the last decade. The use of a nutritive formula as alternative force feeding allowed us to consolidate a steady *Micrurus* snake population exceeding the average lifespan 1.4 years and a median survival time of 91 days, including captive specimens with a lifespan of 8 years.

The accumulative experience teaches us that the two main success factors were: (1) The composition of the nutritive formula; and (2) the technical improvement over time that encompassed the experience gained by the snake keepers in handling snakes, introducing a Nelaton catheter into the digestive tract, and accurate performance and following feeding steps using the nutritive formula.

The development of the nutritive formula was the response to the strong constraints to obtaining prey from the wild that allow a continuous feeding schedule according to coralsnake needs, as well as the frequent rejection of live prey in the first months of captivity. The nutritive formula is a hyperproteic solution based on "criolla" chicken eggs (criolla = a hen raised in a natural way in the countryside) mixed with Casilan® (Composition: protein = 95 %, carbohydrates = 1%; fat = 4) plus vitamin and amino acid supplements. Based on the experience of the INS serpentarium

veterinarians, the use of "criolla" chicken eggs is mandatory. The use of other kinds of eggs provoked the rejection of the nutritive formula by the snakes. Because Casilan® disappeared from the market in 2021, the nutritive formula changed, replacing Casilan® by Reptomin® (Composition: protein = 44 %, carbohydrates = 3%; fat = 5%, humidity = 9%, Calcium = 0.3%, Phosphorus = 0.4%) and observing the same previous good results using Casilan®.

The nutritive formula has shown excellent results for most of the coral snakes although this was not an infallible solution. After feeding ten *Micrurus surinamensis* (aquatic coral snake) with nutritive formula that replaced the "criolla" chicken eggs by fish as a source of protein, all snakes exhibited severe life-threatening effects such as dull skin, incomplete ecdysis, and immobility. Therefore, the use of a nutritive formula based on a fish-based diet was contraindicated. In the case of *M. surinamensis*, the nutritive formula based on water plus Reptomin® showed acceptable results, although it is still a challenge.

The preparation and administration of the nutritive formula must be careful and very liquid. Dense consistencies can make digestion difficult. Lumps or bubbles can cause the rupture of the digestive tract during administration. Handling time must be minimized by quick administration of the nutritive formula. Therefore, the introduction of the Nelaton catheter must be quick, gently pushing into the digestive tract. The gauge of the Nelaton catheter must be selected according to the snake's body length. Handling must be done using bare hands to avoid the use of tweezers or any kind of restrictive tool that provokes stress in the snake and consequent rejection of the nutritive formula.

Consumption of the nutritive formula must be registered in the clinical record of each snake. According to accumulated experiences feeding coral snakes, the suitable proportion of nutritive formula to feed snakes is between 10% and 30% of the snake's weight, considering a proportion 1:1 (body weight g/formula mL). However, the proportion of the nutritive formula supplied can vary between individuals because the stage development, medical condition, or body condition of the snake. Clinical veterinary records must be consulted, looking to adjust the percentage according to the historical records of each specimen.

Three snake keepers must perform force-feeding when using the nutritive formula. The technical approach employed to feed snakes using nutritive formula is as follows (Figure 10):

1. The snake keepers must remove the snake from its enclosure using a narrow herpetological hook and put it in a cloth bag for weighing to allow an estimate of the volume of formula to be administered.
2. Preparation of a Nelaton catheter and syringe according to snake body size with nutritive formula (A 3mL syringe with Nelaton catheter of 6 gauge for snake body size less than 20cm; a 20mL syringe with gavage tube of 9 or 10 gauge for snake body size more than 20cm).

3. Performing force-feeding using nutritive formula handling must be done using bare hands, avoiding the use of tweezers or any kind of restrictive tool. The first snake keeper is in charge of capturing and holding the snake, the second keeper takes care of the feeding technique.
4. The first snake keeper introduces the Nelaton catheter up to the first third of the snake's body. It must be introduced with a gentle but quick push into the digestive tract.
5. Immediately after the introduction of the Nelaton catheter, the second snake keeper must introduce the controlled supply of the formula, while a third snake keeper holds the snake's body, performing gentle massaging on the ventral surface of the animal. A steady injection speed of the formula through the syringe must be maintained, avoiding a buildup and bubbles that could provoke indigestion or digestive tract obstruction.
6. Once the entire volume of nutritive formula has been administered, the Nelaton catheter is slowly withdrawn from the digestive tract. The snake's mouth must be washed with water before the snake is returned to its enclosure.
7. Returning the snake to its enclosure. Verify the acceptance of the nutritive formula for 24 hours. In case of regurgitating the formula, clean the enclosure and reprogram a feeding procedure.

Note: This feeding procedure cannot be carried out in specimens that are experiencing ecdysis unless the veterinarian suggests it is ok to do so. If any additional medical procedure is necessary, it must be carried out before the feeding procedure.

The established feeding schedule in the INS serpentarium is monthly for viperids and fortnightly for elapids. Nevertheless, neonates of both viperid and elapid snakes feed each 10 and 15 days due to their high metabolic demands. Other reasons for the change in feeding frequency are due to specific veterinary medical decisions. The feeding schedules, having been established over 10 years, exhibit good performance and results. However, we recommend that each serpentarium according to the species of snake housed must be assessed for the correct feeding frequency.

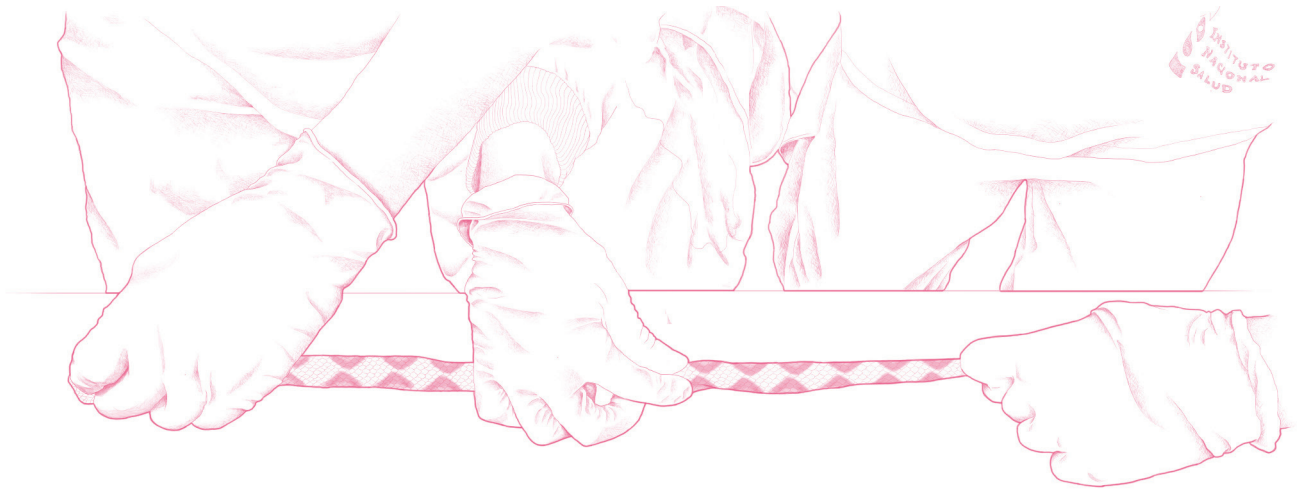




Figure 10. Weighing and feeding process of *Micrurus* species. (A) Opening the snake enclosure using the lid as protective barrier. (B) Using a narrow herpetological hook to shift the snake into an empty white cloth bag (Dacron fabric) for weighing. (C) Folding the white cloth bag and weighing the snake. (D-E) The first snake keeper, observing the snake against the light, locates the snake head. (F) The first snake keeper immobilizes the snake´s head while the second snake keeper gently holds the snake's body. (G) A third snake keeper milks the venom using two capillary tubes. (H) The first snake keeper introduces the Nelaton catheter up to the first third of the snake's body. Insertion must be with a gentle but quick push into the digestive tract. (I) The second snake keeper begins introducing the controlled supply of the formula, while a third snake keeper holds the snake body while performing a gentle massage on the ventral surface of the animal. (J) The Nelaton catheter is slowly withdrawn from the digestive tract. The snake's mouth must be washed with water before being returned to its enclosure. (K)The snake is then returned to its enclosure.

4.2 Lesson two: Iatrogenic deaths

Despite iatrogenic deaths representing less than 2% of snake deaths in the last decade in the INS serpentarium, it is important to describe the most relevant events as learned lesson to be considered in future implementations of a large-scale program for antivenom production based on captive snakes.

Case 1: digestive tract rupture.— Force-feeding using nutritive formula through a Nelaton catheter is an adapted technique from those feeding techniques used to feed murine models in research procedures [39]. Thus, there are no references of its use in snakes, and therefore our learning was done through experience. Force-feeding employing a Nelaton catheter requires an experienced snake keeper for employment; meanwhile experience is gained, mistakes arise, and the most common mistake results in digestive tract rupture.

The main factors that contribute to digestive tract rupture are the following: (1) unsuitable selection of the Nelaton catheter size that mismatches the snake body size; (2) an inadequate entrance of the Nelaton catheter that exceeds the first third of snake's body; (3) an overly rapid and too strong push entering the Nelaton catheter; (4) an uncontrolled supply of the nutritive formula that generates a buildup and bubbles; (5) lack of a gentle massage on the ventral surface of the snake; and (6) fast and uncontrolled extubating. Based on our experience, death by digestive tract rupture can be detected within the first 72 h after the force-feeding procedure.

4.3 Lesson three: environmental enrichment

Environmental enrichment is the set of techniques and management measures used by keepers looking to encourage the expression of natural behaviors of animals in captive conditions [40]. Additionally, these management measures seek to promote and increment the survival, health, and well-being of captive animals [41].

Historically, environmental enrichment of snake captives has been mostly studied in zoos and educational biodioramas rather than serpentariums that are focused on antivenom production [25]. For example, for public environmental educational initiatives, Cardoso et al. [42] demonstrated that enriched environments have significantly positive effects on the welfare and behavior of captive populations of *Micrurus corallinus,* reducing stress and preventing maladaptation syndromes.

Environmental enrichment of serpentariums focused in intensive antivenom production is challenging due to the lack of research that addresses which are the "best" practices. Particularly, environmental enrichment needs to overcome special challenges for standardized animal sanitation protocols in the context of an intensive snake captivity program that seeks replicability and reproducibility [25]. For example, to maintain high standards of sanitation all elements (tree branches, rocks, pieces of tree trunks, plants, etc.), both natural and artificial, must be sanitized to turn them into aseptic elements for the control of pathogenetic sources. Many disinfectants are inactivated in the presence of organic matter, so washing and disinfecting trunks and plants is useless for the snake health.

An intensive snake captivity program for antivenom production that meets the requirements of good environmental enrichment is a challenging task because a high number of snakes must be cared for daily that includes the amount of time that must be invested in milking, as well as the trained staff and resources required to do so. Commonly in this kind of serpentarium there is a trade-off between environmental enrichment and operational snake management. The extremes of this trade-off are, on the one hand, an enriched biodiorama or terrarium ornamented as a faithful replica of the snake's known microhabitat (like exhibition serpentarium), and on the other hand a basic enclosure without snake microhabitat elements, only with water supplied *ad libitum*.

The experience gained during INS serpentarium history suggests that the midpoint between both extremes is the "best" choice despite having different environmental enrichment needs according to species specificity. According to Loughman [43] protocol maintenance of snakes in captivity and the environmental enrichments of its enclosures should be based on the natural history of the snakes. The INS serpentarium follows this approach but adjusts it according to the INS's own rigid and standardized operational guidelines for safety, health, welfare of animals, worker safety, product quality, and traceability. These details are similar to those stated by the Center for the Study of Venoms and Venomous Animals (CEVAP) at São Paulo State University (UNESP) Brazil [25]; and World Health Organization (WHO) guidelines for snake antivenom production [44].

Intensive serpentarium

General captive conditions.— The serpentarium houses 182 specimens, of which 146 are viperids and 36 are elapids. Each specimen has an alphanumeric code that acts as its identification number. Adults of the species that reach large body size have a chip implanted that is no larger than a grain of rice that provides an alphanumeric code. The captive environment maintains steady physical conditions (temperature: 26±0.2 C; humidity relative 65±5 humidity relative). Nevertheless, due to the area of the serpentariums space the relative humidity and temperature is not homogeneous across the room. There are zones that are more humid and other zones are drier, as well as zones that are relatively warmer and others less warm. Therefore, taking advantage of this variability snake enclosures are arranged based on the relative humidity and temperature of the original habitat of the snakes. For example, *Crotalus durissus* enclosures are in the drier zones of the serpentarium while *Micrurus surinamensis* enclosures are in the humid sections. Also, inside the enclosures the relative humidity and temperature varies, indicating that the polypropylene boxes provide a microclimate (Table 6).

Table 6. Records of thermohydrometer inside the snake enclosures

Specie	Relative humidity	Temperature
Crotalus durissus	60.5 (42.3-75.3)	26.6 (25.5-27.8)
Bothriechis schlegelii	87.0 (78.1-93.9)	26.3 (25.2-27.5)
Bothrops asper	62.5 (42.2-81.9)	25.6 (25.0-27.2)
Bothrops atrox	77.1 (45.2-96.9)	26.4 (42.3-75.3)
Lachesis acrochorda	64.6 (43.1-84.1)	25.1 (23.7-28.9)
Micrurus surinamensis	93.9 (73.4-100)	25.4 (24.5-26.4)

Enclosure.— Snakes are housed in individual translucid polypropylene boxes of varying sizes (20 cm long x 15 cm wide and 20 cm high used in snakes less than 30 cm SVL; up to boxes of 60 cm long x 40 cm wide and 35 cm high used for large viperids such as snakes of the genera *Bothrops* and *Crotalus* up to 2 m SVL). The advantages are safety, ease of cleaning, and maintenance. In this manner, boxes are easily monitored with relation

to feeding, and health. For special cases of large snakes like *Lachesis,* the size of the enclosures is 100 cm long x 45 cm wide and 35 cm high, built with stainless steel shelves with rigid translucent acrylic walls.

Substrate.— The substrate that covers the floor of the box must meet four criteria: (1) offer comfort to the snake; (2) help to maintain conditions of humidity in the enclosure; (3) facilitate cleaning of the enclosure; and (4) be deep enough to allow snakes with semi-fossorial habits to hide. A 1:1 mixture of shavings (poplar shavings) and an inert sanitized vermiculite (hydrous phyllosili-cate mineral) fills the floor of the box 2 cm deep, generating bedding that allows the maintenance of relative humidity at about 70%. In the case of fossorial snakes, the floor of the box is filled up to 5 cm deep. Other substrates like natural soil, natural leaf litter, or sawdust are not recommended since they are common sources of pathogens and are difficult to sanitize. Unlike all other snake species in the serpentarium, *Crotalus durissus* requires the driest microhabitat, so besides placing the enclosure in the drier zone of the serpentarium, corrugated cardboard is used as a substitute of mixed vermiculite to reduce the relative humidity inside the enclosure.

Water supply.— The enclosures of adult snakes have a circular plastic drinker of 15 cm in diameter and 10 cm high, while enclosures of juvenile or neonate used have 5-ounce disposable plastic cups embedded in the substrate and attached to the floor of the enclosure. Water is administering *ad libitum,* and it is changed every fifteen days, however, this time may decrease depending on whether it becomes dirty due to defecating or substrate.

Enclosure ornamentation.— The arrangement of ornamentation inside the enclosures, in addition to providing an environment similar to the natural habitat, must allow for the safe handling for serpentarium staff, always reducing snakebite risk during the routine procedures of cleaning, feeding, and milking. Therefore, elements of ornamentation in the enclosures are the minimum necessary according to the natural history of the snakes. For example, the plastic water container works as a shelter for coral snakes, neonates, and juveniles of viperid species. Additional to the mixed vermiculite substrate and plastic water container, the enclosure of arboreal snakes like *Bothriechis schlegelii* or *Bothrops bilineatus* are ornamented with a set of sanitized tree branches assigned per specimen in such a way that each snake always uses the same set of branches, avoiding cross contamination.

In the case of very large snakes (over 2,000 mm snout-vent length) such as *Lachesis*, experience has taught us that enclosures with fallen objects help in shedding for this kind of snake. So, the enclosures are enriched with heavy wooden logs and daily water sprinkling. Both shelters and tree branches must be strategically placed to allow the snake to feel hidden while it remains visible to the serpentarium staff.

Rattlesnakes are one of the most representative species in the INS serpentarium with the highest median survival time among all species

(9.7 years), as well as being one of the most numerous historically in the collection (54 specimens). No ornamented elements are used to enrich the captive environment of *Crotalus durissus*, only a corrugated cardboard and a plastic water container are inside their enclosure. The inclusion of elements that accumulate humidity (e.g., vermiculite, wooden logs, or plastic shelters) has been associated with pathologies like mycosis and incomplete ecdysis. Therefore, this is an example illustrating that not all snakes require complex environmental enrichment, some species need only a few elements to adapt to captivity and enjoy well-being.

4.4 Lesson four: unexpected survivors

Several snakes housed in the INS serpentarium were donations from Colombia's Environmental Regional Autonomous Corporations (CARs, Spanish acronym), Environmental police, Fire Department, obtained through rescues, confiscation, or forfeitures. In several cases previous snake handling and captivity were improper, causing deleterious effects on the initial condition of the snake before admission to the serpentarium. Consequently, several pathologies arose significantly impacting the snake's survival probability. Nevertheless, there are remarkable cases of injured snakes that survived for a long time, despite wounds or pathologies. This lesson illustrates the relevant events of snake survivors after improper handling procedures when the animals exhibited exceptional recovery that teaches us that, despite poor conditions of some snakes, the will to live never gives up.

Case 1: Micrurus dumerilii INSV-SR-109

Snake's health condition during admission.— An adult female snake (SVL:680 mm; TL: 65 mm; weight: 59.8 g) from Viota, Cundinamarca, Colombia, admitted in July of 2016 presenting a musculoskeletal injury in the first third of the snake's body, showing a skin wound with a depression or convexity possibly caused by crushing or a strike with a blunt object, causing ataxia and constraining the natural serpentine movements after the wound. The snake remained in abnormal anatomical positions in the enclosure, elongating and inverting body position (body ventral surface visible from above) after the injury, without the possibility of displaying normal behaviors such as hiding in the substrate.

Veterinary considerations in the captive management.— One month after admission, the snake presented weight loss (normal at the beginning of captivity). A structure compatible with a rib was observed protruding from the skin, exhibiting necrosis surrounding the wound. In accordance with veterinary medical criteria, the snake was kept under observation, no food was offered but water was provided *ad libitum*. No additional medical procedure was performed.

In the second month of captivity, a yellow coloration was observed in the rib (non-viable rib bone) protruding from the skin, showing an evident skin retention in the wound area. The skin retained was manually removed and the entire detached rib. Clotrimazole topical was applied, and the

snake was returned to its enclosure without evidence of improvement in body mobility.

In the third month of captivity (October) the snake was entered into the production scheme, performing procedures of capture, handling, milking, and feeding using force feeding with nutritive formula. At this point, the snake had lost 36.8% of its initial weight (weight: 43 g).

In the following months, the snake regurgitated, rejecting the nutritive formula on several occasions, causing a dramatic weight loss until the eighth month. In the nineth month weight recovery was observed, as well as the first signs of acceptance of the nutritive formula; recovery of health and body condition reaching the admission weight approximately in May 2017 (tenth month).

The snake weight and health were recovered in the following five months, as well as acceptance of force feeding using nutritive formula, showing good adaptation to the captive environment. The wound healed without need of medical treatment or intervention, leaving a marked scare in the area of injury. However, ataxia continued without improvement in body mobility after the injury, constraining normal behavior in the enclosure (inability to hide in the substrate or artificial shelters). Due to the injury, normal ecdysis was obstructed making it necessary for the snake keepers to help in shedding.

Adaptation, survival, and death expectation.— Due the severity of the injuries the initial diagnostic was negative. Besides, according to accumulated experience with captive *Micrurus* species, the predicted life expectancy was less than two months, which could have decreased more after veterinary intervention to detach the rib. However, despite all expectations, the snake lived for six years (until June 22 of 2022) as part of the production scheme, including it in the routine activities of milking, and feeding.

Necropsy did not provide a conclusive cause of death. However, during the last ten months before death, the snake showed a deterioration of health, exhibiting weight loss and shedding retention in the entire body, as well as progressive general immobility, which are clear signs of aging for geriatric reptiles [45].

Case 2: Micrurus dumerilii INSV-SR-113

Snake's health condition during admission.— An adult female snake (SVL:690 mm; TL: 82 mm; weight: 60.3 g) from San Onofre, Sucre, Colombia, admitted in September 17 of 2017, presenting good conditions of health.

Veterinary considerations in captive management.— One month after admission, the snake presented weight loss (normal at the beginning of captivity), exhibiting opacity in the internasal and parietal scales. Nevertheless, a complete ecdysis had been observed during the first month of captivity, so it seemed unlikely that the opacity was due to shedding retention. In the following months, the snake showed signs of maladapta-

tion syndrome, such as rejection of the nutritive formula (regurgitation), excessive ecdysis (two events of shedding per month), weight loss, opacity in both head and dorsal scales.

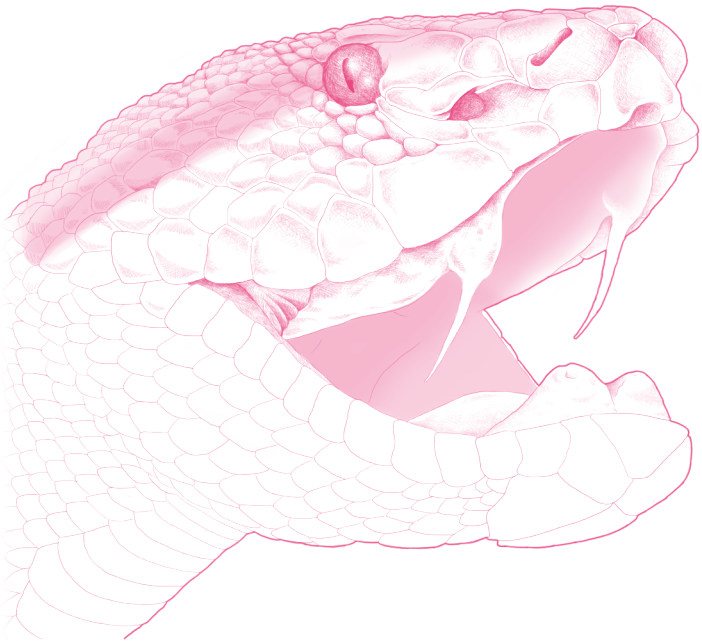
Since the maladaptation syndrome persisted, force-feeding using nutritive formula was performed during ecdysis to attempt to stop the weight loss and depressed body condition of the snake, despite the risks generating shedding retention. Opacity in both head and dorsal scales was clinically attended as a mycotic infection; however, the treatment had no effect. Thus, scale opacity became a chronic condition of the snake. Ecdysis had to be aided by the snake keepers in each shedding event. This captive management technique was maintained for a long time and the weight loss and depressed body condition of the snake was stopped, but not the chronic signs of excessive ecdysis and scale opacity.

Adaptation, survival, and death expectation.— This case exemplifies how a snake admitted to the serpentarium to a captivity environment with good health conditions and veterinary diagnosis, quickly developed maladaptation syndrome that threatened its survival. However, thanks to suitable veterinary care and management, this trajectory was reversed, prolonging its life expectancy, despite the snake never having suitable adaptation to the captive environment. The snake lived four years in captivity and was included in the routine production scheme of the INS serpentarium till it died, though no other signs or pathologies were observed.

Appendix: Material and Methods

Based on the history of the data available from 1990 to 2021 of the captive snakes employed for antivenom production housed in the serpentarium of the Colombian National Health Institute, we described, analyzed, and reported their most remarkable data and special cases. Additionally, we performed a standard survival analysis through the Kaplan-Meier estimator and Cox PH models (considering constant covariates) for censored data [46,47], to assess the survival probability of the captive snakes and the associated variables . All analyses were run using the software Rwizard 4.3 [48], and the R package survival [49].

The use of a nutritional formula for feeding, as well as the enrichment of the enclosure through the incorporation of vermiculite as a substrate component, were contributions made by biologist Jairo Maldonado, who, during his tenure at the INS, shared his expertise in the handling, care, and welfare of coral snakes. Based on his observations and recommendations, the technical team at the INS serpentarium has refined and perfected these practices



References

1. Vargas-salinas, F.; Angarita–Sierra, T.; Ospina-L, A.; Rocha-Úsuga, A.; Rueda-Solano, L. Comunicación y ecología reprodcutiva. In *Biología de los anfibios y reptiles en el bosque seco tropical del norte de Colombia*; **2019**; pp. 248–295 ISBN 9789586603416.
2. Ospina-Sarria, J.J.; Angarita-Sierra, T. A New Species of *Pristimantis* (Anura: Strabomantidae) from the Eastern Slope of the Cordillera Oriental, Arauca, Colombia. *Herpetologica* **2020**, *76*, 83–92.
3. Angarita-Sierra, T.; Montaño-Londoño, L.F.; Bravo-Vega, C.A. ID please: Evaluating the utility of Facebook as a source of data for snake research and conservation. *An. Acad. Bras. Cienc.* **2022**, *94*, 1–25, doi:10.1590/0001-3765202220211043.
4. Duellman, W.E. *Cusco Amazónico/ the Lives of Amphibians and Reptiles in an Amazonian Rainforest.*; Comstock Publishing Associates: Ithaca, **2005**.
5. Lynch, J.D. El contexto de las serpientes de Colombia con un análisis de las amenazas en contra de su conservación. *Rev. la Acad. Colomb. Ciencias Exactas, Físicas y Nat.* **2012**, *36*, 435–449.
6. Castillo-Beltrán, M.C.; Hurtado-Gómez, J.P.; Corredor-Espinel, V.; Ruiz-Gómez, F.J. A polyvalent coral snake antivenom with broad neutralization capacity. *PLoS Negl. Trop. Dis.* **2018**, *13*, 1–14, doi:10.1371/journal.pntd.0007250.
7. Lovich, J.E.; Gibbons, J.W. Age at Maturity Influences Adult Sex Ratio in the Turtle *Malaclemys terrapin*. *Oikos* **1990**, *59*, 126, doi:10.2307/3545132.
8. Braz, H.B.; Rocha, M.M.T.; Furtado, M.F.D. Maintaining rear-fanged snakes for venom production: An evaluation of mortality and survival rates for *Philodryas olfersii* and *P. patagoniensis* in captivity. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2012**, *18*, 164–172, doi:10.1590/S1678-91992012000200006.
9. Warwick, C.; Arena, P.; Steedman, C. Spatial considerations for captive snakes. *J. Vet. Behav.* **2019**, *30*, 37–48, doi:10.1016/j.jveb.2018.12.006.
10. Warwick, C. Reptilian ethology in captivity: Observations of some problems and an evaluation of their aetiology. *Appl. Anim. Behav. Sci.* **1990**, *26*, 1–13, doi:10.1016/0168-1591(90)90082-O.
11. Henao Duque, A.M.; Núñez Rangel, V. Maintenance of red-tail coral snake (*Micrurus mipartitus*) in captivity and evaluation of individual venom variability. *Acta Biológica Colomb.* **2016**, *21*, 593, doi:10.15446/abc.v21n3.51651.
12. Murphy, J.B.; Armstrong, B.L. *Maintenance of Rattlesnakes in captivity*; University of Kansas: Lawrence, **1978**; ISBN 0-89338-006-7.
13. Van Waeyenberge, J.; Aerts, J.; Hellebuyck, T.; Pasmans, F.; Martel, A. Stress in wild and captive snakes: Quantification, effects and the importance of management. *Vlaams Diergeneeskd. Tijdschr.* **2018**, *87*, 59–65, doi:10.21825/vdt.v87i2.16082.
14. Rataj, A. V.; Lindtner-Knific, R.; Vlahović, K.; Mavri, U.; Dovč, A. Parasites in pet reptiles. *Acta Vet. Scand.* **2011**, *53*, doi:10.1186/1751-0147-53-33.
15. Grego, K.F.; Vieira, S.E.M.; Vidueiros, J.P.; de Oliveira Serapicos, E.; Barbarini, C.C.; da Silveira, G.P.M.; de Souza Rodrigues, F.; de Carvalho Francisco Alves, L.; Stuginski, D.R.; Rameh-De-Albuquerque, L.C.; et al. Maintenance of venomous snakes in captivity for venom production at Butantan Institute from 1908 to the present: A scoping history. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2021**, *27*, 1–11, doi:10.1590/1678-9199-JVATITD-2020-0068.

16. Rangel-Ch, O.J. *Colombia Diversidad Biotica I*; Rangel-Ch, O.J., Ed.; nstituto de Ciencias Naturales. Universidad Nacional de Colombia,: Bogotá, **1995**.
17. Dunham, A.; Grant, B.; Overall, K. Interfaces between biophysical and physiological ecology and the population ecology of therretrial vetrebrate ectotherms. *Physiol. Zool.* **1989**, *62*, 338–355.
18. Mader, D.R. *Reptile medicine and surgery*; Saunders Elsevier, **2006**.
19. Warwick, C. The Morality of the Reptile "Pet" Trade. *J. Anim. Ethics* **2014**, *4*, 74, doi:10.5406/janimalethics.4.1.0074.
20. Warwick, C.; Grant, R.; Steedman, C.; Howell, T.J.; Arena, P.C.; Lambiris, A.J.L.; Nash, A.E.; Jessop, M.; Pilny, A.; Amarello, M.; et al. Getting it straight: Accommodating rectilinear behavior in captive snakes—A review of recommendations and t heir evidence base. *Animals* **2021**, *11*, 1459, doi:https://doi.org/10.3390/ani11051459.
21. Chacón, D.; Rodríguez, S.; Arias, J.; Solano, G.; Bonilla, F.; Gómez, A. Maintaining Coral Snakes (*Micrurus nigrocinctus*, Serpentes: Elapidae) for venom production on an alternative fish-based diet. *Toxicon* **2012**, *60*, 249–253, doi:10.1016/j.toxicon.2012.04.332.
22. Mendes, G.F.; Stuginski, D.R.; Loibel, S.M.C.; Morais-Zani, K. De; Da Rocha, M.M.T.; Fernandes, W.; Sant'anna, S.S.; Grego, K.F. Factors that can influence the survival rates of coral snakes (*Micrurus corallinus*) for antivenom production. *J. Anim. Sci.* **2019**, *97*, 972–980, doi:10.1093/jas/sky467.
23. Sasa, M.; Arias Ortega, J.; Bonilla-Murillo, F. Assessing survival of wild-caught snakes in open venom production systems. *Toxicon* **2017**, *138*, 49–52, doi:10.1016/j.toxicon.2017.08.001.
24. Castro-Sandoval, C.A. Propuesta de manejo y supervivencia en cautiverio de serpientes de género Micrurus para la producción de suero antiofídico en el Instituto Nacional de Salud, Universidad Nacional Abierta y a Distancia, https://repository.unad.edu.co/bitstream/handle/10596/44654/cacastrosan.pdf?sequence=1&isAllowed=y, **2021**.
25. Santos, L.; Oliveira, C.; Vasconcelos, B.M.; Vilela, D.; Melo, L.; Ambrósio, L.; da Silva, A.; Murback, L.; Kurissio, J.; Cavalcante, J.; et al. Good management practices of venomous snakes in captivity to produce biological venom-based medicines: achieving replicability and contributing to pharmaceutical industry. *J. Toxicol. Environ. Heal. - Part B Crit. Rev.* **2021**, *24*, 30–50, doi:10.1080/10937404.2020.1855279.
26. Campbell, J.A.; Lamar, W.W. The Venomous Reptiles of the Western Hemisphere (Vol. 1); Comstock Publishing: New York, **2004**; ISBN 978-0801441417.
27. Duque Henao, A.M.; Rangel Núñez, V. Maintenance of red-tail coral snake (*Micrurus mipartitus*) in captivity and evaluation of individual venom variability. *Acta Biológica Colomb.* **2016**, *21*, 593–600.
28. Mason, G.J. Species differences in responses to captivity: Stress, welfare and the comparative method. *Trends Ecol. Evol.* **2010**, *25*, 713–721, doi:10.1016/j.tree.2010.08.011.
29. Goodman, G. Common dermatoses in reptiles. *In Pract.* **2007**, *29*, 288–293, doi:10.1136/inpract.29.5.288.
30. McAloose, D.; Colegrove, K.M.; Newton, A.L. Wildlife necropsy. In *Pathology of Wildlife and Zoo Animals*; Terio, K.A., McAloose, D., Judy St., L., Eds.; Elsevier Inc., **2018**; pp. 1–20 ISBN 9780128053065.
31. Mans, C. Clinical update on diagnosis and management of disorders of the digestive system of reptiles. *J. Exot. Pet Med.* **2013**, *22*, 141–162,

doi:10.1053/j.jepm.2013.05.006.
32. Banzato, T.; Hellebuyck, T.; Van Caelenberg, A.; Saunders, J.H.; Zotti, A. A review of diagnostic imaging of snakes and lizards. *Vet. Rec.* **2013**, *173*, 43–49, doi:10.1136/vr.101450.
33. Driggers, T. Respiratory diseases, diagnostics, and therapy in snakes. *Vet. Clin. North Am. Exot. Anim. Pract.* **2000**, *3*, 519–530.
34. Ramsay, E.C.; Munson, L.; Lowenstine, L.; Fowler, M.E. A Retrospective Study of Neoplasia in a Collection of Captive Snakes Published by : American Association of Zoo Veterinarians content in a trusted digital archive . We use information technology and tools to increase productivity and facilitate new forms. **1996**, *27*, 28–34.
35. Page, L.A. Diseases and Infections of Snakes: A Review. *Bull. Wildl. Dis. Assoc.* **1966**, *2*, 111–126, doi:10.7589/0090-3558-2.4.111.
36. Kauffeld, C.F. Herpetologists' League Methods of Feeding Captive Snakes. *Methods* **2010**, *9*, 129–131.
37. Donoghue, S.; McKeown, S. Nutrition of captive reptiles. *Vet. Clin. North Am. Exot. Anim. Pract.* **1999**, *2*, 69–91, doi:10.1016/S1094-9194(17)30140-8.
38. Roze, J. *Coral Snakes of the Americas: Biology, identification, and Venoms*; Krieger Publishing Company, **1996**; ISBN 0894648470.
39. Wheatley, J.L. A gavage dosing apparatus with flexible catheter provides a less stressful gavage technique in the rat. *Lab Anim. (NY).* **2002**, *31*, 53–56, doi:10.1038/5000176.
40. Burghardt, G.M. Environmental enrichment and cognitive complexity in reptiles and amphibians: Concepts, review, and implications for captive populations. *Appl. Anim. Behav. Sci.* **2013**, *147*, 286–298, doi:10.1016/j.applanim.2013.04.013.
41. Mellor, D.J. Enhancing animal welfare by creating opportunities for positive affective engagement. *N. Z. Vet. J.* **2015**, *63*, 3–8, doi:10.1080/00480169.2014.926799.
42. Cardoso, S.R.T.; Silva, A.G.; Pourto, G. Maintenance and reproductive behavior of *Micrurus corallinus* in captivity. In *Advances in Coralsnake Biology: with Emphasis on South America*; da Silva-Jr, N.J. da, Porras, L.W., Aird, S.D., da Costa Prudente, A.L., Eds.; PUC Goia´s, Eagle montain Publishing: Goiás, **2021**; pp. 353–369 ISBN 9780972015462.
43. Loughman, Z.J. Utilization of natural history information in evidence based herpetoculture: A proposed protocol and case study with *Hydrodynastes gigas* (false water cobra). *Animals* **2020**, *10*, 1–20, doi:10.3390/ani10112021.
44. World Health Organization Guidelines for the production, control and regulation of snake antivenom immunoglobulins. *WHO Tech. Rep. Ser.* **2013**, *964*.
45. Paré, J.A.; Lentini, A.M. Reptile Geriatrics. *Vet. Clin. North Am. - Exot. Anim. Pract.* **2010**, *13*, 15–25, doi:10.1016/j.cvex.2009.09.003.
46. Bewick, V.; Cheek, L.; Ball, J. Statistics review 12: Survival analysis. *Crit. Care* **2004**, *8*, 389–394, doi:10.1186/cc2955.
47. Therneau, T. Using Time Dependent Covariates and Time Dependent Coefficients in the Cox Model. **2012**, 1–8.
48. Guisande-Gonzáles, C.; Vaamonde-Liste, B.; Barreiro-Felpeto, A. Rwizard **2014**, 1–452.
49. Therneau, T. A Package for Survival Analysis in R. **2024**, 1–191.

Chapter 9

Ecoepidemiology and clinical outlook of the snakebites in the Colombian context

Carlos Andres Bravo-Vega, Lina Maria Peña Acevedo, Teddy Angarita-Sierra, Diana Marcela Walteros Acero, Katterine Isabel Urieles Sierra

Abstract: Colombia is a tropical country, with an elevated diversity of animals, plants, and human cultures. As a tropical country, snakebite is an important public health issue that affects mainly poor, rural populations. However, the country has a strong health surveillance system, where snakebite is an event of mandatory reporting since 2004. Nowadays, there is an average of 4,469 reported events that place Colombia as the third country in South America. Thanks to data gathered by the national surveillance system (SIVIGILA), this chapter presents an ecological analysis of the trends of snakebite during the last decade. Here the spatio-temporal behavior of the event, the trend of reporting behavior, demography, rural and urban burden, clinical traits, and the use of antivenom usage are dilucidated from SIVIGILA's data. In addition, this chapter makes recommendations on how to improve data gathering that will help to empower communities with the knowledge of snakebite ecology and how to enhance medical personnel capacitation that will reduce snakebite mortality as recommended by the World Health Organization global guidelines for snakebite.

Keywords: Snakebite; epidemiology; neglected tropical disease; envenomation; Colombia; neurotoxicity.



Citation: Bravo-Vega CA, Peña Acevedo LM, Angarita-Sierra, T, Walteros-Acero DM, Urieles-Sierra KS. Chapter 9. Ecoepidemiology and clinical outlook of the snakebites in the Colombian context. In Book: *Bites, venoms, and venomous snakes of Colombia*; Angarita-Sierra, T., Ruiz-Gomez, F.J, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 385–432.
doi: 10.33610/808870pfqmln

Copyright: © 2024 by the authors. Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0) license (http://creativecommons.org/licenses/by/4.0/).

Illustrations by:
Oscar A. Ramirez Ruiz

1. A decade of snakebites in Colombia: where are we now and where are we going?

During the last ten years (2010-2020) in Colombia about ~49,143 snakebite accidents have occurred, most of them taking place in rural environments (77%), with an annual average of 4,468.68 reported events, and an average per capita incidence of 9.27 events per 100,000 persons (Figure 1A). As a result, among South American countries, Colombia ranks third in the number of snakebites per year and sixth in snakebite incidents per 100,000 inhabitants [1]. During this decade, snakebite accidents showed a total annual average growth of 14.72% with a cumulative variation of 1.38%, with 2018 and 2019 as the years with the highest snakebite events reported. This means that during the period 2010-2020 snakebites increased an average of 65.76 ±6.16 events per year.

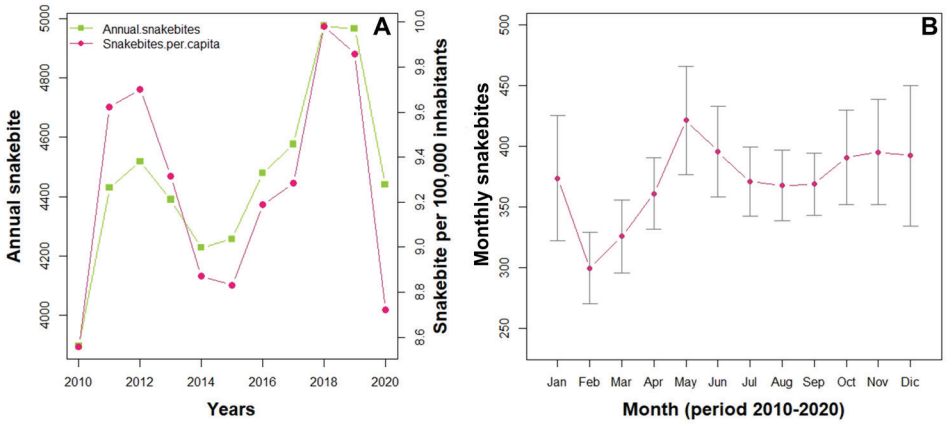


Figure 1. Snakebite incidence in Colombia. (A) Line chart depicting the variability of the absolute annual snakebite cases and snakebite incidence rate per 100,000 inhabitants during the period 2010-2020. (B) Line chart depicting the variability of absolute monthly snakebites cases per month during the period 2010-2020. Dots represent mean per month, and whiskers represent standard deviation (SE).

During the months of January, February, and March the lowest number of snakebite events were reported; and during May and November the highest number were reported (Figure 1B). This pattern coincides with the bimodal climate regimen of the Colombia regions that report the highest annual snakebites events (Figure 2) [2]. After performing a timeseries analysis including a seasonal and trend decomposition using loess (STL), which decompose timeseries in a series which capture trend and another one which captures seasonality, we found that there were two seasons with high risk, around April-June, and around October-December.

This corresponded to the bimodal rainfall pattern found in the Andean region of the country, that is more evident during this analysis in comparison with the description in Figure 1B. There is a conspicuously low risk season during January, corresponding to the marked dry season throughout the country. In Colombia, the nationally aggregated bimodal climate is characterized by two rainy seasons (April-June and October-November) and two dry seasons (mid-November to March and between July and mid-September).

So, annual snakebites at the national level suggest they are seasonal, with a major case reporting beginning in the rainy season and then decreasing at the end of the dry season. This relationship between incidence and rainfall has been deeply explored in Colombia by Bravo-Vega et al.

[3], where the authors show that dry seasons are the mechanisms behind snakebite incidence and rainfall association: Rainfall acts as a limiting resource, decreasing incidence during marked dry seasons. In addition, time-series analysis shows an interannual increase in cases between 2018 and 2020, that may correspond to the latest The La Niña meteorological phenomenon where average rainfall increases; and the decay shown in the trend after 2020 can be explained by the lockdown because of the COVID-19 pandemic [4,5]. Finally, the per capita snakebite incidence does not show a steady annual increase. Conversely, across the decade it exposes a heterogenous behavior, depicting two peaks in 2013 and 2019 (Figure 1), suggesting an interannual cyclical trend which can be associated with macroclimatic fluctuations such as el Niño and la Niña (Figure 2).

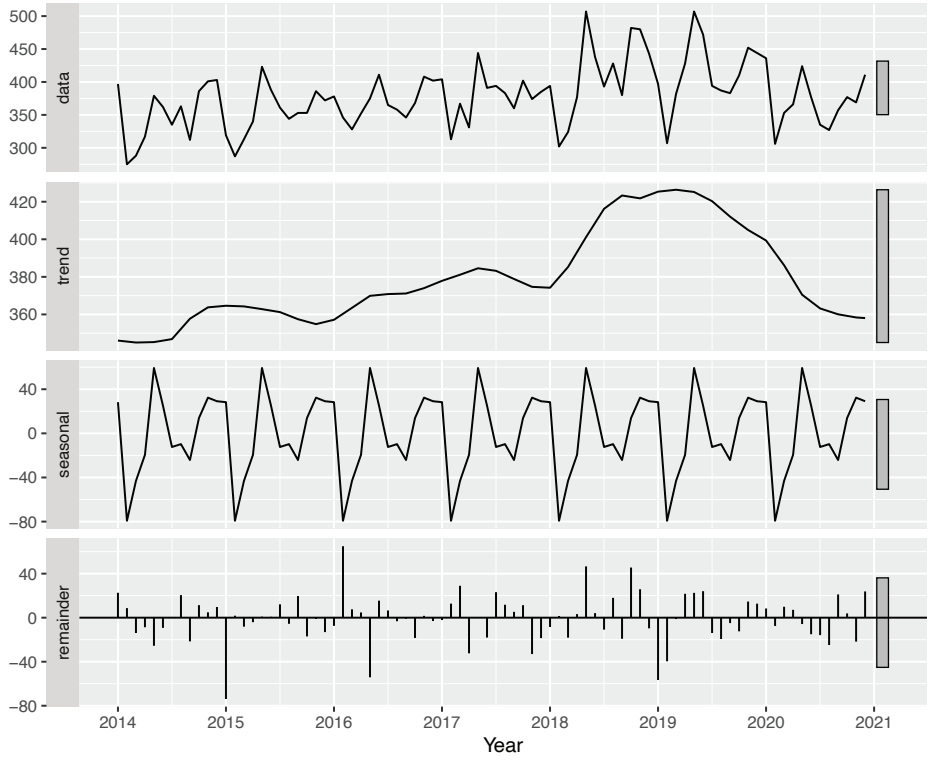


Figure 2. Seasonal and trend decomposition using loess (STL decomposition) of cases timeseries. We used monthly data and a frequency of data sampling = 12 to capture seasonal variation between months, thus capturing in the seasonal component intra-annual seasonality and the trend towards inter-annual variation. At the beginning of each year the seasonal component decreases conspicuously because of the marked dry season in the national aggregated rainfall. In addition, after 2018 the trend starts increasing until 2020, that corresponds to the latest La Niña phenomenon, and to the beginning of the COVID-19 pandemic.

1.1 Accumulative snakebite incidence in Colombia

The accumulated average of snakebite incidence has shown conspicuous inter-annual and intra-annual differences. Each timespan tends towards two different mathematical functions, indicating that they belong to different distributions, and exhibiting a distinctive behavior across time. Inter-annual snakebite incidence fits well with a saturation function (Fig. 3A, r^2= 0.88, P<0.0001) with its residuals fitting a normal distribution (D = 0.17, P= 0.47), meaning that the entire model is also significant and well-supported. Therefore, the theorical annual snakebite incidence expected in Colombia is about 4,401 events per year. However, the uncertainty of the regression model is about 12%, meaning the annual snakebite incidence can range from 3,872 to 4,929 snakebite events, that agree with the variability observed across the decade assessed.

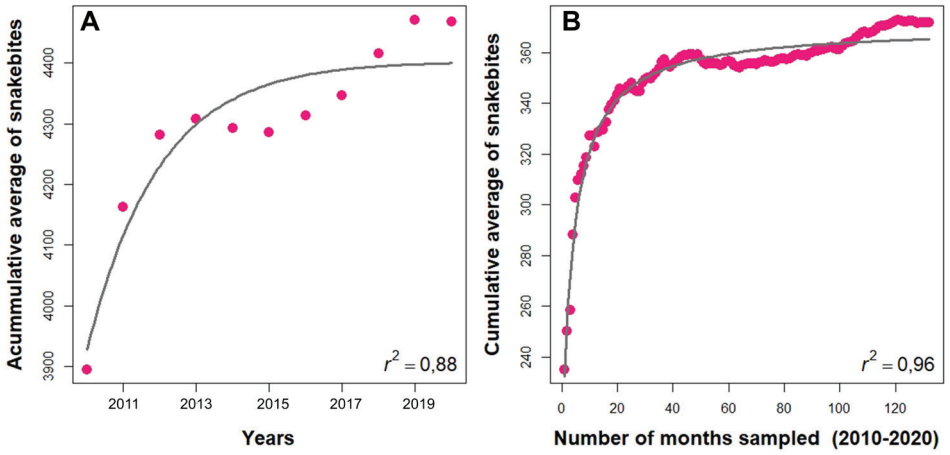


Figure 3. Theoretical predictions of snakebite incidence in Colombia. (A) Inter-annual snakebite incidence prediction by the saturation regression model. (B) Intra-annual snakebite incidence prediction by the rational regression model. Pink dots depict the annual and month cumulative average of snakebites. Black line depicts regression models.

The intra-annual snakebite incidence fits very well with a rational regression model (Fig. 3B, r^2= 0.96, P<0.0001), but its residuals did not fit a normal distribution (D = 0.096, P= 0.004), indicating that the model is significant but not well-supported. This fact does not invalidate the model since its predictive capacities are high (high r^2). However, its predictive power is constrained because the model cannot assure that the probability value shown is the correct one [6]. According to the rational regression model, the theoretical monthly snakebite incidence expected in Colombia is about 365.3 events per month with uncertainty ranging from 351 to 380 snakebite events per month. However, due to the poor fit of residuals, the monthly snakebite incidence predicted by the model may be close to 96% of real data.

Despite differences between inter or intra-annual snakebite incidences, as well as the constraints of mathematical predictions, both models conclude that the National Public Health Surveillance System: SIVIGILA, Spanish acronym) captures to a high degree (> 88% based on the analysis of the saturation function used to fit data) the completeness of the snakebite cases occurring during the last decade in the country. Thus, if the snakebite notification continues this tendency or it is enhanced for a short time, in a few years the reports could reach an asymptotic trend. This result can be seen as a national achievement whether during the timespan between 1975 to 1999 underreporting was huge with 70.8 cases per year, whilst before 2010 2,161 events per year were reported [7,8].

1.2 Spatial distribution of snakebites in Colombia

The geographically weighted regression (GWR) shows that bioclimatic and socioeconomic variables drive the spatial heterogeneity of the distribution of snakebite cases in Colombia (see appendix A and B for details). By dividing the incidence into urban and rural cases, the predictive capability of the model increases (Table 1). The difference between the R-squared of an ordinary multiple linear regression and the GWR is remarkable, thus the spatial structure of the cases plays an important role driving incidence.

Table 1. Results of snakebite incidence models

Population	R-squared*	GWR R-Squared	Explanatories variables
Total**	0.19	0.56	Population, NBI, B1, B4, B7, B12, B15, B19
Urban	0.05	0.53	Population, Human footprint index, B12, B18
Rural	0.36	0.67	Population, Human footprint index, B1, B4, B7, B12, B18, B19

*This value corresponds to a multiple linear model that does not account for spatial structure. B1, B4, B7, B12, B15, B18 and B19 correspond to bioclimatic variables obtained from the Worldclim server. (Appendix A and B for details). ** Total population means urban + rural populations in which snakebite incidence occurs.

We calibrated models by using all variables, and with Akaike information criterion (AIC), that compensate the model's predictive capacity by the number of parameters; and we selected the "best" model. The independent variables that explained better snakebite incidence changed between areas (Table 1), that may be related to the distinctive drivers of snakebite incidence affecting urban and rural populations. The precipitation (B12) and population were the only explanatories variables shared by urban, rural, and total (urban+rural) populations assessed. The distribution of the GWR coefficient for both variables can be seen in Figure 4. (The distribution for the other variables is shown in appendix, as well as cases at a municipality scale are shown in appendix B).

These differences underline the importance of analyzing discriminated data, because by using aggregated data several associations and important behaviors could be masked. For example, the human footprint does not drive total incidence, but it is important in the rural and urban incidence (see Figure S2 in appendix B). In fact, the coefficient is negative in rural cases, suggesting that an increase in this index will decrease the cases, but it is positive for urban cases. (Human footprint index = 0-100, where zero (0) indicates no human impact or footprint, and 100 indicates total human impact or footprint that has completely transformed the original ecosystem)

Thus, a complex relationship is behind the human footprint and snakebite, that may be explained by the spatial distribution of the coefficient. For urban populations, the positive association is mainly in Meta department, that encompasses Orinoco savannas. Due to its proximity to the country's capital, this department has experienced a rapid expansion of both urban and agricultural frontiers, leading to habitat loss for snakes. Consequently, this has reduced the likelihood of snakebite incidents within urban areas. However, it has simultaneously increased the frequency of snakebites in peri-urban zones where such frontier expansion is occurring. On the other hand, the negative association observed in rural areas located in the northern Pacific region, the middle Orinoco River basin, and the Amazon indicates that the probability of snakebite accidents increases when rural settlements are situated near or within natural snake habitats. This is further exacerbated by the exposure of farmers and rural workers to activities that directly disturb these habitats. Even so, this complex effect must be detailed to understand the association between anthropic intervention and snakebite incidence, and its relevance to public health.

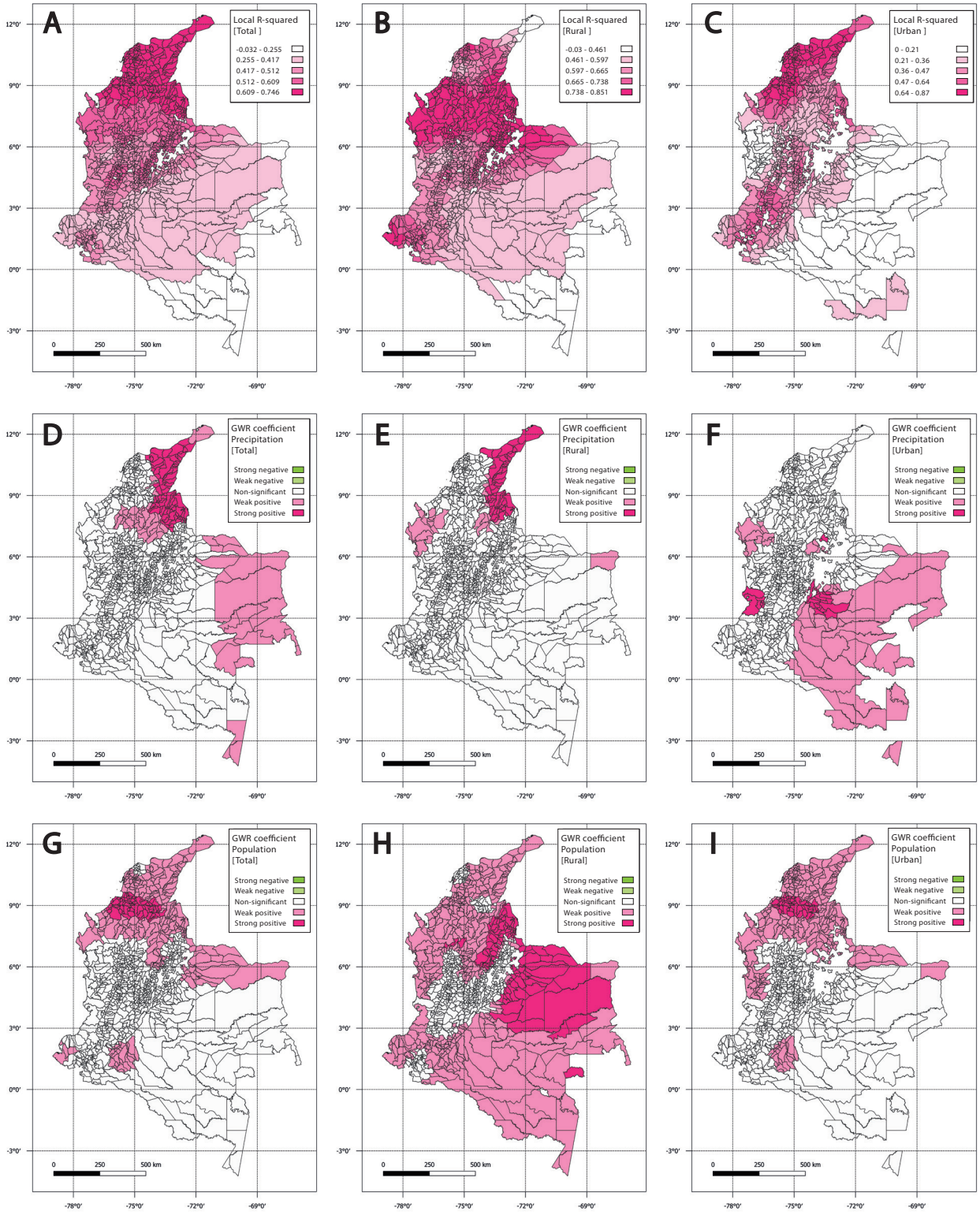


Figure 4. R-squared and GWR coefficients spatial distribution. (A-C) Local R-squared for the GWR model in the three populations (Total, Rural and Urban). Note the spatial variation of the values, where urban and total population adjust the Caribbean coast, but rural populations adjust better in the southern and northern Pacific, the middle Magdalena valley, and the northern Orinoco plains. (D-F) Illustrate GWR coefficient for precipitation. Note the differences between the spatial distribution, where for rural populations the precipitation strongly affects the incidence on the northern Caribbean coast and in the Catatumbo region, whilst it affects urban cases in most of the Orinoco and Amazonian region. (G-I) Depict GWR coefficient for the population. Note how the rural population drives the incidence in most of the country, whilst urban populations drive the incidence mostly in the northern Pacific and the Caribbean coast.

1.3 Per capita snakebite incidence

Per capita snakebite incidence showed wide variability across Colombian departments and ecoregions (see https://ofidismo.ins.gov.co/). Despite the trans-Andean departments of Antioquia, Bolivar, Norte de Santander, and Cesar having the highest annual number of snakebites (Figure 5), the cis-Andean departments of Vaupes, Guaviare, Amazonas, Caqueta, Putumayo (Amazon ecoregion), Arauca, Vichada, and Casanare (Orinoquia ecoregion) exhibited the highest per capita incidence (Table 2).

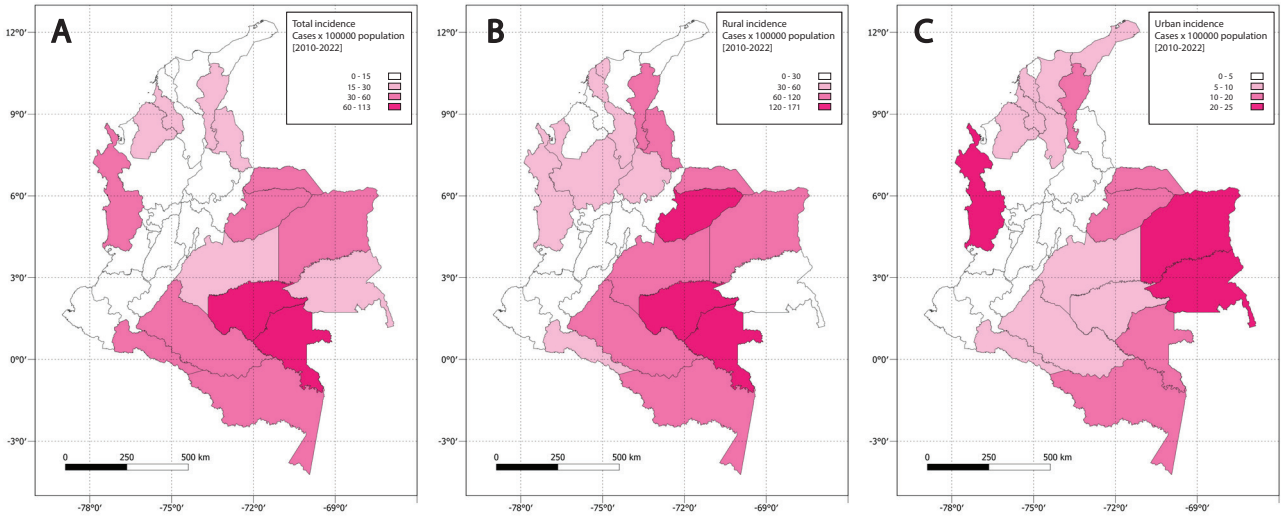


Figure 5. Incidence per 100,000 inhabitants during 2010-2020. (A) Total snakebite incidence. (B) Urban snakebite cases. (C) Rural snakebite incidence.

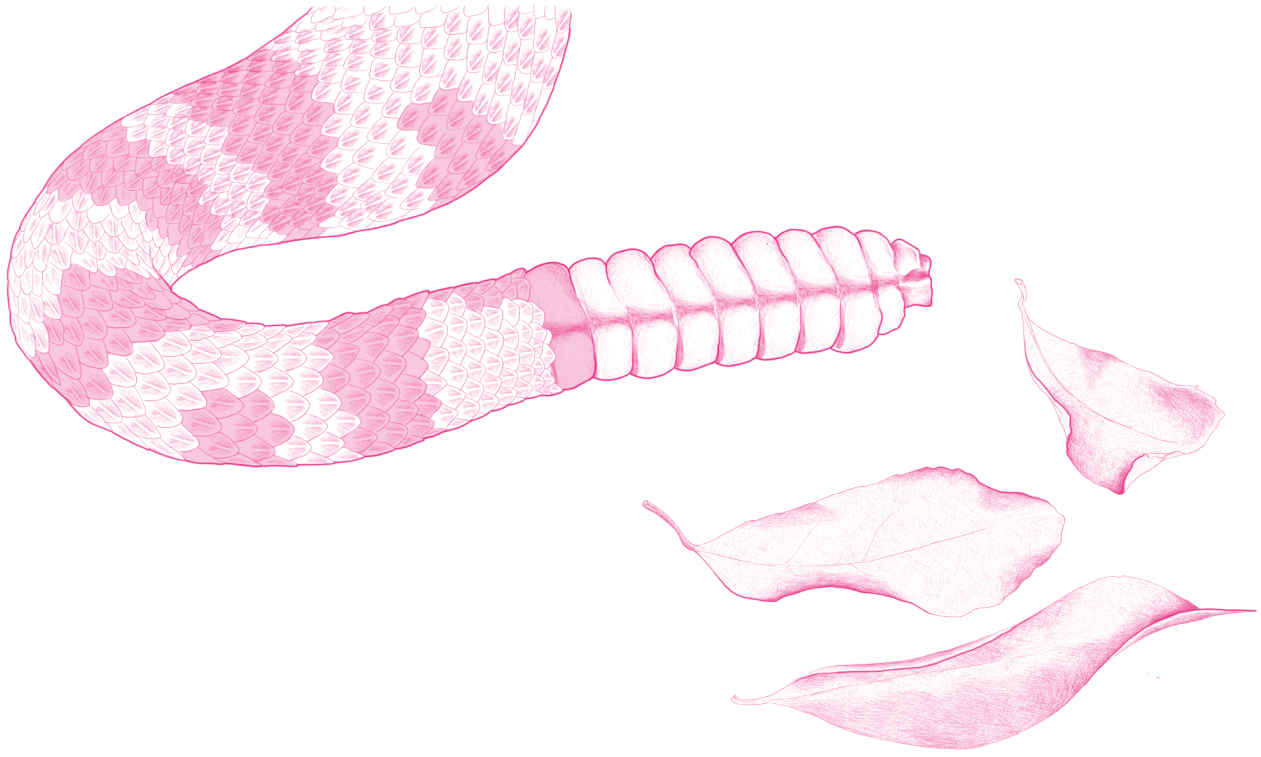


Table 2. Snakebite incidence per 100,000 inhabitants (2010-2020) divided by rural, urban, and total (rural+urban) populations inhabiting Colombian territorial entities.

Department	Mean per capita rural incidence	Mean per capita urban incidence	Mean per capita total
Amazonas	82.8	19.5	59.7
Antioquia	37.1	1.5	9.3
Arauca	101.9	12.1	45.4
Atlantico	38.1	3.8	5.3
Bogotá D.C	1.1	0.0	<0.001
Bolivar	39.3	6.3	13.8
Boyaca	12.0	0.7	5.5
Caldas	18.3	1.2	6.0
Caqueta	67.6	9.2	33.0
Casanare	133.5	11.9	43.8
Cauca	11.8	2.6	8.2
Cesar	66.7	12.5	26.3
Choco	50.4	24.0	37.5
Cordoba	25.5	6.4	15.4
Cundinamarca	6.1	0.5	2.3
Guainia	29.6	23.4	27.8
La Guajira	17.4	5.5	10.9
Guaviare	164.6	5.7	71.6
Huila	24.5	2.2	11.1
Magdalena	28.6	8.3	13.7
Meta	81.5	7.8	25.7
Nariño	10.3	1.7	11.1
Norte Santander	84.3	4.6	16.8
Putumayo	58.6	8.8	34.4
Quindio	7.3	0.8	1.6
Risaralda	17.9	0.7	4.4
San Andrés archipelago	0.0	1.8	1.3
Santander	30.8	2.3	9.3
Sucre	27.0	9.7	15.4
Tolima	16.7	2.4	6.9
Valle del Cauca	10.1	0.7	1.9
Vaupes	170.2	19.1	111.9
Vichada	70.3	23.6	50.0
Mean	46.7	7.3	22.3
National per capita incidence			9.3
National per capita incidence, disaggregated by population group	5.0	0.78	2.4

All per capita incidences for each department are of the annual average of the per capita incidences during the period 2010-2020. The national per capita incidence was calculated based on the national population per 100,000 inhabitants during the period 2010-2020.

The cis-Andean departments share the lowest human population in the country mainly living in rural areas (~60-63%) rather than urban settlements (~37-40%) and having close interaction with intact forest landscapes [9]. Hence, in a demographic sense, these departments depict an opposite pattern to that observed at the national level in which ~70% of Colombians live in urban settlements rather than rural areas [9,10]. This fact, in addition to the snakebite occurrence described above, highlights the Colombian rural population as the highest vulnerability for snakebite accidents.

Nevertheless, as global a standard, in the Colombian epidemiological surveillance system the per capita snakebite incidence is calculated based on the total population per 100,000 inhabitants rather than considering rural and urban populations separately [11,12]. Given that in Colombia most snakebite envenomation patients live in rural environments, calculating the per capita snakebite incidence following the global standard raises significant bias about the real snakebite incidence in the country.

Previous assessments in Colombia have employed the standard measurement of incidence, with some misunderstanding the data, and reporting a per capita snakebite incidence ranging from 6.3 events for lowest populated localities to 20 events in the most populated departments or municipalities [13,14]. Others underestimate the incidence per department or ecoregion since rural and urban populations were not considered separately [15]. After analyzing snakebite events during the period 2010-2020, we found a significant differences in the per capita snakebite incidence between rural and urban populations (Figure 6; $H_{(2)} = 325$, $P < 0.0001$). Indeed, rural population was two to three-fold higher than the total and urban per capita snakebite incidence. Moreover, the per capita snakebite incidence by department ranged 0–170.2 ($\bar{x} = 46.7$) events for rural populations, 0–24 ($\bar{x} = 7.3$) for urban populations, to 0-11.2 ($\bar{x} = 22.3$) events in the total departmental population.

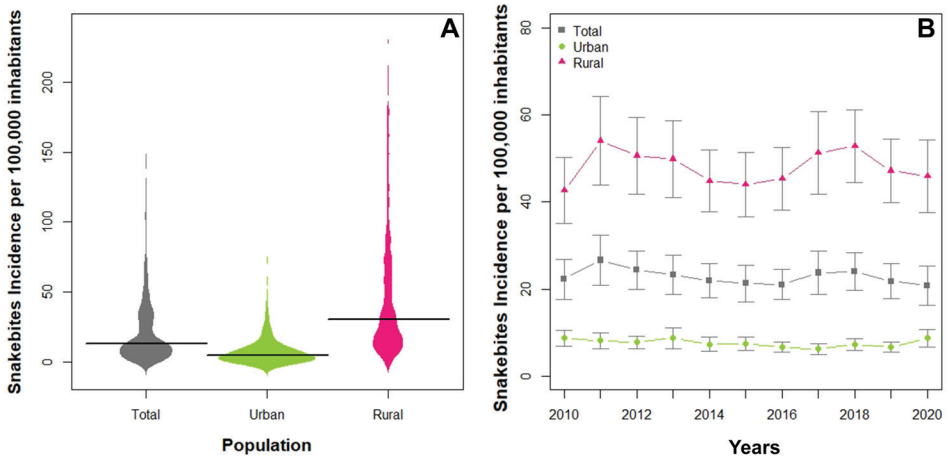


Figure 6. Comparisons of per capita snakebite incidence between rural, urban, and total department (standard calculus) populations clustering by years. (A) Bean plot depicting the per capita snakebite incidence distribution by population. The bold horizontal line depicts median. (B) Line chart depicts per capita snakebite incidence by population across timespan studied (2010-2020). Symbols represent mean and whiskers show the standard error (SE).

The average rural per capita snakebite incidence is five-fold the national per capita incidence reported by previous assessments 9.3 events [13–16]. These results indicate that the standard measurements under-

estimate the impact of the annual snakebites in vulnerable rural populations. Furthermore, the standard incidence measurement masks the association between occupational, environmental, or behavioral factors that could be impacting snakebite accidents due to the lack of differentiation between the areas in which the accidents occur.

An accurate estimation of the annual per capita snakebite incidence is of paramount importance to improve the prevention of snakebites and to control the incidence [11]. Therefore, we recommend calculating the per capita snakebite incidence, considering rural and urban populations separately. In this fashion, epidemiological interventions and strategies that seek to mitigate and prevent snakebite accidents can be addressed based on a better understanding of the incidence.

One of the most serious constraints in the estimation of the incidence of snakebites is robust data acquisition. Snakebite epidemiological surveillance of developing countries like Colombia frequently have significant shortfalls due to the lack of resources, trained health staff, or technological infrastructure. However, since 2004 when snakebite was included in the SIVIGILA as a mandatory report [13], continuous improvements have been made to accelerate the learning curve, as well as to enhance the national epidemiological strategy [8,18]. Currently, the growing number of snakebite accidents reported between 2010-2020 reflect the past efforts to improve the Colombian epidemiological surveillance strategy. Additionally, throughout the decade studied, a total of 26 atypical values of per capita snakebite incidence were observed in the departments of Vaupes, Guaviare, Casanare, and Atlantico. These snakebite events were negatively associated with time (R^2= -0.67, P= 0.025), indicating that as the snakebite notification system improved its data acquisition, extreme values dropped.

Nevertheless, due to large and isolated rural populations in Colombia and their limited access to the national health system [19], currently, SIVIGILA continues having a significant underestimation of snakebite incidence. Even though epidemiological data gathered via medical records is the most cost-effective way to obtain reliable data [20], several socio-demographic biases (e.g., patient seeking for traditional healers, or low family income) constrain the accurate account of annual total snakebite events reported. To overcome these difficulties for estimating accurate snakebite incidence and to reduce uncertainties due underreporting, mathematical models have been proposed that can estimate the geographic patterns of snakebite incidence, as well as the underreporting [20].

Particularly in Colombia, recent research has developed a Bayesian estimation framework, using a generalized linear mixed effect model based on theoretical models to estimate snakebite events underreported in the country [21]. This tool focuses on snakebites of *Bothrops asper* (Common lancehead pitviper) and *B. atrox* (Mapanare), the two most medically important venomous snake species in the country that cause ~62% of the snakebite events [14,22,23]. This research estimated that around 10% of

the total cases are not reported, so around 447 cases are not reported yearly. This number can be considered as small but given that mortality and morbidity will increase in these cases the absence of medical attention, amputations and deaths will be underestimated.

These models showed that the Orinoco and Amazonian basin (cis-Andean region) share the highest snakebite risk and the highest underreporting in the country. These results agree with per capita incidence assessments discussed above, as well as high rural per capita incidence observed in the Colombian departments located in the cis-Andean region (Table 2). Further, these models show that the higher snakebite risk values and reported incidence tend to cluster in deprived populations located in departments characterized by low population densities, low coverage of road infrastructure, presence of indigenous populations, low urbanization, and in which *B. atrox* is distributed (cis-Andean region).

Therefore, despite most of the annual envenomation reports in Colombia coming from the Antioquia department and Caribbean region (trans-Andean region), that are densely populated and where *B. asper* is distributed, the people living in departments located in the cis-Andean region in which *B. atrox* is distributed are more prone to suffer a snakebite.

Statistical inference models rise as an effective and cost-effective way to estimate the snakebite burden in tropical countries like Colombia, constrained by sociodemographic and infrastructure factors. Nevertheless, mathematical models cannot replace the incidence data gathered via medical records since they have limitations, as explained below.

Data sampling.— Most species occurrence data is based only on species presence records, causing bias in the niche or distribution models (see https://ofidismo.ins.gov.co/). As most of the tropical areas and species, Colombian snake species lack large sets of data including both presence and absence. Hence, niche or distribution models commonly exhibit strong spatial biases in survey efforts, causing in most cases spatial autocorrelation [24,25]. This bias can have a deep impact on the spatial correlation between eco-epidemiological variables, as well as on the quality of the model that seeks to explain the variability of the snakebite incidence. Therefore, species occurrence models based on presence-only data must be carefully employed by users, and statistical adjustment must be carried out to obtain the “best” model possible [24].

Exposure of population assumptions.— In Andean tropical countries like Colombia, several municipalities have cities and towns located at high elevations where nobody or very few venomous snakes are found, but the incidence is calculated by clustering the cases by municipality without considering the elevational gradient in which snakebite events occur. This causes a serious bias in the correlation between the geographic distribution of snakebite incidence and snake species distributions, as well as in the correlation with environmental variables associated with the dynamics of snakebite envenomation [21].

Sympatric species and crypticity.— In Colombia, like most of the megadiverse tropical countries worldwide, several species share and overlap their distributions (see Chapter 1). Hence, closely related or species resembling each other can generate bias in taxonomic species identification during the procedure of filing medical reports. Particularly, due to the difficulties of taxonomic species identification, the Colombian notification snakebite sheet only identifies most of the genera of venomous snakes (e.g., *Bothrocophias* genus is missing), but not species when snakebite is reported.

Hence, despite snakebite accidents being caused by species-specific negative interactions, SIVIGILA cannot directly detect the involved species. The misidentification of snake species generates significant bias for snakebite frequencies, as well as in the total number of snakebites per species. Furthermore, due to the parameter of models defining the probability that an encounter with a snake species causes a bite must be calibrated, when snakebite is caused by multiple species, it is necessary to perform a multiple linear regression based on measured encounter frequency for each species separately [20].

Despite their limitations, mathematical models are powerful predictive tools when used synergistically with data collected by epidemiological surveillance systems like SIVIGILA, helping to determine the snakebite incidence at local, regional, and national levels, as well as the real demand of antivenom, and selecting important epidemiological parameters and factors underlying the snakebite ecoepidemiology [14,20,26].

1.4 Snakebite demography

As expected, in Colombia men are significantly more prone to suffer snakebite than women ($t_{(12.05)} = -23.68$, $P < 0.0001$), with a female to male annual ratio of snake-bitten victims of 1: 2.3–2.7. This pattern agrees with the general accident rate pattern by gender observed in the Americas due to the cultural labors done in rural environments, as well as the patterns observed in developing countries [1,27]. In both genders, most of snakebites occur in young people who range 12-29 years old and that comprise the labor force in rural areas (Figure 7A). However, snakebites in Colombia occurs from early years (infants of 0.5-5 years old) to elder ages (elders of 85-110) and are frequent in infants (~4.0%) and infrequent in elders (< 1%).

During the period 2010-2020, 239 foreign persons received medical care in Colombian health institutions due snakebite. Snakebite events followed a similar pattern by gender observed in the Colombia population. Most snakebites occurred in young people ranging from 12-29 years old (Figure 7). However, in the foreigner population there was a marked heterogeneity within the female age-ranks, in which women of middle age (38–55-year-old) were prone to suffer snakebite (Figure 7B).

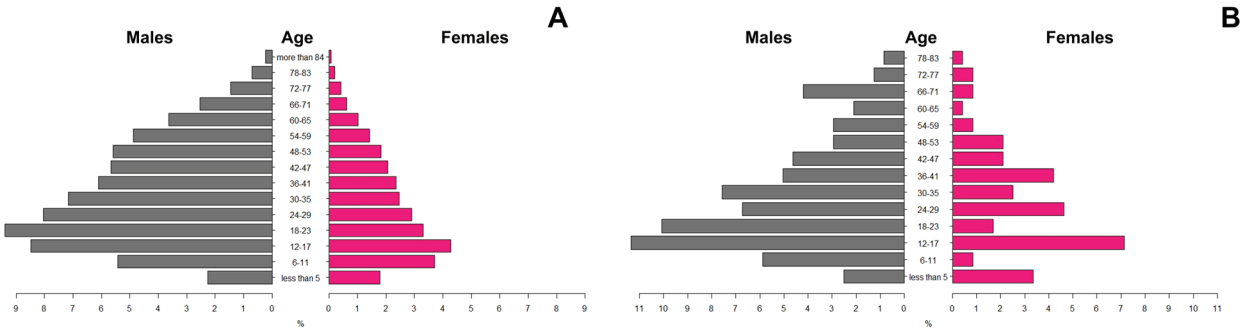


Figure 7. Snakebite accident distribution per age rank. (A) Pyramid plot depicting the age rank distribution of snakebite accidents in the Colombian population during the period 2010-2020. (B) Pyramid plot depicting the age rank distribution of snakebite accidents of the foreigner population that received medical care in theColombian health institutions during the period 2010-2020.

A detailed revision of snakebites of the Colombian social-vulnerable populations showed that among ethnic groups present in the country, indigenous and afro-Colombian populations cluster 97% of the snakebites (N= 8,888 events), the highest frequency of snakebites is found in the Indigenous population (53%) as compared to afro-Colombians (44%) populations. Moreover, infants have an annual incidence average of 178.7 bites per year and are bitten mainly in rural environments (74%). The infants belong to communities of farmers with no ethnicity declared (78%) and to indigenous (14%) and afro-Colombian (8%) communities. In contrast, about 4% of the total snakebites reported in the last decade were suffered by the remaining social-vulnerable populations: handicapped, displaced, migrants, prisoners, pregnant, Indigents, orphans, community mothers, demobilized, psychiatric patients, and violence victims.

Most of the snakebites in the country occurred in persons belonging to socioeconomic strata 1 and 2, clustering respectively about 84% and 15 % of total snakebites in the country. Nevertheless, these proportions may be underestimated or have biases because around 75% of the snakebite notification records lacked socioeconomic strata assignments. The houses or properties of strata 1 and 2 represent the lowest socioeconomic strata in the country [28], meaning that the people live in homes with the lowest resources and livelihoods carry the most burden of snakebites in Colombia.

Most of the snakebite accidents encompassed during the period 2010-2020 (~49,143 events) were persons that belong to subsidized health schemes (71.5%), or they were uninsured (11.6%), while the remaining 16.9% were persons belonging to contributing health scheme (14.5%), or specials or excepted health schemes (1.9%; soldiers, police, and other occupations). This means that, in addition to the burden carried by low-income population living in rural areas, snakebite accidents represent a significant load on the national health system because most affected persons belong to subsidized schemes.

Regrettably, all these trends described above have not changed from the national evaluation of snakebite incidence made in 2010 [14], indicat-

ing that national efforts made in the last decade to reduce the snakebite accidents in Colombia and its burden on deprived populations and the health system, have not had a relevant impact. Therefore, the Colombian government must continue strengthening and enhancing its public health strategies to face snakebite accidents because it remains a neglected tropical disease.

2. The clinical picture of snakebite envenomation in Colombia

After analyzing SIVIGILA information regarding snakebite symptomatology between 2010 and 2020, we found that most bites were caused by snakes from the family Viperidae. Snakebite cases caused by species of the genera *Bothrops, Bothriechis,* and *Porthidium* were grouped under the category of bothropic envenomation (62%, Table 3). In all types of envenomation, mild envenomation represents 56-60 % of the total cases reported by SIVIGILA, while severe cases represent 6.4-10%, (Table 4). We propose a general algorithm for the diagnose of the type of envenomation in Colombia based on snakebite patient symptomatology (Figure 8).

Table 3. Frequency of the type of snake envenomation reported in Colombia by SIVIGILA during 2010-2020.

Envenomation type	Number of cases reported during 2010-2020	%
Pitvipers		
Bothropic	30,528	62.4
Crotalic	1,317	2.7
Lachesic	254	0.5
Elapids		
Micruric	601	1.2
Hydrophic	65	0.1
Colubrids	345	0.7
Other	3,004	6.1
Not identified	1,2821	26.2
Total	48,935	100

Table 4. Envenomation type and severity frequency reported during 2010-2020.

Severity	Bothropic	%	Crotalic	%	Lachesic	%	Micruric	%
Mild	16,966	56	767	58.2	135	53.1	380	63.2
Moderate	11,138	36	461	35	73	28.7	157	26.1
Severe	2,424	8	89	6.7	46	18.1	64	10.6

Bothropic: Envenomations caused by species of the genera *Bothrops*, *Bothriechis*, *Bothrocophias* and *Porthidium*. Crotalic: Envenomations caused by *Crotalus durissus*. Lachesic: Envenomations caused by *Lachesis acrochorda* and *L. muta*. Micruric: Envenomations caused by species of the genus *Micrurus*.

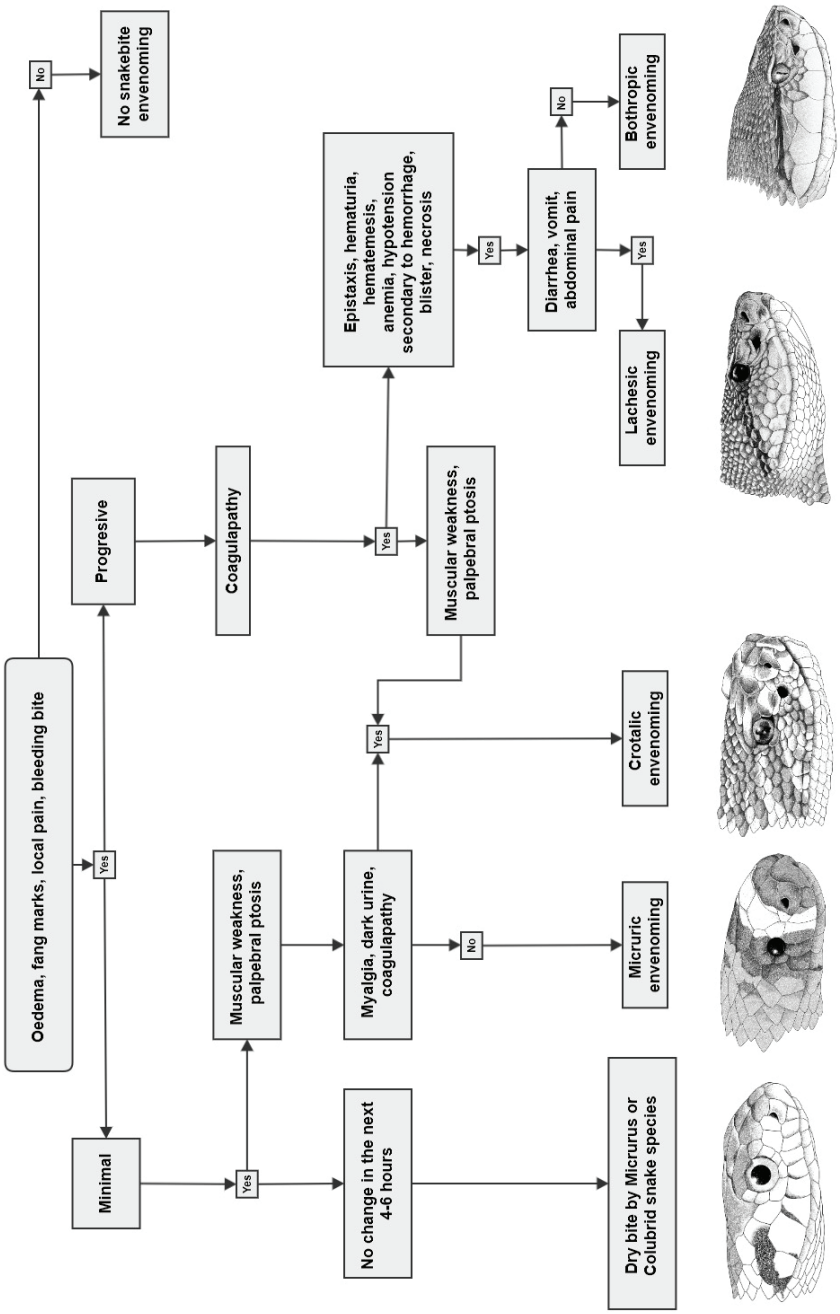


Figure 8. General algorithm for the diagnose of envenomation type in Colombia. Illustrations by Oscar A. Ramirez Ruiz.

2.1 Envenomation caused by pit viper snakes

Bothropic envenomation

Snake venom from the genera *Bothriechis, Bothrocophias, Bothrops* and *Porthidium* have toxins that together cause similar symptomatology, making it possible to clinically cluster their envenomation under a single category named bothropic envenomation. Some of these toxins are phospholipase A_2, metalloproteinases, serine proteases, and l-amino acid oxidases, which affect coagulation, destroy tissues, and promote inflammation [29] (See Chapter 5). According to SIVIGILA data for 2010-2020 the most frequent local symptoms are fang marks, pain, edema, and erythema, occurring in 91.6%, 90.9%, 85.4% and 38.4% of the cases (Table 5)

Table 5. Local symptoms caused by pit viper snakebites.

	Bothropic cases	%	Crotalic cases	%	Lachesic cases	%
Total cases	30,528		1,317		254	
Symptoms						
Fang marks	27,986	91.6	1,010	87.4	222	76.6
Pain	27,762	90.9	1,042	87.4	222	79.1
Oedema	26,091	85.4	1,113	78.3	199	84.5
Erythema	11,730	38.4	533	39.3	100	40.4
Equímosis	47,98	15.7	147	15.7	40	11.1
Blisters	1,949	6.3	76	9	23	5.7
Necrosis	688	2.2	23	2.4	6	1.7

Bothropic: Envenomations caused by species of the genera *Bothrops*, *Bothriechis*, *Bothrocophias* and *Porthidium*. Crotalic: Envenomations caused by *Crotalus durissus*. Lachesic: Envenomations caused by *Lachesis acrochorda* and *L. muta*.

At least 21.7% of patients had systemic symptoms. The venom of these snakes typically causes symptoms of anticoagulation, such as disseminated intravascular coagulation (DIC), epistaxis, hematuria, hematochezia, hematemesis, anemia, hypotension secondary to hemorrhage or third spacing; but the incidence of these symptoms in the last decade was low. Each one of these symptoms occurred in less than 5% of the patients. The most common hemotoxic symptom was gingival bleeding, representing 6.2% of the cases. Another important systemic symptom was acute kidney failure, that was present in 1.02% of cases (312 cases). Finally, necrosis and compartmental syndrome, associated with local severity, only occurred in 2.25% and 0.75% of the cases. Additionally, the most common infectious symptoms were cellulitis (20.6%), and abscesses (3.26%). Table 6 shows the specific results for each type of envenoming.

Table 6. Frequency of systemic symptoms by viperid envenomation according to SIVIGILA during 2010-2020.

Systemic symptoms	Bothropic	%	Lachesic	%	Crotalic	%
Nausea	8,462	27.7	70	27.6	361	27.4
Vomit	4,690	15.4	42	16.5	207	15.7
Sialorrhea	764	2.5	7	2.8	40	3.0
Diarrhea	421	1.4	7	2.8	16	1.2
Bradycardia	474	1.5	9	3.5	20	1.5
Hypotension	1,091	3.6	12	4.7	43	3.3
Abdominal pain	1,640	5.4	22	8.7	70	5.3
Neurological facies	198	0.6	2	0.8	18	1.4
Visual disturbance	490	1.6	6	2.4	26	2.0
Sensory disturbance	348	1.1	4	1.6	16	1.2
Muscular weakness	2,506	8.2	20	7.9	114	8.7
Oliguria	463	1.5	4	1.6	15	1.1
Cyanosis	305	1.0	4	1.6	13	1.0
Epistaxis	354	1.2	219	86.2	12	0.9
Gingival bleeding	902	6.2	17	6.7	46	3.5
Hematemesis	836	2.7	10	3.9	22	1.7
Hematuria	693	2.3	6	2.4	27	2.0
Hematochezia	102	0.3	1	0.4	4	0.3
Vertigo	1,564	5.1	10	3.9	68	5.2
Eyelid ptosis	74	0.2	1	0.4	9	0.7
Speech difficulty	409	1.3	5	2.0	24	1.8
Dysphagia	111	0.4	0	0	9	0.7
Cellulitis	6,294	20.6	47	18.5	277	21.0
Abscess	997	3.3	3	1.2	46	3.5
Necrosis	688	2.2	6	2.4	23	1.7
Myonecrosis	122	0.4	1	0.4	5	0.4
Fasciitis	224	0.7	4	1.6	3	0.2
Circulatory disorders	648	2.1	7	2.8	27	2.0
Compartment syndrome	228	0.7	3	1.2	10	0.8
Anemia	393	1.3	4	1.6	9	0.7
Hypovolemic shock	250	0.8	6	2.4	14	1.0
Septic shock	146	0.5	1	0.4	3	0.2
Acute respiratory infection	312	1.0	6	2.4	8	0.6
Disseminated intravascular coagulation	188	0.6	3	1.2	3	0.2
Subarachnoid hemorrhage	99	0.3	2	0.8	1	0.1
Cerebral edema	99	0.3	2	0.8	7	0.5
Ventilatory failure	173	0.6	4	1.6	18	1.4
Coma	58	0.2	0	0	1	0.1
Death	249	0.8	4	1.6	7	0.5
Septic shock	6,294	20.6	47	18.5	277	21.0

N: Number of cases reported by SIVIGILA.

Lachesic envenomation

The symptoms for this envenomation are like bothropic envenomation, but its severity is stronger due to the large amounts of venom injected by *Lachesis* species, as well as given the cholinergic nature of the venom. Typical symptoms of envenomation are bradycardia, diarrhea, and hypotension [22]. The percentage of cases caused by *Lachesis* between 2010-2020, was 0.5%; only 18% of these accidents were classified as severe. Besides, 55% of the bites were in the lower limbs, 38% were in the upper limbs and the rest in other locations. The most frequent manifestations were visible fang marks (87.4%), pain (87.4%), epistaxis (86.2%), and edema (78.3%). Others, such as hypotension, bradycardia, and diarrhea, were reported in only 4.7%, 3.5%, and 2.7%, respectively. The most frequent type of local infection was cellulitis, which was reported in 18.5% of the cases (Table 5 and 6).

Crotalic envenomation

In Colombian, crotalic envenomation is caused by *Crotalus durissus* and is characterized by the typical coagulopathy symptoms observed in snakebites caused by species of the Viperidae family. However, local symptoms are less critical than those present in bothropic and lachesic envenomation. Crotalic envenomation have singular symptoms such as rhabdomyolysis, dark urine, secondary kidney failure, and neuromuscular paralysis, that are considered as characteristics of crotalic bites [22].

Throughout the assessed period from 2010 to 2020, crotalic envenomation demonstrated rare hemorrhagic manifestations, occurring in fewer than 4% of patients. Neurotoxic effects such as ventilatory failure, palpebral ptosis, slurred speech, or neurotoxic facies were described in less than 2% of the patients; and muscle weakness showed up in 8.6% of the cases. Those related to rhabdomyolysis and secondary renal failure occurred in less than 1% of cases. The most frequent type of local infection was cellulitis reported in 21% of cases (Table 6).

The percentage of cases of crotalic envenomation throughout the last decade were 2.7%, of which 56% were in the lower limbs, 31% in the upper limbs, and the rest in other parts of the body. The most frequent manifestations in this type of envenomation were local: edema (84.5%), pain (79.1%), fang marks (76.6%), and erythema (40.4%). In addition, ecchymosis, blisters, and necrosis were described in 11%, 5.7%, and 1.7% of cases (Table 5).

2.2 Envenomation caused by elapids

Micruric and Hydrophic envenomation

The venom of New World elapids is neurotoxic. These toxins affect the functioning of the neuromuscular junction both at the pre- and postsynaptic level (see Chapter 5). The clinical picture of neurotoxic envenomation is characterized by muscle weakness that is initially seen in the smallest muscles (eyelids, oculomotor muscles, phonation, and swallow-

ing), progressing to larger muscle groups until it leads to respiratory failure and death in few hours [30].

About 63% of the cases of neurotoxic envenomation corresponded to mild envenomation (only local manifestations), 26% moderate and 10% severe; 50% of the envenomations was in the lower limbs; and 38% was in the upper limbs. bites on the fingers and toes occurred in 6% and 3% of the cases, respectively. Body areas that traditionally have been considered unlikely for a Micrurus snakebite due to the size and limited capacity of the snake's mouth to open (such as the head, neck, thorax, back and abdomen; see Chapter 5), occurred in less than 1% of the cases.

In neurotoxic envenomation the local symptoms are scarce, but according to the SIVIGILA data, in more than 75% of the cases, there were findings such as fang marks (78.9%) and pain (80.9%), while less than 5% of the cases showed characteristic symptoms such as slurred speech, palpebral ptosis, visual disturbances, sialorrhea, dysphagia, neurotoxic facies, and ventilatory failure (Table 7). Slurred speech, muscle weakness, drooling, dysphagia, and visual disturbances were the most frequent manifestations in moderate poisoning, and ventilatory failure occurred in severe cases (91%) (Table 8).

Table 7. Distribution of symptom frequencies caused by *Micrurus sp.* envenomings during 2010-2020.

Symptoms	Number of cases reported during 2010-2020	%
Fang marks	474	78.9
Pain	486	80.9
Oedema	350	58.2
Erythema	244	40.6
Nausea	168	27.9
Paresthesia	156	26.0
Muscular weakness	79	13.1
Vomit	59	9.8
Sialorrhea	24	4.0
Vertigo	31	5.2
Slurred speech	27	4.5
Palpebral ptosis	25	4.2
Ventilatory failure	22	3.7
Dysphagia	12	2.0
Neurotoxic facies	22	3.7
Blisters	11	1.8
Gingival bleeding	6	1.0
Epistaxis	3	0.5
Visual disturbance	28	4.7
Sensory disturbance	23	3.8
Total cases reported by SIVIGILA (2010-2020) = 601.		

Table 8. Distribution of systemic symptom frequencies caused by *Micrurus sp.* Envenomings divided by severity during 2010-2020.

	Moderate		Severe	
Symptoms	**Cases reported**	**%**	**Cases reported**	**%**
Paresthesia	42	26.9	23	14.7
Muscular weakness	32	40.5	23	29.1
Vomit	32	54.2	14	23.7
Sialorrhea	10	41.67	11	45.8
Vertigo	10	32.3	2	6.4
Slurred speech	13	48.1	12	44.4
Palpebral ptosis	9	36	14	56
Ventilatory failure	2	9.1	20	90.9
Dysphagia	5	41.7	6	50
Visual disturbance	12	42.9	7	25
Sensory disturbance	6	26.1	9	39.1
Neurotoxic facies	7	31.8	14	63.6

In 131 cases (21 %) the common name of the snake was related to pit vipers rather than coral snakes. Thus, it is highly possible that symptoms such as gingival bleeding, epistaxis and blisters were reported in the SIVIGILA form, given the species likely misidentification. Even so, coral snake' venom does have phospholipases and there are some reports of coagulopathy [31], but these symptoms are rarely related to bleeding.

However, Rodríguez-Vargas et al. [82] demonstrated that the venoms of *Micrurus helleri*, *M. medemi*, and *M. sangilensis* contain significant percentages of metalloproteases (9.8-13.8%) in their proteome, along with marked enzymatic and protease activity. These findings have serious implications for public health and the clinical management of patients for the following reasons: (1) these snakes inhabit urban and peri-urban areas; (2) The neutralizing capacity of the antivenoms available on the market is limited; and (3) medical staff and SIVIGILA, who diagnose the cases and classify micruric envenoming, base their criteria on the absence or mild formation of edema, absence of hemorrhages, and absence or mild manifestation of coagulopathies. Therefore, we recommend that soon, significant efforts should be invested in characterizing the venoms of the remaining endemic species in Colombia, linking their proteomic profiles with the clinical signs observed in patients, as well as their geographical distribution, ecological, and phylogenetic signals.

Hydrophis platurus (yellowbelly sea snake) is the only sea snake present in Colombia. Across the epidemiological snakebite surveillance history, envenomation caused by this species has been controversial. According to the SIVIGILA dataset during the period 2010-2020 there were 65 cases reported of bites caused by this snake. Nevertheless, this information is not reliable because the geographical location of the bites did not correspond to the known distribution for this species and is probably the result of misidentification of the snake that caused the envenomation (See Chapter 1).

For example, 61 reported cases occurred in mainland localities within the Andes cordillera, or on the Caribbean coast, outside of the known historical range (See Chapter 1). Another four cases were reported from localities from the Pacific Ocean, that is the known distribution for this species, but the common name reported corresponds to common names for local pit vipers rather than the common sea snakes' names. Also, the symptoms of these cases were local such as pain or edema, and there were no descriptions of any neurotoxic symptoms that would be is expected in an envenomation caused by sea snakes [32]. So, we conclude that during the period 2010-2020 in Colombia there are not official cases of envenomation caused by *Hydrophis platurus*. Nevertheless, there are historical records of envenomation caused by *H. platurus* in localities such as Guapí (mouth of the Guapí River, Cauca), Gorgona Island; Tumaco and El Charco, municipalities on the Pacific coast of Nariño [13,30,43].

2.3 Antivenom use, non-medical practices, and snakebite risk

Antivenom use

During the last decade snakebite cases with antivenom application exhibited significant differences between snake genera, as well as within the categories of envenomation severity by genus. The national toxicological guideline stated by the Colombian Health Ministry indicates the required antivenom dosage based on the snake's genus, severity and antivenom manufacturer [35]. Despite this in all medically important genera considered on the national snakebite notification sheet, (*Bothrops*, *Crotalus*, *Lachesis, Porthidum*, and *Micrurus*) the number of envenomation cases without antivenom application was considerable (Table 9).

Table 9. Chi-square test evaluating the differences between snake genera, as well as withing categories of envenomation severity by genus.

Genus	Severity	Antivenom usage		Chi-square
		No	Yes	
Bothrops	Mild	3,065	13,945	585.9, df = 2, p < 0.0001
	Moderate	946	10,261	
	Severe	206	2,238	
Crotalus	Mild	230	540	19.793, df = 2, p <0.0001
	Moderate	100	367	
	Severe	11	78	
Lachesis	Mild	33	103	7.89, df = 2, p < 0.019
	Moderate	9	64	
	Severe	4	42	
Porthidium	Mild	575	1,649	13.58, df = 2, p < 0.001
	Moderate	174	689	
	Severe	9	50	
Micrurus	Mild	179	200	12, df = 2, p < 0.0018
	Moderate	51	107	
	Severe	20	44	

df= degrees of freedom; p = probability under the assumption of no effect or no difference.

This demonstrates that medical practitioners have been avoiding the use of antivenom therapy when the patients are classified as mildly envenomation. In contrast, a significantly high number of cases with antivenom application was observed in moderate and severe bothropic envenomation, as well as severe lachesic envenomation (Figure 9). Envenomation caused by *Porthidium* species, considered, and treated clinically as bothropic envenomation, exhibited a significantly high number of cases with antivenom application when the patient was classified as mildly envenomation, but significantly low when the patient was classified as moderately or severely envenomation. Similarly, most micruric envenomations are only treated with antivenom when patients are classified as moderately or severely envenomation. Nevertheless, in micruric snakebites the data provided by SIVIGILA does not allow an accurate association between envenomation severity and the treatment applied, and the incidence output (alive/death patient).

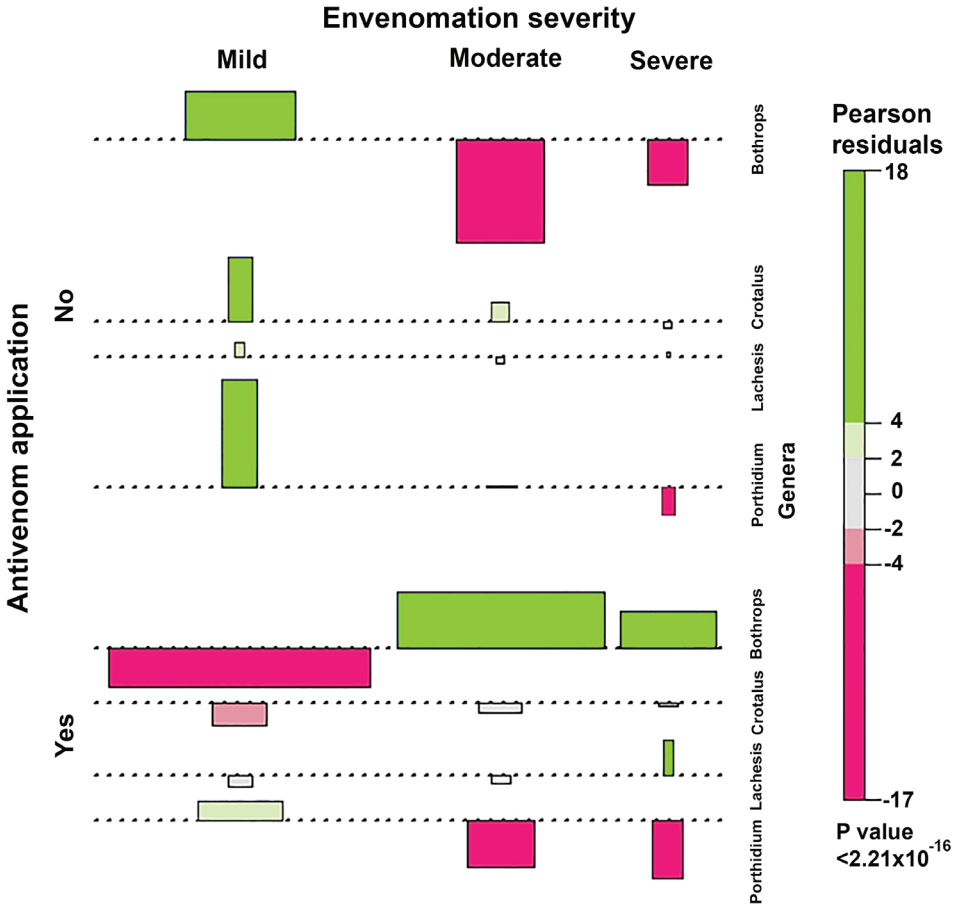


Figure 9. Chi-square for polytomous variables .Plot depicting the differences between snake genera, as well as withing categories of envenomation severity by genus.

Non-medical practices for snakebite that are used in Colombia

Most of the non-medical practices occur in bothropic, crotalic, and lachesic envenomation, and the cases are significantly high in bothropic envenomation that is categorized as moderate and severe (Table 10). Particularly, prayers and sucking are significantly high in bothropic envenomation

in all severities, while herbal poultices and potions are significantly high in moderate and severe envenomation. The number of bothropic envenomation cases without non-medical practices was significantly low. There were no significant differences between non-medical practices in crotalic, lachesic and micruric envenomation for patients categorized in any envenomation category. In contrast, envenomation caused by *Porthidium* species has a significantly high number of cases with non-medical practices for all envenomation categories.

Non-medical practices are common for snakebites caused by pitviper species, but these practices are particularly frequent among Colombian patients that suffer bothropic envenomation, representing 21% of total reported cases. Non-medical practices such as the use of potions (30%), herbal poultices (24%), magic blessing or prayers (22%) represent the most frequent practices, which agrees with literature [36–39]. Most of these practices have been described as controversial, ineffective, or dangerous because traditional treatments performed by shamans and healers have been shown to be inadequate for combating the envenomation caused by *Bothrops* snake species [40–42]. Particularly, non-medical practices have been contraindicated because these delay in time before receiving suitable treatment for the patient, as well as an antivenom therapy that will reduce the clinical complications and possible sequelae [35,43].

Nevertheless, in most cases due to a lack accessibility of medical assistance in rural areas, shamans and healers are the first responders. Despite traditional medicines or first aid practices not having direct effects on envenomation caused by snakebites [38], there is little research proving claimed clinical efficacy of traditional medicines and practices with respect to physical symptoms like pain, edema, erythema, nausea, paresthesia, blurry vision, body aches, headaches, weakness, and tiredness the body. Also, there is little evidence supporting the effect of these practices on physical or psychological symptoms such as post-traumatic stress disorder and depressive disorder [44–49]. More research must be done to generate evidence about the effectiveness and possible use of traditional medicine to deal with snakebite envenomation, but nowadays the only supported treatment is still antivenom administration.



Table 10. Non-medical practices against snakebite used in Colombia by envenomation severity.

Genus	Severity	Herbal poultices	Potions	Magic blessing or Prayers	Suction	Other	Chi-square
Bothrops	Mild	1,076	1,361	1,062	152	1,252	105.1, df = 8; p < 0.0001
	Moderate	916	1125	841	95	725	
	Severe	266	351	294	20	150	
Crotalus	Mild	47	75	48	16	42	10.63, df =8; p = 0.22
	Moderate	40	54	28	5	34	
	Severe	8	7	10	0	4	
Lachesis	Mild	8	9	5	1	13	5.79, df =8; p = 0.67
	Moderate	3	6	4	1	4	
	Severe	3	11	6	0	6	
Porthidium	Mild	191	273	104	14	142	37.4, df =10; p<0.0001
	Moderate	105	122	48	15	66	
	Severe	9	7	6	2	3	
Micrurus	Mild	9	241	20	6	25	6.21, df =8; p = 0.62
	Moderate	6	10	12	1	9	
	Severe	5	4	5	0	5	

Chi-square tests depicting the differences between envenomation severity per genus. df= degrees of freedom; p = probability under the assumption of no effect or no difference.

Survival probability during bothropic envenomation

Commonly, snakebites in rural and peri-urban areas are seen as a death sentence. Since bothropic envenomation represents ~62% of Colombian cases, the estimation of the survival probability during a bothropic envenomation provides a useful approach for understanding the explanatory variables of the risk of death due to bothropic envenomation. We performed a logistic regression based on the bothropic envenomation dataset (2010-2020) provided by SIVIGILA (see appendix A for details). The logistic model correctly predicts 97.9% of all snakebite cases caused by Colombian pitviper and, more important, it was able to correctly predict 99.9% of the cases in which the patient survives. The most relevant explanatories variables that works as survival predictors facing a snakebite by Colombian pitvipers are age ($p < 0.0001$), severity ($p < 0.0001$), antivenom application ($p < 0.0001$), systemic symptoms ($p < 0.0001$), local symptoms ($p = 0.04$), gender ($p = 0.01$), and non-medical practices ($p= 0.01$).

In addition, according to the classification and regression tree model the most important variables for predicting the outcome of a snakebite caused by pitviper is snakebite severity, followed by antivenom use, age, non-medical practices, systemic symptoms, and gender (Figure 10). Both models highlight the paramount importance of the correct medical diagnosis of the snakebite and antivenom application to increase the patient survival probability. Likewise, the models showed how performing non-medical practices decrease survival probability of the patient, rein-

forcing that traditional medicines and practices must be contraindicated. Besides, gender and age are determinant indicators of patient survival when antivenom application and non-medical practice were or were not performed.

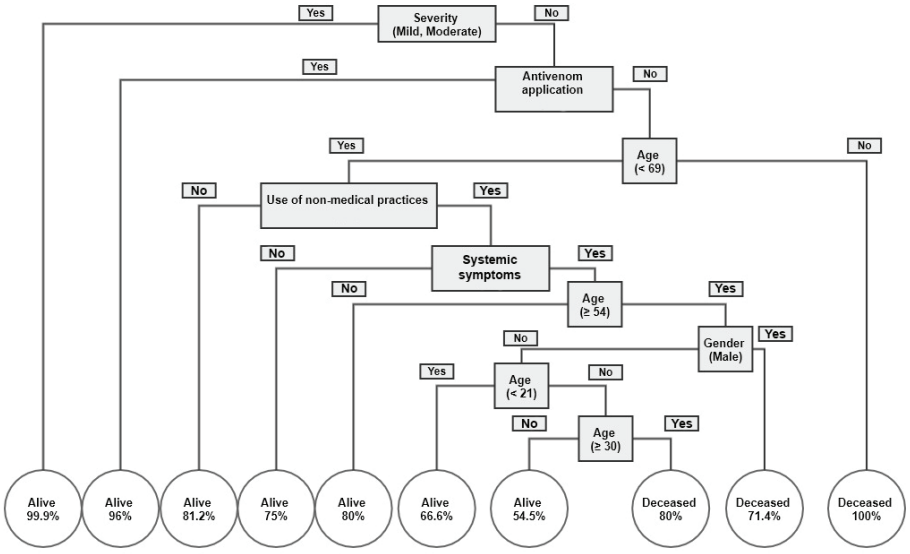


Figure 10. Classification and regression tree model depicting the survival probability of patients after a bite by a Colombian pit viper. Double negatives produce an affirmative response [e.g., Systemic symptoms (No)+logical branch (No) = Systemic symptoms were observed (Yes)].

Snakebite mortality

Between 2010 and 2020 snakebite mortality showed a decreasing trend, where minimum mortality occurred in 2019 and maximum mortality in 2020, and an average of 0.07 per capita deaths. Furthermore, during this period the asymmetry coefficient was -0.21; showing a kurtosis of -0.88, and a variation coefficient of 17.34% indicating that the dynamics behind the mortality trend are far from stable. During the years 2013, 2019, and 2020 the deaths caused by snakebites showed extreme rates outside of the confidence intervals (Figure 11A). The atypical behavior during 2020 may be related to COVID-19 pandemic and lockdown. This unfortunate event caused the Colombian health system to collapse, limiting its capacity to surveil snakebites in the country. In contrast, extreme mortality rates observed in 2013 and 2019 need further research to explore the possible factors behind the strong variability reported.

Mortality in urban and rural settings exhibited strong scale differences being highest in rural settings, representing about 70%-80% of total per capital deaths caused by snakebites in the country (Figure 11B). This was an expected result given the known worldwide pattern in which snakebite mortality is concentered in rural populations [11,42]. Likewise, rural populations showed a decreasing trend during the last decade while urban populations exhibited a steady trend (Figure 11B).

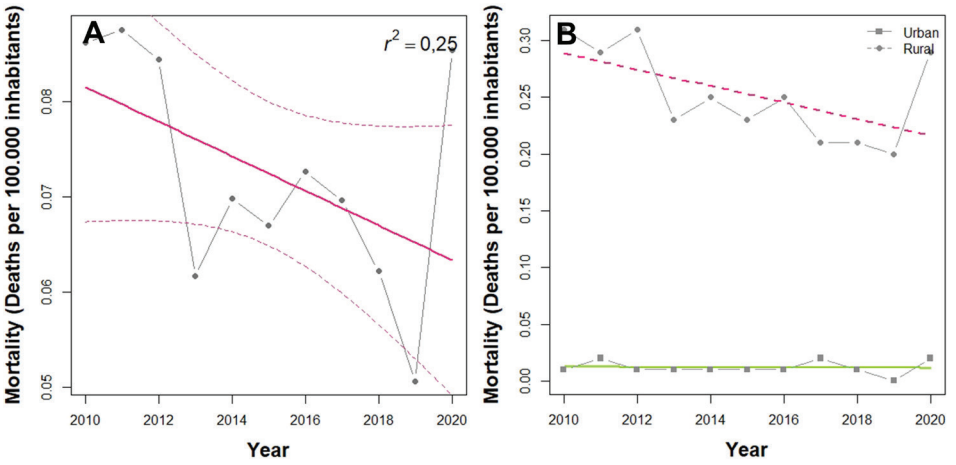


Figure 11. Snakebite mortality trends across the period 2010-2020 in Colombia. (A) Mortality rate. Solid line depicts linear regression model while dashed line confidence intervals of 95%. (B) Mortality rate split between urban and rural areas. Both solid and dashed lines depict the linear regression model.

During the last decade, the mean snakebite mortality rate in Colombia was 0.3 per capita deaths. Vaupes was the single department of the country that maintained its mortality rate above national-average throughout the decade (Figure 12A). Vaupes exhibited the highest record of snakebite mortality (8.8 per capita deaths) as well as the highest yearly variability, followed by Amazonas (4.5 per capita deaths), Guainia (4.2 per capita deaths), and Guaviare (2.7 per capita deaths) (Figure 12). In contrast, departments, or territorial units like Bogotá D.C., Quindio, Huila, Barranquilla, and Archipelago of San Andres and Providence have no reported deaths caused by snakebites during the same period. In conclusion, the mortality rate, considered along with the results of the snakebite incidence analysis described above (see section 1 of this chapter) show us that Colombian populations living in the cis-Andean region, in particular the Amazonian region, are the most at-risk for snakebite.

Since the cis-Andean region is the most affected area by incidence underreporting in the country [21], mortality reports also can be underestimated, further aggravating the truthfulness and accuracy of SIVIGILA. Thus, the Orinoco and Amazonian regions urgently require actions that reduce, mitigate, and prevent snakebite accidents, as well as a prioritization in the nation health plans and programs that focus on neglected tropical diseases.

Despite this unfortunate scenario, our results agree with previous studies that reported the same trends, annual averages, and variability of mortality rates, highlighting the low mortality in Colombia with respect to other countries of the region like Bolivia, Panama, Costa Rica, Nicaragua, Guyana, and French Guyana [1]. However, the trend observed during 2010-2020, including both total mortality rate and mortality rate differentiated by urban or rural areas, did not show a correlation with time (F_{global}= 2.947, df = 1-9, p = 0.12; F_{rural}= 0.22, df = 1-9, p = 0.88; F_{urban}= 4.70, df = 1-9, p = 0.06), indicating that the Colombian surveillance system has significant shortcomings for reporting the yearly per capita deaths caused by snakebites, probably due to underreporting (Figure 11B). Therefore, the SIVIGILA must invest significant efforts for enhancing the way that the system detects, and reports deaths caused by snakebite, as well as to boost the understanding of the factors behind the dynamics of underreporting.

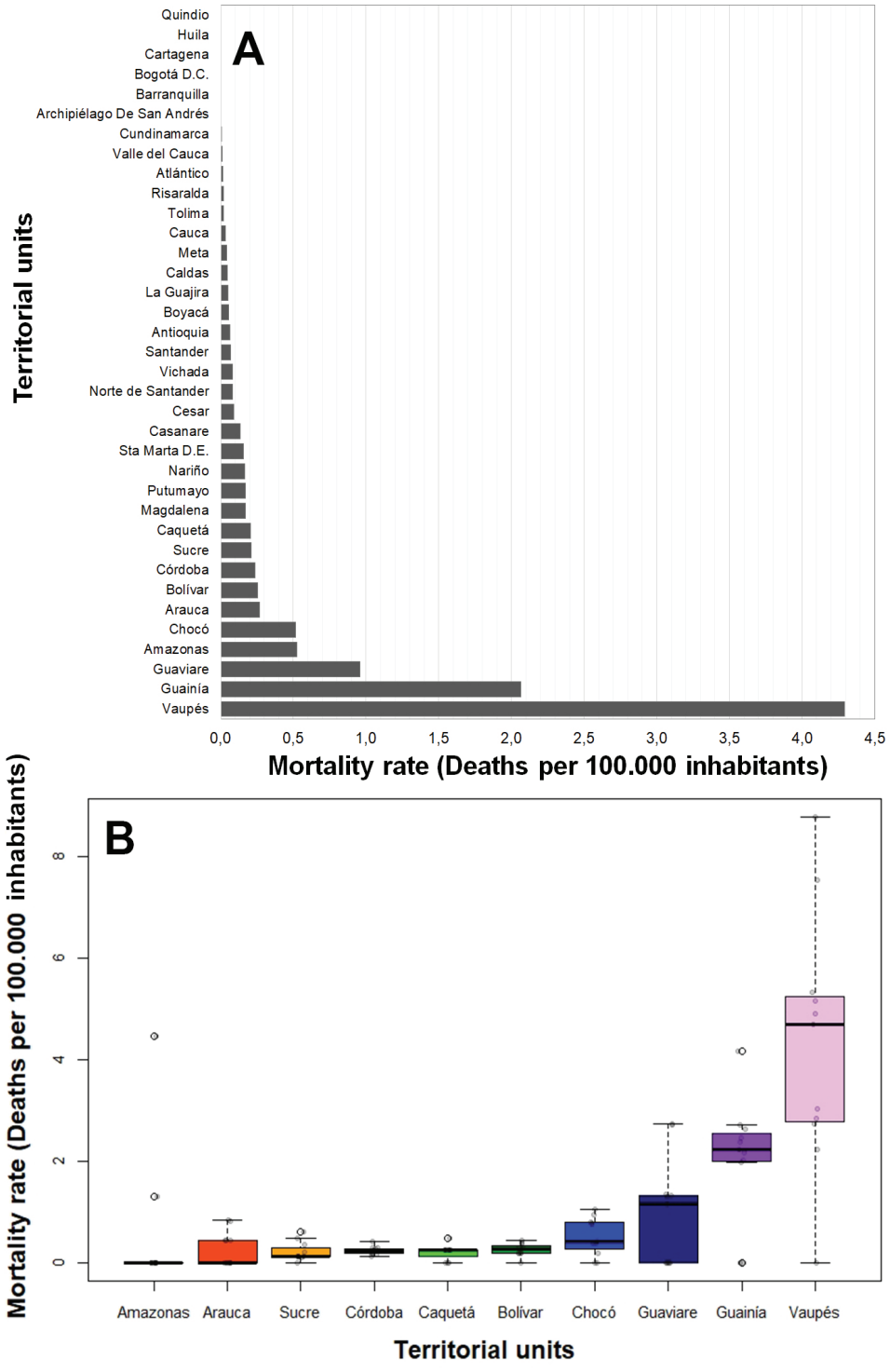


Figure 12. Mortality rate variability by territorial units. (A) Mean mortality rate by territorial units during 2010-2020. (B) Boxplot depicting the mortality rate variability of the ten territorial units with the highest mortality rates during 2010-2020.

Snakebite lethality

The snakebite lethality rate shows a decreasing trend across the decade. An average lethality of 0.73% per snakebite was reported for the last decade (Figure 13A), exhibiting a strongly scaled difference between urban and rural areas; the highest trend was found in rural areas and representing about 70%- 80% of total deaths due to reported snakebites

(Figure 13B). However, when snakebite lethality rate is divided splitted urban and rural areas, the tendencies showed opposite trends, showing an increase in reported lethality in urban areas and a decrease in rural areas (Figure 13B). Based on the data available, this contrasting behavior is difficult to explain, where most of it could be due to the underlying dynamics of underreporting that is poorly understood. Thus, readers must be cautious employing this result in an analysis or in decision making until a comparative analysis can be carried out, or other data can shed light on these trends. Similar to observations of snakebite mortality, neither urban nor rural snakebite lethality showed correlations with time, indicating significant shortcomings in the yearly report, possibly due underreporting (F_{global}= 20.38 df= 1-9, p= 0.18; F_{rural}= 3.15 df= 1-9, p= 0.11; F_{urban}= 0.4 df= 1-9, p= 0.83).

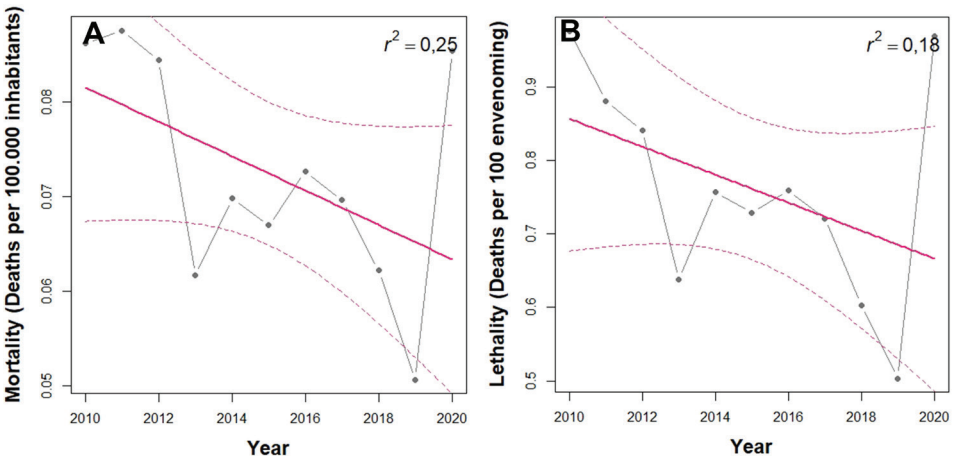


Figure 13. Snakebite lethality rate trends across the period 2010-2020 in Colombia. (A) Mortality rate. Solid line depicts linear regression model while dashed line shows confidence intervals of 95%. (B) Lethality rate. Both Solid and dashed lines depict the linear regression model.

Among all territorial units only 20 of the 32 Colombian departments reported deaths from snakebite: Vaupes, Nariño, Santa Marta, Caldas, and Arauca were the top five territorial units with the highest snakebite lethality rate, ranging from 0% to 7,9% during 2010-2020. Once again, Vaupes department stands out as the riskiest in Colombia. Thus, urgent efforts must be made to reduce the incidence, mortality, and lethality in this department because this department is characterized by high poverty rates, and poor and absent roads, two of the main factors that increase the risk for snakebite. as well as its underreporting [21].

3. Clinical traits of snakebite in Colombia: learnings and future directions

Kasturiratne et al. [50] gathered global information from different countries governments, hospitals report, scientific papers, other sources of gray literature, and then they extrapolated the data. This research reported for the Americas an average of between 83,012 and 132,942 cases per year, with between 547 and 2,305 deaths each year using data from 1985 to 2007.

Recently, Chippaux [1] presented new data for the Americas by gathering information from the 20th century to the year 2016, retrieving informa-

tion from scientific publications, governments, and hospital reports. He estimated an average of 57,500 cases each year with 370 yearly deaths, which is 30% less than the Kasturiratne *et al*. estimation [50]. Colombia had third place in the number of cases in the Americas (7.1%, N = 4,082), after Brazil (first place at 47.3%, N = 27,200) and the United States (second place at 8.7%, N = 5,000). Additionally, Colombia had fourth place in yearly deaths. with an average of 20.8 deaths per year, Brazil had first place (N = 145 deaths), then Mexico (N = 50), and third place was occupied by Bolivia (N = 40) [1].

According to SIVIGILA data, the lethality in Colombia for 2010-2020 was 0.73% (365 cases); 68% of these deaths (245 cases) corresponded to bothropic envenomations. The country's lethality has been significatively decreasing with respect to data collected between 1992 and 1998 by previous studies that reported a mortality rate of 5% [51]. This reduction in lethality may be related to the good reporting system, reliable national antivenom production, and higher territorial antivenom distribution (see Chapter 6). Reports of quality can be improved to enhance reliability and to obtain more epidemiological information relevant to a decrease in this lethality, which is aligned with the WHO strategy aiming to reduce snakebite mortality by 50% in 2030 [52].

Viperidae is the family that causes most of the envenomation in South America, and species of *Bothrops* cause 80%–90% of total envenomations. *Bothrops asper* and *B. atrox* cause between 50% and 80% of the envenomations [29,53]. Until 2017, data from SIVIGILA showed a higher incidence from *Bothrops* species reaching 70-80% of total incidence, but after 2018 this percentage dropped to 62%, significantly lower than data reported until 2017 in Colombia, as well as in surrounding countries of the continent [54]. Interestingly, after 2018, 32% of the cases were caused by snakes in groups defined as “other species” or “species not identified”. The common name reported for 33% of the bites caused by these undefined groups correspond with family *Viperidae*. Furthermore, most of the clinical symptoms are compatible with the expected symptoms of envenomation caused by species from this family. Therefore, it is plausible that this drop in the incidence since 2018 was caused by some modifications done this year in the reporting sheet, as well as the low capacity of the medical practitioners for making suitable identification of venomous snakes.

Currently in the country, there are few practical tools and guidelines to face snakebite envenomation, as well as handbooks to help in the suitable identification of venomous snakes. However, those that exist have had low use [55]. This aspect must be improved by policies decisions and normative actions looking to improve snakebite epidemiological surveillance and knowledge of health personnel in this regard [52].

Common signs of envenomation

After envenomation caused by species of Viperidae, alterations caused by blood coagulation are the most common systemic alteration, and they are the main cause of death [56]. This venom action is called hemotoxicity,

and it is explained by toxins affecting coagulation, fibrinolysis, vascular endothelium, and platelet dysfunction [54]. The effects eventually cause abnormal coagulation tests, hemorrhage of different organs and systems or both. The symptoms have been traditionally classified as disseminated intravascular coagulation (DIC), but recently this concept has been revised and redefined as venom-induced consumption coagulopathy (VICC). VICC may be present in 54% of the cases, and systemic bleeding in 15% [57].

Diagnosis of VICC is done by a demonstration of alterations in coagulation times by using activated partial thromboplastin time (aPTT), prothrombin time (PT), international normalized ratio (INR), the increase of D-dimer, and the decrease of fibrinogen and thrombocytopenia [57,58]. Unfortunately, SIVIGILA data cannot clarify the incidence of VICC because not all medical centers, especially those located in remote areas, can perform coagulation times tests, and this procedure is not requested in the SIVIGILA's data collection form. As an alternative, the 20-min whole blood clotting test has been used globally to look for hemotoxic effects, diagnose VICC and to define the use of antivenom in countries or regions where routinely other kinds of coagulation tests are not available, and Colombia has not been the exception [58,59].

Acute kidney injury (AKI) is defined as an increase in serum creatinine ≥ 0.3 mg/dL (26.5 μmol/L) in 48 hours; the increase ≥ 1.5 times of its basal level in the last 7 days; or a urinary volume <0.5 mL/(Kg * h) for 6 hours [60]. The global estimation of AKI incidence varies between 8% and 60% of the patients, and this problem is called snakebite-associated acute kidney injury (SAKI). Of these cases, between 15% and 92% will require kidney replacement therapy. SAKI mortality is estimated at 45%, and 50% will develop chronic kidney disease (CKD) [61].

In South America SAKI occurs in between 1.4% and 38.5% of total cases, varying between species. *Bothrops asper* exhibits the highest value (38.5%), while *B. atrox* is at 6% [62]. Since the late 1990s, it has been proposed that kidney damage that develops from snake envenomation whose venom is hemotoxic is due to the formation and accumulation of fibrin microthrombi in the renal tubules (thrombotic microangiopathy - TMA). This condition has been defined as the presence of microangiopathic hemolytic anemia (MAHA), thrombocytopenia and AKI [63].

In Colombia, SIVIGILA data showed that the incidence of AKI during 2010-2020 is lower than reported in the previous studies [62], and the incidence of TMA is unknown. This may because: (1) TMA diagnostics are not being performed; (2) there is lack of knowledge in medical personnel; (3) there is difficulty in measuring creatinine and other laboratory parameters in remote hospitals or health centers; or (4) the data that define the diagnosis are not recorded in the SIVIGILA reporting form.

Strict quantification of urine volume as a guiding measure of the development of kidney failure, as well as a peripheral blood smear showing spherocytes and microschistocytes, and platelet count, are feasible

in minimally complex hospitals and should be strictly done. Also, the data collection form must be adjusted so that this type of data can be recorded.

Data from studies in neighboring countries, with similar snakebite' characteristics and clinical behavior, and data from previous reports for Colombia, found necrosis in 2% – 38.5% of cases [29,51], compartment syndrome in 6% – 7.7% [51,64]. Rhabdomyolysis in 31% [65], and the presence of cellulitis, fasciitis, and abscesses in 7% – 30.8% [29,51]. Another study found even higher incidences: abscesses (69.1%), necrotizing fasciitis (16.4%), and cellulitis (21.8%) [65]. The information obtained from SIVIGILA data are quite inferior when compared to what has been previously reported in the specialized literature.

For lachesic envenomation clinical effects are similar to bothropic envenomation, where the envenomation severity is mainly severe (60% of cases), and the mortality is close to 10% [22,66]. In addition, it has vagal symptoms such as bradycardia, profuse diarrhea, abdominal pain, and hypotension, where these symptoms are considered as manifestations of severe envenomation and can help differentiating between this envenomation and bothropic envenomation [67].

In Colombia there are scarce knowledge around lachesic envenomation because of the low incidence of snakebites caused by this genus. Even so, it is important to remark that 86% of patients under this envenomation presented epistaxis as a systemic manifestation of coagulopathy, while only 1.5 % of bothropic envenomation cases presented this manifestation, thus epistaxis can show the strong fibrinolytic effect of *Lachesis* venom [68].

Data used in this analysis contrasts with that previously reported in the literature since mortality was very low (1.5%), only 18% of the accidents were severe and vagal manifestations as a severe clinical picture were described in less than 5% of the cases [65,68]. Low mortality could be due to a greater availability of antivenom serum and the change in the country's political conditions that have allowed people to move more easily from remote areas to hospitals, since the data available for the country in the specialized literature, are from the last 20 years of the last century.

Compared to other genera of viperids, the venom of the South American rattlesnake (*Crotalus durissus*) has low capacity to produce edema and hemorrhage. However, rhabdomyolysis, secondary renal failure and neuromuscular paralysis are very characteristic findings [22,69]. A description of the incidence of the manifestations of this type of envenomation in Colombia was not found in the specialized literature, however, that between 27%-40% of severe crotalic envenomations develop ARF [22,69]. SIVIGILA data only reported 0.6%. The most frequent manifestations in crotalic envenomation were local, coinciding with reports on this type of envenoming in Brazil, where pain and edema occurred in 91% and 73% of the cases [69]. In addition, ecchymosis, blisters, and necrosis were reported in the SIVIGILA data, findings not previously described for South American crotalic envenomation, since their venom lacks a dermonecrotic

effect [22], although this has been described for Central and North American rattlesnakes [70].

These findings open new questions and hypotheses to be tested in the future. For example, recently, Rodriguez-Vargas et al. [71] evaluated the biochemical and biological differences in the venom of *C. durissus* from three ecoregions of Colombia, showing a remarkable variability in the proportions, presence/absence of the main toxins, as well as in their biological activity's intensity. Thus, this strong venom shows geographic variability that could explain the atypical reports of clinical manifestation in the SIVIGILA dataset.

Another hypothesis that could explain these findings is an erroneous identification of the snake by the patient or by a lack of knowledge or misidentification of clinical manifestations by the health personnel. Additionally, these described effects can be not previously described because of the low incidence of this event. In this analysis, neurological and hemorrhagic manifestations had a low incidence, when compared to what is described in the literature, where manifestations present in SIVIGILA' data such as muscle weakness or neurotoxic facies, or hemorrhages were found in up to a quarter of the patients (24%) [69]. However, comprehensive studies will be required to link clinical manifestation and the remarkable geographic variability of toxins observed in Colombian *C. durissus* populations.

The venom of coralsnake species of the *Micrurus* genus is of the neurotoxic type. Its toxins affect the functioning of the neuromuscular junction both at the pre- and post-synaptic level. The incidence of this type of envenomation is very low [72], and what is known about the clinical behavior in our country and other regions of America has been obtained from the description of cases. The low incidence is related to the fact that these snakes are not aggressive, have small fangs, and produce and inject small amounts of venom (see Chapter 2 and 5), as well as being rarely encountered in the wild, thus encounters between coral snakes and humans are low [72] (see Chapter 1). The capacity to produce edema or proteolysis is minimal; this is why the local effects are very inconspicuous from the clinical point of view [22].

In this analysis pain (80.8%) and edema (58%) were the most common local symptoms and paresthesias were reported in 26%, while literature mentions that pain and paresthesias are the local symptoms that occur more frequently [72–74]. Systemic neurotoxicity manifestations were described in a small number of cases, muscle weakness in 13% of them, the other manifestations only occurred in less than 5% of all cases. Both in Colombia and in other countries, a higher incidence of systemic neurotoxicity has been reported (50-91%) [22,72].

According to the SIVIGILA data, manifestations such as gingivorrhagia, epistaxis, and blisters were found in a minimum percentage of cases (1%, 0.5% and 1.8%, respectively). However, in 131 cases classified as micruric envenomation, the reported common name of the snake corresponded

to those used for species in *Viperidae*. Although coral snakes have phospholipases in their venom, and cases of coagulation and platelet alterations have been described in some cases of New World elapid bites [31], it is very rare that these alterations could be related to some degree or type of bleeding [31]. Nevertheless, recently Rodríguez-Vargas et al. [82] demonstrated that two Colombian endemic coral snake species (*Micrurus medemi* and *M. sangilensis*) have a considerable quantity of metalloproteinases in their venom composition. This finding raises serious implications in clinical manifestations of envenoming caused by coral snakes, pointing out that our understanding of snakebite envenomation, is far to be accomplished with sufficiency.

4. Conclusions

The reporting system and data recompilation by SIVIGILA has improved significantly over the last decades, and nowadays it is one of the strongest in South America. Our results show how this system has captured most expected snakebite cases (96%). This great effort done by the vigilance division of the National Institute of Health led us to reach all the conclusions presented in this chapter. SIVIGILA' data show that 26,324 cases of envenomation were treated by antivenom (53.8 %), and a total of 3,195 (12.13%) patients showed adverse reactions to antivenom. Of these reactions, 51% (N = 1,612) were generalized and the rest were localized, and about 70% of these reactions were observed during bothropic envenomation.

Spatial analyses show how important it is to discriminate cases between urban and rural population. We found that variables explaining the spatial variation of incidence changed between the urban or rural settings. This is a crucial finding because it tells us that the dynamics of snakebite accidents differ depending on the spatial contexts in which they occur, so prevention and management strategies must be adjusted to this reality. Furthermore, we found that the spatial distribution of snakebite cases is an essential component for understanding the viability of this public health event, as well as its association with the environmental and social variables that explain it. In particular, the GWR models showed better predictive capacity (higher r^2 values) than the multiple linear models that considered the same set of explanatory variables. As expected, closer municipalities will share same snakebite dynamics, but our explanatory variables are not enough to capture all the spatial heterogeneity of the distribution of cases. It is necessary to find more variables that can explain this heterogeneity, and to start including variables related mechanistically to venomous snake abundance.

Snakebite incidence has temporal trends with seasonal biannual peaks that corresponds to rainfall seasons. This behavior occurs at a national scale, but at a finer scale it is not homogeneous. Rainfall could be acting as a limiting factor: rainfall only modulates snakebite incidence in areas with marked dry seasons such as the Orinoco basin and the Caribbean coast [3]. Sadly, natural history data for reproductive cycles of venomous snakes in Colombia don't exist. Thus, it is difficult to attribute these behaviors to snakes'

phenology. On the other hand, we found an increase in the average of cases between 2018 and 2019, that may be caused by La Niña. This analysis should be done to understand the interannual variation of incidence. Similarly, the decrease of cases during 2019 could be caused by the COVID-19 pandemic. These analyses should be the next step to understand the temporal heterogeneity of snakebite incidence and to start generating monitoring and predicting tools that allow us to enhance the way as we confront this NTDS.

We found that the highest burden associated with snakebite envenomation occurs in the Orinoquia-Amazonian region of the country, and it affects mainly rural population. It is important to understand why this region shows this high burden, because it is remote and have high underreporting, thus its population is more vulnerable to this health problem [21]. Particularly, the departments of Vaupes, Amazonas, Guainia, Guaviare, Arauca, and Choco should be prioritized for the formulation and implementation of prevention and mitigation strategies for snakebite accidents, because these are the regions of the country at the highest risk.

Mortality and lethality have been decaying, but lethality in urban population has been increasing. This must be understood because there should be a higher antivenom accessibility in urban areas. Another interesting result is that a correct determination of envenomation severity is crucial to reduce mortality caused by pitvipers' snakebites, and in severe cases the absence of antivenom usage and the preference for non-medical practices are the main explicative variables of these fatalities. Finally, it is important to keep compilating data for years posterior to COVID-19 pandemic, because this unfortunate event affected snakebite dynamics and reporting system, thus generating atypical data that can bias our presented analysis.

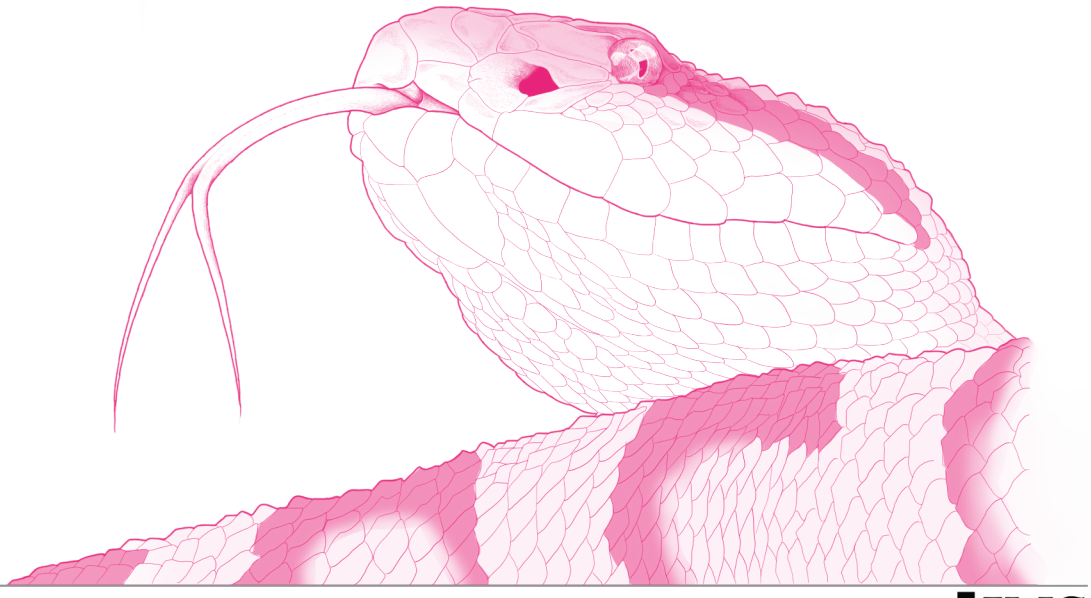
We want to offer some recommendations after writing this chapter that we hope will help to exploit all the potential that our reporting system offers.

First, the mandatory form to report snakebite should be simplified. There should be specific boxes for each symptom, thus data can be homogenized between different hospitals. Secondly, the current form doesn't allow a correct classification of the severity of envenomation because the present frequency of severe and local symptoms is low compared to similar countries. Capacitation for medical practitioners on snakebite symptom diagnosis, characteristics, and treatment is fundamental. Furthermore, the increase in reporting quality will strengthen SIVIGILA reporting system and will enhance our knowledge of the geographic distribution of these clinical manifestations. The snakebite accident reporting form should be able to be filled out online or on a web platform that centralizes the data, allowing for better surveillance and monitoring of the event, reducing information duplication, speeding up administrative management, and ensuring traceability.

Another paramount issue is the detailed information on the times between envenomation, medical attention, and antivenom administration.

We could not determine times between the bite and antivenom administration because the data is not accurate, or it is deeply incomplete. There are spaces for different digits in the format, but there is no concise organization of the units of these digits. Thus, information is just a number, and it is impossible to know if it relates to days, hours, or minutes. It is necessary to homogenize the scales of time for each one of the cells in the form to perform detailed analysis of severity, symptoms, and time to antivenom administration. Finally, the 20-min whole blood clotting test should be a mandatory test to determine VICC, which will help determining the family of snake that caused the envenomation. This would reduce the high number of unidentified cases, and it will help to determine which kind of antivenom should be used.

After characterizing the behavior of envenomation symptoms of venomous snakes in the country, we found that it is substantially different from that previously described for Colombia and neighboring countries. This highlights the need for correct capacitation of medical practitioners to determine symptoms and the correct identification of venomous snake' that caused the envenomation. It is also important to start homogenizing the common English and Spanish names of venomous snakes to compile a central database that can overcome detected limitations. This training can help to improve surveillance, that will help to detail the causes and consequences of snakebites in the country to implement strategies proposed by the World Health Organization to reduce snakebite mortality by 50% [52].



Appendix A: Material and Methods

Mathematical models
We retrieved 19 bioclimatic variables for the country at a resolution of around 1 km x 1 km from the worldclim server (https://worldclim.org/ [75]). Afterwards, we removed collinear variables by using a Pearson correlation cutoff of 0.8 to select independent layers. We did this reduction by using the package virtual species in the R environment [76]. We selected eight variables: temperature (B1), mean diurnal temperature range (B2), temperature seasonality (B4), temperature annual range (B7), precipitation (B12), precipitation seasonality (B15), precipitation of warmest quarter (B18), and precipitation of coldest quarter (B19). Then we computed the average value for each municipality of the country.

We extracted discriminated data for total, urban, and rural population and poverty (NBI) estimates at municipality scale from the national statistics department DANE, and we used the average value from 2010 to 2020 because this timespan corresponds with the national surveillance system (SIVIGILA) data coverage. We used human footprint maps for 2009 obtained from (https://wcshumanfootprint.org/ [77], and we computed its average value for each municipality. Finally, SIVIGILA reported data corresponding to the national mandatory reports of snakebite from 2010 to 2020.

We performed the geographical weighted regressions (GWR) in the R environment by using the GWmodel package [78]. This package allows comparison between the spatial-structured linear model and a null classic linear model without this spatial structure. We first computed the bandwidth for the geographic structure by using an adaptive gaussian kernel, based on an Akaike information criterion (AIC) approach that selects the bandwidth that minimizes this value. Afterwards, we computed all possible models resulting from combining the defined independent variables (We used total population and NBI for total incidence, urban population, and NBI for urban incidence, and the same for rural areas); and we selected the model with the lowest AIC. Finally, we determined significant areas for each GWR coefficient by defining a significance threshold of 0.05.

Antivenom use, non-medical practices, and snakebite risk analyses
We evaluated the differences of the envenomation severity among the snake genera, and the differences in the non-medical practices against snakebite used in Colombia using Pearson's Chi-square for polytomous variables and the G-test of likelihood ratio.

We employed a logistic regression model to estimate the survival probability of patients after a snakebite. Based on the bothropic envenomation dataset (2010-2020) provided by SIVIGILA, we used the snakebite outcome codified as alive/deceased as dependent variable. We hypothesized as explanatory variables for the occurrence of the alive/deceased outcome after a bite by viperid snakes using the following

features of the snakebite records: gender, age, hospitalization, initial non-medical treatment, non-medical practice, bite site, snake genus, local envenomation symptoms, systemic envenomation symptoms, and snakebite severity (Table 5). The dataset of snakebites by Colombian pitvipers encompasses a total of 32,918 records, however, after missing data depuration, we employed a subset of 16,495 records to perform all statistical analyses.

Before running the model, we tested multicollinearity between the different independent variables throughout inflation factors of the variance (VIF) to select uncorrelated variables, employing a cut-off value of 10. Afterwards, we performed a stepwise regression to eliminate those variables that were not significant using the Akaike information criterion (AIC) to define which were the variables to exclude. Finally, variables such as hospitalization, initial non-medical treatment, snake genus, and bite site were excluded by the VIF and AIC; and we used the logistic regression model using the remaining variables selected.

Additionally, we performed a classification and regression tree model to identify independent variables with the greatest power of discrimination, allowing an estimation of the survival probability of a patient after a bite by Colombian pit viper snakes. The tree was built employing five observations by partition and terminal node, as well as a pruning complexity of 0.0001. All statistical analyses were performed using the software Rwizard 4.3 [6], and the R packages rpart [79], stat [80], and MASS [81].

Table S1. Coding of the independent explanatory variables used in the logistic regression model and Classification and regression tree model.

Variable	Coding
Gender	Male / Female
Age	Age in years
Hospitalization	Yes / No
Initial non-medical treatment (Bleeding, limb immobilization, mechanic suction, patient immobilization, Puncture, tourniquet, other)	Yes / No
Non-medical practice (Herbal poultices, potions, prayers, sucking, other)	Yes / No
Bite site	Abdomen, back, toes and fingers, genitals, gluteus, head, lower limbs, upper limbs, neck, thorax.
Snake genus	*Bothrops* / *Crotalus* / Lachesis / *Porthidium*
Local envenomation symptoms	Yes / No
Systemic envenomation symptoms	Yes / No
Snakebite severity	Mild / Moderate / Severe

Appendix B

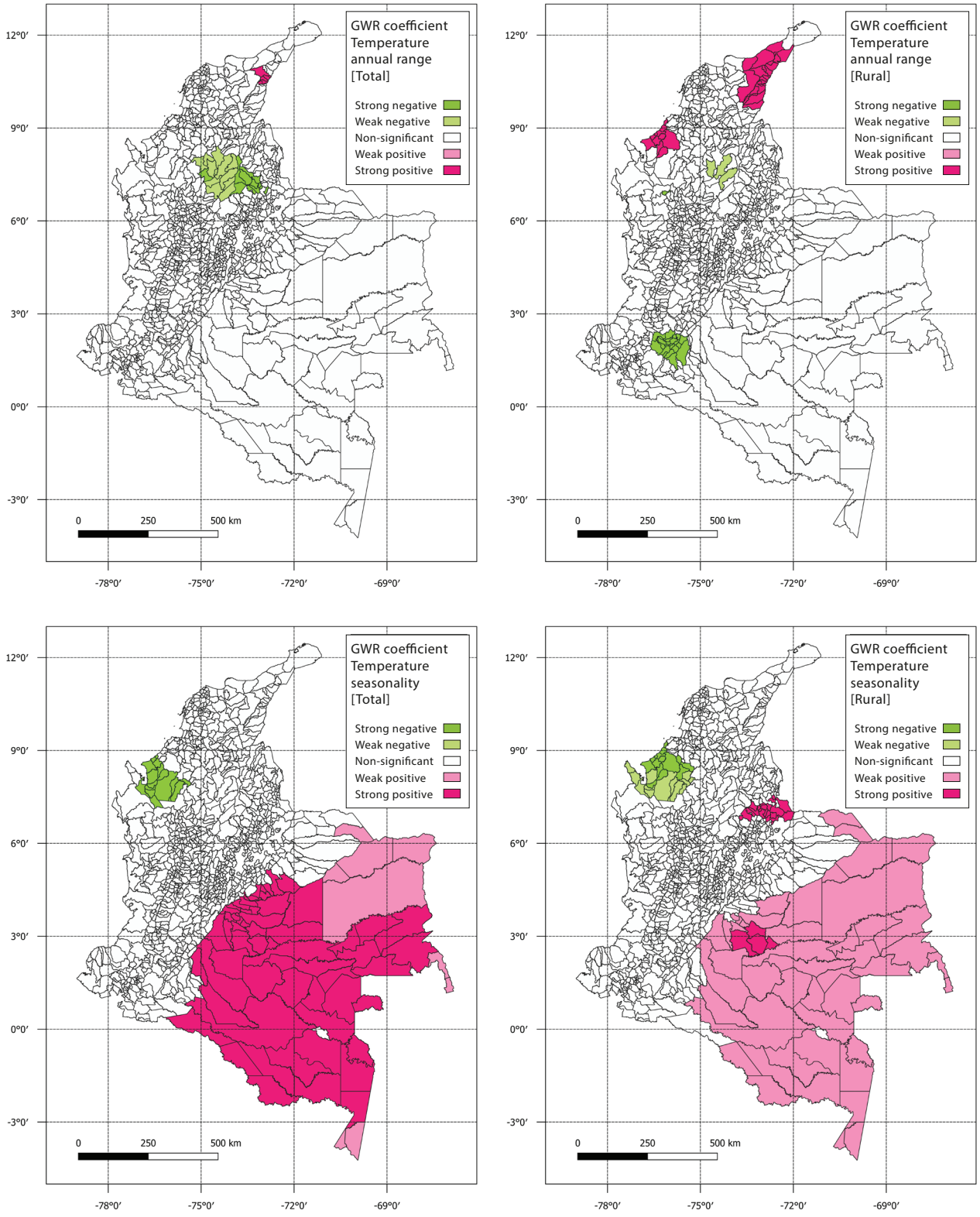


Figure S1. GWR coefficients for variables shared between total and rural areas.

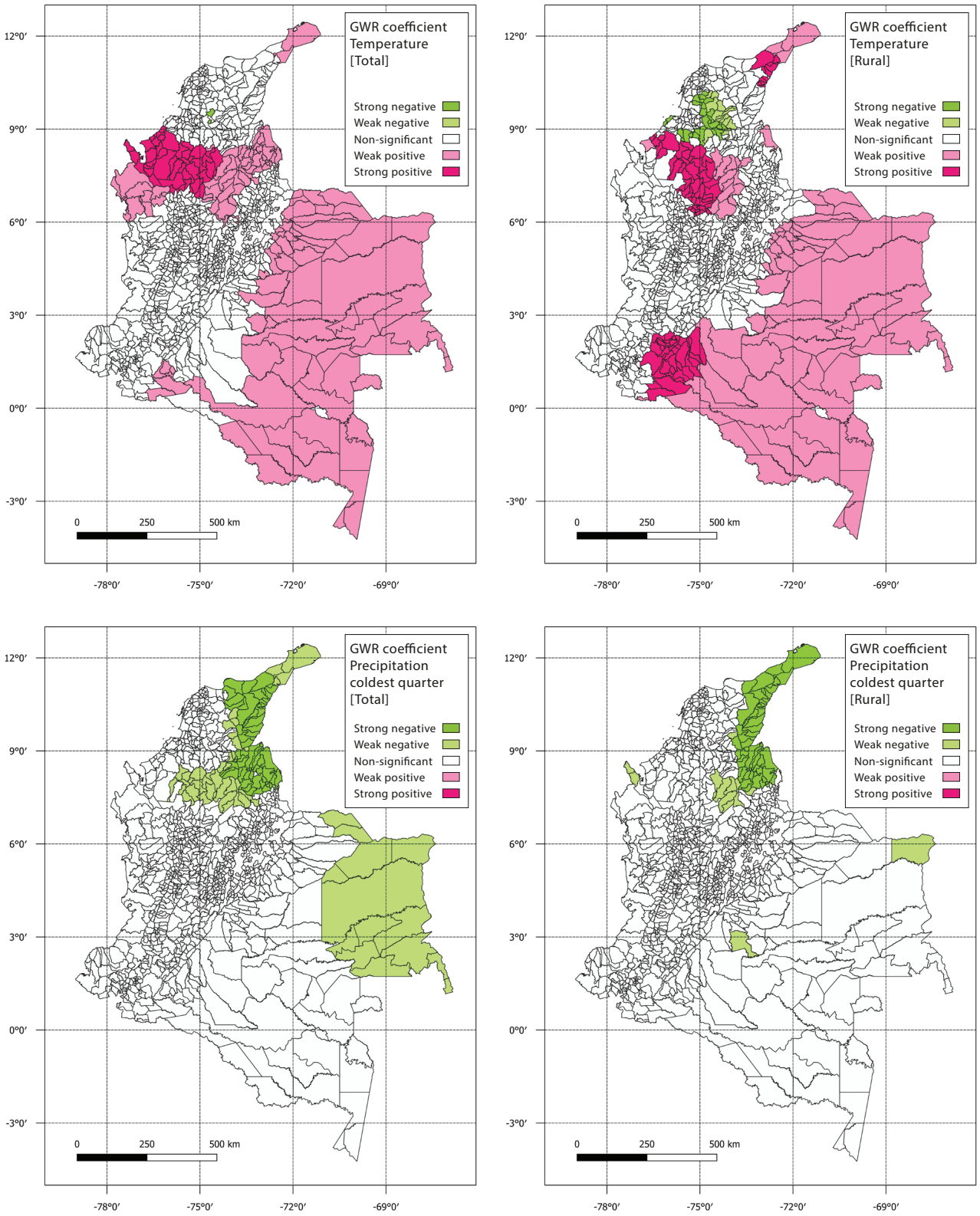


Figure S1. GWR coefficients for variables shared between total and rural areas.

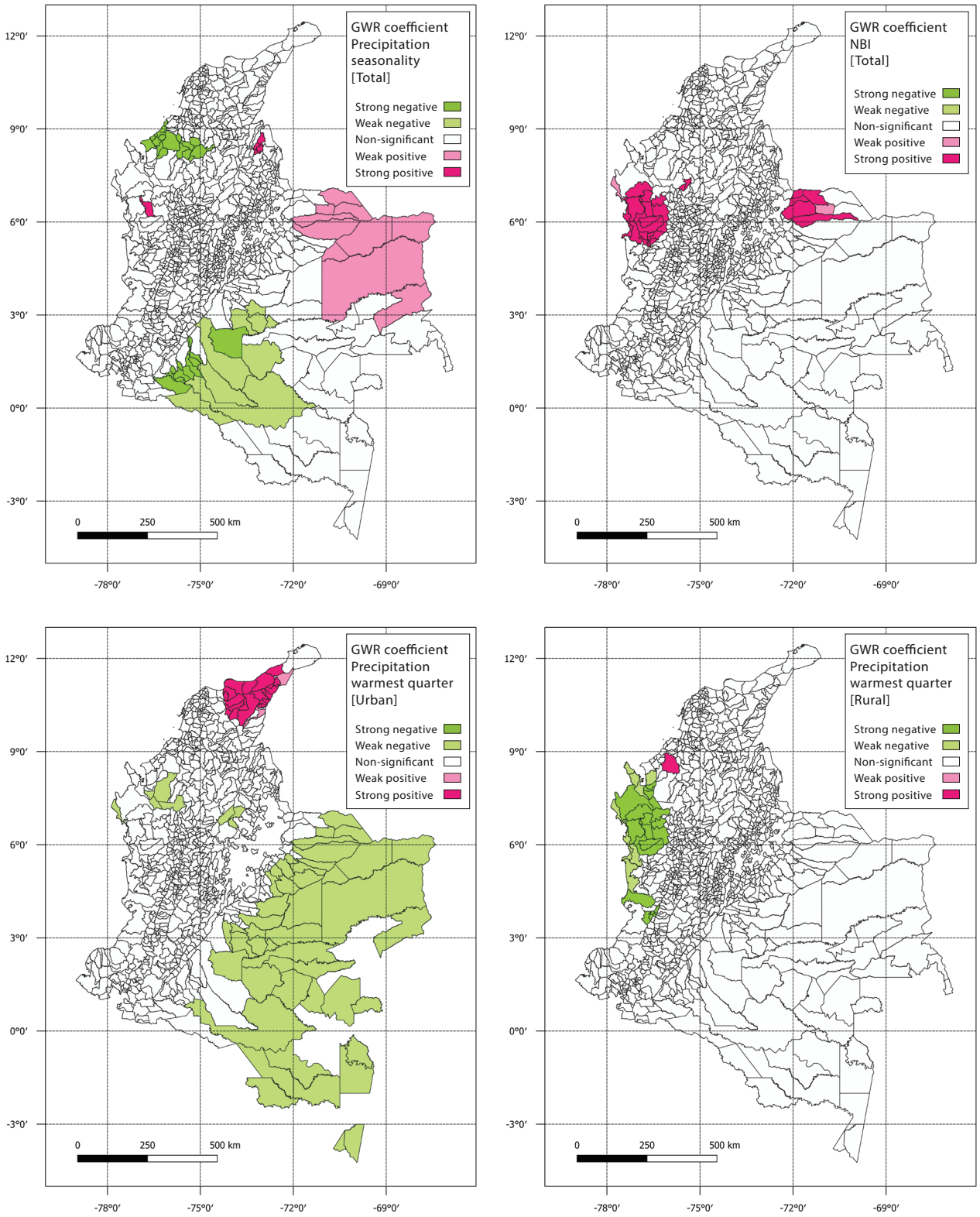


Figure S2. GWR coefficients spatial distribution of two variables only present in urban areas and variables shared between urban and rural areas.

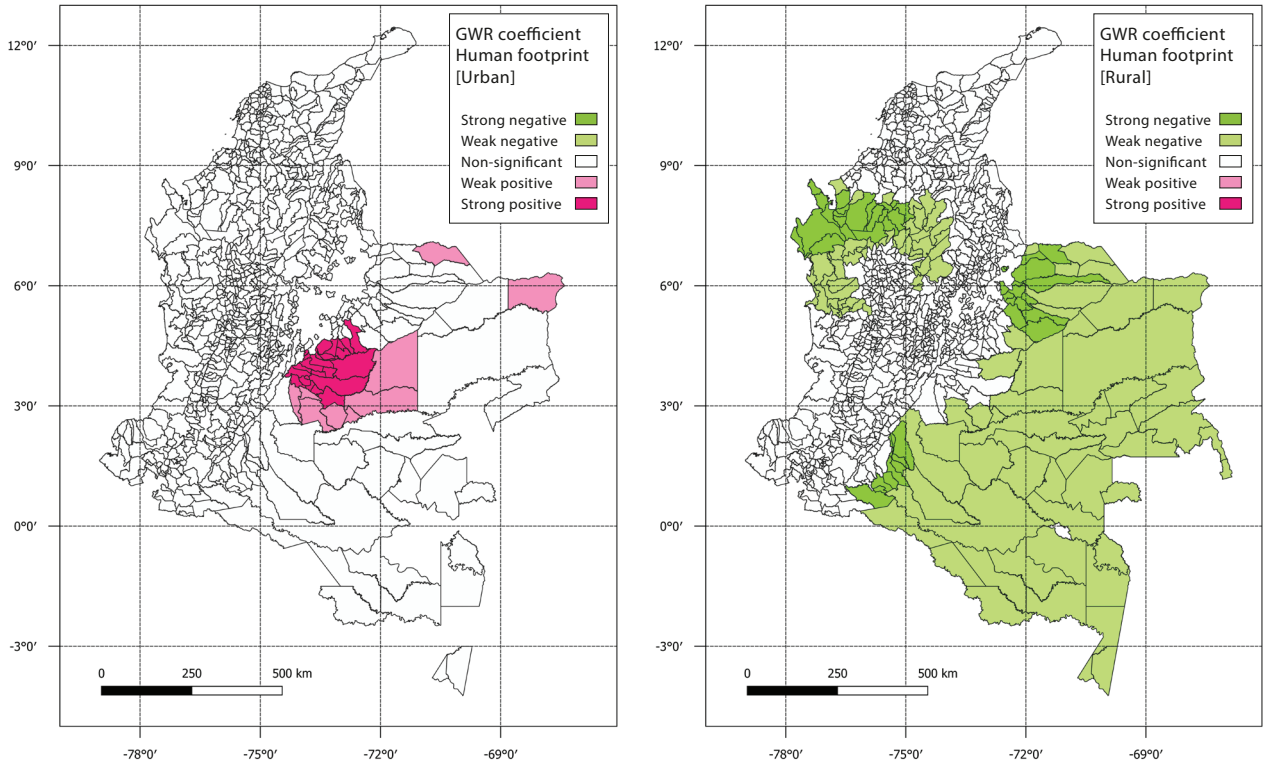


Figure S2. GWR coefficients spatial distribution of two variables only present in urban areas and variables shared between urban and rural areas.

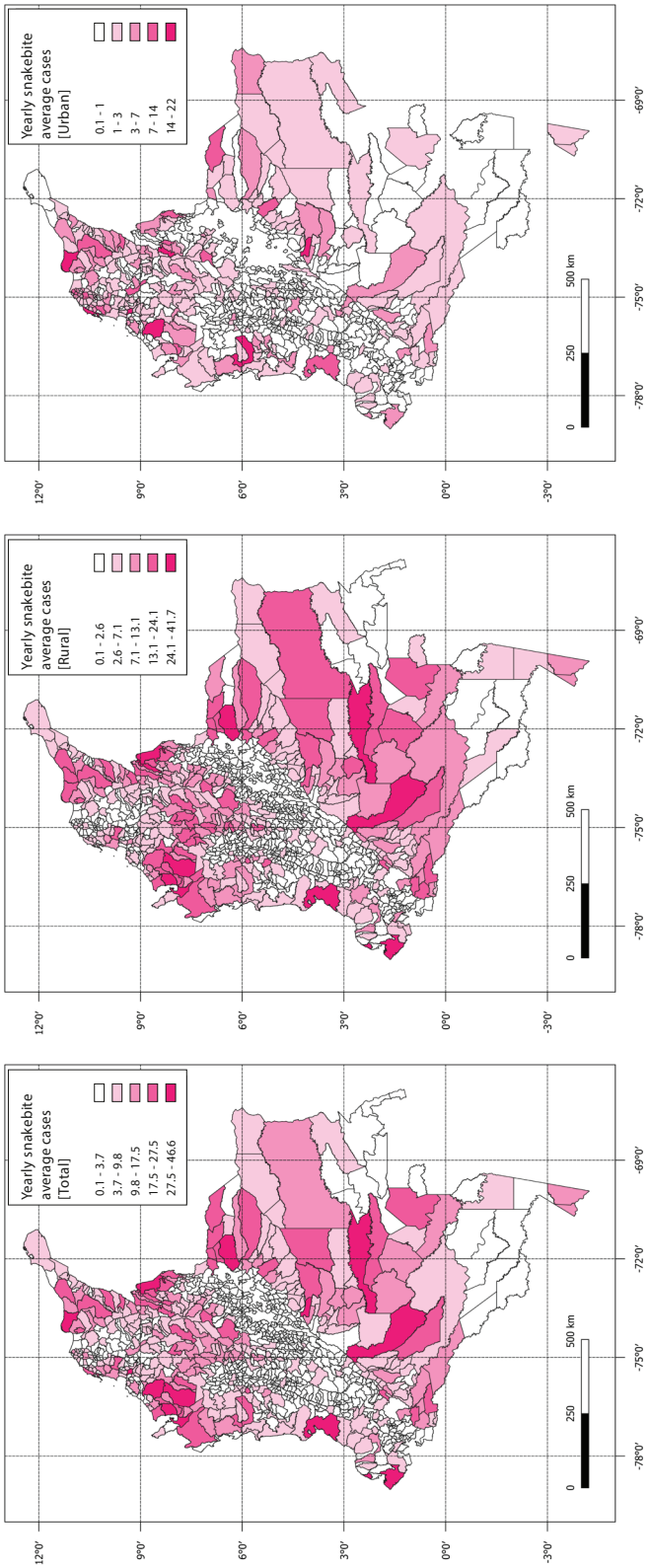


Figure S3. Spatial distribution of snakebite cases

References

1. Chippaux, J.-P. Incidence and Mortality Due to Snakebite in the Americas. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005662, doi:10.1371/journal.pntd.0005662.
2. Angarita–Sierra, T.; Lozano–Daza, S.A. Life Is Uncertain, Eat Dessert First: Feeding Ecology and Prey-Predator Interactions of the Coffee Snake *Ninia atrata*. *https://doi.org/10.1080/00222933.2019.1655105* **2019**, *53*, 1401–1420, doi:10.1080/00222933.2019.1655105.
3. Bravo-Vega, C.I.; Santos-Vega, M.I.; Manuel Cordovez, J.I. Disentangling Snakebite Dynamics in Colombia: How Does Rainfall and Temperature Drive Snakebite Temporal Patterns? *PLoS Negl. Trop. Dis.* **2022**, *16*, e0010270, doi:10.1371/JOURNAL.PNTD.0010270.
4. Chaves, L.F.; Chuang, T.-W.; Sasa, M.; Gutiérrez, J.M. Snakebites Are Associated with Poverty, Weather Fluctuations, and El Nino. *Sci. Adv.* **2015**, *1*, e1500249–e1500249, doi:10.1126/sciadv.1500249.
5. Xiang, Y.; Jia, Y.; Chen, L.; Guo, L.; Shu, B.; Long, E. COVID-19 Epidemic Prediction and the Impact of Public Health Interventions: A Review of COVID-19 Epidemic Models. *Infect. Dis. Model.* **2021**, *6*, 324–342, doi:10.1016/J.IDM.2021.01.001.
6. Guisande-Gonzáles, C.; Vaamonde-Liste, B.; Barreiro-Felpeto, A. Rwizard **2014**, 1–452.
7. Charry-Restrepo, H. Epidemiología Del Accidente Ofídico En Colombia. *Temas de Toxinologia* **2006**, 1–14.
8. Nuñez León, L.J. Informe Del Evento Accidente Ofídico, Colombia, 2016. *Inst. Nac. Salud- SIVIGILA* **2016**, 33.
9. Manuel Santos Calderón, J.; Vargas Lleras, G.; Fernando Cristo Bustos, J.; Cárdenas Santamaría Ministro de Hacienda Crédito Público Yesid Reyes Alvarado, M.; Carlos Pinzón Bueno Ministro de Defensa Nacional Aurelio Iragorri Valencia, J.; Gaviria Uribe, A.; Eduardo Garzón Ministro de Trabajo Tomás González Estrada, L.; Álvarez-Correa, C.; Parody Ministra de Educación Nacional Gabriel Vallejo López, G.; Felipe Henao Cardona Ministro de Vivienda, L.; et al. *Documento Conpes: POLÍTICA NACIONAL PARA CONSOLIDAR EL SISTEMA DE CIUDADES EN COLOMBIA*; Bogotá, **2014**.
10. DNP: DDUPA; Ministerio de Ambiente, V. y D.T. *Documento Conpes: LINEAMIENTOS PARA OPTIMIZAR LA POLÍTICA DE DESARROLLO URBANO*; Bogotá, **2004**.
11. World health organization *Snakebite Envenomation a Strategy for Prevention and Control*; WHO Geneva, **2019**; ISBN 9789241515641.
12. World Health Organization Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins. *WHO Tech. Rep. Ser.* **2013**, *964*.
13. Sevilla-Sánchez, M.J.; Mora-Obando, D.; Calderón, J.J.; Guerrero-Vargas, J.A.; Ayerbe-González, S. Accidente Ofídico En El Departamento de Nariño, Colombia: Análisis Retrospectivo, 2008-2017. *Biomédica* **2019**, *39*, 715–736, doi:10.7705/BIOMEDICA.4830.
14. Nuñez León, L.J.; Camero-Ramos, G.; Gutierrez, J.M. Epidemiology of Snakebites in Colombia (2008-2016). *Rev. Salud Pública* **2020**, *22*, 1–8, doi:10.15446/rsap.V22n3.87005.

15. Estrada-Gómez, S.; Quintana Castillo, J.C.; Vargas Muñoz, L.J. Accidente Ofídico En Animales de Pastoreo: Acercamiento Epidemiológico, Clínico y de Manejo. *Rev. Med. Vet.* **2014**, *27*, 149–161.
16. Paredes Medina, A.E. *Informe del evento accidente ofidico, Colombia, 2013*; Bogotá, **2014**.
17. Rojas Bárcenas, A.M. *Informe de evento accidente ofídico, Colombia*, *2017*; **2018**.
18. Rojas Bárcenas, A.M. *Informe de Evento Accidente Ofidico, Colombia, 2018*; **2019**.
19. Vargas-Lorenzo, I.; Luisa Vázquez-Navarrete, M.; Mogollón-Pérez, A.S. Acceso a La Atención En Salud En Colombia. *Rev. Salud Pública* **2010**, *12*, 701–712, doi:10.1590/S0124-00642010000500001.
20. Bravo-Vega, C.A.; Cordovez, J.M.; Renjifo-Ibáñez, C.; Santos-Vega, M.; Sasa, M. Estimating Snakebite Incidence from Mathematical Models: A Test in Costa Rica. *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007914, doi:10.1371/journal.pntd.0007914.
21. Bravo-Vega, C.; Renjifo-Ibañez, C.; Santos-Vega, M.; Nuñez, L.J.L.; Angarita-Sierra, T.; Cordovez, J.M. A Generalized Framework for Estimating Snakebite Underreporting Using Statistical Models: A Study in Colombia. *PLoS Negl. Trop. Dis.* **2023**, *17*, e0011117, doi:10.1371/JOURNAL.PNTD.0011117.
22. Otero-Patiño, R. Snake Bites in Colombia. *Clin. Toxinology Aust. Eur. Am. Toxinology* **2018**, doi:10.1007/978-94-017-7438-3_41.
23. Pineda, D.; Ghotme, K.; Aldeco, M.E.; Montoya, P. Accidentes Ofídicos En Yopal y Leticia, Colombia, 1996-1997. *Biomédica* **2002**, *22*, 14, doi:10.7705/biomedica.v22i1.1135.
24. Phillips, S.J. Transferability, Sample Selection Bias and Background Data in Presence-Only Modelling: A Response to Peterson et Al. (2007). *Ecography.* **2008**, *31*, 272–278, doi:10.1111/J.0906-7590.2008.5378.X.
25. Peterson, A.T.; Papes, M.P.; Muir, P.; Peterson, E.A.T.; Papes and, M.; Papes and, P.; Eaton, M. Transferability and Model Evaluation in Ecological Niche Modeling: A Comparison of GARP and Maxent. *Ecography.* **2007**, *30*, 550–560, doi:10.1111/J.0906-7590.2007.05102.X.
26. Rodríguez-Vargas, A.L.; Rodriguez-Buitrago, J.; Diaz, G.J. Comportamiento General de Los Accidentes Provocados Por Animales Venenosos En Colombia, 2006-2010. *Rev. salud pública.* **2012**, *14*, 1001–1009.
27. Tochie, J.N.; Temgoua, M.N.; Njim, T.; Celestin, D.; Tankeu, R.; Nkemngu, N.J. The Neglected Burden of Snakebites in Cameroon: A Review of the Epidemiology, Management and Public Health Challenges. *BMC Res. Notes* **2017**, *10*, 405, doi:10.1186/S13104-017-2718-2.
28. Grupo de Estratificación, D. de G. *Metodología de estratificación socioeconómica urbana para servicios públicos domiciliarios*; **2015**
29. Silva, F.S.; Ibiapina, H.N.S.; Neves, J.C.F.; Coelho, K.F.; Barbosa, F.B.A.; Lacerda, M.V.G.; Sachett, J.A.G.; Malheiro, A.; Monteiro, W.M.; da Costa, A.G. Severe Tissue Complications in Patients of *Bothrops* Snakebite at a Tertiary Health Unit in the Brazilian Amazon: Clinical Characteristics and Associated Factors. *Rev. Soc. Bras. Med. Trop.* **2021**, *54*, doi:10.1590/0037-8682-0374-2020.

30. Cañas, C.A.; Castro-Herrera, F.; Castaño-Valencia, S. Envenomation by the Red-Tailed Coral Snake (*Micrurus Mipartitus*) in Colombia. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2017**, *23*, 1–4, doi:10.1186/S40409-017-0100-4/TABLES/1.
31. da Silva, A.M.; da Fonseca, W.L.; de Araujo Valente Neto, E.; Bisneto, P.F.; Contreras-Bernal, J.; Sachett, J.; Monteiro, W.M.; Bernarde, P.S. Envenomation by *Micrurus anellatus bolivianus* (Peters, 1871) Coral Snake in the Western Brazilian Amazon. *Toxicon* **2019**, *166*, 34–38, doi:10.1016/J.TOXICON.2019.05.008.
32. Ramos-Rodríguez, V.; Montero-Carvajal, E.; Corrales, G.; Gómez, A. Clinical Report of a Human Bite by the Pelagic Sea Snake *Hydrophis Platurus* (Serpentes: Elapidae), in the Pacific Ocean of Costa Rica. *J. Clin. Images Med. Case Reports* **2021**, *2*, 1091.
33. Hernández-Camacho, J.I.; Álvarez-León, R.; Renjifo-Rey, J.M. Pelagic Sea Snake *Pelamis Platurus* (Linnaeus, 1766) (Reptilia: Serpentes: Hydrophiidae) Is Found on the Caribbean Coast of Colombia. *Mem. la Fund. La Salle Ciencias Nat.* **2006**, *164*, 7–16.
34. Briski, E.; Gollasch, S.; David, M.; Linley, R.D.; Casas-Monroy, O.; Rajakaruna, H.; Bailey, S.A. Combining Ballast Water Exchange and Treatment to Maximize Prevention of Species Introductions to Freshwater Ecosystems. *Environ. Sci. Technol.* **2015**, *49*, 9566–9573, doi:10.1021/ACS.EST.5B01795/SUPPL_FILE/ES5B01795_SI_001.PDF.
35. Rodríguez-Vargas, A. Accidente Ofídico. In: *Guía para el manejo de emergencias toxicologícas*; Alejandro, M.P., Álvaro, C.Q., Ana, D.A.V., Ana María, R.M., Andrés Felipe, E.A., Eds.; Ministerio de Salud y la Protección Social: Bogotá D.C, **2017**; pp. 499–507 ISBN 9789585401334.
36. Bucaretchi, F.; De Capitani, E.M.; Vieira, R.J.; Rodrigues, C.K.; Zannin, M.; Da Silva, N.J.; Casais-e-Silva, L.L.; Hyslop, S. Coral Snake Bites (*Micrurus* spp.) in Brazil: A Review of Literature Reports. *Clin. Toxicol.* **2016**, *54*, 222–234.
37. da Silva, C.J.; Jorge, M.T.; Ribeiro, L.A. Epidemiology of Snakebite in a Central Region of Brazil. *Toxicon* **2003**, *41*, 251–255.
38. Otero, R.; Fonnegra, R.; Jimenez, S.L.; Nunez, V.; Evans, N.; Alzate, S.P.; Garcia, M.E.; Saldarriaga, M.; Del Valle, G.; Osorio, R.G.; et al. Snakebites and Ethnobotany in the Northwest Region of Colombia: Part I: Traditional Use of Plants. *J. Ethnopharmacol.* **2000**, *71*, 493–504.
39. Michael, G.C.; Aliyu, I.; Grema, A.B.; De-Kaa, N.L.P. Prehospital Care Practices for Venomous Snakebites in Resource-limited Settings: A Narrative Review. *Arch. edicine Heal. Sci.* **2017**, *7*, 237–241, doi:10.4103/amhs.amhs.
40. Otero-Patino, R. Epidemiological, Clinical and Therapeutic Aspects of *Bothrops Asper* Bites. *Toxicon* **2009**, *54*, 998–1011, doi:10.1016/j.toxicon.2009.07.001.
41. Otero-Patiño, R.; Nuñez, V.; Barona, J.; Díaz, A.; Saldarriaga, M. Características Bioquímicas y Capacidad Neutralizante de Cuatro Antivenenos Polivalentes Frente a Los Efectos Farmacológicos y Enzimáticos Del Veneno de *Bothrops asper* y *Porthidium nasutum* de Antioquia y Choco. *Iatreia* **2002**, *15*, 1–15.
42. World Health Organization *Snakebite Envenomation*; **2019**; Vol. 3; ISBN 9789241515641.

43. Ayerbe González, S.A. *SERPIENTES | Accidentes & Soluciones*; Ayerbe doc serpientes: Popayán, **2021**; ISBN 9789584924872.
44. Kasturiratne, A.; Lalloo, D.G.; Janaka de Silva, H. Chronic Health Effects and Cost of Snakebite. *Toxicon X* **2021**, *9–10*, 100074, doi:10.1016/j.toxcx.2021.100074.
45. Kasturiratne, A.; Wickremasinghe, A.R.; de Silva, N.; Gunawardena, N.K.; Pathmeswaran, A.; Premaratna, R.; Savioli, L.; Lalloo, D.G.; de Silva, H.J. The Global Burden of Snakebite: A Literature Analysis and Modelling Based on Regional Estimates of Envenomation and Deaths. *PLoS Med.* **2008**, *5*, e218, doi:10.1371/journal.pmed.0050218.
46. Fita, D.S.; Neto, E.M.C.; Schiavetti, A. "Offensive" Snakes: Cultural Beliefs and Practices Related to Snakebites in a Brazilian Rural Settlement. *J. Ethnobiol. Ethnomed.* **2010**, *6*, 1–13, doi:10.1186/1746-4269-6-13.
47. Williams, S.S.; Wijesinghe, C.A.; Jayamanne, S.F.; Buckley, N.A.; Dawson, A.H.; Lalloo, D.G.; de Silva, H.J. Delayed Psychological Morbidity Associated with Snakebite Envenomation. *PLoS Negl. Trop. Dis.* **2011**, *5*, e1255, doi:10.1371/journal.pntd.0001255.
48. Carrasco, P.A.; Grazziotin, F.G.; Cruz Farfán, R.S.; Koch, C.; Antonio Ochoa, J.; Scrocchi, G.J.; Leynaud, G.C.; Chaparro, J.C. A New Species of *Bothrops* (Serpentes: Viperidae: Crotalinae) from Pampas Del Heath, Southeastern Peru, with Comments on the Systematics of the *Bothrops neuwiedi* Species Group. *Zootaxa* **2019**, *4565*, 301–344, doi:10.11646/zootaxa.4565.3.1.
49. Bhaumik, S.; Kallakuri, S.; Kaur, A.; Devarapalli, S.; Daniel, M. Mental Health Conditions after Snakebite: A Scoping Review. *BMJ Glob. Heal.* **2020**, *5*, doi:10.1136/bmjgh-2020-004131.
50. Kasturiratne, A.; Wickremasinghe, A.R.; de Silva, N.; Gunawardena, N.K.; Pathmeswaran, A.; Premaratna, R.; Savioli, L.; Lalloo, D.G.; de Silva, H.J. The Global Burden of Snakebite: A Literature Analysis and Modelling Based on Regional Estimates of Envenomation and Deaths. *PLOS Med.* **2008**, *5*, e218.
51. Otero, R.; Gutiérrez, J.; Beatriz Mesa, M.; Duque, E.; Rodríguez, O.; Luis Arango, J.; Gómez, F.; Toro, A.; Cano, F.; María Rodríguez, L.; et al. Complications of *Bothrops*, *Porthidium*, and *Bothriechis* Snakebites in Colombia. A Clinical and Epidemiological Study of 39 Cases Attended in a University Hospital. *Toxicon* **2002**, *40*, 1107–1114, doi:10.1016/S0041-0101(02)00104-6.
52. Williams, D.J.; Faiz, M.A.; Abela-Ridder, B.; Ainsworth, S.; Bulfone, T.C.; Nickerson, A.D.; Habib, A.G.; Junghanss, T.; Fan, H.W.; Turner, M.; et al. Strategy for a Globally Coordinated Response to a Priority Neglected Tropical Disease: Snakebite Envenomation. *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007059, doi:10.1371/JOURNAL.PNTD.0007059.
53. Mora-Obando, D.; Pla, D.; Lomonte, B.; Guerrero-Vargas, J.A.; Ayerbe, S.; Calvete, J.J. Antivenomics and in Vivo Preclinical Efficacy of Six Latin American Antivenoms towards South-Western Colombian *Bothrops asper* Lineage Venoms. *PLoS Negl. Trop. Dis.* **2021**, *15*, e0009073, doi:10.1371/JOURNAL.PNTD.0009073.
54. Wedasingha, S.; Isbister, G.; Silva, A. Bedside Coagulation Tests in Diagnosing Venom-Induced Consumption Coagulopathy in Snakebite. *Toxins.* **2020**, *12*, doi:10.3390/TOXINS12090583.
55. Lynch, J.D.; Angarita-Sierra, T.; Ruiz-Gómez, F.J. *Programa Nacional Para La Conservación de Las Serpientes Presentes En Colombia*; Minis-

terio de Ambiente y Desarrollo Sostenible, Colombia, Universidad Nacional de Colombia - Instituto de Ciencias Naturales, Instituto Nacional de Salud: Bogotá, **2014**; ISBN 9789588901183.
56. Albuquerque, U.P.; Fernandes Cruz da Cunha, L.V.; Paiva de Lucena, R.F.; Nóbrega Alves, R.R. *Methods and Techniques in Ethnobiology and Ethnoecology. Springer Protocols Handbooks*; Springer Protocols Handbooks. Humana Press: New York, **2013**; ISBN 9781461486350.
57. Silva de Oliveira, S.; Campos Alves, E.; dos Santos Santos, A.; Freitas Nascimento, E.; Tavares Pereira, J.P.; Mendonça da Silva, I.; Sachett, J.; dos Santos Ibiapina, H.N.; Santos Sarraf, L.K.; Contreras Bernal, J.C.; et al. Bothrops Snakebites in the Amazon: Recovery from Hemostatic Disorders after Brazilian Antivenom Therapy. *Clin. Toxicol. (Phila).* **2020**, *58*, 266–274, doi:10.1080/15563650.2019.1634273.
58. Park, E.J.; Choi, S.; Kim, H.H.; Jung, Y.S. Novel Treatment Strategy for Patients with Venom-Induced Consumptive Coagulopathy from a Pit Viper Bite. *Toxins (Basel).* **2020**, *12*, doi:10.3390/TOXINS12050295.
59. Maduwage, K.; Isbister, G.K. Current Treatment for Venom-Induced Consumption Coagulopathy Resulting from Snakebite. *PLoS Negl. Trop. Dis.* **2014**, *8*, doi:10.1371/JOURNAL.PNTD.0003220.
60. Khwaja, A. KDIGO Clinical Practice Guidelines for Acute Kidney Injury. *Nephron. Clin. Pract.* **2012**, *120*, doi:10.1159/000339789.
61. Sarkar, S.; Sinha, R.; Chaudhury, A.R.; Maduwage, K.; Abeyagunawardena, A.; Bose, N.; Pradhan, S.; Bresolin, N.L.; García, B.A.; McCulloch, M. Snake Bite Associated with Acute Kidney Injury. *Pediatr. Nephrol.* **2021**, *36*, 3829–3840, doi:10.1007/S00467-020-04911-X.
62. Albuquerque, P.L.M.M.; Paiva, J.H.H.G.L.; Martins, A.M.C.; Meneses, G.C.; Da Silva, G.B.; Buckley, N.; Daher, E.D.F. Clinical Assessment and Pathophysiology of *Bothrops* Venom-Related Acute Kidney Injury: A Scoping Review. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2020**, *26*, doi:10.1590/1678-9199-JVATITD-2019-0076.
63. Mohan, G.; Guduri, P.R.; Shastry, S.; Kandasamy, D. Thrombotic Microangiopathy in Hematotoxic Snakebites and Its Impact on the Prognosis: An Entity Often Overlooked. *J. Thromb. Thrombolysis* **2019**, *48*, 475–482, doi:10.1007/S11239-019-01868-Z.
64. Valente-Aguiar, M.S.; Da Costa E Silva, B.G.; Magalhães, T.; Dinis-Oliveira, R.J. Compartment Syndrome Following *Bothrops* Snakebite Leads to Decompressive Fasciotomies. *Case Rep. Med.* **2019**, *2019*, doi:10.1155/2019/6324569.
65. Houcke, S.; Resiere, D.; Lontsingoula, G.R.; Cook, F.; Lafouasse, P.; Pujo, J.M.; Demar, M.; Matheus, S.; Hommel, D.; Kallel, H. Characteristics of Snakebite-Related Infection in French Guiana. *Toxins (Basel).* **2022**, *14*, doi:10.3390/TOXINS14020089.
66. Davidson, T.M.; Schafer, S.F.; Moseman, J. Central and South American pit vipers. *J Wilderness Med*. **1993**, 4(4), 416–40.
67. Diniz-Sousa, R.; Moraes, J.N.; Rodrigues-da-Silva, T.M.; Oliveira, C.S.; Caldeira, C.A. A brief review on the natural history, venomics and the medical importance of bushmaster (*Lachesis*) pit viper snakes. *Toxicon X*. **2020**, 7.
68. Rosenthal, R.; Meier, J.; Koelz, A.; Müller, C.; Wegmann, W.; Vogelbach, P. Intestinal Ischemia after Bushmaster (*Lachesis muta*) Snake-

bite a Case Report. *Toxicon,* **2002**, *40*, 217–220, doi:10.1016/S0041-0101(01)00203-3.
69. Asato, M.S.; Cruz Carbonell, R.C.; Martins, A.G.; Mendonça de Moraes, C.; Chávez-Olórtegui, C.; Apolonia da Costa Gadelha, M.; Pereira de Oliveira Pardal, P. Envenomation by the Rattlesnake *Crotalus durissus* Ruruima in the State of Roraima, Brazil. *Toxicon X,* **2020**, *8*, 100061, doi:10.1016/J.TOXCX.2020.100061.
70. Corbett, B.; Clark, R.F. North American Snake Envenomation. *Emerg. Med. Clin. North Am.* **2017**, *35*, 339–354, doi:10.1016/J.EMC.2016.12.003.
71. Rodríguez-Vargas, A.; Vega,N.; Reyes-Montaño, E.; Corzo, G.;Neri-Castro, E.; Clement, H.;Ruiz-Gómez, F. Intraspecific Differences in the Venom of *Crotalus durissus* cumanensis from Colombia. Toxins **2022**, 14, 532.
72. Bisneto, P.F.; Alcântara, J.A.; Mendonça da Silva, I.; de Almeida Gonçalves Sachett, J.; Bernarde, P.S.; Monteiro, W.M.; Kaefer, I.L. Coral Snake Bites in Brazilian Amazonia: Perpetrating Species, Epidemiology and Clinical Aspects. *Toxicon* **2020**, *175*, 7–18, doi:10.1016/J.TOXICON.2019.11.011.
73. Bucaretchi, F.; Hyslop, S.; Vieira, R.J.; Toledo, A.S.; Madureira, P.R.; De Capitani, E.M. Bites by Coral Snakes (*Micrurus* spp.) in Campinas, State of São Paulo, Southeastern Brazil. *Rev. Inst. Med. Trop. Sao Paulo* **2006**, *48*, 141–145, doi:10.1590/S0036-46652006000300005.
74. Bisneto, P.F.; Araújo, B. dos S.; Pereira, H. da S.; Mendonça da Silva, I.; Sachett, J. de A.G.; Bernarde, P.S.; Monteiro, W.M.; Kaefer, I.L. Envenomations by Coral Snakes in an Amazonian Metropolis: Ecological, Epidemiological and Clinical Aspects. *Toxicon* **2020**, *185*, 193–202, doi:10.1016/J.TOXICON.2020.07.009.
75. Hijmans, R.J.; Cameron, S.E.; Parra, J.L.; Jones, P.G.; Jarvis, A. Very High Resolution Interpolated Climate Surfaces for Global Land Areas. *Int. J. Climatol.* **2005**, *25*, 1965–1978, doi:10.1002/joc.1276.
76. Leroy, B.; Meynard, C.N.; Bellard, C.; Courchamp, F. Virtualspecies, an R Package to Generate Virtual Species Distributions. *Ecography.* **2015**, *39*, 599–607, doi:10.1111/ecog.01388.
77. WCS, W.C.S.-; University, C. for I.E.S.I.N.-C.-C. Last of the Wild Project, Version 2, 2005 (LWP-2): Global Human Footprint Dataset (Geographic) **2005**.
78. Gollini, I.; Lu, B.; Charlton, M.; Brunsdon, C.; Harris, P. GWmodel: An R Package for Exploring Spatial Heterogeneity Using Geographically Weighted Models. *J. Stat. Softw.* **2015**, *63*, 1–50, doi:10.18637/JSS.V063.I17.
79. Milborrow, S. Plot "rpart" Models: An Enhanced Version of "Plot.Rpart" **2021**, 25.
80. Bolar, K. STAT: Interactive Document for Working with Basic Statistical Analysis **2019**.
81. Ripley, B.; Venables, B.; Bates, D.M.; Hornik, K.; Gebhart, A.; Firth, D. Package ' MASS ' **2022**, 170.
82. Rodríguez-Vargas, A.; Franco-Vásquez, A.M.; Bolivar-Barbosa, J.A.; Vega, N.; Reyes-Montaño, E.; Arreguín-Espinosa, R.; Carbajal-Saucedo, A.; Angarita-Sierra, T.; Ruiz-Gómez, F. Unveiling the Venom Composition of the Colombian Coral Snakes *Micrurus helleri*, *M. medemi*, and *M. sangilensis*. Toxins **2023**, 15, 622. https://doi.org/10.3390/toxins15110622

Chapter 10

The dose makes the poison: A review of the pharmacology of snake venoms

Carolina Zona, Teddy Angarita-Sierra, Jaime Andres Pereañez

Abstract: There has always been a close link between snakes and human perceptions of life and death in human history. Snake venoms have been used in many forms, from enhancing weapons to medieval remedies. From ancient civilization to the present, snake venoms have been useful for creating new molecules, from the first antivenom to captopril®, a new drug. We carried out online searches from April to June of 2021. We performed the searches following the Prisma methods and recommendations. Therapeutic properties attributed to venoms can be grouped into the following pharmacological uses: first alterations of the cardiovascular system and nervous system; and other indications such as cancer treatment, antimicrobial therapy, and management of coagulation disorders. The therapeutic potential of Colombian snake venoms is immense, and they can help generate new treatments for multiple pathologies. Using "omics" technologies will allow identifying more potential molecules in the venoms of snakes from Colombia. However, we must continue conventionally studying the venom components, while "omics" technologies can be performed in a massive form and at a reasonable cost for Colombian researchers.

Keywords: snake venoms, pharmacological activity, drug discovery, medicinal applications, *Bothrops atrox*, *Crotalus durissus*



Citation: Zona C.; Angarita-Sierra T.; Pereañez JA. Chapter 10: The dose makes the poison: A review of the snake venoms used in pharmacology with special interest in Colombian snakes. In Book: *Bites, venoms, and venomous snakes of Colombia*; Angarita-Sierra T., Ruiz-Gomez FJ, Eds.; Instituto Nacional de Salud: Bogota D.C., Colombia, 2024; pp. 433–480.
doi: 10.33610/890573oeutlw

Copyright: 2024 by the authors. Open access publication under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND 4.0) license (http://creativecommons.org/licenses/by/4.0/).

Illustrations by:
Oscar A. Ramirez Ruiz

1. From ancient potions to current drugs: A short journey thru the history of venoms used in medicine.

There has always been a close link between snakes and human perceptions of life and death in human history. Snake venoms have been used in many forms, from enhancement for weapons to medieval remedies. In some languages, the same word is used both for life and for the snake. This double meaning explains why in so many cultures snakes were seen as a healing creatures or like a life-given God [1].

Since ancient civilizations, snakes have been seen as a symbol of life and health. This explains why snakes are the symbol of medicine. In Mesoamerica cultures, Quetzalcoatl (In Nahua, *quetzalli* means "bird" and "*coatl*" means snake); a feathered serpent stands as a symbol for Earth and sky, time and space, fertility and creation, as well as being the guardian of snake medicine [2]. Today in the Sierra of Jalisco, Mexico, traditional healers of the Wixarika community stay devout to the plumed serpent through song and dance [2].

In Greek civilization, snake represented air, earth and was an attribute of Athena; and its meaning was fecundity, health, continuity, and eternity. It was said that in the temples of *Asklepios* at Epidaurus, Rhodes, Cnides, and Cos, the touch of the snake's tongues could heal blind people. It is also said that Aristotle described the properties of venom and their uses [3]. For example, Aristotle said that some vipers produce a venom that causes septic symptoms and immediate death; and others refer to the asp as that which makes the skin rotten. He also thought that poisonous animals ate another venomous animal to obtain its venom. Finally, he describes that some humans in Egypt and Libya obtained those venoms to poison other animals and enemies [4].

In Africa, ancient Egyptians represent the cycle of life, death, and resurrection in the Ouroboros symbol that depicts a snake eating its tail [5]. This ancient civilization referred to snakes as Atoum, the creator of the air and the Earth. Also, the golden cobra (*Naja haje*) named Uraeus was the symbol of life, knowledge, and sovereignty [5]. It is said that Cleopatra was especially interested in snake venom effects [6]. She tested venoms on condemned prisoners to see the different body reactions and to find the toxic limits [6].

Moreover, she compared symptoms of envenomation between species of vipers (*Cerastes* spp, *Echis* spp) and elapids (*Naja* spp, *Walterinnesia* spp). This information later served another purpose when she committed suicide. After many years of study of the symptoms and reactions of many snake venoms, she learned that the death produced by a viper was more violent and painful than elapids. Afterward, she chose the royal Uraeus (*Naja haje*), a cobra snake of the Pharaohs, and died from its silent bite [6]. Nevertheless, Cleopatra's death is a subject of controversy. There are other theories, including murder or suicide using her knowledge of poisonous plants, but snake envenomation is one of the most likely methods that she used [6].

After Christianity and considering the Bible's lousy reputation given to snakes, it is clear why in Europe snake venom was not frequently used. There are only a few references about this. One of them is an exciting tale from de 19th century from the German chemist Kekule (1829-1896), who in one of his dreams saw a snake with its tail in its mouth. Later he related this dream to the structure of the benzene rings (aromatic rings composed of six carbon atoms used in the preparation of dyes or of detergents) [3].

Fascination with the toxicity of snake venom has led humanity to use it to kill and heal. The uses of snake venoms can be summarized in two paths: as a weapon to silently defeat enemies and its role in therapies and remedies to heal dying patients. One of the best-known weapons in which snake venom was used were arrows contaminated with venom. These weapons have their origins in Greek mythology and were first used by Heracles. In addition, many ancient soldiers and warriors used venom dipped arrows from southern Africa to Siberia [3].

Since 326 BC, Alexander the Great was attacked in India with these lethal projectiles. Symptoms described by the soldiers suggested that it was poison from Russell's vipers (*Daboia russelii*). Later in the V century BC, Scythian warriors used venom as a coating for arrow tips to kill enemies. Nevertheless, this culture went further by exploring the venom healing properties. Some ancient writings describe the technique employed by Scythians to obtain the venoms to create antivenoms and medicines [7].

The Scythian healer Agari joined King Mithridates (more later) to research snake venoms. They concluded that venom could be digested in small amounts, but it was lethal if it enters the bloodstream [8]. Also, they used to say that every poisonous substance has its natural antidote. That is why they commonly combined ingredients such as cinnamon, honey, castor, garlic, charcoal, with snake venom to create treatments. One common use was the *electuary*. This was a paste made with honey and a minute amount of venom alongside the *Theriac* (=*Theriaque*), the first known antivenom. It was believed to boost the immune system [9]. Finally, snake venom was also used to treat war wounds and hemorrhages for its anticoagulant properties [10].

Similarly, Carthaginian Hannibal Barca (247-181 BC), a general who led the fight against the Roman Republic during the Second Punic War, once ordered his soldiers to throw pots full of snakes at enemy ships, causing significant damage to them. However, this biological attack led to the creation of the first "antivenom" by mixing snake meat with a variety of components and was named "*Theriaque*" [7].

In the Black Sea empire of the first century BC, using Cleopatra's methodological approaches, King Mithridates VI of Pontus (120–63 BC), known as the first toxicologist, formulated a universal antidote that contained a mixture of toxins and antidotes. King Mithridates proclaimed himself the discoverer of the antidote (*Theriaque*) useful in the treatment for every

venomous reptile and poisonous substance. He also used prisoners to explore antidotes by subjecting them to snake envenomation [11].

It was said that this substance the "*Theriaque"* was able to neutralize all snake venoms. The technique recalled the principles of immunization by inoculating small amounts of venom and thus "priming" the immune system's response to a snake bite [12]. Later in 67 A.C., Mithridates suffered a severe sword wound to the leg that produced profuse bleeding putting him at risk of death. But one of his disciples stopped the bleeding with snake venom, saving his life. This is the first reported use of snake venoms for their coagulating effects. This discovery was recently used by scientists in the new field of "venomics" [12] (Chapter 5).

However, *Theriaque* was widely used for multiple illnesses by cultures and civilizations of the ancient world until the XVII century when the famous French apothecary, Moyse Charas, published the formula for *Theriaque,* thus ending its monopoly. Currently, the original formula for *Theriaque* is in disuse. It can be found in the market as a food supplement that maintains its name, but it is reportedly used as an over-the-counter treatment for food poisoning, purifying the body of traces of bewitchment, treating digestive spasms; and it is helpful in obsessive-compulsive disorder and psychological crises [11]. Please never use it as antivenom therapy, but it may be helpful in cases of bewitchment.

Ancient wars led to the development of various medicines and healing substances. Some of them are based on snake venom. This is clear in the almost obsessive interest in snakes in the medieval medical literature. As mentioned above, the *Theriaque* was one of the first questionable antivenoms. Later this substance was produced in Paris, but a new, much simpler and still questionable one called *Orvietan* was also created along with another called *Bezoard*, containing snake liver and fat, both used as a panacea against poisonings with criminal intent, as well as snake envenomation [3].

Also, a toxic powder called *Viperine* was obtained by Lucien Bonaparte in 1843. He precipitated the venom of *Vipera berus* (common adder) with alcohol and ether, and compared its effects to digestive enzymes [13]. Later in 1868, the American Mitchell isolated the *Crotaline* toxin from rattlesnakes and measured its effects on animals, the main output being symptoms of paralysis [14]. A few decades later, Pelder proposed that snake venoms had a high protein content. Wolfenden later validated this theory in 1886, obtaining a substance like albumin for the venom. Years after these discoveries, Martin separated *Pseudechis porphyriacus* (Red-bellied black snake) venom into two main components. One of these caused hemorrhages, while the other stopped the breathing of animals when injected [14]. These findings represent the first descriptions of the biological activities of venoms (Figure 1).

Another important use of venom was the induction of immune response in animals. In 1890, Louis Pasteur met the French physician Al-

bert Calmette (1863–1933), who was invited to create and direct the first daughter Pasteur Institute in Saigon (now Ho Chi Minh City, Vietnam), French Indochina, aiming to protect the local population against rabies and smallpox. This Pasteur Institute opened in 1891, and Calmette performed research on snake venoms. He carried out several attempts to induce an immune response in animals, but they were unsuccessful [15-16]. In 1894, Calmette used repeat venom inoculations in rabbits, and he produced an anticobra serum. Calmette presented his results to the French Society of Biology in the same year [17]. Finally, in the Pasteur Institute in France, Calmette began the production of anticobra serum for therapeutic use by injecting snake venom in horses in a repeated schedule the production of which was first suggested by Sewall [18].

In Latin America, the pioneer of antivenom production was Vital Brazil, who read the manuscript published by Calmette [5]. One year later, Brazil entered the Institute of Bacteriology, and immediately started injecting animals with small doses of Brazilian snake venoms [18,19]. In 1901, Vital Brazil published his findings of snake venoms and serum production [20,21]. In February 1901, the Institute Butantan was officially opened under the name of the Institute of Serumtherapy with Vital Brazil as Director [6].

During the 20th century, the physiological effects and compositions of the venoms of different snake species were isolated and became known. Snake venoms were classified by their properties; for example, the venoms of *Naja naja, Crotalus adamanteus* (eastern diamondback rattlesnake), and *Daboia russellii* (Russel's viper) were found to be effective for curare as well as depressor and coagulating effects. Also, a new term *ophiotoxin* was added to the substances present in venoms [3].

Later, some experiments were conducted, showing that the venoms of *Crotalus adamanteus, Agkistrodon piscivorus* (cottonmouth), and *Naja atra* (Chinese cobra) could affect the nervous system, producing respiratory paralysis. The venoms with coagulant proprieties were the ones from the viperids *Daboia russelii, Echis carinatus* (phoorsa), *Trimeresurus gramineus* (bamboo viper) and *Crotalus adamanteus;* and the elapids *Pseudechis porphyriacus* and *Acanthophis antarcticus* (common death adder) [3].

In the second half of the 20th century, Sergio Ferreira reported a venom fraction from *Bothrops jararaca* (Jararaca) that could inhibit the conversion of angiotensin I to II, potentiating the action of bradykinin (see in detail later). This fraction was named the bradykinin-potentiating factor (BPF) [22]. In parallel, Ferreira joined Jhon Vane's Lab as a Post-doc and Vane became a consultant for the Pharmaceutical Squibb. Simultaneously but separately, Ferreira et al. [23], and Ondetti et al. [24], another researcher from Squibb, continued working to obtain more accurate information about BPF, leading to isolation and characterization of the active peptides of the fraction [23,24].

These peptides did not have enough potential to be a pharmaceutical product since they lacked oral stability, and their massive production was

expensive. Furthermore, Cushman et al. [25] published the characterization of some analogs of the original peptides and the specific region that binds to the active site of the angiotensin-converting enzyme I (ACE-I), blocking the conversion of angiotensin II, and resulting in vasodilatation. Then, Squibb spent millions of dollars to generate an orally active form of the drug and on animal and clinical studies, so that finally in the early 1980s Captopril® was approved by the Food and Drug Administration as a drug for the treatment of hypertension. This made it possible to manage and avoid the sequelae of arterial hypertension such as kidney injury, heart attack, encephalopathy, etc., [26].

Late in the 1980s to 2000s, two snake venom disintegrins have been used as lead compounds for the development of two anti-thrombotic drugs (Eptiftibatide, Integrilin®, Millenium Pharmaceuticals) and Tirofiban (Agrastat®, Merck & Co) [27]. Equally, snake venom toxins have shown multiple biological activities, such as antitumor, antibacterial, antiviral, hypotensive activity, anticoagulant, etc. [28–30]. Likewise, snake venoms have been employed as tools in biomedical research. For example, some toxins have been essential for describing receptors or the mode of action of enzymes, as well as venom proteins as essential reagents in clinical chemistry [28,31].

So, disciplines such as biochemistry, physiology, pharmacology, and immunology provide new knowledge about these toxic substances and their structure, and their effects and reactions on organic systems. This knowledge allows the synthesis of active ingredients and much more selective and effective drugs. From this outlook, snake venoms are an essential source of molecular components that can modulate several physiologic processes. Nowadays, there are a variety of active principles derived from snake venoms. These can be classified by their effects, such as cancer, anticoagulation, pain management, antibiotics, antihypertensive, etc., (Figure 2). The amazing therapeutic potential and biomedical use of snake venom toxins are covered in this chapter.

2. Pharmacological role of snake venoms

Knowledge about pharmacological properties of snake venoms has bloomed with the advancement of science and technology. Currently, a great variety of active principles derived from these substances can be found. Snake venoms are a mix of active substances such as enzymes, proteins, and peptides (see Chapter 5). The main components of venoms include about 19 protein families such as PLA_2, serine proteases, metalloproteinase, lectins, l-amino-acid oxidases, bradykinin potentiating factors, natriuretic factors, and integrin antagonists, etc. [32]. In addition, these substances have biological activities that could have therapeutic properties by interacting with endogenous receptors (e.g., cell membrane receptors G protein-coupled and ion channels, among others) and membrane and coagulation proteins, affecting the neuromuscular and cardiovascular system, blood coagulation function, and can cause significant alterations in the body [33].

Nevertheless, their target specificity, affinity, and selectivity provide an excellent opportunity for new pharmacological interventions with more effective action mechanisms, fewer negative interactions, and adverse effects. These features are essential in an "ideal drug" that could be produced from these toxins [34]. Some examples of possible new drugs controlling hypertension, antithrombotic, anticoagulant, antiviral, analgesic, neuromodulator, and fibrinolytic problems and effects have been studied for human uses (Table 1). Although these toxins are helpful as a drug, they can also be helpful in diagnosis and studies of cellular mechanisms [35].

The therapeutic properties that are attributed to venoms can be grouped into the following pharmacological uses: first, alterations of the cardiovascular system and nervous system; and other indications, such as cancer treatment, antimicrobial therapy, management of coagulation disorders. These therapeutic properties will be addressed in the following sections (Figure 2)



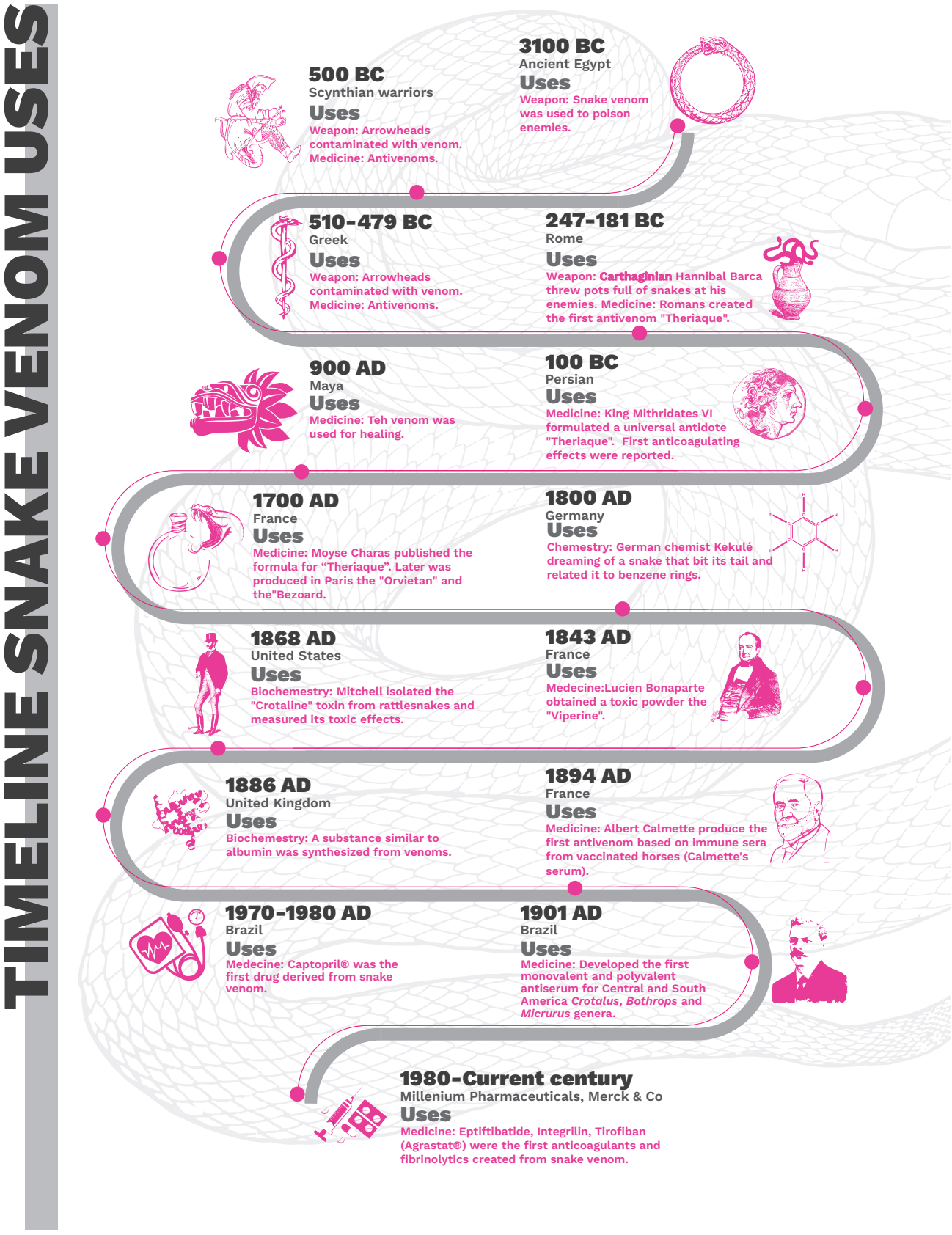


Figure 1. Timeline infographic depicting the main snake venom uses across human history.

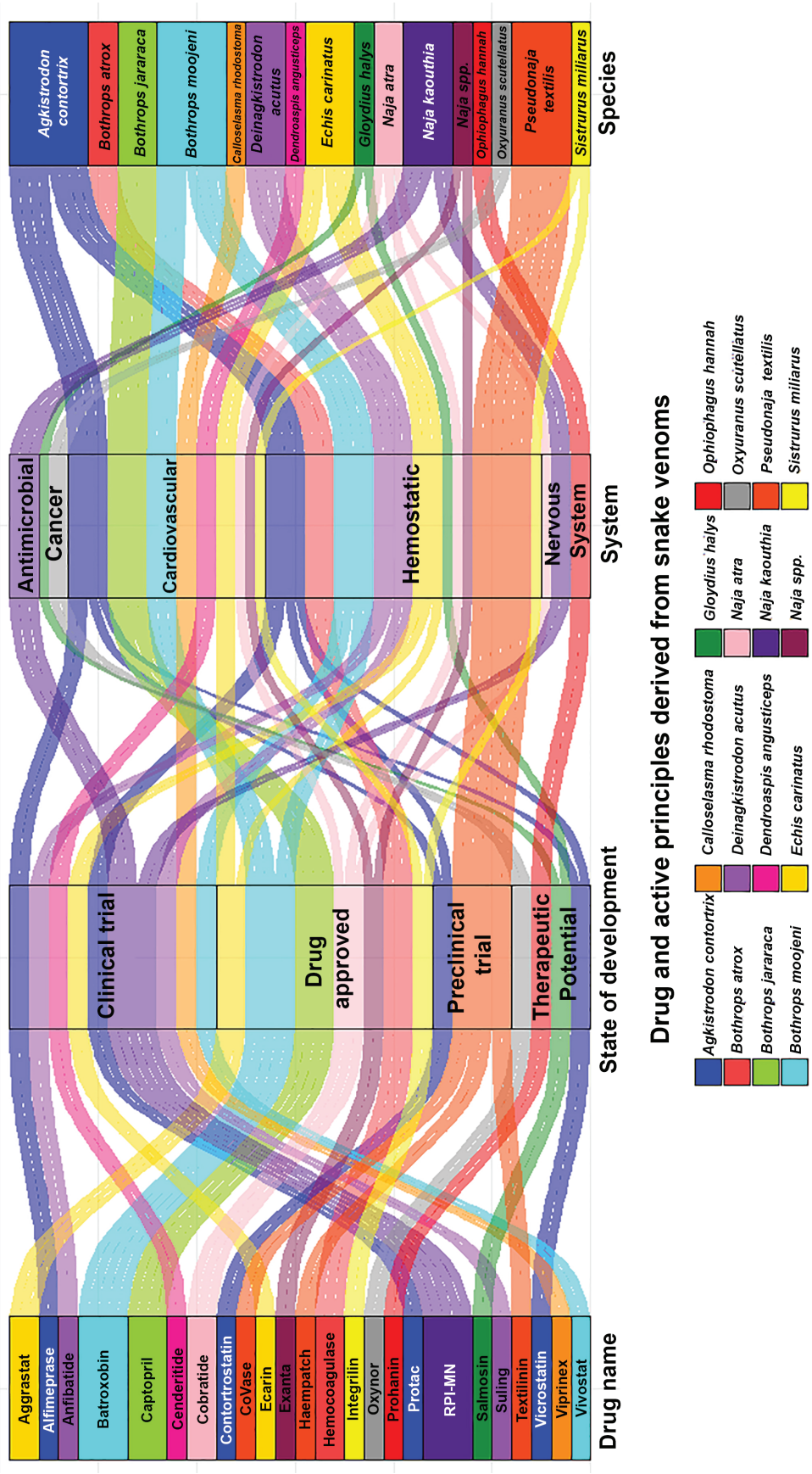


Figure2. Alluvial diagram depicting the relationship between drugs, snake venom, human systems, and development state.

Table 1. Biological activities of some snake venom toxins and their pharmacological potential.

Toxin	Biological activity	Potential	References
PLA_2	Myotoxicity, neurotoxicity, modulation of platelet aggregation, anticoagulation, edema-forming, and hypotension	Cytotoxic, anti-viral, and bactericidal activities	[36–40]
SVMPs	Hemorrhage, myotoxicity, edema-forming, blister formation, and dermonecrosis	Activation of blood coagulation factors such as V and X	[41–43]
SVSPs	Hemostatic disturbances and hypotension	Blood clot hydrolysis, ischemic stroke, and reagents in clinical chemistry	[44–46]
LAAOs	Hemorrhage, edema-forming, and modulation of platelet aggregation	Antibacterial, antileishmanial, anti-viral, and cytotoxic activities	[47,48]
Disintegrins	Inhibition of platelet aggregation	Anti-cancer and antithrombotic agents	[27,49]
Lectins	Hemostatic disturbances and inflammatory responses	Anti-cancer and antithrombotic agents	[46,50]
3FTxs	Neurotoxicity, cardiotoxicity, hemostatic disorders, and interactions with ion channels	Analgesic activity and agents useful for the study of structure and function of muscarinic receptors (parasymatic receptors)	[51–53]

For the mode of action of these toxins see Chapter 5 in this book. The biological potential will be described in this chapter.

Cardiovascular system

Cardiovascular system alterations are one of the most frequent diseases worldwide. These include high blood pressure, ischemic heart diseases, and cerebrovascular diseases. For example, among the natural causes of death for men and women in Colombia are as follow: 36.9 % ischemic heart diseases, 14.7% cerebrovascular diseases, and 8.2% hypertension [54].

Considering the variability of the etiology and mechanisms involved in the onset of these pathologies, their management becomes a challenge for the health care system. Some of these diseases are associated with lesions in the blood vessels, dyslipidemias, heart failure, alterations in the rhythm and heart rate, atherosclerosis, etc. [55]. Additionally, the occurrence of these pathologies can be connected to genetic or external risk factors and associated with each person's habits. This means that managing these pathologies requires a wide range of specific medications to treat each condition that led to the pathology [55].

Arterial systemic hypertension is a chronic condition characterized by a vascular alteration that causes abnormally high blood pressure. This disease leads to atherothrombotic vascular events (e.g., myocardial infarction, cerebrovascular attack, etc.), heart failure, or kidney failure. However, in most cases, the cause is not identified, and it requires chronic treatment that includes different mechanisms [56,57].

The approach to treating hypertension requires changes in vascular tone and blood volume. This can be achieved by inhibiting or blocking vasoconstrictor substances (e.g., adrenaline, noradrenaline, vasopressin, angiotensin II, aldosterone, antidiuretic hormone) or by potentiating vasodilator agents (e.g., nitric oxide, histamine, bradykinin, substance P, and vasoactive intestinal peptide) [58]. To change blood volume diuretic drugs are used. These medicines cause water elimination and some electrolytes (sodium, potassium, magnesium, calcium, among others) depending on the selected drug [58].

Bradykinin potentiating peptides (BPP)

As explained above, Captopril® was the first Food and Drug Association-approved drug derived from the bradykinin potentiating peptide from snake venoms (*Bothrops jararaca;* Table 2*)*. Since then, a great variety of drugs that belong to the angiotensin-converting enzyme inhibitors group have been synthetized [26]. Bradykinin potentiating peptides are present in viperid and elapid venoms from genera like *Agkistrodon*, *Bitis*, *Bothrops*, *Crotalus*, *Lachesis, Hydrophis Oxyuranus, and Pseudonaja* [59].

Bradykinin potentiating peptide acts like angiotensin-converting enzyme (ACE) inhibitors and is one of the most utilized groups of molecules for treating hypertension by causing relaxation of blood vessels and a decrease in blood volume [60]. Also, they prevent the conversion of angiotensin I to angiotensin II, avoiding its vasoconstrictor, as well as sodium and water retention effects. This inhibition also enhances the vasodilators and anti-fibrosis effects of bradykinin that accumulates when ACE is inhibited. This action is especially important in patients with renal disease [60]. Likewise, cytoprotective effects from BPP-derived drugs have been observed in the heart muscle and could be beneficial in heart failure and acute myocardial infarction [60].

Benefits from the BPP are not limited to acting as ACE inhibitors. These peptides promote the synthesis of nitric oxide (endogenous vasodilator agent). Also, BPP can bind to muscarinic receptors M1 causing vasodilatation by activating the parasympathetic system response [61]. This leads to a more sustained decrease in blood pressure and a possible new molecule to treat hypertension [26].

Additionally, BPP affects the central nervous system and modifies the release of neurotransmitters such as GABA and glutamate. These neurological effects could modulate autonomic functions like blood pressure, heart rate, and release of neurotransmitters. Even though more research is needed, these findings offer new opportunities to treat hypertension and other autonomic conditions [26].

Gouda et al. [62] raise the hypothesis that a drug derived from these BPP could be effective for treating COVID-19, given the role of ACE in the replication mechanism of the virus. Some evidence suggests the virus could alter the function of ACE, affecting bradykinin production. Therefore, inhibiting ACE with BPP from snake venoms could result in an effective drug for treating COVID-19. Although this hypothesis requires further evidence, the physiological processes demonstrate biological plausibility [62].

Natriuretic peptides

Natriuretic peptides (atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide) are vasoactive agents that cause regulation of vascular permeability, an increase of venous capacitance (the ability of veins to store blood), inhibition of the renin-angiotensin and aldosterone system (in charge of regulating the retention and elimination of sodium and water, vascular tone, etc.), diuresis and natriuresis. In other words, natriuretic peptides have an important role in regulating vascular tone, retention, or elimination of water and sodium, through the modification of different mechanisms [61].

Recently, natriuretic peptides have been found in the venom of the snake *Dendroaspis angusticeps* (green mamba). This peptide causes vasodilation stimulating the guanylate cyclase and calcium channels in aortic myocytes resulting in vascular smooth muscle relaxation [46,61]. Moreover, natriuretic peptides have been isolated from the venom of *Crotalus oreganus* (Northern Pacific rattlesnake), showing a vasodilator effect by increasing nitric oxide production by stimulating potassium channels. These peptides can also be found in the venom of diverse and unrelated species such as *Bungarus flaviceps* (red-headed krait), *Bungarus multicinctus* (many-banded krait), *Crotalus durissus* (South American rattlesnake), *Lachesis muta* (South American bushmaster), *Micrurus corallinus* (painted coral snake), *Oxyuranus microlepidotus* (inland taipan), *Pseudonaja textilis* (eastern brown snake), *Pseudechis australis* (mulga snake), *Pseudocerastes persicus* (Persian horned viper), and *Protobothrops flavoviridis* (habu snake) [46,59].

Despite the different snake venom sources, natriuretic peptides have the same vasodilatation effects allowing study and synthesis of this molecule using different species. Medicines derived from this peptide can be used to treat pathologies of the cardiovascular system. Since the studies carried out to date show that they have a cytoprotective effect on cardiac cells and their vasodilator effect these properties benefit patients with congestive heart failure and ischemic heart disease [46,61,63].

Calcium channel blockers

Calcium plays an essential role in muscle contraction. When this ion is released through calcium channels, actin and myosin interact to produce muscle contraction. Although there are different calcium channels (e.g., T, L, N, P, Q, R), some molecules from snake venom such as 3FTx protein and PLA_2 act specifically in L and T type channels [40]. On the other hand, the L-type calcium channel cardiovascular functions include smooth muscle contraction and cardiac peacemaking. T-type calcium channels are

involved in the action potential on cardiac myocytes [38]. By blocking calcium channels (L-type), calcium transport in the cardiac muscle and vascular smooth muscle is impeded, causing vasodilatation, heart rate decrease, and cardiac contractibility. This is especially useful in managing high blood pressure, angina, arrhythmia, and heart failure [64].

Given the paramount importance that drugs must be highly selective, snake venom toxins raise a great opportunity to develop highly selective medicine targeting calcium channels. This development will avoid the adverse effects of currently available drugs due to their lack of selectivity facing multiple cardiovascular and neurological alterations. For example, 3FTx proteins from elapid snake venoms like calciseptine, FS2 toxin, C10S2C2, and S4C8 have shown high selectivity by binding and blocking L-type calcium channels.

Moreover, active principles synthesized from these toxins can be used to treat diseases of the cardiovascular system mentioned above. Therefore, these toxins have the potential to replace drugs like amlodipine or verapamil (calcium channel blockers), among others, avoiding adverse effects such as palpitations, hypotension, edema in feet and ankles, fatigue, vertigo, headaches, drowsiness, nausea, dyspepsia and dry mouth [63].

Adrenergic Receptors

Adrenergic receptors are G protein-coupled membrane receptors responsible for sympathetic responses to adrenaline. This means that they have a key role in regulating heart rate, cardiac contraction, vascular tone, lipolysis, insulin release, bronchodilation, kidney function, etc. These receptors are divided into α and β with 9 subtypes (six for α1A, α1B, and α1D; α2A, α2B, α2C; and three for β1, β2, and β3). Adrenergic receptors are present in several tissues and intervene in different physiological sympathetic responses [65].

In the cardiovascular tissues, α1, α2, and β2 receptors are mainly present on the blood vessels' smooth muscle cells [65]. Also, α1 and β1 are also found on the heart muscle. The activation of β receptors leads to increased heart rate, contractility, and conduction velocity of the heart. The α1 receptors also increase cardiac contractility, causing vasoconstriction, while β2 produces smooth muscle relaxation. As a result, drugs like beta-blockers (e.g., propranolol, carvedilol, metoprolol) are widely used in angina, congestive heart failure, hypertension, irregular heartbeat, heart attack, tachycardia, coronary heart disease, as well as migraine, anxiety, and essential tremor, in combinations with other treatments [65].

Therefore, some β-cardiotoxins (3FTx) from the venom of *Ophiophagus hannah* have been characterized thru β-cardiotoxins that bind or block β1 and β2 receptors causing a lower heart rate and being useful for cardiovascular diseases such as heart failure, alterations in the rhythm, and ischemic heart diseases, but lacking the hemolytic and lytic effects observed in currently used drugs. Likewise, AdTx1 and UniProtKB P85092 toxins (3FTx from elapids) are α1 receptor blockers and could be used as vasodilators [35,46].

Moreover, toxins such as UniProtKB C0HJR1, UniProtKB, and C0HJR2 (3FTx) are present in *Micrurus mipartitus* (redtail coral snake) venom and have been shown to modulate the neurotransmitter GABA, lowering blood pressure and heart rate from a central mechanism [63].

Finally, some cardiotoxins like those found in king cobra venom (*Ophiophagus hannah*) increase heart rate. These substances are known to cause cell membrane perturbation and have lytic factors. They are also named cytotoxins and have been studied for cancer treatment, and could have some antimicrobial properties (this will be addressed later) [35].

Nervous system

There are snake venoms that contain neurotoxic components that interrupt communication between the axon and the motor plate, producing immobilization and loss of coordination in the prey. In addition, these neurotoxic agents act on physiological mechanisms that are important for neurotransmission, such as ion channel receptors on cell membranes [34,66]. Fatal effects such as muscle paralysis can be found in both Viperidae (*Crotalus* species) and Elapidae (sea snakes: Hydrophiinae; coral snakes: *Micrurus* species). Venoms from elapid snakes are mainly rich in PLA_2 and 3FTx, potent neurotoxins that intervene in neuromuscular transmission at presynaptic or postsynaptic levels [32,67].

Presynaptic Neurotoxins

The presynaptic effects are mostly attributed to β-neurotoxins. These act through the inhibition of acetylcholine release by eliminating acetylcholine vesicles, resulting in a blockage of the neuromuscular transmission without interfering with the sensitivity of the motor plate to acetylcholine (Ach) [66,68]. This feature could be useful, and a drug synthesized from these toxins could be a good candidate to replace the current available neuromuscular blockers (e.g., depolarizing succinylcholine, non-depolarizing rocuronium, vecuronium, atracurium, cisatracurium, mivacurium). Furthermore, these new drugs could avoid the common side effects of hemodynamic instability (tachycardia, bradycardia, hypertension, or hypotension); bronchospasm, malignant hyperthermia; hyperkalemia, impaired neuromuscular transmission, muscular injury weakness and their interactions [67,69].

Postsynaptic Neurotoxins

On the other hand, α-neurotoxins have postsynaptic activity, reversibly blocking cholinergic receptors. In addition, these toxins bind to the postsynaptic nicotinic acetylcholine receptors preventing the ion channels' opening and interrupting the neuromuscular transmission at the motor plate [66,68]. This mechanism has been helpful to elucidate the physiopathology of some neuromuscular diseases such as myasthenia gravis that causes weakness in the skeletal muscles that worsens after periods of activity and improves after periods of rest [68].

Analgesic toxins

Another use of the interaction between these neurotoxins and the cholinergic pathways is an anti-nociceptive beneficial effect on treating chronic

pain. For example, cobra toxin isolated from typical cobra venom (*Naja naja*) has analgesic properties. This toxin is a specific ligand for the cholinergic receptor a1 nAChR, causing analgesic effects by a non-opiate-dependent pathway [35,53]. Likewise, the cobra toxin binds with high affinity to the a7 nAChR, producing the same effect. Moreover, it has been reported that this toxin can be replaced with morphine, helping manage the morphine-abstinence syndrome [70]. Currently, the potential analgesic activity from a-neurotoxin from the king cobra (*Ophiophagus hannah*) is under study. Crotamine from the South American rattlesnake (*Crotalus durissus*) has demonstrated an anti-nociceptive effect more potent than morphine [33,67,71].

Otherwise, neurotoxins (dendrotoxin) from green mamba venom (*Dendroaspis angusticeps*) selectively binds to the muscarinic acetylcholine receptors. The muscarinic receptors have an essential role in treating neurodegenerative diseases like Alzheimer's and Parkinson's. These receptors' selective blockade could help to restore normal movement in these diseases [71,72]. Likewise, a toxin (Glu-Val-Trp) described from common lancehead or mapaná (*Bothrops atrox*) has shown neuroprotective and pro-neuroplasticity properties. Particularly, dopaminergic cell cultures treated with this toxin have shown a significant decrease in cell viability. This effect has the potential to treat pathological neuronal degeneration and could be useful in therapies used for Alzheimer's and Parkinson's diseases [73].



Table 2. Drugs or active principles approved by the FDA for use in humans.

Drug	Mechanism	State	AC	System	Indication	Origin	Reference
Captopril	Inhibiting angiotensin-converting enzyme. (ACE inhibitor)	FDA approved	Yes	Cardiovascular	Hypertension, congestive heart failure, myocardial infarction, and diabetic neuropathy	*Bothrops jararaca*	[29,32,35]
Aggrastat (Tirofiban)	Glycoprotein IIb/IIIa inhibitors.	FDA approved	Yes	Cardiovascular, hemostatic	Heart attack, acute coronary disease, and antithrombotic therapy.	*Echis carinatus*	[29,61,71]
Integrilin (Eptifibatide)	Glycoprotein (GP) IIb/IIIa inhibitors.	FDA approved	Yes	Cardiovascular, hemostatic	Acute coronary disease and antithrombotic therapy	*Sistrurus miliarus.*	[29,33,35]
Defibrase/Reptilase (Batroxobin)	Converts fibrinogen into fibrin	FDA approved	No	Cardiovascular, hemostatic	Stroke, pulmonary embolism, deep vein thrombosis and myocardial infarction.	*Bothrops atrox* & *B. moojeni.*	[32,33,71]
Hemocoagulase	Catalyzes the blood coagulation	FDA approved	No	Hemostatic	Plastic surgery, abdominal surgery, and human vitrectomy.	*Bothrops atrox*	[33,35,74]
Exanta (Ximelagatran).	Direct thrombin inhibitors	FDA approved	No	Cardiovascular, hemostatic	Thromboembolic complications of atrial fibrillation	Cobra venom (*Naja spp*)	[29,32,74]
Cobratide (Ketongning, cobratoxin)	Blockage of nicotinic receptors.	No FDA approval, but currently used in China.	No	Cardiovascular, hemostatic, nervous system.	Chronic arthralgia, sciatica, neuropathic headache.	*Naja kaouthia* *Naja atra*	[29,32,71]

AC: Available in Colombia

Cancer

Cancer is one of the commonest diseases in the world. In Colombia, according to the Ministerio de Salud y Protección Social, by 2020 the incidence was 182 cases per 100,000 inhabitants. Hence, early detection and adequate treatment reduce mortality and is a priority for health systems [75].

By definition, cancer is a cellular disorder in which cells divide abnormally, forming lesions that grow, damaging surrounding tissues, using the body's energy resources, and negatively affecting physiology [76]. This process is called carcinogenesis [76]. In addition, cancer cells can migrate from their original tissue invading other systems, causing abnormal growth at any site. This process is known as metastasis [77]. The alteration produced by the overgrowth of these abnormal tissues and its effect on the body systems ultimately causes complications and death in some cases [78].

The genetic alteration in the cell is the result of the interaction between the patient's genetic factors and external agents, such as: physical carcinogens like ultraviolet and ionizing radiation, chemical carcinogens like asbestos, components of tobacco smoke, aflatoxins (contaminants in food) and arsenic (contaminants in drinking water), and biological carcinogens like certain viruses, bacteria and parasites [79]. The genetic damage or mutations caused by these factors allow the cells to divide at a higher rate, generating clones with the same genetic mutation or damage. Then the "daughter" cells accumulate various mutations that allow them to create different types of clones. These have a greater growth and proliferation capacity, compared to normal cells [80].

Under normal conditions, the immune system can control tumor cells through a process called tumor immunosurveillance. However, some clones have the ability to evade the control mechanisms, causing neoplasia (increase in the number of new cells) [81]. Moreover, some alterations in the immune system can cause disbalances between the cellular proliferation and reproductive processes and between growth control and apoptosis mechanisms (programmed mechanism of cell death) [82].

Therefore, the processes involved in cancer are sustained proliferative signaling, growth suppressor evasion, immune destruction evasion, apoptosis evasion, replicative immortality, induction of angiogenesis (new blood vessels that feed the tumor), invasion and metastasis, mutation and genomic instability, tumor-promoted inflammation, and cellular energy dysregulation [83]. Hence, cancer treatment is focused on interrupting the processes of cell replication, altering the cancer cell's environment, activating processes of cell death, apoptosis, autophagy, necrosis, and finally alerting the immune system to the presence of the tumor [84,85].

Although the pathology alone causes significant damage to the body, treatments such as chemotherapy, radiotherapy, and immunotherapy in most cases hurt several body systems [86]. Moreover, these medical in-

terventions inhibit the growth or cause the death of healthy cells, producing important side effects (hair loss, weak nails, bleeding, failure in other systems such as the kidney, liver, etc.). Given the difficulty of producing more selective and specific drugs, there is currently paramount interest in finding specific drugs to treat each kind of cancer known [86].

Current research has created safer drugs for cancer patients by improving their selectivity and specificity. As seen above, snake venom toxins are promising candidates to achieve this goal. Numerous studies have shown that these toxins can induce a direct lytic effect on tumor cells or alter the environment created by the tumor for its survival (e.g., angiogenesis). Moreover, some toxins trigger an inflammatory response that helps alert the immune system to the presence of the tumor [87–90]. There are several studies that have shown that snake venom toxins have specific effectiveness against different types of cancer, such as cervical cancer, breast cancer, pancreatic cancer, and ovarian cancer [91–95]. In the following paragraphs, we briefly describe the known anticancer action of snake venom toxins.

Phospholipase A2 (PLA_2)
Among toxins present in South American viper venoms such as *Bothrops asper, B. jararacussu, B. pauloensis, and B. moojeni*, PLA_2 toxins have been found to be useful in fight cancer [84,87,89]. Particularly, PLA_2 antitumor activity is given by its cytotoxicity, its phospholipid hydrolysis and mobilization of arachidonic acid properties, resulting in inhibition of tumor growth, DNA damage, apoptosis, autophagy, and suppression of metastasis (Table 3). In addition, these properties interfere with vascular proliferation, affecting tumor angiogenesis (formation of new blood vessels) [88,96].

Crotoxin (a kind of PLA_2) from the venom of the South American rattlesnake (*Crotalus durissus*) has shown specific cytotoxic activity towards cancer cells [87,96]. This toxin interferes with the epidermal growth factor receptor that is highly selective. In some phase I and II trials, the crotoxin showed a reduction of the disease in different types of cancer such as breast cancer, melanoma, and leukemia [88–90,95].

Cardiotoxin-3
As described above, the cardiotoxin present in elapid venom has cytotoxic properties useful in cancer treatment by inducing apoptotic cell death and regulating the processes of proliferation [89]. In addition, some studies have shown that cardiotoxin activates apoptosis in the endoplasmic reticulum and in the mitochondrial pathway, being effective as an antineoplastic drug [88]. Currently, the molecule VRCTC-310-Onco, composed of crotoxin (from *Crotalus durissus*) and cardiotoxin (from *Naja atra*) is being tested in skin and breast cancer, showing that in 80% of the cases, tumor reduction is observed [88,95,96].

Disintegrins
Disintegrins (non-enzymatic proteins, e.g., Salmosin) from Haly's pit viper (*Gloydius halys*) have shown anticancer properties inhibiting tumor behavior. They block cell to cell interaction, along with cell-matrix communica-

tion and signaling transmission [88], stopping the growth of metastatic and solid tumors. This effect has also been found in snake venoms from other non-closely related vipers [88,89].

Contortrostatin and vicrostatin disintegrins extracted from the copper head (*Akistrodon contortrix*), Phoorsa (*Echis carinatus*), and McMahons desert viper (*Eristicophis macmahoni*) have been shown to impede cancer cell mobility, invasion, metastasis, and tumor angiogenesis [90,92,93,96,97].

L-amino acid oxidases (LAAOs)
The toxin LAAOs found in the venoms of king cobra (*Ophiophagus hannah*) and Chinese moccasin *(Deinagkistrodon acutus*) have been shown to induce a reduction in cellular proliferation and apoptosis [88,89,96]. Similarly, toxins from the venom of the Malayan pit viper (*Calloselasma rhodostoma*) have shown anticancer properties. CR-LAAO toxin causes DNA damage and has been described in cancer cells; likewise, the toxin BjussuLAAO-II from jararacussu snakes (*Bothrops jararacussu*) has genotoxic and cytotoxic effects in cancer cells [84,85,94].

Several studies have described that LAAOs from species of genera *Bothrops*, *Calloselasma*, *Cerastes*, *Crotalus*, *Cryptelytrops*, *Bungarus*, and *Micrurus* could induce autophagy, apoptosis, and necrosis in normal and cancer cells [84]. In addition, these toxins can also activate proinflammatory cytokines, inducing cancer cell death by activation of the immune system [84,85,90,94].

Antimicrobial defense
Several components of the venoms have been reported to have very effective antimicrobial activity. These are the main groups of natural antimicrobial peptides with powerful microbicidal properties against bacteria, fungi, and some viruses like defensin and cathelicidin. The most common proteins with antimicrobial activity are PLA_2s and LAAOs (see Chapter 5). These toxins destabilize the membrane of the microorganism and produce local concentrations of H_2O_2 that are toxic for the microbe (Table 3). PLA_2s and LAAOs have also demonstrated activity against gram-positive and gram-negative bacteria, parasites, and other viruses [33,98–100].

Other non-enzymatic toxins such as C-type lectins, 3FTXs, and cathelicidins have been reported as compounds with antimicrobial activity. For example, the C-type lectin from the venom of the whitetail lancehead (*Bothrops leucurus)* has been effective against bacteria like *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus subtilis* [101]. Likewise, proteins belonging to the same family, isolated from the venom of the jararacussu (*Bothrops jararacussu*), acts against *S. aureus* [102]. Similarly, C-type lectins from the venom of the South American rattlesnake (*Crotalus durissus*) have shown activity against *Xanthomonas axonopodis* and *Clavibacter michiganensis* (cancer-inducing bacteria) [103].

Some 3FTXs have also been reported as antimicrobial agents, specifically those isolated from the venom of cobras (*Naja* spp). The binding of

these toxins to lipopolysaccharide and lipoteichoic acid, the major components in the bacterial wall, was described as the mode of action [104,105].

Peptides with antimicrobial activity are the cathelicidins that have been identified in several venom transcriptomes of several species. These peptides have activity against multi-drug-resistant *Acinetobacter baumannii* (MRAB) and methicillin-resistant *S. aureus*, whose control has become a challenge for the current commercialized drugs. The most studied cathelicidins are those from Chinese cobra (*Naja atra*), banded krait (*Bungarus fasciatus*), and king cobra (*Ophiophagus hannah*) venoms [106–110]. The action of all described molecules is believed to allow snakes to block the proliferation of infectious microorganisms present in prey, facilitating predation.

Hemostatic system

Antithrombotic agents

The development of Captopril®, as well as the design of the antithrombotic agents, are the most successful cases of drug development from snake venoms (Table 2). As mentioned above, Eptiftibatide (Integrilin®, Millenium Pharmaceuticals) and Tirofiban (Agrastat®, Merck & Co) were obtained from snake venom disintegrins [20]. Nowadays, they are available to treat acute myocardial infarction, acute coronary syndrome, and percutaneous coronary intervention (Table 2).

Echistatin drug, isolated from the venom of the saw-scaled viper (*Echis carinatus*) was an effective antagonist of fibrinogen-induced platelet aggregation [111]. This disintegrin contains an RGD motif (see Chapter 5) and can bind several integrins in the range of nanomolar. Thus, the drug requires minimal quantities to block integrins and impede platelet aggregation. However, considering that the distance between arginine (R) and aspartate (D) is a structural determinant for the activity of these toxins, Merck & Co performed some modifications, including the insertion of tyrosine at the site of a 4-(4-piperidinyl) butyl group in the N-terminal and (S)-butylsulfonylamino group in the C-terminal. The modifications led to developing a compound with a great potency, named MK-0383 that is called Tirofiban that can inhibit platelet aggregation (Table 3). This drug is used in acute myocardial infarction, acute coronary syndrome, and percutaneous coronary intervention [112,113].

Likewise, barbourin, isolated from the venom of the pygmy rattlesnake (*Sistrurus miliarius*), was discovered after screening several snake venoms for their ability to inhibit platelet aggregation [114,115]. Barbourin has the KGD motif; several modifications were needed to obtain the Eptiftibatide, including peptide cyclization and derivatization of the lysine side chain in the KGD motif (Table 3). This drug is also used in acute myocardial infarction, acute coronary syndrome, and percutaneous coronary intervention [116,117].

Another group of toxins that have potential as antithrombotic agents is the C-type lectins that bind to a different receptor on the platelet's sur-

face (see Chapter 5). For example, Vixapatin, isolated from the venom of the Ottoman viper (*Montivipera xanthina*), has great potential as an inhibitor of platelet aggregation by blocking the α2β1 receptor [118]. After some modifications, this toxin leads to the design of Vipegitide. The capacity to antagonize the mentioned receptor on the platelet has been demonstrated, and it is considered a new type of template of an antithrombotic agent derived from snake venoms [119].

Biomedical uses of snake venom toxins in the hemostatic system

Due to the action of snake venom toxins on the blood clotting factors and platelets, they can be used as tools to study coagulation and be employed as strategies in the chemical clinic as alternatives to conventional reagents. For example, the snake venom thrombin-like enzyme batroxobin (Reptilase®), isolated from the venom of the common lancehead (*Bothrops atrox*) is used to determine the reptilase time (a blood test used to detect deficiency or abnormalities in fibrinogen, especially in cases of heparin contamination). This test is usually performed to confirm or exclude the suspicion of dysfibrinogenemias or alternative in samples containing heparin [120]. Another protein with important activity is Ancrod, isolated from the venom of the Malayan pit viper (*Calloselasma rhodostoma*). Both toxins either Reptlase or Ancrod, are also employed in the antithrombin III assay, for which plasma must be free of fibrinogen, and thrombin cannot be added because its reaction with antithrombin III could interfere with the test [121].

Other proteins of the Viperidae and Elapidae snake venoms with biomedical potential are the prothrombin activators [122]. These toxins have several applications, including meizothrombin preparation (one of the main products of the prothrombin activation), non-enzymatic production forms of thrombin, or meizothrombin, and in studies of prothrombin hydrolysis [120,123–125]. For example, the production of meizothrombin by ecarin, an SVMP isolated from the venom of the saw-scaled viper (*Echis carinatus*) is used as a diagnostic tool for the anticoagulant from lupus [126]. Moreover, ecarin time is also used for monitoring the anticoagulant properties of dabigatran etexilate, a drug indicated in the venous thromboembolic event [127].

Moreover, snake venoms also contain activators of other blood clotting factors, such as factors V and X. For example, the venom of the Russell viper (*Daboia russelli*) has RVV-V and RVV-X, toxins that activate factor V and factor X, respectively. Therefore, RVV-V is used in routine assays of factor V. And RVV-X is utilized in tests to quantify the factor X of the coagulation cascade and differentiate the deficiencies of factors VII and X [128–131].

Table 3. Drugs or active principles from snake venoms used in clinical trials.

Drug	Target	State	System	Indication	Origin	Reference
Alfimeprase	Thrombolytic activity	Clinical trial	Cardiovascular	Acute peripheral arterial occlusion	*Agkistrodon contortrix*	[29,32,35]
Viprinex (Ancrod)	Defibrinogenating agent	Clinical trial	Cardiovascular	Acute ischemic stroke	*Calloselasma rhodostoma*	[29,61,71]
Protac/protein C activator	protein C activator	Clinical trial	Hemostatic	Clinical diagnosis of hemostatic disorder	*Agkistrodon contortix*	[29,33,35]
Ecarin	Prothrombin activator	Clinical trial	Hemostatic	Diagnostic	*Equis carinatus*	[32,33,71]
Vivostat	Snake venom thrombin-like enzyme/Serine protease	Clinical trial	Hemostatic	Anticoagulant	*Bothrops moojeni*	[33,35,74]
Anfibatide	C-Type lectin like protein/ Proteinase	Clinical trial	Hemostatic	Thrombolytic and antithrombotic activities	*Deinagkistrodon acutus*	[29,32,74]
Cenderitide	Natriuretic and hypotensive peptide	Clinical trial	Cardiovascular	Hypertension	*Dendroaspis angusticeps*	[29,32,71]
RPI-MN/ RPI- 78M	Antiviral, neuromodulatory and analgesic activities	Clinical trial	Antimicrobial defense, nervous system	Drug resistant HIV strains, treatment of multiple sclerosis, muscular dystrophy, myasthenia gravis and amyotrophic lateral sclerosis	*Naja kaouthia*	[29,32,35]
Suling	Snake venom thrombin like enzyme/ Serine Protease	Clinical trial	Hemostatic	Anticoagulant	*Deinagkistrodon acutus*	[29,61,71]
Contortrostatin	Disintegrin	Preclinical studies	Hemostatic, cardiovascular	Inhibits platelet aggregation	*Agkistrodon contortrix*	[29,33,35]
Textilinin	Kunitz-type serine protease inhibitor/Plasmin inhibitor	Preclinical studies	Hemostatic	Antibleeding agent	*Pseudonaja textilis*	[32,33,71]
Haempatch	Prothrombin activator / Factor Xa-like protein	Preclinical studies	Hemostatic	Clotting agent	*Pseudonaja textilis*	[33,35,74]
CoVase	Pro-coagulation factor/ Factor Va-like protein	Preclinical studies	Hemostatic	Non-compressible hemorrhagic agent	*Pseudonaja textilis*	[29,32,74]
Vicrostatin	Chimeric disintegrin	Therapeutic potential	Hemostatic, cardiovascular	Antithrombotic/ inhibits platelet aggregation	*Echis carinatus, Agkistrodon contortrix*	[29,32,71]
Salmosin	Disintegrin	Therapeutic potential	Cancer, hemostatic	Platelet-dependent hemostasis/ blocking integrin (αvβ3)/ anti-cancer agent	*Gloydius halys*	[33,35,74]
Oxynor	Presynaptic neu- rotoxin (β-taipoxin)/Mitogenic activity	Therapeutic potential	Cancer	Mitogenic/wound healing	*Oxyuranus scutellatus*	[29,32,74]
Prohanin	α-Neurotoxin (Hannalgesin)/ Antinociceptive activity/ analgesic effect	Therapeutic potential	Nervous system	Antinociceptive activity/ analgesic effect	*Ophiophagus hannah*	[29,32,71]

3. Pharmacological and biomedical potential of venoms from Colombian snake species

Historically, the medical or pharmacological uses of venoms from Colombian snake species have been poorly explored. Of the amazing venomous snake diversity inhabiting the country´s ecosystems, most of them are endemic (See Chapter 1); and only in recent decades have national and foreign researches focused on Colombian venomous snake species and the huge pharmaceutical potential of their venoms [132].

Efforts made in recent decades have contributed to knowledge about Colombian endemic species, clinical manifestations of snakebite, and the development of new therapeutic agents. Nevertheless, studies about medical or pharmacological uses continue to be incipient although they are extraordinarily promising. As partially explained above, many indications have been found within the pharmacological uses of venoms from snake species found in Colombia. In this section we present a detailed revision of the therapeutic uses of Colombian snakes and their potential toxins (Table 4).

Viperidae

The venoms of snakes from Colombian species of Viparidae are rich in PLA_2s, SVMPs, SVSPs; and they have moderate amounts of LAAOs, bradikinin-potentiating peptides (BPPs), disintegrins, and C-type lectins (see Chapter 3 and 5). Thus, it is feasible that venoms have antibacterial, antiparasitic, anti-cancer, anti-thrombotic potential, among other activities. So, PLA_2s from the patoco (*Porthidium nasutum*), mapaná (*Bothrops asper*), and Colombian populations of the rattlesnake (*Crotalus durissus*) showed antibacterial and antiplasmodial activities, respectively [133,134].

LAAOs from rattlesnakes and eyelash pit vipers (*Bothriechis schlegelii* species complex) have also demonstrated antibacterial activity [135,136]. The venom of the toadheaded pitviper (*Bothrocopias myersi*) may have great potential as a source of antimicrobials since it has the highest amount of PLA_2s compared to the venoms of the characterized Colombian viperids (see Chapter 5) [137]. However, in all cases mentioned above (PLA_2s and LAAOs), more studies are needed to obtain robust insights into the mode of protein action on bacteria and their toxicity, although PLA_2s from *B. asper* are not lethal to mice at doses as high as 15000 µg/kg, indicating their low toxicity [134].

The venom of the Pacific bushmaster (*Lachesis acrochorda*) deserves special attention. Among characterized venoms of the Viperidae from Colombia, Pacific bushmaster venom has the highest quantity of BPPs. So, it should be studied as a potential source of new antihypertensive drugs. Additionally, *L. acrochorda* venom has the unique characteristic of having a higher content of SVSPs than other pit viper venoms from Colombia. The pharmacological and biomedical potential of the SVSPs has been described above, and this is an important fact to consider for the potential of bushmaster venom. Moreover, one type of SVSPs is the kallikrein-like enzymes that are responsible for releasing bradykinin and potentiating the hypotensive actions of other toxins that can be considered other sources of antihy-

pertensive molecules [138]. These proteins have already been isolated from Brazilian populations of Amazon bushmaster venom (*Lachesis muta)* [139].

Except for *Crotalus durissus* and *Lachesis acrochorda*, the venoms of the remaining Colombian pit viper snakes have disintegrin as part of their composition. As described above, disintegrins are used as leads for developing anti-thrombotic agents. However, currently there are no studies from Colombian pit viper snakes that explore disintegrin in pharmacological applications. Thus, the isolation and characterization of these types of proteins from Colombian venoms must be performed to increase our knowledge about them and to clarify their pharmacological potential.

Likewise, C-type lectins, present in all Colombian Viperidae venoms characterized up until now have not been studied pharmacologically in Colombia. We suggest that the venoms with more potential are those with higher concentrations of this kind of toxins. For example, the golden lancehead (*Bothrops punctatus*) has 16.7% of C-type lectins [140], and *B. asper* only 8.54% [141]. The C-type lectin toxins inhibit platelet aggregation and can be primers for developing anti-thrombotic drugs.

Another important case is the Nasulysin-1, an SVMP isolated from the venom of *Porthidium nasutum*. This toxin provokes specific apoptosis-inducing activity (programmed cell death) in Jurkat and K562 cells, a T-cell of acute lymphocytic leukemia (ALL), and a chronic myeloid leukemia (AML) cell model without affecting the viability of human lymphocyte cells. Furthermore, this SVMP activates caspase-3 and the apoptosis-Inducing factor (AIF) [142]. Nevertheless, further studies are needed to obtain information about the toxicity of Nasulysin-1 and structural determinants of cytotoxic activity in order to evaluate its real potential.

Notably, among Colombian pit vipers, *Crotalus durissus*, the single rattlesnake present in the country, possesses crotoxin that is a neurotoxin built from a basic subunit that is a PLA_2 (CB) and an acidic (CA) subunit formed by three peptides (α, β, and γ) bound by seven disulfide bonds (see Chapter 5). It acts as a chaperon for the CB subunit [143]. Crotoxin, isolated from *C. durissus,* has anti-tumoral activity demonstrated in cell culture and with patients with solid tumors that are refractory to conventional therapy [144–147]. A recent study proposed that crotoxin could be a tool against thrombosis development by lowering the levels of procoagulant proteins and increasing those of anticoagulant proteins [148]. Finally, the chain α from subunit CA of the crotoxin has analgesic activity performed by several mechanisms (see above). For all these reasons, *Crotalus durissus* has huge pharmacological potential.

Although studies about medical or pharmacological uses of venoms are incipient in Colombia, we can precisely find several molecules with proven therapeutic activity from venomous species inhabiting Colombian ecosystems (Figure 3). The effects and indications of such molecules are mentioned below, encompassing medically important species of viperids, elapids, and colubrids.

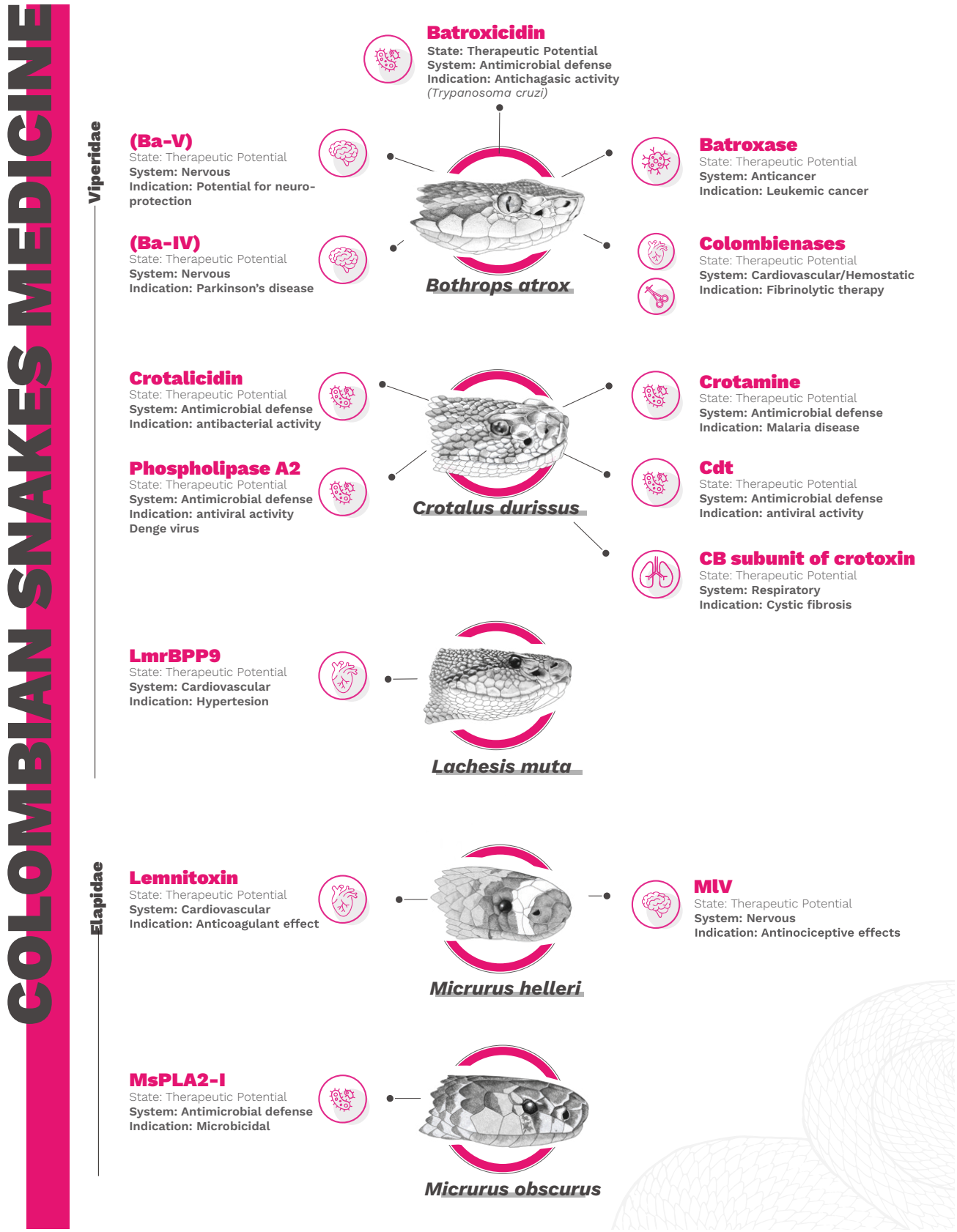
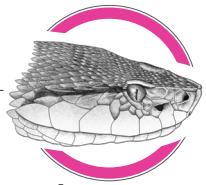


Figure 3. Infographic of pharmacological uses of venoms from Colombia snake species

COLOMBIAN SNAKES MEDICINE

Viperidae

Flaviviridae
State: Therapeutic Potential
System: Antimicrobial defense
Indication: Virucidal activity
Reference:5



Bothrops asper

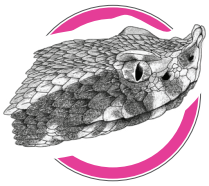
Lansbermin-I
State: Therapeutic Potential
System: Anticancer
Indication: Breast Cancer Cells
Reference:16



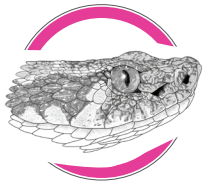
PIlans–II, an acidic Asp49–PLA2
State: Therapeutic Potential
System: Anticancer
Indication: Cervical carcinoma
Reference:18

Porthidium lansbergii

Acidic PLA2
State: Therapeutic Potential
System: Antimicrobial defense
Indication: Microbicidal
Reference:17



Porthidium nasutum



Btae TX-I
State: Therapeutic Potential
System: Anticancer
Indication: Cervical carcinoma
Reference:19

Bothrops taeniata

BsLAAO
State: Therapeutic Potential
System: Antimicrobial defense
Indication: Antibacterial activity
Reference:20



Bothriechis schlegelii

Figure 3. Infographic of pharmacological uses of venoms from Colombia snake species

Bothrops atrox.— Batroxicidin shows antibacterial activity. This molecule has antichagastic activity by inducing necrosis and apoptosis to *Trypanosoma cruzi* (Chagas disease). It was compared to the currently available drugs (benznidazole), but batroxidin shows effectiveness against the benznidazole-resistant strain of *T. cruzi*. This finding makes batroxicidin a molecule that can potentially treat chagas disease and similar blood parasites [149].

Low-molecular-mass peptides Ba-V from *B. atrox* venom have neuroprotective benefits. These peptides have therapeutic potential for neurodegenerative diseases such as Parkinson's and Alzheimer's by inhibiting mitochondrial permeability transition, presumably by altering calcium flow. This process plays a significant role in the pathophysiology of neurodegenerative diseases, and Ba-V's inhibition of this pathway could prevent cell brain death [150]. In addition, a similar molecule, Ba-IV, has shown neuroprotective effects in neurodegenerative diseases such as Parkinson's disease. The Ba-IV inhibits the apoptotic proteases like caspase- 9 and caspase-3 that prevent cell death in dopaminergic neurons, the primary process presents in Parkinson's disease. Therefore, Ba-V and Ba-IV might make it possible to prevent the progression of neurodegenerative diseases [151].

Bothrops asper.— From the venom of *B. asper* two molecules such as Mt–I and Mt-II, PLA_2-like isoforms have been isolated. Both have been tested as potential drug-facing diseases by flaviviruses (viral hemorrhagic fevers). Due to their enzymatic activity Mt–I and II could permeabilize the virus envelope, explaining their virucidal activity. Mt–I has strong antiviral activity, while Mt-II does not show the same results. These findings could lead to an effective treatment against flaviviruses such as Denge [152].

Other toxins from *B. asper* venoms with therapeutic potential are colombienases I and II, and batroxase. These could be helpful as a thrombolytic agent being used in cardiovascular diseases. These enzymes have several biological activities that are hemorrhagic, fibrinogenolytic, proteolytic, hemolytic, edematogenic, and cytotoxic. For example, they degrade fibrinogen without activating the fibrinolytic system (plasminogen/plasmin) [153]. In addition, batroxase modulates pro-inflammatory cells in human blood and induces cytotoxic effects in tumoral blood cells. This activity could regulate cancer cell proliferation present with leukemia, and a new treatment could be derived from this molecule [154]

Bothrops taeniatus.— The enzymatic activity from Btae TX-I, a PLA_2 molecule isolated from *B. taeniatus* venom, has similar properties as an anticancer molecule seen in other PLA_2 toxins from other toxins described amongst its congeners. Further, the potential as an anticancer drug was also seen in the Btae TX-I molecule [155].

Crotalus durissus.— The South American rattlesnake *Crotalus durissus* is one of the most promising Colombian species in the pharmacological exploration of new drugs. Currently, there have been at least five toxins with pharmacological uses tested. The polypeptide toxin crotamine iso-

lated from *C. durissus* venom has shown antiparasitic effects, especially involving *Plasmodium falciparum* infections (Malaria). Since crotamine enters the parasite's nucleus, it alters pH regulation modifying ambient acidity, leading to changes in the parasite's membrane. This effect is achieved without affecting healthy blood cells, demonstrating the excellent potential to treat malaria [156]. In addition, the same mechanism has been studied for anticancer activity against melanoma, pancreatic cancer, and carcinoma, in which crotamine has shown effectiveness [157].

Cytotoxin (Cdt), a molecule isolated from PLA_2 of *C. durissus* venom, has virucidal activity. Cdt has been described as useful against the measles virus by blocking the cellular receptors that the virus needs for its adsorption. However, more research is needed regarding effectiveness after infection because this molecule was only shown to prevent the virus from entering the cell. Neveretheless, this finding may elucidate new mechanisms of action for virucidal drugs [158].

The CB subunit of crotoxin was also isolated. This toxin has been shown to regulate the cell membrane Cl- channel influx. Therefore, crotoxin could lead to medical applications such as treating cystic fibrosis, a disease characterized by a genetic mutation that leads to alterations in the regulation of chloride channels. This leads to systemic alterations and considerably limits a patient life expectancy [159].

Also, antibacterial activity from crotalicidin has been described. This molecule was tested against *Escherichia coli* and *Pseudomonas aeruginosa,* causing rupture of the cell membrane of the bacteria leading to bacterial death. One advantage of crotalicidin against infections is that effective concentration does not cause damage to healthy human cells. This finding represents excellent news for antibacterial resistance to current drugs; and in the future, it could help produce a new generation of non-resistant antibiotics [157,160]. Furthermore, antiviral activity, especially for the dengue virus, was found in phospholipase A_2 from *Crotalus durissus*. The mechanism is related to the enzymatic activity of phospholipase A_2 that causes degradation of the virus envelope, exposing the RNA virus, enabling its degradation by the RNase enzyme [161].

Finally, another therapeutic potential has been described from *C. durissus* toxin venom. The peptide crotalphine has an antinociceptive activity that could be important in treating chronic pain, such as cancer treatments. Furthermore, crotalphine effects are induced by opioids kappa and delta receptors, leading to long-lasting pain management compared to morphine. Therefore, this molecule could be beneficial to avoid the administration of opioid drugs, preventing abstinence syndrome [162]. In addition, a similar molecule, crotapotin, has also shown analgesic effects, and is being studied for encephalomyelitis pain in multiple sclerosis, establishing it as a promising molecule to treat this kind of pain [163].

Lachesis muta.— The peptide named LmrBPP9 was synthesized from the Amazonian bushmaster. This molecule has ACE inhibitory activity like

the BPPS used to create captopril. Moreover, LmrBPP9 effectively lowers blood pressure, comparable to captopril. This LmrBPP9 result opens up new sources of BPPS that can help to create drugs to treat hypertension [164].

Porthidium species complex.— Currently, two components from *Porthidium lansbergii* venom have been found to have anticancer activity. First, PLA_2 (Pllans-II) has been tested against cervical cancer and showed effectiveness without damaging healthy tissues [165]. Also, a disintegrin (Lansbermin-I) isolated from *P. lansbergii* venom had anticancer activity in breast cancer cells. This toxin affects angiogenesis and the migration of breast cancer cells. All these molecules offer new alternatives for research and for the creation of new drugs for cancer treatment [91,166].

An acidic PLA_2 toxin isolated from *Porthidium nasutum* exhibited antibacterial activity. Like other PLA_2 toxins, the mechanism also involves the rupture of the bacterial membrane, and this makes it a powerful bactericide, adding it to the molecules with the potential to create new generations of antibiotics [133].

Bothriechis schlegelii species complex.— Despite this arboreal species being frequently seen across varied elevations of Colombian Andes (0-3200 m asl), few pharmacological studies have been done. Despite this, the activity of BsLAAO was described as a potent antibacterial activity similar to other LAAOs present in viperids snake species. Mainly, its effects on *Staphylococcus aureus* and *Acinetobacter baumanni* were studied. *Acinetobacter baumannii* is one of the bacteria with the highest antibiotic resistance. Therefore, infection by this bacterium presents a high mortality rate. This makes the compounds derived from these toxins useful for creating a new generation of antibiotics that help control antibiotic resistance and reduce mortality rates [167].

Elapidae

Coral snake venoms are less studied than pit viper venoms due to the difficulty of obtaining snake venom samples, an adequate venom volume extracted per specimen, and the purification of the proteins. Only the venom proteome and the composition from three Colombian species have been characterized; these are *Micrurus mipartitus, M. dumerillii,* and *M. helleri* [168–170]. All these venoms have significant amounts of 3FTx, PLA_2, and LAAO, making them potential sources of compounds that could be active against cardiovascular disease, antibacterial antiviral, and antiparasitic infection, as well as analgesic drugs. Nevertheless, several toxins have been purified from the venoms of *M. mipartitus* and *M. dumerillii.* However, their either analgesic or modulation of ion channel activities have not been tested perhaps these activities are similar to other 3FTxs that have been studied.

Micrurrus helleri.— Explorations of the pharmacological potential of *M. helleri* venom result in the isolation of a PLA_2 molecule named MlV. This compound has antinociceptive activity, demonstrating a superior effect in comparison to conventional drugs [morphine (opioid) and indomethacin

(NSAIDs)] without affecting the locomotor system. The MlV effects are manifested by blockading opioid receptors. This discovery allows an excellent opportunity to create new drugs to treat chronic pain [164].

Another PLA_2 molecule isolated from *M. helleri* venom is lemnitoxin. It has exhibited myotoxic, cytotoxic, pro-inflammatory, and anticoagulant activities. This last effect has been studied due to the therapeutic potential for new anticoagulant drugs [171].

Micrurus mipartitus.— This is the most common coral species inhabiting low to moderate elevations of the Colombian Andes (400-2000 m a.s.l.). Despite this, few pharmacological studies have been done. Recent pharmacological explorations have isolated a toxin from the LAAO protein family with potent bactericidal activity against two critical bacteria (*Staphylococcus aureus* and *Escherichia coli*) [172]. In addition, several toxins of PLA_2s from the *M. mipartitus* snake venoms have been isolated and characterized, but their pharmacological potential has not yet been evaluated [173].

Micrurus obscurus.— Pharmacological explorations of *M. obscurus* snake venoms from Colombian populations have not yet been done. However, a toxin $MsPLA_2$ that exhibits a phospholipase activity was isolated from the venom of *M. spixii* from Brazilian populations (the closest related species, see [174]). This PLA_2 effect showed a similar result found in *Bothrops* venoms. Moreover, cytotoxic studies of $MsPLA_2$ have shown microbicidal and antitumor activities and effects against *P. falciparum* and liver cancer. Although its microbicidal and antitumor effects have been demonstrated, they do not exceed the effectiveness of currently available medications. However, its potential can help understand the biological processes of these pathologies and potentially generate new molecules [175].

Colubridae

Few Colombian Colubrid venoms have been characterized by their toxin content using proteomic techniques (see Chapter 4). However, essential knowledge has been generated in recent years (see, for example, the venoms from the banded cat-eyed snake (*Leptodeira annulata*), double-banded coral snake mimic (*Erythrolamprus bizona*) and, Neuwiedd's false boa (*Pseudoboa neuwiedii)* have been characterized [176–178] we assessed the enzymatic properties and biological activities of *Leptodeira annulata* (banded cat-eyed snake. From a pharmacological view, all these secretions show great potential as cytotoxic, antibacterial, antiviral, and antiparasitic compounds due to their significant composition of PLA_2 and SVNP activities. Moreover, the venom of *Leptodeira annulata* shows strong α-fibrinogenase and fibrinolytic activities that suggest the presence of SVMPs which leads to molecules for the development of thrombolytic agents [178]. Nevertheless, its pharmacological potential has not yet been evaluated.

In addition, three-finger toxins from this family were characterized that are not toxic to human cells. As explained above, three-finger toxins have several activities applicable to medical use. However, more research is

needed. In addition, this toxin has the potential to explore its pharmacological uses [179]. Some examples are the molecules *H. t. texana* and *T. b. Lambda* isolated from *Philodryas baroni*, *P. olfersii*, *P. patagoniensis* that exhibit PLA_2 activity and anti-leishmanial activity. Even though they are not as potent as currently available drugs (amphotericin B), they represent new anti-leishmanial drug opportunities [180].

As shown in this chapter, the therapeutic potential of Colombian snake venoms is immense. They can help generate new treatments for multiple pathologies. Using "omics" technologies will allow identifying more potential molecules in the venoms of snakes from Colombia. However, we must continue studying the venom components conventionally, while "omics" technologies can be performed in a massive form and at a reasonable cost for Colombian researchers. The biomedical and pharmacological potentials of snake venom from Colombia have been presented in this chapter. Due to the complex nature of these venoms and the findings discovered up until now, it is clear that the discovery of more potential molecules is possible. However, more resources are needed to increase our knowledge about Colombian snake venoms and to establish clear bioprospection of these substances.

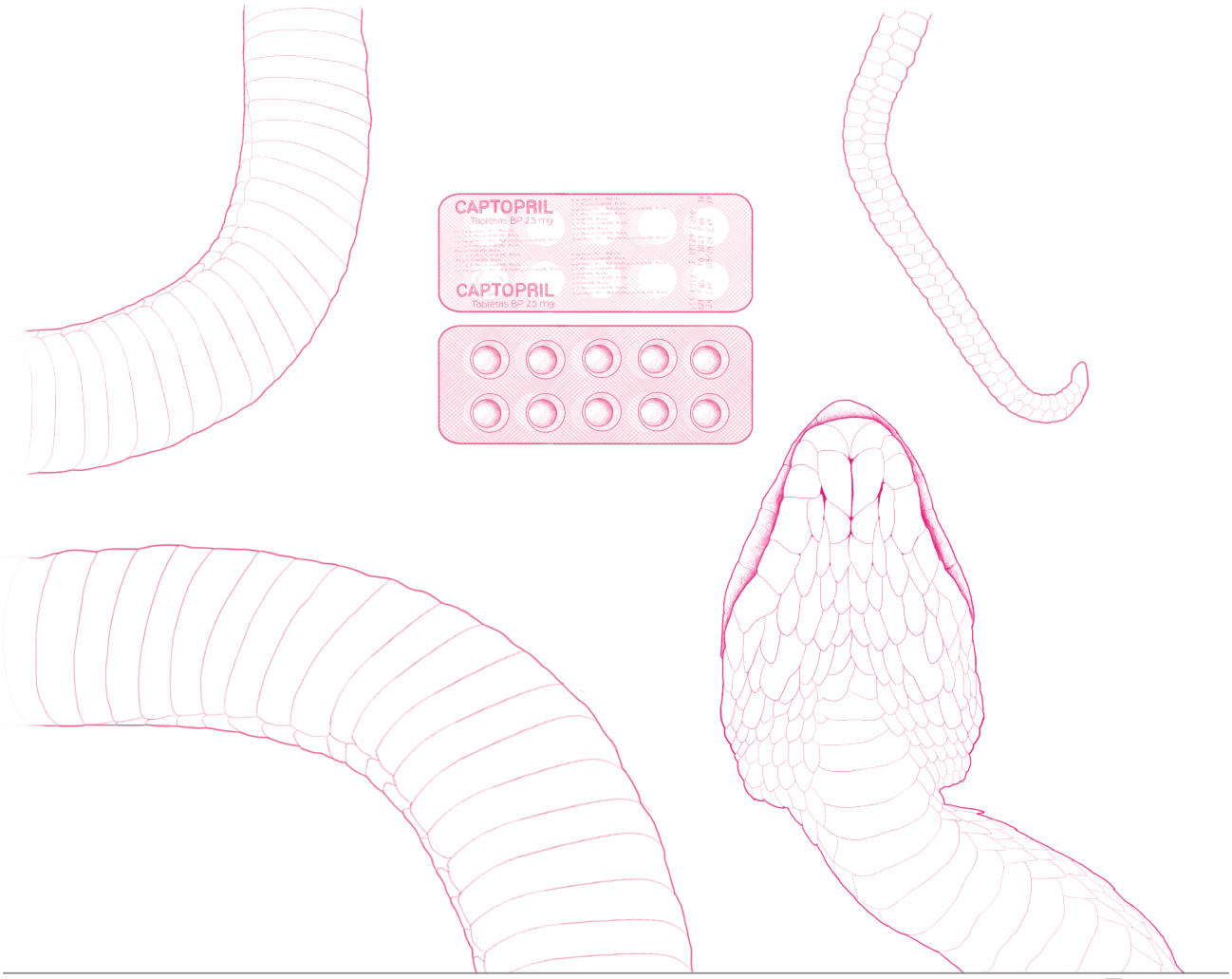


Table 4. Molecules with therapeutic potential derived from Colombian snake venoms.

Family	Origin species	Drug	Mechanism	Systems	Indication	Reference
Viperidae	*Bothrops atrox*	Batroxicidin	Induces necrosis and apoptosis in *T. cruzi*.	Antimicrobial defense	Antichagasic activity (*Trypanosoma cruzi*)	[149]
	Bothrops asper	Colombienases	Degrades fibrinogen but do not activate the fibrinolytic system (plasminogen/ plasmin)	Cardiovascular/ Hemostatic	Fibrinolytic therapy	[153]
	Bothrops atrox	Batroxase	Cytotoxicity in tumoral blood cells.	Anticancer	Leukemic cancer	[154]
	Bothrops atrox	(Ba-V)	Inhibit mitochondrial permeability transition, presumably altering calcium flow	Nervous system	Potential for neuroprotection	[150]
	Bothrops asper	Mt–I	Permeabilize the virus envelope due to enzimatic activity.	Antimicrobial defense	virucidal activity	[152]
	Bothrops atrox	(Ba-IV)	Inhibiting apoptotic proteases such as caspase-9 and caspase-3 that prevents cell death in dopaminergic neurons.	Nervous system	Parkinson´s disease	[151]
	Crotalus durissus	Crotamine	Disrruption of H+ balance, and membrane pertubation.	Antimicrobial defense/ Anticancer activity	Malaria / Melanoma, pancreatic cancer, and carcinoma.	[149,156]
	Crotalus durissus	Cdt	Blocks receptors in the cell membrane, impeding the absorption of the virus.	Antimicrobial defense	antiviral activity	[158]
	Crotalus durissus	crotalicidin	Rupture of the bacterial cell membrane.	Antimicrobial defense	Antibacterial activity	[157,160]
	Crotalus durissus	Phospholipase A_2	Degradation of the virus envelope, exposing the virus RNA.	Antimicrobial defense	Antiviral activity Denge virus	[161]
	Crotalus durissus	CB subunit of crotoxin	Regulation of Cl- channels.	Respiratory system	Cystic fibrosis	[159]
	Lachesis muta	LmrBPP9	ACE inhibition.	Cardiovascular system	Hypertension	[164]
	Porthidium lansbergii	Lansbermin-I	Disintegrin activity.	Anticancer	Breast Cancer Cells	[91,166]
	Porthidium nasutum	Acidic PLA_2	Phospholipase activity.	Antimicrobial defense	Microbicidal	[133]

Viperidae	*Porthidium lansbergii*	Pllans–II, an acidic Asp49–PLA_2	Phospholipase activity.	Anticancer	Cervical carcinoma	[182]
	Bothrops taeniata	Btae TX-I	Phospholipase activity.	Anticancer	Cervical carcinoma	[155]
	Bothriechis schlegelii	BsLAAO	LAAO activity	Antimicrobial defense	Antibacterial activity	[167]
Elapidae	*Micrurus helleri*	MlV	Blocks opioid receptors.	Nervous system	Antinociceptive effects	[181]
	Micrurus spixii	$MsPLA_2$-I	Phospholipase activity.	Antimicrobial defense/ anticancer activity	Microbicidal/ antitumoral	[175]
	Micrurus helleri	Lemnitoxin	Phospholipase activity.	Cardiovascular system	Anticoagulant effect	[171]

Appendix: Material and Methods

Search methods for identification of studies: Electronic searches.

The searches were carried out from April to June 2021. We performed the searches as follow:

1. Weekly searches of the Cochrane Central Register of Controlled Trials (CENTRAL)
2. Weekly searches of MEDLINE (Ovid).
3. Weekly searches of Embase (Ovid).
4. Weekly searches of Scielo;
5. Weekly searches of Scopus.
6. Weekly searches of Google Scholar.
7. Weekly searches of clinicaltrial.gov

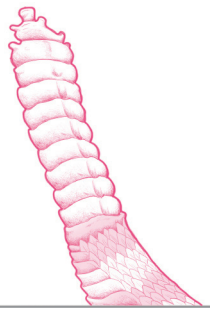
The search strategies contained the following keywords and Mesh were combined in different ways using the boolean connectors AND OR: snake venoms, *Bothrops atrox*, *Crotalus*, *Bothriechis*, *Porthidium*, *Lachesis*, *Micrurus*, *Xenodon*, *Leptophis*, *Erythrolamprus*, *Oxybelis*, *Helicops*, *Bothrocophias*, *Thamnodynastes*, *Leptodeira*, Philodryas, and drug development.

Searching other resources

We checked relevant cited studies while reviewing the reports identified by the electronic searches, as well as reference lists from any directly relevant reviews identified. We did not apply language or date restrictions and included studies regardless of the publication type (e.g., conference abstract, trial registry entry, journal article, book).

We searched March 2021 and identified 1087 articles for potential inclusion. In addition, we identified a total of 320 articles for the title and abstract screening, of which experts suggested 39 articles, 46 studies were duplicates, and 40 were excluded for other reasons.

We obtained the full text of the 234 remaining articles for inclusion. Details of the flow of studies for this review are recorded in a PRISMA diagram in Figure 4.



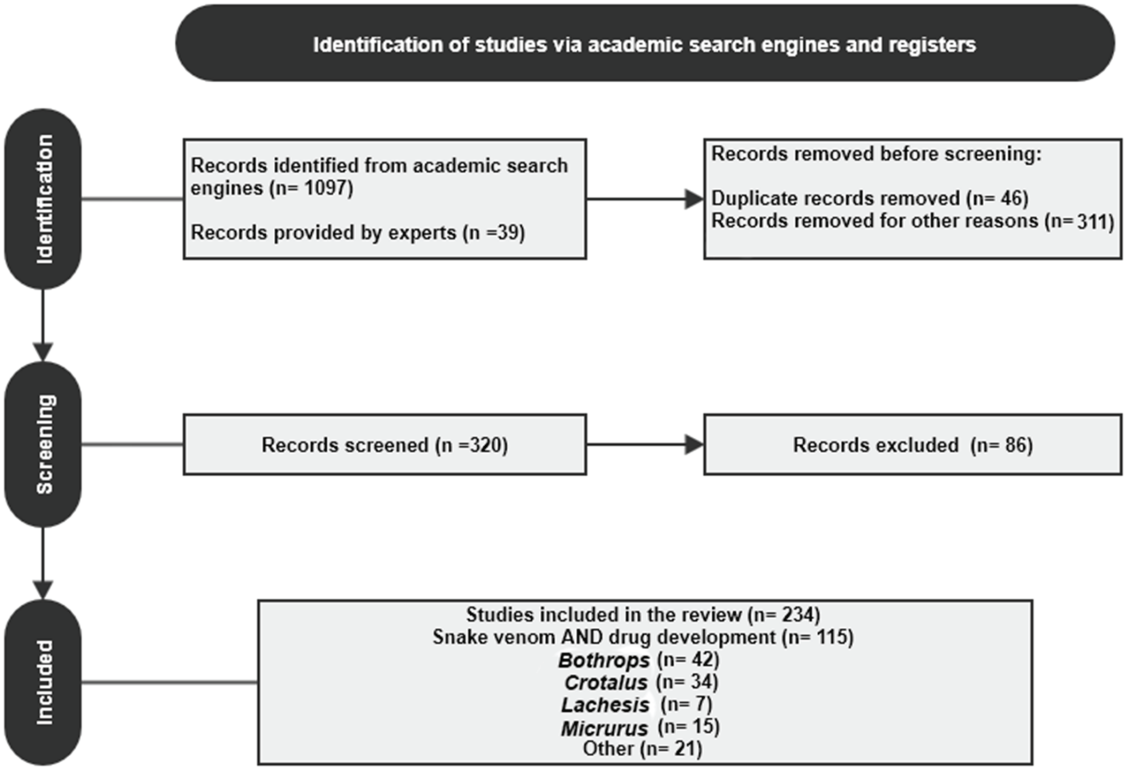
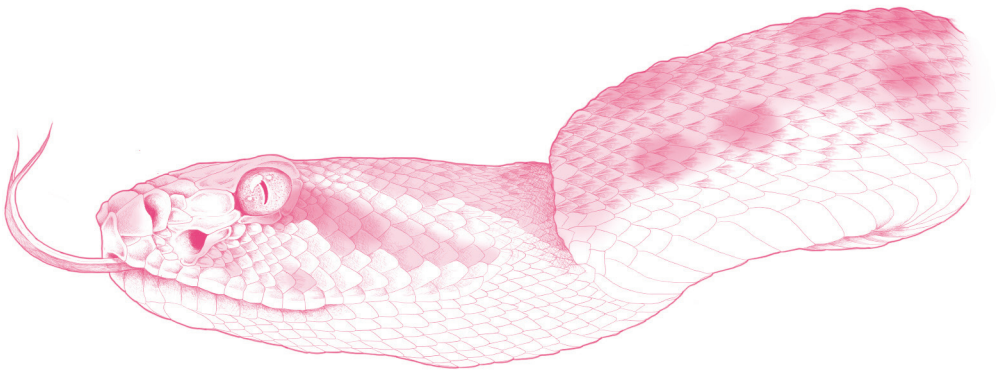


Figure 4. PRISMA approach employed.



References

1. Couchoud, P.-L. J.-G. Frazer et Le rameau d'or. *Bull. l'Association Guillaume Budé* **1924**, *4*, 46–50.
2. García, S.A.; Márquez, C.I. Cultivating positive health, learning, and community: The return of Mesoamerica's Quetzalcoatl and the Venus star. *Genealogy* **2021**, *5*, 53.
3. Boquet, P. History of snake venom research. In *Snake venoms*; Springer, **1979**; pp. 3–14.
4. Creswell, R.T. Aristotle's history of animals in ten books. **1902**.
5. Chevalier, J.; Gheerbrant, A. Dictionnaire des symboles. Paris: Robert Laffont. *Jupiter* **1982**, 349–350.
6. Rosso, A.M. Toxicology and snakes in ptolemaic Egyptian dynasty: The suicide of Cleopatra. *Toxicol. reports* **2021**, *8*, 676–695.
7. T'serstevens, A. *Le livre de Marco Polo ou de devisement du monde*; Albin Michel, **1955**.
8. Wexler, P. *History of toxicology and environmental health*; Elsevier, 2014.
9. Wexler, P. *Toxicology in antiquity*; Academic Press, **2018**; ISBN 0128153407.
10. Salazar, C. *The treatment of war wounds in Graeco-Roman antiquity*; Brill, **2018**; ISBN 9004377484.
11. Hodgson, B. *In the arms of morpheus: The tragic history of laudanum, morphine, and patent medicines*; Firefly Books Limited, **2001**; ISBN 155297538X.
12. Totelin, L.M.V. A. Mayor, The poison king: The life and legend of Mithradates Rome's deadliest enemy [Book Review]. *Isis* **2010**, *101*, 639.
13. Bonaparte, L.-L. *Ricerche chimiche sul veleno della vipera;* **1843**.
14. Alper, C.A. Snakes and the complement system. In *Snake Venoms*; Springer, **1979**; pp. 863–880.
15. Hawgood, B.J. Doctor Albert Calmette 1863–1933: founder of antivenomous serotherapy and of antituberculous BCG vaccination. *Toxicon* **1999**, *37*, 1241–1258.
16. Calmette, A. Étude expérimentale du venin de *Naja tripudians* ou cobra capel et exposé d'une méthode de neutralisation de ce venin dans l'organisme. *Ann Inst Pasteur* **1892**, *6*, 160–183.
17. Calmette, A. Contribution à l'étude du venin des serpents. Immunisation des animaux et traitement de l'envenimation. *Ann. Inst. Pasteur* **1894**, *8*, 275–291.
18. Sewall, H. Experiments on the preventive inoculation of rattlesnake venom. *J. Physiol.* **1887**, *8*, 203.
19. Hawgood, B.J. Pioneers of anti-venomous serotherapy: Dr Vital Brazil (1865–1950). *Toxicon* **1992**, *30*, 573–579.
20. Brazil, V. Contribuiçãoaoestudo de venenoophidico. II. Veneno de algumas espécies brazileiras. *Rev Méd São Paulo* **1901**, *4*, 296–300.
21. Brazil, V. Contribuição ao estudo do veneno ophidico. *Rev. Médica São Paulo* **1901**, *4*, 255–260.
22. Ferreira, S.H. A bradykinin-potentiating factor (BPF) present in the venom of *Bothrops jararaca*. *Br. J. Pharmacol. Chemother.* **1965**, *24*, 163–169.
23. Ferreira, S.H.; Bartelt, D.C.; Greene, L.J. Isolation of bradykinin-potentiating peptides from *Bothrops jararaca* venom. *Biochemistry* **1970**, *9*, 2583–2593.

24. Ondetti, M.A.; Williams, N.J.; Sabo, E.; Pluscec, J.; Weaver, E.R.; Kocy, O. Angiotensin-converting enzyme inhibitors from the venom of *Bothrops jararaca*. Isolation, elucidation of structure, and synthesis. *Biochemistry* **1971**, *10*, 4033–4039.
25. Cushman, D.W.; Pluščec, J.; Williams, N.J.; Weaver, E.R.; Sabo, E.F.; Kocy, O.; Cheung, H.S.; Ondetti, M.A. Inhibition of angiotensin-converting enzyme by analogs of peptides from *Bothrops jararaca* venom. *Experientia* **1973**, *29*, 1032–1035.
26. Camargo, A.C.M.; Ianzer, D.; Guerreiro, J.R.; Serrano, S.M.T. Bradykinin-potentiating peptides: Beyond captopril. *Toxicon* **2012**, *59*, 516–523, doi:10.1016/j.toxicon.2011.07.013.
27. Lazarovici, P.; Marcinkiewicz, C.; Lelkes, P.I. From snake venom's disintegrins and C-type lectins to anti-platelet drugs. *Toxins (Basel).* **2019**, *11*, 303.
28. Pereáñez Jiménez, J.A.; Vargas Muñoz, L.J. Toxinas de serpientes con alto potencial terapéutico y su uso en la biomedicina. *Iatreia* **2009**, *22*, 382–391.
29. El-Aziz, T.M.A.; Soares, A.G.; Stockand, J.D. Snake venoms in drug discovery: Valuable therapeutic tools for life saving. *Toxins (Basel).* **2019**, *11*, 1–25, doi:10.3390/toxins11100564.
30. Li, L.; Huang, J.; Lin, Y. Snake venoms in cancer therapy: Past, present and future. *Toxins (Basel).* **2018**, *10*, 1–8, doi:10.3390/toxins10090346.
31. Jolkkonen, M.; Van Giersbergen, P.L.M.; Hellman, U.; Wernstedt, C.; Oras, A.; Satyapan, N.; Adem, A.; Karlsson, E. Muscarinic toxins from the black mamba *Dendroaspis polylepis*. *Eur. J. Biochem.* **1995**, *234*, 579–585.
32. Bordon, K. de C.F.; Cologna, C.T.; Fornari-Baldo, E.C.; Pinheiro-Júnior, E.L.; Cerni, F.A.; Amorim, F.G.; Anjolette, F.A.P.; Cordeiro, F.A.; Wiezel, G.A.; Cardoso, I.A.; et al. From animal poisons and venoms to medicines: Achievements, challenges and perspectives in drug discovery. *Front. Pharmacol.* **2020**, *11*, doi:10.3389/fphar.2020.01132.
33. Chan, Y.S.; Cheung, R.C.F.; Xia, L.; Wong, J.H.; Ng, T.B.; Chan, W.Y. Snake venom toxins: toxicity and medicinal applications. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 6165–6181, doi:10.1007/s00253-016-7610-9.
34. Herzig, V.; Cristofori-Armstrong, B.; Israel, M.R.; Nixon, S.A.; Vetter, I.; King, G.F. Animal toxins—Nature's evolutionary-refined toolkit for basic research and drug discovery. *Biochem. Pharmacol.* **2020**, *181*, 114096.
35. Munawar, A.; Ali, S.A.; Akrem, A.; Betzel, C. Snake venom peptides: Tools of biodiscovery. *Toxins (Basel).* **2018**, *10*, 474.
36. Kini, R.M. Excitement ahead: structure, function and mechanism of snake venom phospholipase A2 enzymes. *Toxicon* **2003**, *42*, 827–840.
37. Tonello, F.; Rigoni, M. Cellular Mechanisms of action of snake phospholipase A2 toxins. In *Snake Venoms*; Inagaki, H., Vogel, C.-W., Mukherjee, A.K., Rahmy, T.R., Gopalakrishnakone, P., Eds.; Springer Netherlands: Dordrecht, **2017**; pp. 49–65 ISBN 978-94-007-6410-1.
38. P Samy, R.; Gopalakrishnakone, P.; G Stiles, B.; S Girish, K.; N Swamy, S.; Hemshekhar, M.; S Tan, K.; G Rowan, E.; Sethi, G.; TK Chow, V. Snake venom phospholipases A_2: a novel tool against bacterial diseases. *Curr. Med. Chem.* **2012**, *19*, 6150–6162.

39. Teixeira, S.C.; Borges, B.C.; Oliveira, V.Q.; Carregosa, L.S.; Bastos, L.A.; Santos, I.A.; Jardim, A.C.G.; Freire, F.M.; Martins, L.; Rodrigues, V.M. Insights into the antiviral activity of phospholipases A_2 (PLA_2s) from snake venoms. *Int. J. Biol. Macromol.* **2020**.
40. Teixeira, S.C.; da Silva, M.S.; Gomes, A.A.S.; Moretti, N.S.; Lopes, D.S.; Ferro, E.A.V.; de Melo Rodrigues, V. Panacea within a Pandora's box: The antiparasitic effects of phospholipases A_2 (PLA_2s) from snake venoms. *Trends Parasitol.* **2021**.
41. Gutiérrez, J.M.; Escalante, T.; Rucavado, A.; Herrera, C. Hemorrhage caused by snake venom metalloproteinases: A journey of discovery and understanding. *Toxins (Basel).* **2016**, *8*, 93.
42. Gutiérrez, J.M.; Rucavado, A. Snake venom metalloproteinases: Their role in the pathogenesis of local tissue damage. *Biochimie* **2000**, *82*, 841–850.
43. Takeya, H.; Nishida, S.; Miyata, T.; Kawada, S.; Saisaka, Y.; Morita, T.; Iwanaga, S. Coagulation factor X activating enzyme from Russell's viper venom (RVV-X). A novel metalloproteinase with disintegrin (platelet aggregation inhibitor)-like and C-type lectin-like domains. *J. Biol. Chem.* **1992**, *267*, 14109–14117.
44. Karapetian, H. Reptilase time (RT). *Methods Mol. Biol.* **2013**, *992*, 273–277, doi:10.1007/978-1-62703-339-8_20.
45. Serrano, S.M.T. The long road of research on snake venom serine proteinases. *Toxicon* **2013**, *62*, 19–26, doi:10.1016/j.toxicon.2012.09.003.
46. Koh, C.Y.; Kini, R.M. From snake venom toxins to therapeutics - Cardiovascular examples. *Toxicon* **2012**, *59*, 497–506, doi:10.1016/j.toxicon.2011.03.017.
47. Ullah, A. Structure–function studies and mechanism of action of snake venom l-amino acid oxidases. *Front. Pharmacol.* **2020**, *11*, 110.
48. Tan, K.K.; Bay, B.H.; Gopalakrishnakone, P. L-amino acid oxidase from snake venom and its anticancer potential. *Toxicon* **2018**, *144*, 7–13.
49. Calvete, J.J. The continuing saga of snake venom disintegrins. *Toxicon* **2013**, *62*, 40–49.
50. Clemetson, K.J. Snaclecs (snake C-type lectins) that inhibit or activate platelets by binding to receptors. *Toxicon* **2010**, *56*, 1236–1246.
51. Utkin, Y.N. Three-finger toxins, a deadly weapon of elapid venom - Milestones of discovery. *Toxicon* **2013**, *62*, 50–55, doi:10.1016/j.toxicon.2012.09.007.
52. Utkin, Y.N. Last decade update for three-finger toxins: Newly emerging structures and biological activities. *World J. Biol. Chem.* **2019**, *10*, 17.
53. Pu, X.C.; Wong, P.T.H.; Gopalakrishnakone, P. A novel analgesic toxin (hannalgesin) from the venom of king cobra (*Ophiophagus hannah*). *Toxicon* **1995**, *33*, 1425–1431.
54. Departamento Administrativo Nacional de Estadística (DANE). Estadísticas vitales: defunciones. Itrim 2022. Bogotá (Colombia): DANE; 2022. Disponible en: https://www.dane.gov.co/files/investigaciones/poblacion/bt_estadisticasvitales_defunciones_Itrim_2022pr.pdf
55. Uthman, O.A.; Hartley, L.; Rees, K.; Taylor, F.; Ebrahim, S.; Clarke, A. Multiple risk factor interventions for primary prevention of cardiovascular disease in low-and middle-income countries. *Cochrane Database Syst. Rev.* **2015**, *8*, CD011163. https://doi.org/10.1002/14651858.

56. Social, M. de S. y P. *Guía de práctica clínica para el manejo de la hipertensión arterial primaria (HTA)*; **2017**.
57. Xie, X.; Atkins, E.; Lv, J.; Bennett, A.; Neal, B.; Ninomiya, T.; Woodward, M.; MacMahon, S.; Turnbull, F.; Hillis, G.S. Effects of intensive blood pressure lowering on cardiovascular and renal outcomes: updated systematic review and meta-analysis. *Lancet* **2016**, *387*, 435–443.
58. Oparil, S.; Schmieder, R.E. New approaches in the treatment of hypertension. *Circ. Res.* **2015**, *116*, 1074–1095.
59. Péterfi, O.; Boda, F.; Szabó, Z.; Ferencz, E.; Bába, L. Hypotensive snake venom components—A mini-review. *Molecules* **2019**, *24*, 2778.
60. Strozzi, C.; Cocco, G.; Portaluppi, F.; Urso, L.; Alfiero, R.; Tasini, M.T.; Montanari, L.; Al Yassini, K.; Rizzo, A. Effects of captopril on the physical work capacity of normotensive patients with stable-effort angina pectoris. *Cardiology* **1987**, *74*, 226–228.
61. Frangieh, J.; Rima, M.; Fajloun, Z.; Henrion, D.; Sabatier, J.-M.; Legros, C.; Mattei, C. Snake venom components: Tools and cures to target cardiovascular diseases. *Molecules* **2021**, *26*, 2223.
62. Gouda, A.S.; Mégarbane, B. Snake venom-derived bradykinin-potentiating peptides: A promising therapy for COVID-19? *Drug Dev. Res.* **2021**, *82*, 38–48.
63. Kini, R.M.; Koh, C.Y. Snake venom three-finger toxins and their potential in drug development targeting cardiovascular diseases. *Biochem. Pharmacol.* **2020**, *181*, 114105, doi:10.1016/j.bcp.2020.114105.
64. Cómo funcionan los bloqueadores de los canales del calcio. *Nursing (Lond).* **2003**, *21*, 42.
65. Kobilka, B.K. Structural insights into adrenergic receptor function and pharmacology. *Trends Pharmacol. Sci.* **2011**, *32*, 213–218.
66. Hodgson, W.C.; Wickramaratna, J.C. In vitro neuromuscular activity of snake venoms. *Clin. Exp. Pharmacol. Physiol.* **2002**, *29*, 807–814.
67. Dutertre, S.; Nicke, A.; Tsetlin, V.I. Nicotinic acetylcholine receptor inhibitors derived from snake and snail venoms. *Neuropharmacology* **2017**, *127*, 196–223, doi:10.1016/j.neuropharm.2017.06.011.
68. Mohamed Abd El-Aziz, T.; Soares, A.G.; Stockand, J.D. Snake venoms in drug discovery: valuable therapeutic tools for life saving. *Toxins (Basel).* **2019**, *11*, 564.
69. Cook, D.; Simons, D.J. Neuromuscular blockade. *StatPearls [Internet]* **2020**.
70. Koh, D.C.I.; Armugam, A.; Jeyaseelan, K. Snake venom components and their applications in biomedicine. *Cell. Mol. Life Sci.* **2006**, *63*, 3030–3041, doi:10.1007/s00018-006-6315-0.
71. Waheed, H.; Moin, S.F.; Choudhary, M.I. Snake venom: From deadly toxins to life-saving therapeutics. *Curr. Med. Chem.* **2017**, *24*, 1874–1891, doi:10.2174/0929867324666170605091546.
72. Camargo, L.C.; Campos, G.A.A.; Galante, P.; Biolchi, A.M.; Goncalves, J.C.; Lopes, K.S.; Mortari, M.R. Peptides isolated from animal venom as a platform for new therapeutics for the treatment of Alzheimer's disease. *Neuropeptides* **2018**, *67*, 79–86.
73. de Oliveira Amaral, H.; Monge-Fuentes, V.; Mayer, A.B.; Campos, G.A.A.; Lopes, K.S.; Camargo, L.C.; Schwartz, M.F.; Galante, P.; Mortari, M.R.

Animal venoms: Therapeutic tools for tackling Parkinson's disease. *Drug Discov. Today* **2019**, *24*, 2202–2211.
74. Akef, H.M. Snake venom: Kill and cure. *Toxin Rev.* **2019**, *38*, 21–40.
75. Social, M. de S. y P. Prevención enfermedades NO transmisibles - Cáncer.
76. Hanahan, D.; Weinberg, R.A. The hallmarks of cancer. *Cell* **2000**, *100*, 57–70.
77. Onuigbo, W.I. A definition problem in cancer metastasis. *Neoplasma* **1975**, *22*, 547—550.
78. Mitrus, I.; Bryndza, E.; Sochanik, A.; Szala, S. Evolving models of tumor origin and progression. *Tumor Biol.* **2012**, *33*, 911–917.
79. Wild, C.; Weiderpass, E.; Stewart, B.W. *World cancer report: Cancer research for cancer prevention*; IARC Press, **2020**; ISBN 9283204476.
80. Lopez-Camarillo, C.; Arechaga-Ocampo, E. *Oncogenomics and cancer proteomics: Novel approaches in biomarkers discovery and therapeutic targets in cancer*; BoD–Books on Demand, **2013**; ISBN 9535110411.
81. Forrester, H.B.; Li, J.; Hovan, D.; Ivashkevich, A.N.; Sprung, C.N. DNA repair genes: Alternative transcription and gene expression at the exon level in response to the DNA damaging agent, ionizing radiation. *PLoS One* **2012**, *7*, e53358.
82. Vesely, M.D.; Kershaw, M.H.; Schreiber, R.D.; Smyth, M.J. Natural innate and adaptive immunity to cancer. *Annu. Rev. Immunol.* **2011**, *29*, 235–271.
83. Loeb, L.A. Human cancers express mutator phenotypes: origin, consequences and targeting. *Nat. Rev. Cancer* **2011**, *11*, 450–457.
84. Urra, F.A.; Araya-Maturana, R. Putting the brakes on tumorigenesis with snake venom toxins: New molecular insights for cancer drug discovery. In Proceedings of the Seminars in Cancer Biology; Elsevier, **2020**.
85. Ma, R.; Kwok, H.F. New opportunities and challenges of venom-based and bacteria-derived molecules for anticancer targeted therapy. In Proceedings of the Seminars in Cancer Biology; Elsevier, **2020**.
86. Valent, P.; Bonnet, D.; De Maria, R.; Lapidot, T.; Copland, M.; Melo, J. V; Chomienne, C.; Ishikawa, F.; Schuringa, J.J.; Stassi, G. Cancer stem cell definitions and terminology: The devil is in the details. *Nat. Rev. Cancer* **2012**, *12*, 767–775.
87. Rodrigues, R.; Izidoro, L.F.; de Oliveira Jr., R.; Soares, A.; Rodrigues, V.; Sampaio, S. Snake venom phospholipases A2: A new class of antitumor agents. *Protein Pept. Lett.* **2009**, *16*, 894–898, doi:10.2174/092986609788923266.
88. Jain, D.; Kumar, S. Snake venom: A potent anticancer agent. *Asian Pacific J. Cancer Prev.* **2012**, *13*, 4855–4860.
89. Siddiqua, A.; Khattak, K.; Nwaz, S. Venom proteins; Prospects for anticancer therapy. *Pak. J. Biochem. Mol. Biol* **2019**, *52*, 15–26.
90. Pérez-Peinado, C.; Defaus, S.; Andreu, D. Hitchhiking with Nature: Snake venom peptides to fight cancer and superbugs. *Toxins (Basel).* **2020**, *12*, 255.
91. Montoya-Gómez, A.; Montealegre-Sánchez, L.; García-Perdomo, H.A.; Jiménez-Charris, E. Cervical cancer and potential pharmacological treatment with snake venoms. *Mol. Biol. Rep.* **2020**, *47*, 4709–4721,

doi:10.1007/s11033-020-05503-6.
92. Swenson, S.; Costa, F.; Minea, R.; Sherwin, R.P.; Ernst, W.; Fujii, G.; Yang, D.; Markland, F.S. Intravenous liposomal delivery of the snake venom disintegrin contortrostatin limits breast cancer progression. *Mol. Cancer Ther.* **2004**, *3*, 499–511.
93. Lucena, S.; Castro, R.; Lundin, C.; Hofstetter, A.; Alaniz, A.; Suntravat, M.; Sánchez, E.E. Inhibition of pancreatic tumoral cells by snake venom disintegrins. *Toxicon* **2015**, *93*, 136–143, doi:10.1016/j.toxicon.2014.11.228.
94. Machado, A.R.T.; Aissa, A.F.; Ribeiro, D.L.; Ferreira, R.S.; Sampaio, S.V.; Antunes, L.M.G. BjussuLAAO-II induces cytotoxicity and alters DNA methylation of cell-cycle genes in monocultured/co-cultured HepG2 cells. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2019**, *25*, 1–9, doi:10.1590/1678-9199-jvatitd-1476-18.
95. Moga, M.A.; Dimienescu, O.G.; Arvătescu, C.A.; Ifteni, P.; Pleş, L. Anticancer activity of toxins from bee and snake venom—an overview on ovarian cancer. *Molecules* **2018**, *23*, 692.
96. Khusro, A.; Aarti, C.; Barbabosa-Pliego, A.; Rivas-Cáceres, R.R.; Cipriano-Salazar, M. Venom as therapeutic weapon to combat dreadful diseases of the 21st century: A systematic review on cancer, TB, and HIV/AIDS. *Microb. Pathog.* **2018**, *125*, 96–107.
97. Schönthal, A.H.; Swenson, S.D.; Chen, T.C.; Markland, F.S. Preclinical studies of a novel snake venom-derived recombinant disintegrin with antitumor activity: A review. *Biochem. Pharmacol.* **2020**, *181*, 114149, doi:10.1016/j.bcp.2020.114149.
98. Teixeira, S.C.; Borges, B.C.; Oliveira, V.Q.; Carregosa, L.S.; Bastos, L.A.; Santos, I.A.; Jardim, A.C.G.; Freire, F.M.; Freitas, L.M.; Rodrigues, V.M.; et al. Insights into the antiviral activity of phospholipases A_2 (PLA_2s) from snake venoms. *Int. J. Biol. Macromol.* **2020**, *164*, 616–625, doi:10.1016/j.ijbiomac.2020.07.178.
99. Almeida, J.R.; Palacios, A.L. V; Patiño, R.S.P.; Mendes, B.; Teixeira, C.A.S.; Gomes, P.; da Silva, S.L. Harnessing snake venom phospholipases A_2 to novel approaches for overcoming antibiotic resistance. *Drug Dev. Res.* **2019**, *80*, 68–85.
100. Paloschi, M. V; Pontes, A.S.; Soares, A.M.; Zuliani, J.P. An update on potential molecular mechanisms underlying the actions of snake venom L-amino acid oxidases (LAAOs). *Curr. Med. Chem.* **2018**, *25*, 2520–2530.
101. dos Santos Nunes, E.; De Souza, M.A.A.; de Melo Vaz, A.F.; de Sá Santana, G.M.; Gomes, F.S.; Coelho, L.C.B.B.; Paiva, P.M.G.; Da Silva, R.M.L.; Silva-Lucca, R.A.; Oliva, M.L.V. Purification of a lectin with antibacterial activity from *Bothrops leucurus* snake venom. *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.* **2011**, *159*, 57–63.
102. Klein, R.C.; Fabres-Klein, M.H.; de Oliveira, L.L.; Feio, R.N.; Malouin, F.; Ribon, A. de O.B. A C-type lectin from *Bothrops jararacussu* venom disrupts Staphylococcal biofilms. *PLoS One* **2015**, *10*, e0120514.
103. Rádis-Baptista, G.; Moreno, F.B.M.B.; de Lima Nogueira, L.; Martins, A.M.C.; de Oliveira Toyama, D.; Toyama, M.H.; Cavada, B.S.; de Azevedo, W.F.; Yamane, T. Crotacetin, a novel snake venom C-type lectin homolog of convulxin, exhibits an unpredictable antimicrobial activity. *Cell Biochem. Biophys.* **2006**, *44*, 412–423.

104. Chen, L.-W.; Kao, P.-H.; Fu, Y.-S.; Lin, S.-R.; Chang, L.-S. Membrane-damaging activity of Taiwan cobra cardiotoxin 3 is responsible for its bactericidal activity. *Toxicon* **2011**, *58*, 46–53.
105. Chen, L.-W.; Kao, P.-H.; Fu, Y.-S.; Hu, W.-P.; Chang, L.-S. Bactericidal effect of *Naja nigricollis* toxin γ is related to its membrane-damaging activity. *Peptides* **2011**, *32*, 1755–1763.
106. Wang, Y.; Hong, J.; Liu, X.; Yang, H.; Liu, R.; Wu, J.; Wang, A.; Lin, D.; Lai, R. Snake cathelicidin from *Bungarus fasciatus* is a potent peptide antibiotics. *PLoS One* **2008**, *3*, e3217.
107. Wang, Y.; Zhang, Z.; Chen, L.; Guang, H.; Li, Z.; Yang, H.; Li, J.; You, D.; Yu, H.; Lai, R. Cathelicidin-BF, a snake cathelicidin-derived antimicrobial peptide, could be an excellent therapeutic agent for acne vulgaris. *PLoS One* **2011**, *6*, e22120.
108. Zhao, H.; Gan, T.-X.; Liu, X.-D.; Jin, Y.; Lee, W.-H.; Shen, J.-H.; Zhang, Y. Identification and characterization of novel reptile cathelicidins from elapid snakes. *Peptides* **2008**, *29*, 1685–1691.
109. Zhou, H.; Dou, J.; Wang, J.; Chen, L.; Wang, H.; Zhou, W.; Li, Y.; Zhou, C. The antibacterial activity of BF-30 in vitro and in infected burned rats is through interference with cytoplasmic membrane integrity. *Peptides* **2011**, *32*, 1131–1138.
110. Zhao, F.; Lan, X.-Q.; Du, Y.; Chen, P.-Y.; Zhao, J.; Zhao, F.; Lee, W.-H.; Zhang, Y. King cobra peptide OH-CATH30 as a potential candidate drug through clinic drug-resistant isolates. *Zool. Res.* **2018**, *39*, 87.
111. Gan, Z.R.; Gould, R.J.; Jacobs, J.W.; Friedman, P.A.; Polokoff, M.A. Echistatin. A potent platelet aggregation inhibitor from the venom of the viper, *Echis carinatus*. *J. Biol. Chem.* **1988**, *263*, 19827–19832, doi:10.1016/s0021-9258(19)77710-2.
112. Lynch, J.J.; Cook, J.J.; Sitko, G.R.; Holahan, M.A.; Ramjit, D.R.; Mellott, M.J.; Stranieri, M.T.; Stabilito, I.I.; Zhang, G.; Lynch, R.J. Nonpeptide glycoprotein IIb/IIIa inhibitors. 5. Antithrombotic effects of MK-0383. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 20–32.
113. Barrett, J.S.; Murphy, G.; Peerlinck, K.; Lepeleire, I. De; Gould, R.J.; Panebianco, D.; Hand, E.; Deckmyn, H.; Vermylen, J.; Arnout, J. Pharmacokinetics and pharmacodynamics of MK-383, a selective non-peptide platelet glycoprotein-IIb/IIIa receptor antagonist, in healthy men. *Clin. Pharmacol. Ther.* **1994**, *56*, 377–388.
114. Scarborough, R.M.; Rose, J.W.; Hsu, M.; Phillips, D.R.; Fried, V.A.; Campbell, A.M.; Nannizzi, L.; Charo, I.F. Barbourin. A GPIIb-IIIa-specific integrin antagonist from the venom of *Sistrurus m. barbouri*. *J. Biol. Chem.* **1991**, *266*, 9359–9362.
115. Scarborough, R.M.; Rose, J.W.; Naughton, M.A.; Phillips, D.R.; Nannizzi, L.; Arfsten, A.; Campbell, A.M.; Charo, I.F. Characterization of the integrin specificities of disintegrins isolated from American pit viper venoms. *J. Biol. Chem.* **1993**, *268*, 1058–1065.
116. Scarborough, R.M.; Naughton, M.A.; Teng, W.; Rose, J.W.; Phillips, D.R.; Nannizzi, L.; Arfsten, A.; Campbell, A.M.; Charo, I.F. Design of potent and specific integrin antagonists. Peptide antagonists with high specificity for glycoprotein IIb-IIIa. *J. Biol. Chem.* **1993**, *268*, 1066–1073.
117. Phillips, D.R.; Scarborough, R.M. Clinical pharmacology of eptifibatide.

Am. J. Cardiol. **1997**, *80*, 11B-20B.
118. Momic, T.; Cohen, G.; Reich, R.; Arlinghaus, F.T.; Eble, J.A.; Marcinkiewicz, C.; Lazarovici, P. Vixapatin (VP12), a c-type lectin-protein from *Vipera xantina palestinae* venom: Characterization as a novel anti-angiogenic compound. *Toxins (Basel).* **2012**, *4*, 862–877.
119. Momic, T.; Katzhendler, J.; Shai, E.; Noy, E.; Senderowitz, H.; Eble, J.A.; Marcinkiewicz, C.; Varon, D.; Lazarovici, P. Vipegitide: A folded peptidomimetic partial antagonist of α2β1 integrin with antiplatelet aggregation activity. *Drug Des. Devel. Ther.* **2015**, *9*, 291.
120. Novoa, E.; Seegers, W.H. Mechanisms of α-thrombin and β-thrombin-E formation: use of ecarin for isolation of meizothrombin 1. *Thromb. Res.* **1980**, *18*, 657–668.
121. Howie, P.W.; Prentice, C.R.M.; McNicol, G.P. A method of antithrombin estimation using plasma defibrinated with ancrod. *Br. J. Haematol.* **1973**, *25*, 101–110.
122. Kini, R.M.; Rao, V.S.; Joseph, J.S. Procoagulant proteins from snake venoms. *Pathophysiol. Haemost. Thromb.* **2001**, *31*, 218–224.
123. Rosing, J.; Tans, G. Meizothrombin, a major product of factor Xa-catalyzed prothrombin activation. *Thromb. Haemost.* **1988**, *60*, 355–360.
124. Rosing, J.; Tans, G. Structural and functional properties of snake venom prothrombin activators. *Toxicon* **1992**, *30*, 1515–1527.
125. Stevens, W.K.; Côté, H.C.F.; MacGillivray, R.T.A.; Nesheim, M.E. Calcium ion modulation of meizothrombin autolysis at Arg55-Asp56 and catalytic activity. *J. Biol. Chem.* **1996**, *271*, 8062–8067.
126. Triplett, D.A.; Stocker, K.F.; Unger, G.A.; Barna, L.K. The textarin/ecarin ratio: a confirmatory test for lupus anticoagulants. *Thromb. Haemost.* **1993**, *70*, 925–931.
127. Gosselin, R.C.; Douxfils, J. Ecarin based coagulation testing. *Am. J. Hematol.* **2020**, *95*, 863–869.
128. Tokunaga, F.; Nagasawa, K.; Tamura, S.; Miyata, T.; Iwanaga, S.; Kisiel, W. The factor V-activating enzyme (RVV-V) from Russell's viper venom. Identification of isoproteins RVV-V alpha,-V beta, and-V gamma and their complete amino acid sequences. *J. Biol. Chem.* **1988**, *263*, 17471–17481.
129. Kisiel, W.; Canfield, W.M. [22] Snake venom proteases that activate blood-coagulation factor V. In *Methods in enzymology*; Elsevier, **1981**; Vol. 80, pp. 275–285 ISBN 0076-6879.
130. Stocker, K. Application of snake venom proteins in the diagnosis of hemostatic disorders. *Med. use snake venom proteins* **1990**, 213–252.
131. Quick, A.J. Thromboplastin generation: Effect of the Bell-Alton reagent and Russell viper venom on prothrombin consumption. *Am. J. Clin. Pathol.* **1971**, *55*, 555–560.
132. Pereañez, J.A.; Preciado, L.M.; Romero, L.E. Toxinology in colombia: Contributions of programa de ofidismo/escorpionismo and other research groups. *Vitae* **2019**, *26,* 120-134.
133. Vargas, L.J.; Londoño, M.; Quintana, J.C.; Rua, C.; Segura, C.; Lomonte, B.; Núñez, V. An acidic phospholipase A2 with antibacterial activity from *Porthidium nasutum* snake venom. *Comp. Biochem. Physiol. part B Biochem. Mol. Biol.* **2012**, *161*, 341–347.
134. Quintana, J.C.; Chacón, A.M.; Vargas, L.; Segura, C.; Gutiérrez, J.M.;

Alarcón, J.C. Antiplasmodial effect of the venom of *Crotalus durissus cumanensis*, crotoxin complex and Crotoxin B. *Acta Trop.* **2012**, *124*, 126–132, doi:10.1016/j.actatropica.2012.07.003.

135. Vargas, L.J.; Quintana, J.C.; Pereañez, J.A.; Núñez, V.; Sanz, L.; Calvete, J. Cloning and characterization of an antibacterial l-amino acid oxidase from *Crotalus durissus cumanensis* venom. *Toxicon* **2013**, *64*, 1–11, doi:10.1016/j.toxicon.2012.11.027.
136. Vargas Muñoz, L.J.; Estrada-Gomez, S.; Núñez, V.; Sanz, L.; Calvete, J.J. Characterization and cDNA sequence of *Bothriechis schlegelii* l-amino acid oxidase with antibacterial activity. *Int. J. Biol. Macromol.* **2014**, *69*, 200–207, doi:10.1016/j.ijbiomac.2014.05.039.
137. Pereañez, J.A.; Preciado, L.M.; Fernández, J.; Camacho, E.; Lomonte, B.; Castro, F.; Cañas, C.A.; Galvis, C.; Castaño, S. Snake venomics, experimental toxic activities and clinical characteristics of human envenomation by *Bothrocophias myersi* (Serpentes: Viperidae) from Colombia. *J. Proteomics* **2020**, *220*, 103758, doi:10.1016/j.jprot.2020.103758.
138. Matsui, T.; Fujimura, Y.; Titani, K. Snake venom proteases affecting hemostasis and thrombosis. *Biochim. Biophys. Acta (BBA)-Protein Struct. Mol. Enzymol.* **2000**, *1477*, 146–156.
139. Felicori, L.F.; Souza, C.T.; Velarde, D.T.; Magalhaes, A.; Almeida, A.P.; Figueiredo, S.; Richardson, M.; Diniz, C.R.; Sanchez, E.F. Kallikrein-like proteinase from bushmaster snake venom. *Protein Expr. Purif.* **2003**, *30*, 32–42.
140. Culma, M.F.; Pereanez, J.A.; Rangel, V.N.; Lomonte, B. Snake venomics of *Bothrops punctatus*, a semiarboreal pitviper species from Antioquia, Colombia. *PeerJ* **2014**, *2*, e246.
141. Mora-Obando, D.; Guerrero-Vargas, J.A.; Prieto-Sánchez, R.; Beltrán, J.; Rucavado, A.; Sasa, M.; Gutiérrez, J.M.; Ayerbe, S.; Lomonte, B. Proteomic and functional profiling of the venom of *Bothrops ayerbei* from Cauca, Colombia, reveals striking interspecific variation with *Bothrops asper* venom. *J. Proteomics* **2014**, *96*, 159–172, doi:10.1016/j.jprot.2013.11.005.
142. Bonilla-Porras, A.R.; Vargas, L.J.; Jimenez-Del-Rio, M.; Nuñez, V.; Velez-Pardo, C. Purification of nasulysin-1: A new toxin from *Porthidium nasutum* snake venom that specifically induces apoptosis in leukemia cell model through caspase-3 and apoptosis-inducing factor activation. *Toxicon* **2016**, *120*, 166–174.
143. Pereañez, J.A.; Gómez, I.D.; Patiño, A.C. Relationship between the structure and the enzymatic activity of crotoxin complex and its phospholipase A_2 subunit: An in silico approach. *J. Mol. Graph. Model.* **2012**, *35*, 36–42.
144. Brigatte, P.; Faiad, O.J.; Ferreira Nocelli, R.C.; Landgraf, R.G.; Palma, M.S.; Cury, Y.; Curi, R.; Sampaio, S.C. Walker 256 tumor growth suppression by crotoxin involves formyl peptide receptors and lipoxin A4. *Mediators Inflamm.* **2016**, *2016*.
145. Corin, R.E.; Viskatis, L.J.; Vidal, J.C.; Etcheverry, M.A. Cytotoxicity of crotoxin on murine erythroleukemia cells in vitro. *Invest. New Drugs* **1993**, *11*, 11–15.
146. Cura, J.E.; Blanzaco, D.P.; Brisson, C.; Cura, M.A.; Cabrol, R.; Larrateguy, L.; Mendez, C.; Sechi, J.C.; Silveira, J.S.; Theiller, E. Phase I and pharmacokinetics study of crotoxin (cytotoxic PLA2, NSC-624244) in

patients with advanced cancer. *Clin. Cancer Res.* **2002**, *8*, 1033–1041.
147. He, J.; Wu, X.; Wang, Y.; Han, R.; Qin, Z.; Xie, Y. Growth inhibitory effects and molecular mechanisms of crotoxin treatment in esophageal Eca-109 cells and transplanted tumors in nude mice. *Acta Pharmacol. Sin.* **2013**, *34*, 295–300.
148. de Andrade, C.M.; Rey, F.M.; Bianchini, F.J.; Sampaio, S. V; Torqueti, M.R. Crotoxin, a neurotoxin from *Crotalus durissus terrificus* snake venom, as a potential tool against thrombosis development. *Int. J. Biol. Macromol.* **2019**, *134*, 653–659.
149. Mello, C.P.; Lima, D.B.; Bandeira, I.C.J.; Tessarolo, L.D.; Sampaio, T.L.; Falcão, C.B.; Rádis-Baptista, G.; Martins, A.M.C. Evaluation of the antichagasic activity of batroxicidin, a cathelicidin-related antimicrobial peptide found in *Bothrops atrox* venom gland. *Toxicon* **2017**, *130*, 56–62.
150. Martins, N.M.; Ferreira, D.A.S.; Rodrigues, M.A.C.; Cintra, A.C.O.; Santos, N.A.G.; Sampaio, S. V; Santos, A.C. Low-molecular-mass peptides from the venom of the Amazonian viper *Bothrops atrox* protect against brain mitochondrial swelling in rat: Potential for neuroprotection. *Toxicon* **2010**, *56*, 86–92.
151. Gazerani, P. Venoms as an adjunctive therapy for Parkinson's disease: Where are we now and where are we going? *Futur. Sci. OA* **2021**, *7*, doi:10.2144/fsoa-2020-0119.
152. Brenes, H.; Loría, G.D.; Lomonte, B. Potent virucidal activity against Flaviviridae of a group IIA phospholipase A_2 isolated from the venom of *Bothrops asper*. *Biologicals* **2020**, *63*, 48–52.
153. Girón, M.E.; Guerrero, B.; Salazar, A.M.; Sánchez, E.E.; Álvarez, M.; Rodríguez-Acosta, A. Functional characterization of fibrinolytic metalloproteinases (colombienases) isolated from *Bothrops colombiensis* venom. *Toxicon* **2013**, *74*, 116–126.
154. Menaldo, D.L.; Costa, T.R.; Ribeiro, D.L.; Zambuzi, F.A.; Antunes, L.M.G.; Castro, F.A.; Frantz, F.G.; Sampaio, S. V Immunomodulatory actions and epigenetic alterations induced by proteases from *Bothrops* snake venoms in human immune cells. *Toxicol. Vitr.* **2019**, *61*, 104586.
155. Romero-Vargas, F.F.; Rocha, T.; Cruz-Höfling, M.A.; Rodrigues-Simioni, L.; Ponce-Soto, L.A. Biochemical characterization of a PLA_2 Btae TX-I isolated from *Bothriopsis taeniata* snake venom: A pharmacological and morphological study. *J Clin Toxicol* **2014**, *4*:3 DOI: 10.4172/2161-0495.1000197
156. Maluf, S.E.C.; Dal Mas, C.; Oliveira, E.B.; Melo, P.M.; Carmona, A.K.; Gazarini, M.L.; Hayashi, M.A.F. Inhibition of malaria parasite *Plasmodium falciparum* development by crotamine, a cell penetrating peptide from the snake venom. *Peptides* **2016**, *78*, 11–16.
157. Falcao, C.B.; Radis-Baptista, G. Crotamine and crotalicidin, membrane active peptides from *Crotalus durissus terrificus* rattlesnake venom, and their structurally-minimized fragments for applications in medicine and biotechnology. *Peptides* **2020**, *126*, 170234.
158. Petricevich, V.L.; Mendonça, R.Z. Inhibitory potential of *Crotalus durissus terrificus* venom on measles virus growth. *Toxicon* **2003**, *42*, 143–153.
159. Faure, G.; Bakouh, N.; Lourdel, S.; Odolczyk, N.; Premchandar, A.; Servel, N.; Hatton, A.; Ostrowski, M.K.; Xu, H.; Saul, F.A. Rattlesnake

phospholipase A_2 increases CFTR-chloride channel current and corrects F508CFTR dysfunction: Impact in cystic fibrosis. *J. Mol. Biol.* **2016**, *428*, 2898–2915.

160. Pérez-Peinado, C.; Dias, S.A.; Domingues, M.M.; Benfield, A.H.; Freire, J.M.; Rádis-Baptista, G.; Gaspar, D.; Castanho, M.A.R.B.; Craik, D.J.; Henriques, S.T. Mechanisms of bacterial membrane permeabilization by crotalicidin (Ctn) and its fragment Ctn (15–34), antimicrobial peptides from rattlesnake venom. *J. Biol. Chem.* **2018**, *293*, 1536–1549.
161. Muller, V.D.; Soares, R.O.; dos Santos-Junior, N.N.; Trabuco, A.C.; Cintra, A.C.; Figueiredo, L.T.; Caliri, A.; Sampaio, S.V.; Aquino, V.H. Phospholipase A_2 isolated from the venom of *Crotalus durissus terrificus* inactivates dengue virus and other enveloped viruses by disrupting the viral envelope. *PLoS One* **2014**, *9*, e112351.
162. Brigatte, P.; Konno, K.; Gutierrez, V.P.; Sampaio, S.C.; Zambelli, V.O.; Picolo, G.; Curi, R.; Cury, Y. Peripheral kappa and delta opioid receptors are involved in the antinociceptive effect of crotalphine in a rat model of cancer pain. *Pharmacol. Biochem. Behav.* **2013**, *109*, 1–7.
163. Teixeira, N.B.; Sant'Anna, M.B.; Giardini, A.C.; Araujo, L.P.; Fonseca, L.A.; Basso, A.S.; Cury, Y.; Picolo, G. Crotoxin down-modulates pro-inflammatory cells and alleviates pain on the MOG35-55-induced experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. *Brain. Behav. Immun.* **2020**, *84*, 253–268.
164. Pinheiro-Júnior, E.L.; Boldrini-França, J.; de Campos Araújo, L.M.P.; Santos-Filho, N.A.; Bendhack, L.M.; Cilli, E.M.; Arantes, E.C. LmrB-PP9: A synthetic bradykinin-potentiating peptide from *Lachesis muta rhombeata* venom that inhibits the angiotensin-converting enzyme activity in vitro and reduces the blood pressure of hypertensive rats. *Peptides* **2018**, *102*, 1–7.
165. Jiménez–Charris, E.; Lopes, D.S.; Gimenes, S.N.C.; Teixeira, S.C.; Montealegre–Sánchez, L.; Solano–Redondo, L.; Fierro–Pérez, L.; Ávila, V. de M.R. Antitumor potential of Pllans–II, an acidic Asp49–PLA2 from *Porthidium lansbergii lansbergii* snake venom on human cervical carcinoma HeLa cells. *Int. J. Biol. Macromol.* **2019**, *122*, 1053–1061.
166. Montealegre-Sánchez, L.; Gimenes, S.N.C.; Lopes, D.S.; Teixeira, S.C.; Solano-Redondo, L.; de Melo Rodrigues, V.; Jiménez-Charris, E. Antitumoral potential of Lansbermin-I, a novel disintegrin from *Porthidium lansbergii lansbergii* venom on breast cancer cells. *Curr. Top. Med. Chem.* **2019**, *19*, 2069–2078.
167. Vargas Munoz, L.J.; Estrada-Gómez, S.; Nunez, V.; Sanz, L.; Calvete, J.J. Characterization and cDNA sequence of *Bothriechis schlegelii* l-amino acid oxidase with antibacterial activity. **2014**, *69*, 200-7. doi: 10.1016/j.ijbiomac.2014.05.039
168. Rey-Suárez, P.; Núñez, V.; Gutiérrez, J.M.; Lomonte, B. Proteomic and biological characterization of the venom of the redtail coral snake, *Micrurus mipartitus* (Elapidae), from Colombia and Costa Rica. *J. Proteomics* **2011**, *75*, 655–667, doi:10.1016/j.jprot.2011.09.003.
169. Rey-Suárez, P.; Núñez, V.; Fernández, J.; Lomonte, B. Integrative characterization of the venom of the coral snake *Micrurus dumerilii* (Elapidae) from Colombia: Proteome, toxicity, and cross-neutralization by antivenom. *J. Proteomics* **2016**, *136*, 262–273, doi:10.1016/j.

jprot.2016.02.006.
170. Sanz, L.; Quesada-Bernat, S.; Ramos, T.; Casais-e-Silva, L.L.; Corrêa-Netto, C.; Silva-Haad, J.J.; Sasa, M.; Lomonte, B.; Calvete, J.J. New insights into the phylogeographic distribution of the 3FTx/PLA 2 venom dichotomy across genus *Micrurus* in South America. *J. Proteomics* **2019**, *200*, 90–101, doi:10.1016/j.jprot.2019.03.014.
171. Casais-e-Silva, L.L.; Teixeira, C.F.P.; Lebrun, I.; Lomonte, B.; Alape-Girón, A.; Gutiérrez, J.M. Lemnitoxin, the major component of *Micrurus lemniscatus* coral snake venom, is a myotoxic and pro-inflammatory phospholipase A_2. *Toxicol. Lett.* **2016**, *257*, 60–71, doi:10.1016/j.toxlet.2016.06.005.
172. Rey-Suárez, P.; Acosta, C.; Torres, U.; Saldarriaga-Cordoba, M.; Lomonte, B.; Núñez, V. MipLAAO, a new L-amino acid oxidase from the redtail coral snake *Micrurus mipartitus*. *PeerJ* **2018**, *2018*, doi:10.7717/peerj.4924.
173. Rey-Suárez, P.; Núñez, V.; Saldarriaga-Cordoba, M.; Lomonte, B. Primary structures and partial toxicological characterization of two phospholipases A2from *Micrurus mipartitus* and *Micrurus dumerilii* coral snake venoms. *Biochimie* **2017**, *137*, 88–98, doi:10.1016/j.biochi.2017.03.008.
174. Nascimento, L.R.S.; Silva, N.J.; Feitosa, D.T.; Prudente, A.L.C. Taxonomy of the *Micrurus spixii* species complex (Serpentes, Elapidae). *Zootaxa* **2019**, *4668*, 370–392, doi:10.11646/zootaxa.4668.3.4.
175. Terra, A.L.C.; Moreira-Dill, L.S.; Simões-Silva, R.; Monteiro, J.R.N.; Cavalcante, W.L.G.; Gallacci, M.; Barros, N.B.; Nicolete, R.; Teles, C.B.G.; Medeiros, P.S.M.; et al. Biological characterization of the Amazon coral *Micrurus spixii* snake venom: Isolation of a new neurotoxic phospholipase A2. *Toxicon* **2015**, *103*, 1–11, doi:10.1016/j.toxicon.2015.06.011.
176. Torres-Bonilla, K.A.; Schezaro-Ramos, R.; Floriano, R.S.; Rodrigues-Simioni, L.; Bernal-Bautista, M.H.; Alice da Cruz-Höfling, M. Biological activities of *Leptodeira annulata* (banded cat-eyed snake) venom on vertebrate neuromuscular preparations. *Toxicon* **2016**, *119*, 345–351, doi:10.1016/j.toxicon.2016.07.004.
177. Torres-Bonilla, K.A.; Floriano, R.S.; Schezaro-Ramos, R.; Rodrigues-Simioni, L.; da Cruz-Höfling, M.A. A survey on some biochemical and pharmacological activities of venom from two Colombian colubrid snakes, *Erythrolamprus bizona* (Double-banded coral snake mimic) and *Pseudoboa neuwiedii* (Neuwied's false boa). *Toxicon* **2017**, *131*, 29–36, doi:10.1016/J.TOXICON.2017.02.030.
178. Torres-Bonilla, K.A.; Panunto, P.C.; Pereira, B.B.; Zambrano, D.F.; Herrán-Medina, J.; Bernal, M.H.; Hyslop, S. Toxinological characterization of venom from *Leptodeira annulata* (Banded cat-eyed snake; Dipsadidae, Imantodini). *Biochimie* **2020**, *174*, 171–188, doi:10.1016/J.BIOCHI.2020.04.006.
179. Heyborne, W.H.; Mackessy, S.P. Identification and characterization of a taxon-specific three-finger toxin from the venom of the Green Vinesnake (*Oxybelis fulgidus*; family Colubridae). *Biochimie* **2013**, *95*, 1923–1932, doi:10.1016/J.BIOCHI.2013.06.025.
180. Peichoto, M.E.; Tavares, F.L.; DeKrey, G.; Mackessy, S.P. A comparative study of the effects of venoms from five rear-fanged snake spe-

cies on the growth of Leishmania major: Identification of a protein with inhibitory activity against the parasite. *Toxicon* **2011**, *58*, 28–34, doi:10.1016/j.toxicon.2011.04.018.

181. Leite dos Santos, G.G.; Casais e Silva, L.L.; Pereira Soares, M.B.; Villarreal, C.F. Antinociceptive properties of *Micrurus lemniscatus* venom. *Toxicon* **2012**, *60*, 1005–1012, doi:10.1016/j.toxicon.2012.07.003.
182. Jiménez-Charris, E.; Montealegre-Sanchez, L.; Solano-Redondo, L.; Mora-Obando, D.; Camacho, E.; Castro-Herrera, F.; Fierro-Pérez, L.; Lomonte, B. Proteomic and functional analyses of the venom of *Porthidium lansbergii lansbergii* (Lansberg's hognose viper) from the Atlantic Department of Colombia. *J. Proteomics* **2015**, *114*, 287–299, doi:10.1016/j.jprot.2014.11.016.



Abbreviations and meaning

Abbreviation	Meaning
3FTx	Three-Finger Toxins
Ach	Acetylcholine
ADP	Adenosine Diphosphate
AKI	Acute Kidney Injury
ALP	Antilonomic Antivenom
AOO	Area Of Occupancy
aPTT	Activated Partial Thromboplastin Time
ATCC	American Type Culture Collection
AV B/C	Bothropic-Crotalid Antivenom
BiL	Basic Isolated Lectin
BJcuL	*Bothrops jararacussu* C-type Lectin
BK	Bradykinin
BM	Basement Membrane
BmLec	*Bothrops moojeni* Lectin
BN-PAGE	Blue Native-PAGE
BpirL	*Bothrops pirajai* Lectin
BPP	Bradykinin-Potentiating Peptides
CA	Crotapotin Subunit A (associated with crotoxin)
CDR	Carbohydrate Recognition Domain
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CK	Creatine Kinase
CKD	Chronic Kidney Disease
CLP	C-Type Lectin Protein
CNBr	Cyanogen Bromide
CNG	Cyclic Nucleotide-Gated Channels
C-NP	C-terminal Neuropeptide
CRD	Cysteine-Rich Domain
CRISP	Cysteine-Rich Secretory Protein
CTL	C-Type Lectin
CTX	Crotoxin
DTT	Dithiothreitol
ECM	Extracellular Matrix
ED_{50}	Median Effective Dose
ELISA	Enzyme-Linked Immunosorbent Assay
ELISA HRP	Enzyme-linked immunosorbent assay using horseradish peroxidase
EOO	Extent Of Occurrence

EPI	Expanded Program on Immunization
F(ab')2	Fragment antigen-binding
Fab	Fragment antigen-binding
FPLC	Fast Protein Liquid Chromatography
GLN	Glutamine
GMP	Good Manufacturing Practices
GWR	Geographically Weighted Regression
H_2O_2	Hydrogen peroxide
HC	Hydrophobic Channel
HR	Health Registration
IAEC	Institutional Animal Ethics Committee
ICGEB	International Centre for Genetic Engineering and Biotechnology
ICP	Clodomiro Picado Institute
IEF	Isoelectric Focusing
IgG	Immunoglobulin G
INR	International Normalized Ratio
INS	Instituto Nacional de Salud
INVIMA	Instituto Nacional de Vigilancia de Medicamentos y Alimentos
IP	Intraperitoneal
IPTG	Isopropyl β-D-1-thiogalactopyranoside
IRA	Immune-Related Activity
ISCOM	Immune Stimulating Complex
IUCN	International Union for Conservation of Nature
IV	Intravenous (injection method)
KDa	Kilodaltons (unit of molecular weight)
KUN	Kunitz-Type Protease Inhibitors
LAAO	L-Amino Acid Oxidase
LD_{100}	Lethal dose for 100% of the animal test
LD_{50}	Median Lethal Dose
LDH	Lactic Dehydrogenase
Lys	Lysine
MAHA	Microangiopathic Hemolytic Anemia
MCD	Minimum Coagulant Dose
MDiS	Membrane Disrupting Site
MDoS	Membrane Docking Site
MHD	Minimum Hemorrhagic Dose
MMD	Minimum Myotoxic Dose
MMP	Matrix Metalloproteinase
MND	Minimum Necrotizing Dose
MmTX1	Myotoxin 1 from *Micrurus mipartitus*
MmTX2	Myotoxin 2 from *Micrurus mipartitus*
MSP	Minimum Stabilization Point
MTα	Metalloproteinase α
NIV	Non-Injectable Vaccine

NGF	Nerve Growth Factor
NTDS	Neglected Tropical Diseases
OH	Hydroxyl Group
ON	Oxidized Nitrogen
PAHO	Pan-American Health Organization
PFA	Purified Fab Antibodies
PHIS	Polyvalent Hyperimmune Serum
PLA_2	Phospholipase A2
PLB	Phospholipase B
PT	Prothrombin Time
PNCS	National Program for the Conservation of Snakes Present in Colombia
RCpLi	Recombinant Chimeric Protein
rFAB	Recombinant F(ab')2 fragment
RP-HPLC	Reverse-Phase High-Performance Liquid Chromatography
SAM	Monovalent Antiviperid Serum
SAP	Polyvalent Antiviperid Serum
ScFv	Single-chain fragment variable
SDD	Spray Drying Dispersion
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
Ser	Serine
SIVIGILA	Public Health Epidemiological Surveillance System
SOP	Standard Operating Procedure
SOPH	State-Owned Pharmaceutical House
SVMP	Snake Venom Metalloproteinase
SVSP	Snake Venom Serine Proteinase
TMA	Thrombotic Microangiopathy
TNF	Tumor Necrosis Factor
Tyr	Tyrosine
VEGF	Vascular Endothelial Growth Factor
VICC	Venom-Induced Consumption Coagulopathy
VIG	Viral Immunoglobulin
VRBPAC	Vaccines and Related Biological Products Advisory Committee
vWF	von Willebrand Factor
WB	Western Blot
WHO	World Health Organization
Biological collections acronyms	
IAvH	Instituto de Investigación de Recursos Biológicos Alexander von Humboldt
ICN	Instituto de Ciencias Naturales Universidad Nacional de Colombia
INSV	Colección de Animales Venenosos del Instituto Nacional de Salud
INSBT	Banco de tejidos de animales venenosos del Instituto Nacional de Salud

INSZ	Colección Zoológica del Instituto Nacional de Salud
SINCHI-R	Colección Reptiles Instituto Sinchi
UVC	Universidad del Valle del Cauca
Collector acronyms for data retrieved from field notes	
JDL	John Douglas Lynch
TAS	Teddy Angarita Sierra
SDCC	Sergio Daniel Cubides Cubillos
Snake abundance categories	
Extremely common	Likely to be seen every day, usually in large numbers
Common	Recorded weekly in densities above five individuals per locality
Frequent	Recorded weekly in densities below five individuals per locality
Uncommon	Unlikely to be seen more than once every few months
Rare	Total average number of reported observations per locality less than ten
Extremely rare	Total average number of reported observations per locality less than three
IUCN Threat categories	
Extinct (EX)	A species is Extinct when there is no reasonable doubt that the last individual has died.
Critically endangered (CR)	A species is Critically Endangered when the best available evidence indicates that it meets any of the criteria A to E for Critically Endangered, and it is therefore considered to be facing an extremely high risk of extinction in the wild
Endangered (EN)	A species is Endangered when the best available evidence indicates that it meets any of the criteria A to E for Endangered, and it is therefore considered to be facing a very high risk of extinction in the wild
Vulnerable (VU)	A species is Vulnerable when the best available evidence indicates that it meets any of the criteria A to E for Vulnerable, and it is therefore considered to be facing a high risk of extinction in the wild
Near Threatened (NT)	A taxon is Near Threatened when it has been evaluated against the criteria but does not qualify for Critically Endangered, Endangered or Vulnerable now, but is close to qualifying for or is likely to qualify for a threatened category in the near future
Least Concern (LC)	A taxon is Least Concern when it has been evaluated against the criteria and does not qualify for Critically Endangered, Endangered, Vulnerable or Near Threatened. Widespread and abundant taxa are included in this category
Data Deficient (DD)	A taxon is Data Deficient when there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status.
Not evaluated (NE)	A taxon is Not Evaluated when it has not yet been evaluated against the criteria

You can find criteria A to E at: https://portals.iucn.org/library/node/10315)

Glossary

Adrenaline/Epinephrine: This hormone activates both alpha- and beta-adrenergic systems, leading to vasoconstriction and increased blood pressure. It stimulates the heart, elevating heart rate and contractile force. Additionally, it induces bronchodilation, facilitating respiration, and promotes cerebral vasodilation, thereby enhancing cerebral blood flow.

Albumin: Water-soluble proteins found in egg whites, blood, lymph, and other tissues and fluids. They coagulate upon heating.

Aldosterone: A hormone secreted by the adrenal cortex that regulates electrolyte and water balance by increasing the renal retention of sodium and the excretion of potassium.

Allopatry: Allopatry refers to the condition in which populations or species are geographically separated from one another. This separation is often due to physical barriers such as mountains, rivers, or distance, preventing interbreeding between the groups.

Angiotensin I: A decapeptide that is cleaved from precursor angiotensinogen by renin. Angiotensin I has limited biological activity. It is converted to angiotensin II, a potent vasoconstrictor, after the removal of two amino acids at the C-terminal by angiotensin converting enzyme.

Anticoagulant: Certain toxins present in snake venoms (e.g., genus *Bothrops*) act as anticoagulants by inhibiting coagulation, destroying platelets, or activating fibrinolysis. These effects can lead to hemorrhage and impair clot formation. However, some toxins from these venoms show potential for development as anticoagulant drugs for medical therapies.

Antidiuretic hormone/ Vasopressins: Antidiuretic hormones released by the neurohypophysis of all vertebrates (structure varies with species) to regulate water balance and osmolarity. In general, vasopressin is a nonapeptide consisting of a six-amino-acid ring with a cysteine 1 to cysteine 6 disulfide bridge or an octapeptide containing a cystine. All mammals have arginine vasopressin except the pig with a lysine at position 8. Vasopressin, a vasoconstrictor, acts on the kidney collecting ducts to increase water reabsorption, increase blood volume and blood pressure.

Aposematism: Aposematism is a biological phenomenon in which an organism possesses bright and conspicuous coloration or other warning signals to indicate that it is toxic, unpalatable, or otherwise harmful to potential predators. The concept is derived from the Greek words “apo,” meaning “away,” and “sema,” meaning “sign,” essentially translating to a warning signal.

Atherosclerosis: A thickening and loss of elasticity of the walls of arteries that occurs with formation of atherosclerotic plaques within the arterial intima.

Biodiversity (biological diversity): Refers to the variety and variability of life on Earth, encompassing all levels of its organization, from the genes of individuals that make up a local population or species, to the species that constitute all or part of a local community, which forms the living component of the many ecosystems and landscapes of the world.

Bloodstream/ Blood Circulation: The movement of the blood as it is pumped through the cardiovascular system.

Bootstrap support (BS): The bootstrap method is a statistical technique used to estimate the distribution of a statistic (such as a mean, variance, or regression coefficient) by resampling with replacement from the original data. In phylogenetics, the bootstrap method is used to assess the reliability and robustness of phylogenetic trees. These trees represent the evolutionary relationships among a set of species or genes. Bootstrapping provides a way to quantify the confidence in each branch (or clade) of the tree. Thus, the bootstrap support value represents the frequency with which a particular branch or clade appears in these trees, indicating the level of confidence in the inferred relationship. Bootstrap values can be interpreted as follows.

Excellent bootstrap values (≥ 90%): Indicate strong support for the clade and suggest that it is likely to be a true feature of the underlying phylogeny.

High bootstrap values (≥ 80-90%): Indicate moderate support for the clade and suggest that it is possible to be a true feature of the underlying phylogeny.

Moderate bootstrap values (≥60-80%): Indicate week support and suggest that the clade is somewhat reliable but should be interpreted with caution.

Low bootstrap values (< 60%): Indicate poor support and suggest that the clade may not be a reliable feature of the phylogeny.

Bradykinin: A nonapeptide messenger that is enzymatically produced from kallidin in the blood where it is a potent but short-lived agent of arteriolar dilation and increased capillary permeability. Bradykinin is also released from mast cells during asthma attacks, from gut walls as a gastrointestinal vasodilator, from damaged tissues as a pain signal, and may be a neurotransmitter.

Carcinogenesis: The origin, production or development of cancer through genotypic and phenotypic changes which upset the normal balance between cell proliferation and cell death. Carcinogenesis generally requires a constellation of steps, which may occur quickly or over a period of many years.

Cis-Andean region: Refers to the tropical region of South America located east of the Andes and below 1,000 meters in elevation. In Colombia includes the Orinoquia and Amazon regions.

Clade: A clade is a piece of a phylogeny (a branch of evolutionary tree) that includes an ancestral lineage and all the descendants of that ancestor.

Convergent selection: Convergent selection, commonly referred to as convergent evolution, is the process where different species independently evolve similar traits. This phenomenon occurs when species that do not share a recent common ancestor face similar environmental pressures and natural selection, leading to the development of analogous structures or functions. These traits are similar in form or function but evolved independently in different species.

Co-optation: It is a process that occurs when natural selection finds new uses for existing traits, including genes, organs, and other body structures.

Cryptic diversity: Refers to the presence of multiple distinct species or lineages that are morphologically, indistinguishable or very similar. Despite their similar appearance, these species exhibit genetic, ecological, or behavioral differences that are not apparent through traditional morphological characterization alone.

Coagulant: Agents that cause clotting.

Digestive enzymes: The main digestive enzymes made in the pancreas include Amylase (made in the mouth and pancreas; breaks down complex carbohydrates), Lipase (made in the pancreas; breaks down fats), and Protease (made in the pancreas; breaks down proteins)

Diuresis: A fundamental physiological mechanism in which the kidneys perform filtration and excretion of surplus water, electrolytes, and metabolic waste products via urine formation.

Dysecdysis: Abnormal or incomplete shedding of the outer skin (epidermis) in reptiles.

Dyslipidemias: Abnormalities in the serum levels of lipids, including overproduction or deficiency. Abnormal serum lipid profiles may include high total cholesterol, high triglycerides, low high density lipoprotein cholesterol, and elevated low density lipoprotein cholesterol.

Ecdysis: Refer to the periodic process of shedding or molting the outer layer of skin, exoskeleton, or cuticle in animals. This process is most commonly observed in reptiles, such as snakes and lizards.

Ecosystem: A cluster of communities of organisms together with their physical environment, viewed as a system of interacting and interdependent relationships and including such processes as the flow of energy

through trophic levels and the cycling of chemical elements and compounds through living and non-living components of the system.

Edema (swelling): This inflammatory swelling results from venom-induced endothelial damage and increased capillary permeability, mediated by metalloproteinases and other toxins in Bothrops venoms. The subsequent plasma extravasation leads to characteristic local manifestations including pronounced edema, pain, and erythema at the envenomation site.

Evolution: Refers to the changes in the heritable characteristics of biological populations over successive generations as an ongoing process of descent with modification. This process considers mechanisms of genetic inheritance and the role of natural selection and other evolutionary forces such as reproductive isolation and genetic divergence, etc.

GABA/gamma-Aminobutyric Acid: The most common inhibitory neurotransmitter in the central nervous system.

Glutamate/Glutamic Acid: A non-essential amino acid naturally occurring in the L-form. Glutamic acid is the most common excitatory neurotransmitter in the central nervous system.

Haplotype: A haplotype is a physical grouping of genomic variants (or polymorphisms) that tend to be inherited together.

Histamine: Histamine is an amine derived from the enzymatic decarboxylation of histidine, which may be present in certain snake venoms. This biogenic amine acts as: (1) a potent bronchial smooth muscle constrictor, (2) a vasodilator, and (3) a central neurotransmitter. In cases of snakebite envenomation, excessive histamine release may contribute to toxic effects including bronchospasm, hypotension, and severe allergic reactions, thereby complicating the clinical presentation and exacerbating systemic toxicity.

Human footprint: The human footprint measures energy quantities, resources, and products consumed by a human during his/her lifetime. Thus, Human Footprint Index expresses as a percentage the relative human Influence in every biome on the land's surface.

Hyperhumid forest: It is a type of forest ecosystem characterized by extremely high levels of humidity and precipitation, typically exceeding 4,000 mm (157 inches) of rainfall annually. These conditions create a consistently moist environment throughout the year, with minimal seasonal variation in rainfall.

Immune system: The body's defense mechanism against foreign organisms or substances and deviant native cells. It includes the humoral immune response and the cell-mediated response and consists of a complex of interrelated cellular, molecular, and genetic components.

Immunodepleted: Refers to cells, molecules, or components of the immune system that can be selectively depleted or removed, typically through specific interventions. This process is often used in research or clinical settings to study the function of particular immune cells or to modulate the immune response for therapeutic purposes.

Immunogen: Immunogens represent a class of substances capable of inducing adaptive immune responses upon systemic exposure. When present in snake venoms, these molecules (typically proteins) stimulate B-cell activation and subsequent IgG production. The generated immunoglobulins exhibit high-affinity binding to cognate venom antigens, mediating toxin neutralization through epitope masking and Fc-dependent clearance mechanisms.

Lifespan: Refers to the total duration of time an organism is expected to live, from birth to death, under natural or typical circumstances. It can apply to individuals, species, or even biological components like cells.

Lowland tropical forest: Refers to those forested environments from sea level to 1,000 meters elevation.

Metastasis/Neoplasm Metastasis: The transfer of a neoplasm from one organ or part of the body to another remote from the primary site.

Mimicry: Mimicry is a biological phenomenon where one organism, the mimic, evolves to resemble another organism, the model, to gain an advantage, usually related to survival or reproduction. Mimicry often involves aspects such as appearance, behavior, sound, scent, or even location, and it serves various functions, primarily in predator-prey interactions or reproductive strategies.

Molecular marker: A molecular marker is a molecule, typically a DNA/RNA sequence, that can be identified and is heritable. These markers are found at specific locations within the genome and can be used to detect DNA polymorphisms, which are variations in the DNA sequence among individuals.

Monophyletic group: A monophyletic group is a set of organisms that includes a common ancestor and all its descendants.

Natriuretic/ Natriuretic Peptides: Peptides that regulate the water-electrolyte balance in the body, also known as natriuretic peptide hormones. Several have been sequenced (atrial natriuretic factor; brain natriuretic peptide; c-type natriuretic peptide).

Necrosis: Necrosis is the premature death of cells and tissues in the body, which occurs due to factors such as infection, toxins, trauma, or ischemia (lack of blood supply). Necrosis is an uncontrolled cell death process that often results in inflammation and damage to the surrounding tissues.

Neuromuscular blockade: Refers to the action of molecules (= medical drugs, or toxins) that block the transmission of nerve impulses to the muscles (see synapse). This results in muscle relaxation and paralysis.

Nitric oxide: A free radical gas produced endogenously by a variety of mammalian cells, synthesized from arginine by nitric oxide synthase. Nitric oxide is one of the endothelium-dependent relaxing factors released by the vascular endothelium and mediates vasodilation. It also inhibits platelet aggregation, induces disaggregation of aggregated platelets, and inhibits platelet adhesion to the vascular endothelium. Nitric oxide activates cytosolic guanylate cyclase and thus elevates intracellular levels of cyclic GMP.

Noradrenaline: Precursor of epinephrine that is secreted by the adrenal medulla and is a widespread central and autonomic neurotransmitter. Norepinephrine is the principal transmitter of most postganglionic sympathetic fibers, and of the diffuse projection system in the brain that arises from the locus ceruleus. It is also found in plants and is used pharmacologically as a sympathomimetic.

Ontogeny, also known as ontogenesis, refers to the development or course of development of an individual organism from the earliest stage to maturity. This process encompasses all the changes an organism undergoes from a single cell (zygote) through its entire lifespan until death. Ontogeny includes various stages such as embryonic development, growth, and aging, representing the biological unfolding of an organism's life cycle.

Orogeny: Orogeny refers to the geological process of mountain formation, typically resulting from the compression and folding of the Earth's crust. This process often occurs at convergent plate boundaries, where the collision and interaction of tectonic plates lead to the uplift and deformation of the crust, creating mountain ranges.

Paralysis: A general term most often used to describe severe or complete loss of muscle strength due to motor system disease from the level of the cerebral cortex to the muscle fiber. This term may also occasionally refer to a loss of sensory function.

Parapatric speciation: Type of species diversification involving contiguous populations occupying different habitats within a continuous geographic range without any particular extrinsic barrier to gene flow.

Paraphyletic group: A paraphyletic group is a taxonomic grouping that includes an ancestral species and some, but not all, of its descendants.

Parsimonious: Refers to a principle or approach that favors the simplest explanation or hypothesis that fits the available evidence. This principle is known as Occam's razor, which suggests that among competing hypotheses, the one with the fewest assumptions should be selected.

Pathologies: Refer to the scientific study of diseases, including their causes (etiology), development (pathogenesis), effects on the body, and clinical manifestations. The term can also describe the specific abnormalities, conditions, or diseases present in an organism, tissue, or organ.

Phylogeny: Phylogeny refers to the evolutionary history and relationships among a species or group of organisms. It describes the lineage and connections between different organisms based on their common ancestry and evolutionary development.

Phylogeography: Phylogeography is the study of the historical processes that may be responsible for the geographic distribution of genealogical lineages, particularly within and among closely related species.

Polytomy: A polytomy in a phylogenetic tree refers to an internal node that branches into three or more lineages, as opposed to a dichotomous split into just two lineages. Polytomies indicate that the evolutionary relationships among the descendant lineages are not fully resolved.

Prezygotic barriers: A prezygotic barrier is a mechanism that blocks reproduction from taking place; this includes barriers that prevent fertilization when organisms attempt reproduction.

Proteome: The proteome is the entire set of proteins expressed by a genome, cell, tissue, or organism at a certain time under specific conditions.

Proteomics: Studying the structure and function of venom proteins and how they interact with biological targets. This includes the use of bioinformatics tools to predict protein function and interactions.

Putative hypothesis: Refers to a provisional or assumed explanation for a phenomenon that is suggested as a basis for further investigation or study. The term “putative” implies that the hypothesis is proposed as likely or plausible but has not yet been confirmed or rigorously tested. It serves as a starting point for scientific inquiry and experimentation.

Respiratory paralysis: Respiratory paralysis is a life-threatening condition caused by neurotoxic envenomation, primarily observed in snakebites from elapid (e.g., Micrurus spp., Naja spp., Bungarus spp.) and some viperid (e.g., *Crotalus durissus*) species. This condition results from the disruption of neuromuscular transmission, leading to progressive muscle weakness and eventual respiratory failure.

Synapse: Synaptic transmission refers to the physiological process by which neurons communicate with either other neurons or muscle fibers, enabling the propagation of signals that coordinate essential functions such as movement and reflexes.

Sympatry: Sympatry refers to the coexistence of related species or populations within the same geographic area, allowing them to interact and frequently encounter one another.

Survival rate: The proportion of a population or species that survives a given time interval.

Trans-Andean region: Refers to the tropical region of South America located west of the Andes and below 1,000 meters in elevation. In Colombia includes the Caribbean cost, inter-Andean Valleys, and Pacific region.

Transcriptomics: Analyzing the RNA transcripts produced by venom glands to understand which genes are being expressed and how this expression changes under different conditions or stages of development.

Venomics: Studying of venom and its components using advanced techniques in genomics, proteomics, transcriptomics, and bioinformatics. This interdisciplinary field aims to understand the complexity of venom composition, its variation among different species, and its potential applications in medicine, pharmacology, and biotechnology.

Vicariance: Refers to the geographical separation and isolation of a population, leading to the differentiation of the original population into new varieties or species. This process can result from various factors such as tectonic plate movements, changes in climate, or the formation of barriers that divide populations, leading to speciation.

Western blot: A widely used analytical technique in molecular biology and immunogenetics for detecting specific proteins in tissue homogenates or extracts.



I am the fear, the time, the cure,
the one who makes love or awakens lust.
I am Aku, Asklepios, Atoum, Ivána, Raìdu,
Quetzalcóatl and Uraeus.
I am the creator of worlds, change,
life, death and resurrection.
I circle the world giving names to peoples,
before time spun their lineages.
I circle the jungles, the seas, the icy lands and deserts,
always in silence, calm, with nocturnal voracity.
I circle the minds, the dreams,
the souls of those who have never seen me,
the fears of those who have received my kisses.
They see me as strange, smooth, warty, cold, colorful,
iridescent, agile and sinuous.
They see me as a banner of war, as a sacred seal,
as the dream of immortality, as the original sin.
Those who fear me see me,
those who seek me do not see me.
I am she, the first, the only one, and for being so,
they kill me to love me forever.

Salud

INSTITUTO NACIONAL DE SALUD