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International Symposium on Coralsnakes

HONORING JANIS A. ROZE

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FOREWORD

The International Symposium on Coralsnakes is a scientific conference addressing all aspects of coralsnake biology that will honor and celebrate the professional contributions of Janis Arnold Roze, the preeminent living authority on the taxonomy and biology of the coralsnakes. The concept of the Symposium was part of some discussions among Brazilian herpetologists, as we believe in the professional ethics to recognize the extremely important role of Janis Roze to better our knowledge of coralsnakes in a wide view of the group and its importance, from biology to medicine. It is a great honor to host the Symposium at the Pontifícia Universidade Católica de Goiás. We, at the Centro de Estudos e Pesquisas Biológicas (CEPB), started working with coralsnakes back in 1983 and in more than 30 years we think that our contribution, as small as it may be, helped us to understand better this beautiful group of snakes.

The Symposium is divided into five areas of interest, each of which will be overseen by a coordinator who will be responsible for organizing that portion of the Symposium and who will handle communications from researchers and academics interested in that subject. After the Symposium, presentations will be prepared as manuscripts, organized by area, and transformed into chapters of a special symposium volume to be published thereafter.

SUBJECT AREAS

1. **Taxonomy and Systematics**
2. **Biology, Ecology and Genetics**
3. **Venoms, Venomics and Proteomics**
4. **Coralsnake Envenomations**
5. **Ecological and Biogeographical Modelling**

PROGRAM

Time	Dates
	17 OCTOBER – MONDAY
08:30 – 09:30	**Opening Ceremony of the Symposium**
09:40 – 10:20	**Magna Lecture: "Our Inner Snake"** Dr. Janis A. Roze (City College of New York City, USA)
10:30 – 11:20	**Lecture: "Biodiversity Discovery, Species Delimitations and Integrative Taxonomy"** Dr. Jack W. Sites Jr. (Brigham Young University, USA)
Lunch	
14:00 – 14:50	**Lecture: "Origin and Evolution of Elapids and Coralsnakes"** Dr. Hussam Zaher (Museu de Zoologia da Universidade de São Paulo)
15:00 – 15:50	**Lecture: "Coralsnakes of Brazil"** Dr. Nelson Jorge da Silva Jr. (Pontifícia Universidade Católica de Goiás)
Interval	
16:00 – 17:10	**Medal Ceremony - PUC Goiás** Dr. Jack W. Sites Jr. and Dr. Steven D. Aird
17:10 – 18:00	**Lecture: "New Discoveries and the Taxonomy and Systematics of Old and New World Coralsnakes (Squamata: Elapidae)"** Dr. Eric N. Smith (University of Texas – Arlington)
18:00 – 18:50	**Lecture: "Main Taxonomic Problems in the New World Coralsnakes"** Dr. Darlan Tavares Feitosa (Pontifícia Universidade Católica de Goiás)
19:00 - 21:00	**Book launching: "As Cobras-Corais do Brasil"**

Time	18 OCTOBER – TUESDAY
08:30 – 09:20	**Lecture: "Taxonomic Revision of Argentinian Coralsnakes"** Dr. Alejandro Giraudo (Instituto Nacional de Limnología, Argentina)
09:30 – 10:20	**Lecture: "Natural History of the New World Elapidae: What we know and what we do not know about them"** Dr. Otávio Augusto Vuolo Marques (Instituto Butantan)
10:30 – 11:20	**Lecture: "The Intense Sexual Activity in the Genus *Micrurus*: Mating Aggregations, Male-Male Fights, Courtship, Sperm Storage and Sperm Competition"** Dr. Selma Maria de Almeida-Santos (Instituto Butantan)
Lunch	
14:00 – 14:50	**Lecture: "Herpetological Collections: A Vision of Future"** Dra. Ana Lúcia da Costa Prudente (Museu Paraense Emilio Goeldi)
15:00 – 15:50	**Lecture: "The Role of Museum Herpetological Collections as the Guardians of Coralsnake Diversity"** Dr. Patrick Campbell (British Museum of Natural History)
Interval	
16:00 – 18:30	**Oral Presentations 1 / Poster Presentations 1**

19:00 – 20:00	***Honoris Causa* Ceremony** Dr. Janis A. Roze

Time	19 OCTOBER – WEDNESDAY
09:30 – 10:30	**Lecture: "Mimicry and Local Variations in Coralsnakes: The Example of *Erythrolamprus* False Coral Species"** Dr. Felipe Franco Curcio (Universidade Federal do Mato Grosso)
10:30 – 11:30	**Lecture: "The Beginnings of the Knowledge on Coralsnakes"** Dr. Ulisses Caramaschi (Museu Nacional do Rio de Janeiro)
Lunch	
14:00 – 14:50	**Lecture: "Venomics of Brazilian Coralsnakes"** Dr. Steven D. Aird (Okinawa Institute of Science and Technology, Japan)
15:00 – 15:50	**Lecture: Venoms of *Micrurus* Coralsnakes: Evolutionary Trends in Compositional Patterns Emerging from Proteomic Analyses.** Dr. Bruno Lomonte (Instituto Clodomiro Picado, Costa Rica)
15:50 – 16:20	**Lecture: "Venom Yield of Brazilian Coralsnakes"** Dr. Nelson Jorge da Silva Jr. (Pontifícia Universidade Católica de Goiás) Dr. Steven D. Aird (Okinawa Institute of Science and Technology, Japan)
16:20 – 18:20	**Oral Presentations 2 / Poster Presentations 2**

Time	20 OCTOBER – THURSDAY
08:30 – 09:10	**Lecture: "Global Overview: Elapid Snakebites in Africa and Asia"** Dr. Julian White (Department of Toxinology, Women's and Children's Hospital, Adelaide, Australia)
09:10 – 09:40	**Lecture: "Coralsnake Bites in Brazil"** Dr. Fábio Bucaretchi (Universidade Estadual de Campinas)
09:40 – 10:10	**Lecture: "Coralsnake Bites: Historical Aspects and the Clinical Experience of Instituto Butantan (1902 – 2013)"** Dr. José Yamin Risk (Instituto Butantan) Dr. João Luiz Costa Cardoso (Instituto Butantan)
10:10 – 10:40	**Lecture: "Coralsnake Bites in Argentina"** Dr. Adolfo Rafael De Roodt (Universidad de Buenos Aires, Argentina)
Interval	
11:00 – 11:30	**Lecture: "Coralsnake Bites in Colombia"** Dr. Rafael Otero-Patiño (Universidad de Antioquia, Colombia)
11:30 – 12:00	**Lecture: "Coralsnake Bites in Central America"** Dr. José María Gutiérrez (Instituto Clodomiro Picado, Costa Rica)
12:00 – 12:30	**Lecture: "Coralsnake Bites in the United States"** Dr. Tamas Peredy (Florida Poison Information Center – Tampa, USA)
Lunch	
14:00 – 14:40	**Lecture: "Global Overview: Elapid Snakebites in Australia and Papua New Guinea"** Dr. Julian White (Department of Toxinology, Women's and Children's Hospital, Adelaide, Australia)

14:40 – 15:10	**Lecture:** para **Coral Snakes Venoms: Toxic Properties, Immunogenicity, Antivenoms Cross Reactivity and Neutralization Potential.** Dr. Denise Vilarinho Tambourgi (Instituto Butantan)
15:10 – 15:40	**Lecture: "Monoclonal-based Antivenomics and Biological Activities Revealing High Variability in Coralsnake Venoms"** Dr. Carlos Correa Netto (Instituto Vital Brazil)
15:40 – 16:00	**Lecture: "Identification of Epitopes for the Development of a New Antivenom Against Coralsnakes (*Micrurus*)"** Dr. Carlos Chávez-Olortegui (Universidade Federal de Minas Gerais)

Time	**20 OCTOBER – THURSDAY**
Interval	
16:40 – 17:10	**Lecture: "Towards a Universal Antielapidic Serum"** Dr. Paulo Lee Ho (Instituto Butantan)
17:10 – 18:30	**Debate: "Continental *Micrurus* Antivenom: Is it Feasible?"** Dr. Adolfo Rafael De Roodt (Universidad de Buenos Aires, Argentina) Dr. Anibal Rafael Melgarejo Gimenez (Instituto Vital Brazil) Dr. Carlos Chávez-Olortegui (Universidade Federal de Minas Gerais) Dr. Carlos Correa Netto (Instituto Vital Brazil) Dr. Denise V. Tambourgi (Instituto Butantan) Dr. Fan Hui Wen (Instituto Butantan) Dr. José María Gutiérrez (Instituto Clodomiro Picado, Costa Rica) Dr. Paulo Lee Ho (Instituto Butantan) Ms. Guilherme Carneiro Reckziegel (Ministério da Saúde)

Time	**21 OCTOBER – FRIDAY**
08:30 – 09:20	**Lecture: "The Mechanism of Venom Inoculation in Coralsnakes"** Dr. Anibal Rafael Melgarejo Gimenez (Instituto Vital Brazil) Marcus Augusto Buononato (Ciência Brasilis)
09:20 – 10:10	**Lecture: "Comparative Study of Coralsnake Cephalic Glands"** Dr. Leonardo de Oliveira (Museu de Zoologia da Universidade de São Paulo)
10:10 – 11:00	**Lecture: "The Mechanism of Action of Coralsnake (*Micrurus*: Elapidae) Venoms"** Dr. José María Gutiérrez (Instituto Clodomiro Picado, Costa Rica)
11:00 – 11:50	**Lecture: " The Relationship Between Complexity, Variability, and Toxicity in North American Coralsnakes"** Mark Margres (Florida State University, USA)
Lunch	
14:00 – 14:40	**Lecture: "Ecological Modelling as a Tool for Coralsnakes Conservation"** Dr. José Alexandre Felizola Diniz-Filho (Universidade Federal de Goiás) Dr. Levi Carina Terribile
15:00 – 17:00	**Oral Presentations 3 / Poster Presentations 3**
18:30	**Closing Ceremony**

ORGANIZING COMMITTEE

Nelson Jorge da Silva Jr. - Escola de Ciências Médicas, Farmacêuticas e Biomédicas. Pontifícia Universidade Católica de Goiás. Goiânia, Brazil.

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Otávio A. V. Marques – Laboratório de Ecologia e Evolução. Instituto Butantan, São Paulo, Brazil.

Steven D. Aird – Okinawa Institute of Science and Technology. Okinawa, Japan.

ABSTRACTS

A. LECTURES

17 OCTOBER 2016 (MONDAY)

OUR INNER SNAKE

Janis A. Roze

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Snakes are much more than the delightfully slithering beasts chased and studied in field, in laboratories, or pickled in museum jars. They have sneaked into our consciousness and subconsciousness as one of the most remarkable animals, perhaps even one of the most powerful animals, evoking fascination and fear. From the ouroboros, the first symbol that bites its tail to creation of the world, to the kundalini snake as the energy dwelling in our inner body; from Cleopatra's deadly bite, to the snake as spirit animals, as in waterholes in Australia. They have penetrated in science, history, psychology, myths, legends, just stories, adventures, religions, magic, beliefs and arts, and in all walks of life. Let us look at them in a playful way. Fascination and fear lie in us, not in the snake. Snakes are in our inner world. Let us have a look at our self- created snake world. Studying snakes, we have neglected to study our inner snakes. They are not known, but are as real (unreal) as our real (unreal) personal world.

Key words: snakes; history; culture; science.

BIODIVERSITY DISCOVERY, SPECIES DELIMITATION, AND INTEGRATIVE TAXONOMY

Jack W. Sites Jr.

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Biodiversity science inherits a long history of research on: the "origin of species", what species are, and how they should be defined. Little progress was made until the introduction of "evolutionary", and then a more inclusive "general lineage" species concepts. Both of these are non-operational definitions, but they could be tested with many classes of empirical data. Biodiversity science then moved forward in many directions, including much debate and discussion about empirical methods of "species delimitation" (SDL), now clearly separated from the long debates over species_definitions. A common SDL approach has been the widespread use of mitochondrial DNA in phylogeographic studies to identify "candidate species"; it is an ideal "hypothesis generating" method. Once "candidate species" are identified, their boundaries can be further tested by a growing array of SDL methods, and multiple classes of data then applied an "Integrative Taxonomic" assessment of species boundaries.

Key words: species concepts; species delimitation; integrative taxonomy.

ORIGIN AND EVOLUTION OF ELAPIDS AND CORALSNAKES

Hussam Zaher[1], Felipe Grazziotin[1], Ana Lúcia da Costa Prudente[2] & Nelson Jorge da Silva Jr.[3]

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Elapids comprise a poorly known radiation despite their well-known sanitary importance due to significant number of human envenomations in tropical and subtropical countries. It was a common belief to a few years ago that Elapids were one the best delimited families of the clade Colubroides, characterized by the presence of a proteroglyphous inoculation system. However, molecular analyzes published lately pointed out the possibility of polyphyletism. On the other hand, the fossil record of elapids is still scarce and poorly represented of the known diversity despite it shed some light on the origin and diversification of the group. We present a phylogeny that corresponds to a divergence time among the representatives of the family Elapidae by penalized likelihood on a maximum likelihood tree based on a molecular matrix of 15 genes for 196 species. The topology of the tree recovers the Elapidae with 98% of bootstrap support still indicating that the more inclusive relationships among elapids remain uncertain with clades supported by bootstrap indices below 70%. The New World coralsnake clade (*Micruroides*, *Leptomicrurus* and *Micrurus*) has a support inferior to 70% posing as a sister group of *Sinomicrurus*. The estimated divergence time for elapids in our phylogeny is concordant with other studies and suggest an Eocene origin, or close to the Eocene – Oligocene and a basal diversification of the group during the Oligocene. Despite the uncertainties related to the biogeographic origin of the family, our phylogeny is concordant with the most recent molecular results that point out an Asian origin for the radiation of the New World coralsnakes. Our phylogeny does not support the monophyly of the Asian coralsnakes but recovers the monophyly of the American coralsnakes (*Micruroides*, *Leptomicrurus* and *Micrurus*). Although some discussions are currently on the subject, we prefer to retain *Micrurus* as paraphyletic as the data used to day (morphological and molecular) are highly inconclusive. The results point to the need of a more detailed revision of the status of the American radiation of coralsnakes that allow a clearer representation of the morphological and genetic diversity of the group and not only the condition of monophyletic group.

Key words: elapids; coralsnakes; phylogeny; origin.

CORALSNAKES OF BRAZIL

Nelson Jorge da Silva Jr., Matheus Godoy Pires & Darlan Tavares Feitosa

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Coralsnakes possess a confusing and poorly resolved taxonomy owing to the conservative nature of some morphological characteristics and the poor knowledge of the intra- and interspecific variability. For this group of venomous snakes it is essential a combination of characters that include, besides scale counts, the hemipenial morphology, cranial osteology, axial osteology, and a very careful evaluation of color and patterns. This morphological variability is better noticed by the taxonomic arrangements of species with several subspecies, which include *Micrurus diastema*, *M. nigrocinctus* (Central America), *M. mipartitus*, *M. dumerilii*, *M. annellatus*, *M. hemprichii*, *M. lemniscatus*, *M. spixii, Leptomicrurus collaris*, and *L. narduccii*. Among the 82 recognized coralsnake species, 33 occur in Brazil, included in the genera *Leptomicrurus* and *Micrurus*. Our study involved more than 7,500 museum vouchers from the major museums in South America, United States of America, and Europe. Tentatively we classified them as *Leptomicrurus* (3): *L. collaris* (Schlegel, 1837), *L. narduccii* (Jan, 1863), and *L. scutiventris* (Cope, 1870). Monadal species group (11): *M. albicinctus* Amaral, 1926, *M. annellatus* (Peters, 1871), *M. averyi* Schmidt, 1939, *M. corallinus* (Merrem, 1820), *M. langsdorffi* Wagler, 1824, *M. pacaraimae* Carvalho, 2002, *M. paraensis* Cunha & Nascimento, 1973, *M. putumayensis* Lancini, 1962, *M. psyches* (Daudin, 1803), *M. remotus* Roze, 1987, and *M. tikuna* Feitosa, Pires, Silva Jr., Zaher & Prudente, 2015. Bicolor group (1): *M. mipartitus* (Duméril, Bibron & Duméril, 1854). South American triadal group (18): *M. altirostris* (Cope, 1860), *M. brasiliensis* Roze, 1967, *M. decoratus* (Jan, 1858), *M. diana* Roze, 1983, *M. diutius* Burger, 1955, *M. filiformis* (Günther, 1859), *M. frontalis* (Duméril, Bibron & Duméril, 1854), *M. hemprichii* (Jan, 1858), *M. ibiboboca* (Merrem, 1820), *M. isozonus* (Cope, 1860), *M. lemniscatus* (Linnaeus, 1758), *M. nattereri* Schmidt, 1952, *M. potyguara* Pires, Silva Jr., Feitosa, Prudente, Alves Filho & Zaher, 2014, *M. pyrrhocryptus* (Cope, 1862), *M. silviae* Di-Bernardo, Borges-Martins & Silva Jr., 2007, *M. spixii* Wagler, 1824, *M. surinamensis* (Cuvier, 1817), and *M. tricolor* Hoge, 1956. Despite this current taxonomy several studies in course reveal that some species with subspecies status will likely be presented differently (e.g. *M. annellatus*, *M. hemprichii*, *M. lemniscatus*, and *M. spixii*), as well as species with a wide geographical range (e.g. *M. ibiboboca*).

Key words: Brazilian coralsnakes; *Leptomicrurus*; *Micrurus*; diversity.

NEW DISCOVERIES AND THE TAXONOMY AND SYSTEMATICS OF OLD AND NEW WORLD CORALSNAKES (SQUAMATA: ELAPIDAE)

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Coralsnakes represent an extraordinary radiation of brightly colored venomous snakes including ~90 species. Almost one third of the alpha taxonomic diversity within the Elapidae comprises coralsnakes. Usually small, shy and fossorial, coralsnakes are amongst the rarest venomous snakes and their sampling in molecular studies has been sparse. Despite the medical importance and wide distribution of coralsnakes very little is known about their phylogeny. Given that suboptimal taxon sampling is known to negatively affect the overall accuracy of reconstructed phylogenies, the traditional limiting factor in studies on higher level elapid phylogenetics may indeed be the availability of coralsnakes, particularly from the Old World because the origin of coralsnakes rests in Asia. Using both mitochondrial and nuclear gene fragments and fossil data we estimate the phylogenetic relationships among Asian coralsnakes and evaluate their position among the Elapidae. The phylogenetic signal in our data set is evaluated using concatenated methods (Bayesian and maximum likelihood) and species tree analyses, and we used multiple calibration points to assess Bayesian divergence times in major Elapid lineages. To assign Miocene coralsnake fossils to coralsnake clades, in an attempt to accurately calibrate our time-tree, we use geometric morphometric vertebrae data and map them onto our phylogeny. Our phylogenetic analysis refutes the monophyly of coralsnakes and reveals the presence of three cryptic, higher-level taxa. According to our divergence analysis, these three higher level taxa are basal to all other elapids and split from them in the late Oligocene/early Miocene. We also present phylogenetic hypotheses for coralsnakes based on ~1.5kb of mtDNA sequence data for nearly 200 coralsnake samples (OTUs), and a reduced set for 60 samples including three mitochondrial and two nuclear genes, mostly from the New World. These data suggest a majority of species are in need of critical review and that both the alpha taxonomy and generic recognitions are in conflict with phylogeny. Examples of recent efforts used to try to solve some of the taxonomic problems are presented, mostly related to species boundaries and their recognition.

Key words: *Calliophis; Hemibungarus; Leptomicrurus; Maticora; Micruroides; Micrurus; Sinomicrurus;* systematics; taxonomy.

MAIN TAXONOMIC PROBLEMS IN THE NEW WORLD CORALSNAKES

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The New World coralsnakes comprise a monophyletic group included in the genera *Micrurus*, *Leptomicrurus* and *Micruroides*. There are 83 recognized species totaling 135 taxa distributed from the southern United States to central regions of Argentina. The genus *Micrurus* is the most diverse with 126 taxa and therefore has the greatest number of problems related to lack of knowledge about the geographical distribution, morphological variations and thus delimiting the taxonomic status of most species. The latest classification proposed to *Micrurus* is based on the combination of the following characters: body and tail color pattern, hemipenial morphology and tail length, included in four morphological groups. We present an overview of the taxonomic situation of *Micrurus* species and relate them to the availability of the species in scientific collections. We analyzed 8,106 specimens deposited in 73 scientific collections, representing 74% of the valid species (58 species), 100% of all triadal and monadal South American species, including 11.5% (9 taxa) with very low representation in collections (≤5 specimens). Several factors contributed to a confusing scenario with direct consequences on the taxonomy of many taxa. In fact, historically, the lack of criteria for the records of collectors and collections contributed for decades to the perpetuation of inconsistent information (e.g. *M. lemniscatus*). Identifications based on the analysis of little comparative material and lack of comparison with type material reflect at least in mistaken geographical distribution records (e.g. *M. tikuna vs*. *M. ornatissimus*). Among the 78 known species of the genus, 20 taxa present subspecific status (6 triadals and 14 monadals). The low representation and availability of materials can compromise finer morphological analysis, which it is also true for the availability of material for molecular analysis (which is much less representative). This may lead to inconsistencies between molecular and morphological analyzes, best exemplified by several studies dealing with the phylogenetic position of *Leptomicrurus* within *Micrurus*, in which the most prudent decision is to maintain the current *status* until it is possible to perform more robust analyzes based on quality and reliability material, especially morphological. It is becoming increasingly clear that better analyzes considering the variability of some morphological characters, such as pattern and color, cranial and axial osteology, and hemipenis can assist in subtle incongruities taxonomic resolution or unresolved in some groups (e.g. *M. annellatus* and *M. frontalis*).

Key words: coralsnakes; *Micrurus;* morphology; taxonomy.

18 OCTOBER 2016 (TUESDAY)

TAXONOMIC REVISION OF ARGENTINEAN CORAL SNAKES *MICRURUS*

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The genus *Micrurus* comprise 80 species of highly venomous coralsnakes widely distributed in the Americas. Many of its species are cryptic and have poorly defined and complex interspecific limits, which generated numerous historical and current taxonomic confusion. In Argentina, the taxonomic status of genus was very confusing, with unstable specific and sub-specific assignations. In the1980s only two species, one of them with three subspecies, were cited in Argentina, and after 30 years is now recognized the presence of six species, including *M. altirostris, M. baliocoryphus, M. corallinus, M . lemniscatus*, *M. pyrrhocryptus* and *M. silviae*. However, several taxonomic problems are still evident when we analyze the variation and distribution of known Argentinean species. We present a new revision of the genus *Micrurus* in Argentina, based on morphological an coloration characters of more than 500 specimens addressing some of the key taxonomic problems, for example: (1) How many species inhabit Argentina?; (2) What is their distribution?; (3) What is the geographical variation and taxonomic status of *M. pyrrhocryptus* and its recently proposed subspecies?; (4) What are the diagnostic characters to distinguish cryptic species? We demonstrated the presence of *M. frontalis* (*sensu stricto*) in Argentina. Additionally, we analyzed the variation of other species, with emphasis on *M. altirostris, M. baliocoryphus* and *M. pyrrhocryptus*, all with type localities in Argentina, considering its possible taxonomic consequences.

Key words: Argentinean *Micrurus*; systematic; geographic variation.

NATURAL HISTORY OF THE NEW WORLD ELAPIDAE

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Elapidae in the New World (represented by the genus *Micruroides* and *Micrurus*) are medium-sized snakes, ranging from 200 to 1,600mm. They are usually characterized for having a pattern of alternating black, red, and white rings, being called coralsnakes. This group comprises around 80 species distributed from southern United States to central Argentina. These snakes inhabit predominantly forest, but also occur in open areas. Microhabitats data indicate that most of the species are primarily cryptozoic, but also terrestrial and fossorial; few species inhabit bodies of water. Apparently most coralsnakes present a diurnal pattern of activity (peaking early morning and late evening) and a few species are nocturnal. Activity is increased during rainy season and reproductive events. These coralsnakes feed mainly on vertebrate elongate prey, as other secretive snakes, caecilians, amphisbaenians, lizards and even fish. They usually find food in the litter or holes following preys' trails. The prey can be held until its immobilization by the venom, but bite, release and relocate prey prior to swallowing is also another strategy used. Snakes are usually swallowed head first, but prey as amphisbaenians and caecilians are also frequently ingested tail-first. Reproductive patterns diverge in the phylogenetic lineages of *Micrurus*. Species with black rings arranged in triads are characterized by males larger than or equal in size to females, male combat behavior, and a broader season of vitellogenesis and oviposition. On the other hand, in species with black rings arranged in monads, females are generally larger than males, there is no male–male combat, and, at least in subtropical areas. Vitellogenesis and mating occurs at the onset of rainy season, egg laying in the mid rainy season, and recruitment at the end of rainy season and the onset of dry season. Reproduction seem to be seasonal in the genus, but climatic influence on the extension of reproductive cycles is evident, with equatorial species exhibiting more continuous cycles, whereas species from cold areas exhibit more seasonal cycles. Falcons and other birds have been recorded as predators of *Micrurus* in the field, but some mammalian species may also feed on these snakes. Defensive tactics includes aposematic (or cryptic) coloration, flee into leaf litter, dorso-ventral flattening, elevate and coil the tail, erratic behavior, hide the head under body coils, pressing the tip of the tail against predator, tanatosis, and false and true strikes.

Key words: Elapidae; New World; coralsnakes; natural history.

THE INTENSE SEXUAL ACTIVITY IN THE GENUS *MICRURUS:* MATING AGGREGATIONS, MALE-MALE FIGHTS, COURTSHIP, SPERM STORAGE AND SPERM COMPETITION

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The secretive habit of most snakes' genus *Micrurus* cause strong constraints to research, since it is difficult to find these animals in the field due to their semifossorial habits. However, despite these habits, coral snakes have been observed to present an intense sexual activity on the ground: aggregations or male-male combats for access to females. In South America, *Micrurus* species are divided into two distinct phylogenetic lineages: the black rings arranged in monads (BRM) and the black rings in triads (BRT). Reproductive data indicates that reproductive strategies differ greatly between the two *Micrurus* lineages. Phylogenetic distribution of male–male combat suggests that this trait has evolved or has been lost many times within snake phylogeny. However, because behavior is often hard to observe in snakes, it is probable that combat is even more widespread than our current records indicate. Male-male combat is frequently associated with male-biased sexual size dimorphism (SSD). Sexual combats are components of the reproductive biology of the four species with triads - *M. frontalis; M. altirostris; M. ibiboboca and M. lemniscatus carvalhoi*. These first species that present fighting males were bigger than females, with the exception of *M. lemniscatus carvalhoi* with significantly reversed SSD. Field observations of monads *M. corallinus* mating were recorded in early spring and during the mating season, ritual combat was not observed. Thus, male activity increases in spring probably due to their searching for females, which could originate aggregation and perhaps competition during mating. Our findings indicate that *M. corallinus* aggregate for mating and it is possible that there is no combat among males, as females are larger than males. Aggregation and female reproductive synchrony could favour polygyny rather than monogamy in *M. corallinus*, probably the most common snake mating system. Females of *M. corallinus* can store sperm outside the mating season. Thus, there is possibly a sexual aggregation behaviour where many males may compete for females, which can promote insemination by several males, allowing the sperm competition in the reproductive tract of females.

Key words: *Micrurus*; dimorphism; phylogenetic; sexual activity.

HERPETOLOGICAL COLLECTIONS: A VISION OF FUTURE

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Until the early nineteenth century, plants and animals were collected by travelers during their journeys in Brazil and shipped to Europe. This material would later be the beginning of the great Brazilian zoological collections, which had their origin and expansion associated with periods of great economic development of Brazil. Over the last hundred years, Brazilian researchers have accumulated significant zoological material in collections. Scientific collections summarize the species information that when logged into computerized databases and managed efficiently it can generate a number of benefits. Brazilian herpetological collections were born with different goals than those of the great European and US similar collections, which was to raise the fauna. Many of the herpetological collections were built up by sole efforts of researchers and institutions who were led by the need to create reference and information sources for their researches and students as well. Just like the rise of those collections, their development and growth also occurred spontaneously and unorientedly and were intesified from the second half of the twentieth century to the present day. There are herpetological collections housed in research institutions and public and private universities across all regions of Brazil, particularly in Southeast Region and Sao Paulo State. Most of the collections consist of material collected in Brazil and bordering countries although some larger collections in more traditional museums house material from other Latin American countries and other parts of the world. Among the Brazilian institutions, we highlight three that gather together more than 450.000 specimens of amphibians and reptiles: The Zoology Museum of Sao Paulo University (MZUSP) with a collection of approximately 260.000 specimens, The National Museum of Rio de Janeiro (MNRJ) with about 115.000, and The Emilio Goeldi Museum of Pará (MPEG) with about 100.000 specimens. The expansion and the increase in herpetological collections occur in Brazil mainly through research projects, e.g. directed inventories, and also through environmental consultancies. Nonetheless important, a smaller number is obtained through exchanges and donations. The great challenge of the collections, both small and big, is to gather a complete set of information associated with the housed material and make it accessible. Part of this challenge can be achieved with proper curator activity (preparation of clear lendings and consultation policies, proper storage and maintenance of specimens and tissue; computerized database, etc.), with goals to expand the geographical coverage of the collected material, with adoption of effective measures to make the information available to the public and with assurance of the physical integrity of the material and intellectual integrity of information. It is possible to highlight some medium and long term actions for the improvement of collections and advancement of herpetological knowledge in Brazil: implementation of programs that promote a continued gathering effort and devoid of bureaucratic restrictions; debureaucratisation of shipping and receiving biological material among institutions, facilitation and promotion of especialists exchange programs for material identification which is essential for organizing and enhancing collections. Other actions include reducing inequalities in the distribution of financial resources among the collections in different regions of Brazil which directly allows for standardization of collections; setting

up training programs and retaining professionals in taxidermy and curation; recognizing the curator as an official post in institutions; opening bids for funding scientific collections.

Key words: amphibians; challenges; scientific collections; reptiles.

THE ROLE OF MUSEUM HERPETOLOGICAL COLLECTIONS AS GUARDIANS OF CORALSNAKE DIVERSITY

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A brief history of the Natural History Museum is presented, its growth from a small private collection to a vast storehouse of some 80 million specimens of animals, plants, fossils, and minerals with a particular focus on the Herpetology Section. It is also the place where the privileged custodians past and present tasked with caring for and preserving the collection, also managing and accurately recording data accompanying the constant influx of specimens, thus helping to safeguard the collections. One of the oldest herpetology collections in the world dating from the late 18th century, it includes important historical collections made by Charles Darwin, David Livingstone, Lionel Walter Rothschild, John D. Godman, Malcolm A. Smith, Frank Wall, Fernand Lataste, Jacques von Bedriaga and many others. The geographical range of the collection is worldwide, excluding the Arctic and Antarctica, altitude and information relating to topography is also recorded where this information is made available by the collector. Although the strength of the collection is Africa, Europe, Arabia, India (and other parts of South East Asia), generally in areas that were old British colonies, the collection also holds important historical specimens from the Americas and Australia, hence the collection is very diverse and ideally placed for examining coralsnake diversity.

Key words: Natural History Museum; herpetology; scientific collections.

19 OCTOBER 2016 (WEDNESDAY)

MIMICRY AND LOCAL VARIATIONS IN CORALSNAKES: THE EXAMPLE OF *ERYTHROLAMPRUS* FALSE CORAL SPECIES

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Coralsnakes mimicry represents an outstanding subject in evolutionary biology. Although not trivial to test, the homoplastic resemblance of color patterns and behavior among sympatric poisonous and non-poisonous species is highly suggestive of the presence of well-established Batesian/Müllerian mimicry complexes. However, investigations on these local phenomena depend not only on experimental incursions, but also on understanding the systematic significance of several instances of local variations. False coral species of the genus *Erythrolamprus* provide indirect evidence that mimetic interactions with sympatric *Micrurus* taxa do play a role in the selection of local phenotypes of uncertain taxonomic significance. When discussed in the light of a long-term research on *Erythrolamprus* systematics (more than 1800 specimens examined), geographic congruence of color patterns suggest that mimicry may act not only towards reducing, but also towards increasing local polymorphisms. Long-term interactions may be significant in allopatric and parapatric speciation processes, as suggested by the Atlantic Forest and Cerrado *Erythrolamprus* populations. On the other hand, Amazonian *Erythrolamprus* indicate that mimicry may also blur morphological indications of taxonomic diversity depending on the variability of models available in sympatry.

Key words: coralsnakes; *Erythrolamprus*; mimicry; taxonomy.

THE BEGINNINGS OF THE KNOWLEDGE ON CORALSNAKES

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The classical knowledge on the Coralsnakes is treated since the discovery of the New World by Columbus and Cabral, passing through the pre-Linnean works of Merian and Seba, the ancient books of Marcgrave and Piso, until the seminal *Systema Natura*e of Linnaeus. The transference of the Portuguese Crown to Brazil and the great expeditions then carried through the Brazilian territory, as those of Spix and Wied, and the importance of the specimens entering the major European museums. The study and early descriptions of the species of Coralsnakes are discussed, according to the leading schools of knowledge in France, with Daudin, Cuvier, Baird, Girard, Duméril, and Bibron; in Italy, with the fundamental Jan and Sordelli; in Germany, with the major contributions of Wagler, Werner, and Peters; in England, with the basilar works of Günther and Boulenger; in Americas, with the important contributions of Cope in the North and Amaral in the South. Some modern authors are treated, with emphasis in the extensive contributions of Roze, and a final general view on the current status of the knowledge on the species of Coralsnakes is presented.

Key words: coralsnakes; history; classical authors; descriptions; taxonomy.

VENOMICS OF BRAZILIAN CORALSNAKES

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To date there have been only three transcriptomic studies of coralsnake venom glands, only one of which pertained to Brazilian coralsnakes. Some pioneering investigation explored the venom chemistry of *Micrurus corallinus* and *Micrurus altirostris* and provided insights into the compositional diversity of these two very different venoms. The present study investigated venom gland transcriptomes and proteomes of six species using state-of-the-art Illumina Technology (Hiseq PE150, ~500 bp reads) to provide the most quantitative data yet on *Micrurus* venom composition. Transcriptomic results were confirmed proteomically. Enzymatic and chemical cleavages of crude venoms were employed and proteins were identified with LC-MS. Species investigated included *M. carvalhoi*, *M. corallinus*, *M. lemniscatus*, *M. paraensis*, *M. spixii*, and *M. surinamensis*. Using *Ophiophagus hannah* as an outgroup, preliminary maximum likelihood analysis of the transcriptomes using both venom and tissue genes, confirmed the presumed close relationships between *M. corallinus* and *M. paraensis* and between *M. carvalhoi* and *M. lemniscatus*. The documented uniqueness of *M. surinamensis* venom was supported, but perhaps most surprisingly, *M. spixii* does not appear closely related to any of the other five species. Partial sequences previously published for toxins with documented pharmacology have been completed and novel structures of Kunitz serine protease inhibitors and other toxin families will be discussed.

Key words: coralsnakes; *Micrurus*; transcriptomic; venomic.

VENOMS OF *MICRURUS* CORALSNAKES: EVOLUTIONARY TRENDS IN COMPOSITIONAL PATTERNS EMERGING FROM PROTEOMIC ANALYSES

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The application of proteomic tools to the study of snake venoms has led to an impressive growth in the knowledge about their composition (venomics), immunogenicity (antivenomics), and toxicity (toxicovenomics). About one-third of all venomic studies have focused on elapid species, especially those of the Old World. The New World elapids, represented by coralsnakes, have been less studied. In recent years, however, a number of venomic studies on *Micrurus* species from North, Central, and South America have been conducted. An overview of proteomic studies on *Micrurus* venoms is presented, highlighting the emergence of some patterns and trends concerning their compositional, functional, and immunological characteristics. Results gathered to date, encompassing 18 out of the approximately 85 species of *Micrurus*, reveal a dichotomy of venom phenotypes regarding the relative abundance of the omnipresent phospholipases A_2 (PLA_2) and 'three-finger' toxins (3FTx): a group of species express a PLA_2-predominant venom composition, while others display a 3FTx-predominant compositional pattern. These two divergent toxin expression phenotypes appear to be related to phylogenetic positions and geographical distributions along a North-South axis in the Americas, but further studies encompassing a higher number of species are needed to assess these hypotheses. The two contrasting phenotypes also show correlations with some toxic functionalities, complexity in the diversity of proteoforms, and immunological cross-recognition patterns. The biological significance for the emergence of a dichotomy of venom compositions within *Micrurus*, in some cases observed even among sympatric species that inhabit relatively small geographic areas, represents a puzzling and challenging area of research which warrants further studies.

Key words: *Micrurus*; snake; venom; proteomics.

VENOM YIELD OF BRAZILIAN CORALSNAKES

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Coralsnakes are known to be small to medium size snakes, generally non-aggressive, but to possess a highly neurotoxic venom. However, human accidents are very rare and when the neurotoxic effect is well characterized the case becomes a guaranteed medical publication. The greatest diversity of coralsnakes occurs in the Amazon region. However, the greatest incidence of *Micrurus* accidents is in northeastern Brazil. Our goal is to contrast the venom yield of coralsnakes, handled by our research group from 1986 to 2010, with the reality of the low number of accidents and the official protocol (Ministry of Health) for antivenom use. This study reports coralsnake sizes and venom yields, and evaluates the use of antielapidic serum, based upon the inoculation capacity, and variability between coralsnake species from different regions of Brazil. Data regarding extracted venom volumes and snake sizes are stored in the venom database of the Center for Studies and Biological Research of the Pontifical Catholic University of Goiás. We analyzed venom samples with specimen total lengths (N=277) and venom samples without specimen data (N=336). Linear regression analysis showed a strong correlation between body size and extracted venom volume. Despite the diversity of coralsnakes in Brazil (33 species) the present antielapidic serum is produced mainly from two species from southeastern Brazil. The Brazilian Ministry of Health recommends that all accidents be treated as severe and that 10 ampoules of antivenom be administered (one ampoule neutralizes 15 mg of venom). Venom yields of the studied species ranged from 8,04 to 54,38 mg. In a direct comparison, the amount of antivenom necessary should be only one to four ampoules. The policy to administer high doses of antielapidic serum in coralsnake accidents should be revised. However, the diverse venom chemistry of coralsnakes raises concerns about the effectiveness of the current antielapidic serum and confounds simplistic solutions.

Key words: coralsnakes; venom; dry weight; antivenom production.

20 OCTOBER 2016 (THURSDAY)

ELAPID SNAKEBITE IN AFRICA AND ASIA

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Elapid snakes, Family Elapidae, are front fanged venomous snakes of great medical significance across many continents, including Africa and Asia. Globally there are about 350 species, all of which are venomous, but many are either too small or not known to cause medically significant envenoming of humans. The principal medically important elapids are the cobras, kraits, mambas, coralsnakes, Australian snakes and the sea snakes. Historically elapid snakebite has been characterized as principally neurotoxic with only minor local effects, in contrast to characterization of viperid bites as coagulopathic and causing local tissue injury. This view is outdated and, in many instances, wrong. Some elapids can cause, even principally cause, significant local tissue injury, sometimes including extensive necrosis (some cobras, mambas, a few Australian elapids). One group of elapids, from Australia and New Guinea, commonly cause severe coagulopathy. While many elapids can cause neurotoxic descending flaccid paralysis, it may be a less common feature of envenoming than other effects, either local, or systemic. A few elapids can cause systemic rhabdomyolysis and its sequelae, including acute renal failure, though the latter occurs in association with coagulopathy for some other species. In Africa the most important elapids are the cobras, either principally neurotoxic, or principally necrotoxic (some also spit venom), followed by the mambas which vary from principally neurotoxic (black mamba) to variably neurotoxic and necrotic (green mambas), while other elapids (coralsnakes, garter snakes, tree cobras) are of lesser severity or importance. In Asia elapids are more diverse, with cobras again the most important in terms of numbers of major bites (both principally neurotoxic and principally necrotoxic species, with some spitters), closely followed by the principally neurotoxic kraits which account for many deaths in some countries. At least some kraits are now known to cause systemic rhabdomyolysis and/or hyponatraemia. King cobras, though spectacular in size and able to cause both neurotoxicity and local tissue injury, are a rare cause of bites. The several species of coralsnakes in Asia appear to cause few or mostly minor bites, though a few cases of major, even lethal (neurotoxic) envenoming have been reported. For those elapids causing principally systemic envenoming, particularly neurotoxicity, antivenom appears to be the mainstay of treatment, though kraits have presynaptic neurotoxins which may prevent antivenom from reversing already established paralysis. The role of antivenom in treating the local necrotic effects of elapid bites, notably by cobras and some mambas, is less clear. Precise epidemiologic data on elapid snakebite in much of Africa and Asia are not readily available, but studies such as those from Thailand indicate that amongst elapids, kraits may be associated with particularly high fatality rates, due to severe neurotoxicity and frequent unwitnessed nocturnal painless bites.

Key words: Africa; Asia; Elapidae; snakebite.

CORAL SNAKE BITES IN BRAZIL

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Coral snake (*Micrurus* spp.) bites are uncommon throughout the Americas, and are the smallest cause of venomous snakebites in Brazil. To review reports of coral snake bites in Brazil in the last ~150 years, electronic bibliographic databases, standard textbooks on toxicology/toxinology, and abstracts published in conference proceedings were searched for reports of envenomation by coralsnakes. Only reports with clinical descriptions of envenomation were considered. The variables recorded included identification of the offending snake, age, sex, bite site, clinical manifestations, treatment, including antivenom, anticholinesterase drugs and mechanical ventilation, and general evolution of the cases. We identified 25 reports (n=150 cases) that met the inclusion criteria (clinical description). Of the 150 cases, 61.3% were from southern Brazil. Two reports (one book chapter and one abstract) involving nine patients that have also been described in a historical case series of patients bitten by *Micrurus* spp. treated at the Instituto Butantan (1902-1945, n=15) and Hospital Vital Brazil (1945-2013, n= 67), in São Paulo city (Risk *et al.,* 2016),[3] were also included. The offending snakes were described in 59 cases (*M. corallinus* 36, *M. frontalis* 12, *M. lemniscatus* 5, *M. hemprichi* 2, *M. filiformis* 1, *M. ibiboboca* 1, *M. spixii* 1 and *M. surinamensis* 1) while in 22 cases only the genus (*Micrurus* spp.) was reported. Of the 143 cases in which the bite site was recorded, most involved the hands (46.2%) and feet (26.6%). The main clinical features were local numbness/paresthesia (52%), local pain (47.3%), palpebral ptosis (33.3%), blurred vision (20.7%), weakness (16.7%), slight local edema and erythema (15.3%), dysphagia (14.7%), inability to walk (10%), dyspnea (10%), myalgia (9.3%) and salivation (8%). Fang marks were described in 45.3% of cases and 14.7% of the bites were classified as asymptomatic. A slight increase in total blood creatine kinase was reported in three children. Therapeutic procedures included the use of antivenom (74%), anticholinesterase drugs (6%) and mechanical ventilation (3.3%). Two patients reported in 1933 developed respiratory failure/paralysis and died 6 h and 17 h post-bite. Four more deaths probably caused by coral snakes were reported (2 in 1867, 1 in 1959, 1 in 1962), but no clinical information was available. Most coral snake bites reported in Brazil have been caused by *M. corallinus* and *M. frontalis*, with several patients showing signs of systemic neurotoxic envenomation (acute myasthenia), including respiratory failure. Local features, mainly numbness/paresthesia and pain, were also frequently reported. The two deaths occurred in conditions where mechanical ventilation was unavailable.

Key words: coralsnakes; Elapidae; envenomation; *Micrurus* spp.; snakebites.

CORALSNAKE BITES: HISTORICAL ASPECTS AND THE CLINICAL EXPERIENCE OF INSTITUTO BUTANTAN (1902-2013)

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Rare, the micruric envenomations is, among the snake accidents in Brazil, the one that offers the highest difficulties with clinical management. The Instituto Butantan holds a centenary archive with information about accidents caused/attributable to elapids in the Southeastern region of Brazil in a historical series since the first decade of the 20th century to this day. The characteristics of coralsnakes and their peculiarities had already been reported by the chroniclers of the recently discovered lands of the region known today as Portuguese America. The survey of the first 15 records of micruric accidents through the bulletins of notification, created by Vital Brazil in 1902, filed at the documentation center of the Instituto Butantan, and also from 1945 (with the foundation of the Hospital Vital Brazil) till 2013, with 1000,747 medical records from which 67 were attributed to micruric accidents. A total of 50 accidents were confirmed by the presence of clinical signs and/or capture of the causal agent. The variables studied include the accident characteristics, patient data, clinical data, therapeutics and evolution of the case. Among the 50 confirmed patients 38 brought the snake (28/38 were *Micrurus corallinus*). In 78% of the cases, upper limbs were the main bite site, always related to the snake handling. With an age average of 35,5 years old, 88% of the patients were male and 16% were children. The predominant local symptoms were pain and paresthesia. Eyelid ptosis occurred in 50% of the confirmed cases, with 60,5% with light symptoms or asymptomatic. In 34,2% of the cases with a positive identification of the snake, without treatment, did not present envenomation symptoms. Two cases with systemic symptoms caused by *M. corallinus* were not treated and there was a spontaneous regression of the signs and symptoms. Two patients were bitten by *M. frontalis* and both had moderate systemic symptoms. Three fourth of the cases caused by *M. lemniscatus* evolved with intense irradiated pain. Respiratory distress occurred only with one patient. All the patients that received antivenom (doses varied from 2 to 26 ampoules) had a good evolution and fast regression of signs and symptoms. No deaths were reported. Two patients were admitted already dead (3% of the 67 cases) with information provided by the families and without the causal agent. However, the medical records are very difficult to provide a technical interpretation. A group of 17 patients, considered doubtful cases, with indefinite clinical symptoms and the absence of the causal agent, were treated by a well-trained medical team the well trained in attending snakebite accidents. This is likely due to the high importance given to the reported coralsnake bite and the dogma of the gravity and rarity of the event. The high lethality verified in this study coexists with the high number of cases without any signs of systemic envenomation, not treated and with good evolution. This complex panorama requires from the attending cases: rigorous observation for 24 hours; to avoid unnecessary intervention in patients only with local symptoms; to treat promptly the confirmed cases.

Key words: clinical cases; envenomation; *Micrurus*; snakebites.

CORALSNAKE BITES IN ARGENTINA

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Envenomation by coral snakes (*Micrurus* sp.) is one of the most dangerous envenomation by snakes through all America. These accidents must be considered as a serious medical emergency due to the risk of neurotoxic envenoming that can lead to respiratory paralysis and death. The specific treatment must be applied fast after the venom injection in order to avoid the severe envenomation and risk of death. Fortunately, the bites by American coralsnakes are not common like viper´s bites through the continent. In Argentina the bites of *Micrurus* represent in the last years under 0.5% of the bites by venomous animals and no deaths are registered at least since 37 years ago. The majority of accidents occurred in men from 31 to 40 years old and primarily in the warmer months. Although seven species of coralsnakes were described for Argentina and *Micrurus* snakes can be observed inclusive in the Patagonia, most cases were reported from the northeast and northwest provinces of the country. The distribution of the different species is established and monitored, however findings and accidents by species not described in some regions and transported passively are not infrequent. Bites of coralsnakes are mostly located on hands or feet and occurred mostly, but not uniquely, during agricultural activities and so mainly involved farmers. In previous studies only four cases occurred as a result of handling snakes. The *Micrurus* antivenom is produced by the Ministry of Health and is freely distributed through all the country. It is developed immunizing horses with pools of venom from *Micrurus* species, mostly *M. phyrrhocryptus* (the most distributed, abundant and found coralsnake in Argentina). The pharmaceutical presentation is liquid in 5 ml vial containing $F(ab´)_2$ fragments of equine immunoglobulins with a neutralizing power over 1 mg/ml of venom. The production is yearly and continuous with a mean production of 2,000 vials. The production of the antivenom is not easy since these snakes, although their finding is not very infrequent, are difficult to maintain in captivity and yield low amounts of venom. The median time lapsed from snakebite to the treatment with antivenom is around 60 min, and the median number of vials applied around 2. Local pain was mentioned and edema was reported in 41% of patients. All patients recovered without sequel. The incidence of *Micrurus* envenoming is low, and low severity of envenomation is registered. However, although no deaths or severe cases have been reported during the last 30 years, given the toxicity of the venom of *Micrurus* snakes, the risk of severe envenomation must be considered and the distribution of the specific antivenom must be maintained.

Key words: antivenom; coralsnakes; envenomation; *Micrurus*.

CORALSNAKE BITES IN COLOMBIA

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Thirty coralsnake species there are in Colombia, distributed up to 2200 m.a.s.l. Nevertheless, only a little group of them arranged into three colour patterns (bicolor, monadal, triads of black rings) and inhabiting the five natural regions of the country, inflicts 0.4%-0.8% of the 4300 snakebites arereported every year in Colombia. Neurotoxicity of early initiation (2-14 hr after the bite) is the main characteristic of this envenomation. New coralsnake bites occurring in different places of Colombia, within a 32-years period, mainly in Antioquia department hospitals, were included in the study. The diagnosis and treatment of patients were always performed by the author or through directions offered by phone or by e-mail to the physicians in the hospitals. The snake were captured alive or dead and transferred to the hospital in some cases. Informed consent was obtained from all patients or their relatives. The following data were registered: age of the patient, anatomical site of the bite, snake species, time interval to arrive to the hospital, signs of envenoming, laboratory results, antivenom administered, complications of envenoming and outcome. Thirty patients from one to 57 years old (36.7% were children), with coralsnake bites, were seen in different hospitals from 10 of the 32 (31.3%) departments, all of them located in the five natural regions of the country, mainly in Antioquia department (60%). The main sites of the bites were the feet (53.4%), the hands (30%), genitals (penis 3.3%) and the patients sought medical attention 5.0 ±3.5 hr after the bite. The responsible coralsnakes were *Micrurus mipartitus* (36.7%); *M. dumerilii* (40%); *M. nigrocinctus* (6.7%); *M. isozonus* (6.7%); *M. dissoleucus* (3.3%); *M. surinamensis* (3.3%) and one case by an unknown snake spp. Sixty per cent had paresis of respiratory muscles (two cases with apnea), 63.3% palpebral ptosis / ophthalmoplegia, 36.7% mild quadriparesis and 53.3% required respiratory support during 3-6 days. Fifteen patients (50%) received the Suero Anti-Coral from ICP (Costa Rica), nine (30%) the Soro Antielapídico from Instituto Butantan (São Paulo, Brasil), three (10%) the Suero Antiofídico Anticoral Liofilizado from Laboratorios Probiol (Bogotá) and three (10%) did not receive antivenom by different reasons. Four patients had complications: atelectasis (6.7%) and pneumonia (6.7%). Five patients (16.7%) died.Coralsnake bites occur in any geographical region of the country and at any group of age. Almost one-half of the affected patients (40%) did not suffer respiratory paralysis, but paralytic manifestations develop earlier in children (2-4 hr) than in adults (4-14 hr). They are life threatening. Twenty seven per cent of patients did not present clinical signs of envenoming as a consequence of early specific treatment (within the first two hr) or by a dry bite. It is urgent to enhance the distribution of antivenoms against coralsnake bites all over the country, to promote the research and education on this field and the rapid manufacture of potent antivenoms against the three groups of coralsnakes inhabiting Colombia.

Key words: coralsnakes; bites; paralysis; Colombia.

CORALSNAKE BITES IN CENTRAL AMERICA

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An estimated number of 4,000 to 5,000 cases of snakebite envenoming occur every year in Central America. Among these, approximately 1 % corresponds to coralsnakes (*Micrurus* sp) bites, i.e. 40 to 50 cases per year. There are 17 species of coralsnakes in this region, but few of them are responsible for the majority of these accidents, mainly *M. nigrocinctus* and *M. diastema*. Bites usually occur in the fingers as a result of manipulation of the snake. Clinical manifestations are similar to those reported for coralsnakes in other regions, i.e. pain at the site of the bite, paresthesias and, in cases associated with systemic manifestations, a descending neuromuscular paralysis. Characteristic signs and symptoms include palpebral ptosis, ophthalmoplegia, diplopia, paralysis of oropharyngeal muscles and, in severe cases, widespread neuromuscular paralysis, including respiratory muscles. The onset of systemic clinical manifestations usually takes several hours, although cases with neurotoxic manifestations of rapid onset have been described. In some cases a moderate increment of serum creatine kinase (CK) activity has been described, although severe rhabdomyolysis, associated with myoglobinuria, is usually not observed. A monospecific antivenom (anti-*M. nigrocinctus*) is manufactured in Costa Rica and distributed throughout the region. This antivenom is effective in the neutralization of the venoms of several species of Central American coralsnakes, but it is ineffective against the venom of *M. mipartitus*/*M. multifasciatus*. Efforts are being performed to expand the spectrum of coverage of this antivenom. Treatment of coralsnake bite envenomings in Central America is based on the parenteral administration of antivenom (5 to 10 vials). If the patient arrives to the health center with evident signs of respiratory difficulty, endotracheal intubation and mechanical ventilation are indicated.

Key words: *Micrurus*; coralsnakes; Central America; neurotoxicity; antivenom.

CORAL SNAKE BITES IN THE UNITED STATES

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Micrurus fulvius (Eastern), *Micrurus tener* (Texas) and *Micruroides euryxanthus* (Arizona) are the northern-most Coral snakes in the Americas and are the only native elapids in the United States. Their color banding pattern is uniform however, mimicked by several native non-venomous species. Coral envenomation represent less than 5% of all reported venomous snakebites in the United States. By far, the most medically important US Coralsnake is the Eastern or Florida coralsnake, *M. fulvius fulvius*. Traditionally, Florida has recorded 60-80 human exposures per year with approximately half demonstrating some clinical effects of envenomation. These numbers recently declined likely due to the reduction of the snake's habitat. Coralsnake envenomation management in the US is challenging because of a critical shortage of the FDA-approved, shelf-life extended Wyeth product, coupled with the current licensee's (Pfizer) lack of new production (despite continued collection of coralsnake venom). Other products are available only through an ongoing research study under compassionate use only. The most common symptoms are discomfort at the bite site, regional pain and paresthesias, vision changes and altered mental status. A fatality has not been reported in Florida in over 10 years.

Key words: Coralsnake bites, *Micrurus*, *Micruroides*.

ELAPID SNAKEBITE IN AUSTRALIA AND PAPUA NEW GUINEA

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Australian and New Guinea snake fauna is dominated by elapids, with viperids absent, and these snakes have developed clinical envenoming profiles distinctly different from other elapids, possibly because they have been able to fill multiple niches mostly occupied by viperids in other continents, thereby stimulating evolution of different venom armamentariums. Having been connected by land bridges, it is not surprising that New Guinea and Australia share a similar elapid snake fauna. It also appears that Australia has been invaded by progressive waves of elapids, corresponding to sea level and climatic changes, a possible explanation for currently disjunct populations of some species. All these elapids have in common dominant systemic rather than local envenoming, with no species causing the major necrotic effects seen with Asian and African cobras, although some species do cause significant local swelling and, rarely, minor local tissue damage. The major systemic effects seen in envenomed humans include; neurotoxic descending flaccid paralysis, in most cases a mixture of presynaptic and postsynaptic neurotoxicity; coagulopathy and secondary bleeding, mostly procoagulant-based with resultant defibrination causing hypocoagulability, but some causing direct anticoagulant coagulopathy; systemic rhabdomyolysis; plus primary or secondary effects such as acute kidney injury (AKI), intravascular haemolysis, microangiopathic haemolytic anaemia (MAHA). In Australia there are 5 principle venom immunotypes, corresponding to the 5 available "monovalent" antivenoms (a polyvalent antivenom covering all 5 is available); brown snake immunotype (genus *Pseudonaja*; defibrination coagulopathy, rarely neurotoxicity, AKI, MAHA); tiger snake immunotype (genus *Notechis*; defibrination coagulopathy, neurotoxicity, rhabdomyolysis, rarely AKI, MAHA, commonly local swelling, bruising; genus *Tropidechis*, similar to *Notechis*; genus *Austrelaps*; neurotoxicity, possibly anticoagulant coagulopathy or rhabdomyloysis; genus *Hoplocephalus*; defibrination coagulopathy); black snake immunotype (genus *Pseudechis*; rhabdomyolysis, anticoagulant coagulopathy, local swelling, pain); taipan immunotype (genus*Oxyuranus*; defibrination coagulopathy, neurotoxicity, rarely rhabdomyolysis, AKI); death adder immunotype (genus *Acanthophis*; neutrotoxicity, rarely mild anticoagulant coagulopathy or rhabdomyolysis, mainly in New Guinea species). In New Guinea, in addition to the taipan and death adder immunotypes, there are rare cases of brown snake bites/immunotype, Papuan black snake immunotype (similar to Australian *Pseudechis*, except for defibrination coagulopathy and neurotoxicity), and small-eyed snake immunotype (*Micropechis ikaheka*; anticoagulant coagulopathy, rhabdomyolysis, neurotoxicity, responds to Australian polyvalent antivenom). While there are many other elapid species in Australia and a smaller number in New Guinea and some Pacific islands, none appear able to cause major envenoming, although some can cause local swelling and a few rarely cause less severe coagulopathy, neurotoxicity, or rhabdomyolysis.

Key words: Elapid; snakebite; viperids.

CORAL SNAKES VENOMS: TOXIC PROPERTIES, IMMUNOGENICITY, ANTIVENOMS CROSS REACTIVITY AND NEUTRALIZATION POTENTIAL

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Micrurus snakebites can cause death by muscle paralysis and respiratory arrest, few hours after envenomation. The specific treatment for coralsnake envenomation is the intravenous application of heterologous antivenom and, in Brazil, it is produced by horse immunization with a mixture of *M. corallinus* and *M. frontalis* venoms, snakes that inhabit the South and Southeastern regions of the country. However, this antivenom might be inefficient, considering the existence of intra- and inter-specific variations in the composition of the venoms. Therefore, the aim of the present study was to investigate the toxic properties of venoms from nine species of *Micrurus*: eight present in different geographic regions of Brazil (*M. frontalis, M. corallinus, M. hemprichii, M. spixii, M. altirostris, M. surinamensis, M. ibiboboca, M. lemniscatus*) and one (*M. fulvius*) with large distribution in Southeastern United States and Mexico. It was also analyzed the immunogenic properties of *Micrurus* spp. venoms, as well as the cross-reactivity and neutralization potential of the horse commercial antivenom, as well as of experimental monovalent and polyvalent sera, produced in different animal species (mouse, rabbit and horse). Analysis of protein composition and toxicity revealed a large diversity of venoms from the nine *Micrurus* species. ELISA and Western blot assays showed a varied capability of the therapeutic antivenom to recognize the diverse species venoms components. Moreover, we showed that *Micrurus* venoms exhibited the same immunogenicity pattern in different animal species and that specific antisera presented cross reactivity when analyzed in ELISA and Western blot assays. Nonetheless, these positive results were not well correlated with the neutralizing potential of the antisera. These results indicate the existence of a large range of both qualitative and quantitative variations in *Micrurus* venoms, probably reflecting the adaptation of the snakes from this genus, to vastly dissimilar habitats. The data also show that the commercial antivenom, used for human therapy in Brazil, as well experimental specific or polyvalent antisera were not fully able to neutralize all *Micrurus* species occurring in the country. Thus, the establishment of a new antigenic mixture to produce novel more efficient therapeutic *Micrurus* antivenom is not a simple task. It suggests that modifications in the immunization scheme, with the inclusion of other venoms in the antigenic mixture, should occur in order to generate effective therapeutic coral snake antivenom.

Key words: antigenic; *Micrurus*; snakebites; venom.

MONOCLONAL-BASED ANTIVENOMICS AND BIOLOGICAL ACTIVITIES REVEALING HIGH VARIABILITY IN CORALSNAKES VENOMS

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The only effective treatment for venomous snakebites consists in administration of appropriate antivenom that should contain a set of antibodies able to neutralize the envenoming. The progress on the knowledge of venom composition, main driven by "omics" technologies, has contributed to design optimized venom mixtures for immunization and identify toxins that escape from neutralizing spectrum of antivenoms (antivenomics). It has been reported that the Brazilian coralsnake antivenom is inefficient to fully neutralize heterologous *Micrurus* venom lethality in animal model. Lethality and neurotoxicity among *Micrurus* species can be ascribed to PLA_2 and 3FTx toxin families. New strategies and immunization approaches are thus needed to generate improved coralsnake antivenom. In this regard, monoclonal antibodies (mAb) can be used as tool to find hot spots for inhibit the toxins and offer the possibility of to supplement antivenoms formulations with specific mAb expanding their therapeutic window. The results here reported show that two PLA_2-specific monoclonal antibodies cross-reacted with all PLA_2 molecules of *M. altirostris* venom, inhibiting their catalytic activity. These anti-PLA_2 mAbs exhibit paraspecificity against PLA_2 molecules from *Naja naja* venom, suggesting conservation of paraspecific epitopes across the Elapidae. Besides, these mAb reveled by ELISA high variability of PLA_2 content between pooled venoms of *M. altirostris*. The results were confirmed by RP-HPLC analyses and a similar variability was identified in pooled venoms of *M. corallinus*. Moreover, individual venoms of *M. lemniscatus* were also analyzed demonstrating specimens with phospholipases A_2 (PLA_2) or 'three-finger' toxins (3FTx) predominant venom composition. These results demonstrated a synergic action of mAbs to inhibit the catalytic activity of PLA_2 from *M. altirostris* and *Naja naja* venoms and significant variability on coralsnakes venom composition.

Key words: antivenom; monoclonal antibodies; PLA_2; venom variability.

IDENTIFICATION OF EPITOPES FOR THE DEVELOPMENT OF A NEW ANTIVENOM AGAINST CORALSNAKES (MICRURUS)

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The use of large amounts of crude venom for antielapidic serum production (immunizations and preclinical potency tests) represent a limitation of this technic for therapeutic purposes. Moreover the immunogens used are highly toxic for animals. Therefore, there is an increasing interest in evaluating the possibility of alternative methods for antielapidic serum production, which would be ideal to replace or reduce the amount of crude venom used. In this work we describe the mapping, by the SPOT-synthesis technique, of potential B-cell epitopes from five putative toxins (3FTx and PLA2) from *M. corallinus* venom. Overlapping peptides from the amino acid sequence of each toxin were synthesized on cellulose membranes and three different antielapidic sera were used to map the epitopes. After analysis of spot-reactive peptides, nine sequences from the five toxins were chemically synthesized and antigenically/immunogenically characterized. The neutralization potential of sera obtained by immunization of rabbits with all synthetic peptides alone or combined with initial doses of *M. frontalis* and *M. corallinus* venoms were analyzed and compared with the antivenom traditionally produced using crude *M. frontalis* and *M. corallinus* venoms as antigens. A good antibody response against individual synthetic peptides and *M. corallinus venom* was achieved. Anti-peptide IgGs were also cross-reactive against *M. frontalis and M. lemniscatus crude venoms. In addition, anti-peptide* IgGs inhibit the lethal and phospholipasic activities of *M. corallinus crude venom. On the other hand, we have verified by ELISA that rabbits immunized with three initial doses of crude venom containing mixtures of M frontalis* and *M. corallinus* followed by six doses of synthetic peptides from *M. corallinus* toxins generate antibodies with the same reactivity as those produced following traditional immunization. Results from in vivo and in vitro neutralization assays showed that the new rabbit sera are able to neutralize the lethal and PLA_2 activities of *M frontalis* and *M. corallinus* venoms. Our results provide a rational basis to the identification of neutralizing epitopes on coralsnake toxins and show that their corresponding synthetic peptides could improve the generation of immuno-therapeutics. The immunization strategy combining crude venoms and synthetic peptide antigens used approximately 60% less crude *Micrurus* venoms compared to the traditional immunization protocol. The use of synthetic peptide for immunization is a reasonable approach, since it enables poly-specificity, low risk of toxic effects and large-scale production.

Key words: *Micrurus* venoms, antielapidic serum, SPOT-synthesis technique, B-cell epitopes, synthetic peptides.

TOWARDS A UNIVERSAL ANTIELAPIDAE SERUM

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Although rare, coralsnake envenomation is a serious health threat in Brazil, because of the highly neurotoxic venom and the scarcely available antivenom. The major bottleneck for antivenom production is the low availability of venom. Furthermore, the available serum is not effective against all coralsnake species found in Brazil. An alternative to circumvent the lack of venom for serum production and the restricted protection of the actually available antivenom would be of great value. We compared the Brazilian coralsnake and the mono and polyvalent Australian antivenoms in terms of reactivity and protection. The immunoreactivity of venoms from 9 coral snakes species were assayed by ELISA and western blot using the Brazilian *Micrurus* and the Australian pentavalent as well as monovalent anti-*Notechis*, *Oxyuranus* and *Pseudechis* antivenoms. Neutralization assays were performed in mice, using 3LD50 of the venoms, incubated for 30 minutes with 100 lL of antivenom/animal. All the venoms reacted against the autologous and heterologous antivenoms. Nevertheless, the neutralization assays showed that the coral snake antivenom was only effective against *M. corallinus*, *M. frontalis*, *M. fulvius*, *M. nigrocinctus* and *M. pyrrhocryptus* venoms. On the other hand, the Australian pentavalent antivenom neutralized all venoms except the one from *M. spixii*. A combination of anti-*Oxyuranus* and *Pseudechis* monovalent sera, extended the protection to *M. altirostris* and, partially, to *M. ibiboboca*. By adding *Notechis* antivenom to this mixture, we obtained full protection against *M. ibiboboca* and partial neutralization against *M. lemniscatus* venoms. Our findings confirm the limited effectiveness of the Brazilian coralsnake antivenom and indicate that antivenoms made from Australian snakes venoms are an effective alternative for coralsnake bites in South America and in the United States where coralsnake antivenom production has been discontinued.

Key words: antiserum; immunoreactivity; *Micrurus*; venom.

21 OCTOBER 2016 (FRIDAY)

THE MECHANISM OF VENOM INOCULATION IN CORALSNAKES

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Despite literature reports as a system of poorly developed venom injection, the venom apparatus of coralsnakes probably had a common origin with Viperidae from an opistoglyphous group. However, it is worth mentioning that both groups evolved independently. We believe that the idea of the poor development had appeared by direct comparison with the highly kinetic skull and large fangs of the solenoglyphous species and not due to its higher or lower evolutionary degree. It seems evident that coralsnakes had suffered serious restrictions to freedom of movements and by their existence linked to the semifossorial and fossorial habitats. The permanent circulation in narrow environments certainly helped the selection of a cylindrical body with rows of 15 dorsal scales without reduction, shortening of the snout and limited cranial kinetic, and opening of the mouth in a maximum of 30º (limiting the size of the fangs to about 3 mm). These features coupled the group to a specialized diet of serpentiform animals (snakes, legless lizards, amphisbaenians, fish, and even extravagances like onychophorans). Therefore, reasonable variations on the skull kinetic may be observed where aquatic species, like *Micrurus surinamensis*, present noticeable differences in the breadth of the quadrate-mandible movements (especially by the extension and development of the supratemporals and quadrate bones), when compared with *M. ibiboboca*, a species from the Caatinga in the northeast region of Brazil. Differences with the Viperidae venomous apparatus begin with a venom gland without lumen (or extremely reduced) to accumulate secretions, which in turn is generally more viscous, and the compressor muscles of the gland that, by origin and disposition, are also different from the Viperidae. Coralsnakes are snapping animals in predation as well as in defense and they use a slow bite (chewing movements) not only due to the viscosity of the venom but that some secretions might occur during the biting action. Owing to this and other conditions we consider that the coralsnakes venom apparatus is far from being less developed but well-adjusted to their life style of these animals that in most cases is still surrounded by mysteries to science. Certainly, this venomous apparatus is adjusted to deal with and facilitate the feeding biology of coralsnakes and not less important to their defense, that the complex color pattern is always a provocative subject to evolutionary studies.

Key words: coralsnakes; *Micrurus*; venom; Viperidae.

COMPARATIVE STUDY OF CORALSNAKE CEPHALIC GLANDS

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Snake cephalic glands have been object of numerous investigations with different purposes. Despite the wide diversity and obvious medical importance of the New World coralsnakes, little is known about their glandular morphology. This work aims the morphological study of cephalic glands, especially venom and labial glands in *Micrurus* (*M. corallinus*, *M. frontalis*, *M. hemprichii, M. lemniscatus*, *M. spixii* and *M. surinamensis*) and *Leptomicrurus narduccii*. Heads were dissected for examinations of cephalic glands and histological sections of whole heads, and dissected isolated glands were analyzed. Heads were also contrasted with iodine and submitted to computed tomography (CT-Scan). In examined species, the venom glands are quite similar to that described for other elapid snakes. These glands are constituted by large tubules converging to the central area, although a central lumen is not observed. The accessory mucous gland lies along the venom duct in continuation to the main gland. The other cephalic glands (Harder, sublingual and nasal), although less studied than the venom glands, have also a conserved morphology compared to the other elapids previously studied. The infralabial glands morphology, although rarely studied, seems to differ from the coralsnake pattern and are divided into two portions. The anterior portion corresponds to the true infralabial glands and shows a pocket-like structure where the front-fangs are allocated in the mandibular region. Mucous cells predominantly constituted this glandular portion. The posterior portion corresponds to the inferior rictal glands constituted by wide serous acini, with a large duct extending along its medial surface and reaching the posterior region of the mouth at the level of rictal area. The results indicate the great morphological variation in cephalic glands and evidence a protein-secreting gland poorly known, particularly in elapids, which may suggest additional adaptive role besides the usual mucus production of the typical labial glands.

Key words: infralabial glands; inferior rictal glands; front-fanged pocket; venom glands.

THE MECHANISMS OF ACTION OF CORALSNAKE (*MICRURUS*: ELAPIDAE) VENOMS

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Envenomings by coralsnakes (*Micrurus* sp.) occur throughout the Americas, where between 1% to 2% of all snakebite cases are caused by these elapids. The main clinical manifestation of coral snake envenomings is descending neuromuscular paralysis, owing to the action of neurotoxins. These venoms contain low molecular mass neurotoxins of the three-finger toxin family (3FTx), some of which have a high affinity for the nicotinic cholinergic receptor of the motor end-plate in muscle fibers, provoking a blockade of post-synaptic nature, i.e. α-neurotoxicity. In addition, some *Micrurus* sp venoms contain neurotoxic phospholipases A_2 (PLA_2s), which inhibit the neuromuscular transmission by acting at the presynaptic site, as a consequence of the hydrolysis of the phospholipids of the nerve terminal plasma membrane, thus inducing β-neurotoxicity. The relative role of pre- or post-synaptically-acting neurotoxins varies depending on the species, in agreement with the proteomic profiles which underscore a dichotomy between 3FTx-rich or PLA_2–rich venoms. *Micrurus* sp venoms also contain myotoxic PLA_2s, which in mice cause acute systemic myotoxicity, i.e. rhabdomyolysis, associated with widespread myonecrosis, increments in serum creatine kinase (CK) levels, and myoglobinuria. However, although myotoxicity also occurs in humans, it is generally mild, probably due to the low amount of venom injected. A unique intravascular hemolytic effect, caused by a PLA_2, has been described in the venom of the North American species *M. fulvius*. This effect occurs in mice and dogs, but not in humans, and this difference is likely to depend on the different phospholipid composition of the plasma membrane of erythrocytes from these species. Envenomings by *Micrurus* sp are also characterized by local pain, often described as very strong, and an algogenic heterodimeric molecular complex of a PLA_2 homologue and a Kunitz-type proteinase inhibitor has been identified in various venoms. Other venom components, which are not toxic in mammals, but whose ecological role remains unknown, have been purified from coralsnake venoms, such as 3FTxs in the venom of *M. mipartitus* which modulate $GABA_A$ receptor activity. The different mechanisms of action of toxins isolated from *Micrurus* sp venoms will be reviewed in this presentation, and the possible ecological roles of these toxins will be also discussed.

Key words: *Micrurus*; coralsnakes; venom; neurotoxicity; myotoxicity; mechanism of action.

THE RELATIONSHIP BETWEEN COMPLEXITY, VARIABILITY, AND TOXICITY IN NORTH AMERICAN CORALSNAKES

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In certain venomous taxa, simpler venoms are often more toxic to mammalian, non-prey species than more complex venoms (e.g., type I and type II venoms in rattlesnakes), but whether this trend between complexity and toxicity holds in other venomous taxa, including coralsnakes, is uncertain. We used venom-gland transcriptomics and quantitative mass spectrometry to compare venom complexity across the eastern coral snake (*Micrurus fulvius*) and the Texas coralsnake (*Micrurus tener*) and used median lethal dose assays to assess the relationship between complexity and toxicity. We found that *M. tener* venom was much more complex than *M. fulvius,* but was significantly less toxic to lab mice, demonstrating that the relationship between simplicity and toxicity may be a pattern across all venomous snakes rather than being rattlesnake-specific. This phenotypic simplicity, however, could affect the evolvability of *M. fulvius* venom; simplicity could decrease evolvability by providing fewer mutational targets, but could also eliminate certain costs that limit a trait's adaptive potential as described in Fisher's geometric model and, therefore, be more evolvable. We sampled 49 *M. fulvius* from 11 discrete geographical regions in Florida to determine if *M. fulvius* exhibited significant variation in protein expression and/or toxicity; we found no variation in expression levels or in toxicity across all 11 populations, suggesting that *M. fulvius* may have experienced a species-wide selective sweep, has undergone a recent range expansion, or, that although simpler venoms are often more toxic, they may be less evolvable.

Key words: coralsnake; *Micrurus fulvius*; *Micrurus tener*; transcriptomics.

ECOLOGICAL MODELLING AS A TOOL FOR CORALSNAKES CONSERVATION – PART A

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Predicting species' distributions has become an important component of conservation planning in recent years. The most common strategy for estimating the actual or potential geographical of a species is to characterize the environmental conditions that are suitable for the species, and to then identify where suitable environments are distributed in space. This approach is called Ecological Niche Modelling (ENM), and is based on associations between aspects of climate and known occurrences of species across landscapes of interest to define sets of conditions under which species are likely to maintain viable populations. The theoretical framework underlying ENM dates from the Hutchinsonian concept of ecological niches, which defines the fundamental niche of a species as the set of environmental conditions (i.e., the environmental space) within which a species can survive and persist. The duality between the environmental and the geographic space allows transferring this set of environmental conditions to the geographic space to depict the areas of potential distribution for the species, although some of these areas may not be inhabited due to biotic interactions of dispersal limitations. A wide variety of modelling techniques have been developed for this purpose, ranging from very simple bioclimatic envelope models up to complex machine learning-based methods. These techniques also differ in the type of biological data used to describe the known distribution of a species, which can be *presence-only* (when only records of sites where the species has been observed are available), or *presence-absence* methods (when records of presence and absence of the species at samples sites are available). One of the simplest techniques is Bioclim, which is a *presence-only* method based on bioclimatic envelope to define the environmental tolerance of a species for multiple environmental predictors. A more complex and largely used technique is Maxent, which is based on a principle from statistical mechanics and information theory. Statistical methods of parametric and non-parametric regression such as GLM (generalized linear model) and GAM (generalized additive model) that use *presence-absence* data are also frequently used to model species niches and predict potential distributions. Ecological niche modeling has a range of applications, including discovery of new populations or previously unknown species, identifying potential areas for disease outbreaks, examining niche evolution, identifying historical refugia for biodiversity, conservation planning, and forecasting effects of climate change on species distribution. In particular for coralsnakes, we showed that ENM could be a very useful tool when the geographic distribution of a species is poorly documented to predict possible suitable locations that are not documented owing to insufficient sampling. Also, the suitability scores provided for ENM can be applied to identify highly suitable areas for species conservation. Finally, considering the poor dispersal abilities of these species, potential range shifts and the impact of climate changes on species distribution could be anticipated using ENM.

Key words: ecological niche modelling; species distribution; conservation; climate change.

ECOLOGICAL MODELLING AS A TOOL FOR CORALSNAKES CONSERVATION – PART B

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Models that predict distributions of species by combining known occurrence records with environmental variables have much potential for application in conservation. Ecological niche models (ENM) offer the opportunity fill important gaps in the knowledge about the distribution of species, to detect disjoint populations and ever to discover unknown species. In addition, the temporal transferability of niche models allows estimating the impacts of climate change on species distribution. Here, we used niche models to generate a rank of habitat suitability for ten South American coralsnakes species - *Micrurus altirostris, M. baliocoryphus, M. brasiliensis, M. corallinus*, *Micrurus decoratus*, *M. frontalis*, *M. ibiboboca, M. pyrrhocryptus, M. silviae,* and *M. surinamensis* – and four taxa of the *Micrurus lemniscatus* complex – *M. lemniscatus lemniscatus, M. lemniscatus helleri, M. lemniscatus carvalhoi*, and *M. diutius*. Our aim was to estimate the potential geographic distribution of the species and evaluate impacts of future climate change in species' ranges. We also assess the effectiveness of the current network of protected areas to represent species habitat suitability at present and future scenarios of climate change. Species occurrence records were compiled from museum specimens and combined into the niche models with climatic variables characterizing current climatic conditions. These models were then projected for future using climatic variables representing future scenarios of climate change. We used twelve different ENMs, including six methods of presence-only data and six methods of presence-absence. The frequency of prediction resulting from the combination of climate models and ENM was used as surrogate of habitat suitability and distribution for each species. Finally, we compare the proportion of overlapping between the network of protected areas and the habitat suitability values through an index of species representation for present and future. The results of our analyses were presented by each group as oral presentations.

Key words: coralsnakes; habitat; impacts; niche.

B. ORAL PRESENTATIONS

18 OCTOBER 2016 (TUESDAY)

THE TAXONOMIC STATUS OF *MICRURUS LEMNISCATUS* (SERPENTES, ELAPIDAE) AND RELATED FORMS

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The genus *Micrurus* Wagler, 1824 comprises currently more than 120 taxa (species and subspecies). Within this genus, three morphological assemblages are recognized, among them the South American triadal group with 24 species with a robust indication of its monophyletism. Within this group, *M. lemniscatus* complex (*M. l. lemniscatus* Linnaeus, 1758; *M. l. helleri* Schmidt & Schmidt, 1925; *M. l. carvalhoi* Roze, 1967; *M. diutius* Burger, 1955, *M. frontifasciatus* Werner, 1927, *M. potyguara* Pires *et al*., 2014, and *M. serranus* Harvey, Aparicio-E. & González-A., 2003), present evidence of polyphyletism, which led us to perform the present study aiming its taxonomic revision based on the morphology of the external and internal features of these taxa. Head and body color pattern, pholidosis, biometry, cranial and genital morphology were sampled over 1060 specimens of the aforementioned taxa and treated by descriptive, univariate, multivariate statistical analysis and comparatively aiming recognition of distinctive characters. Our results support the recognition of *M. carvalhoi* as distinct species from *M. lemniscatus*, the synonymy of *M. l. helleri* with *M. lemniscatus* and reiterates the specific status recognition for *M. diutius*, *M. potyguara* and *M. serranus*. *M. frontifasciatus* is too poorly represented in our sample, so we kept it within synonymy of *M. lemniscatus* besides its peculiarities. Historical background for type specimens were also addressed, aiming to elucidate type specimens origins, history, traceability and fitness within the presented diagnosis.

Key words: biogeography; Elapidae; history; Neotropical; taxonomy.

PHYLOGEGRAPHY OF *MICRURUS SURINAMENSIS* AND *MICRURUS LEMNISCATUS*

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The coralsnake genus *Micrurus* is distributed from southern North America to southern South America, with approximately 80 currently recognized species and more than 100 described taxa. Despite its medical (venom) and evolutionary (mimicry evolution) importance, there has yet to be a phylogenetic study of the genus. Past phylogenetic studies have been based on immunological, paleontological, meristic, allozyme, and mtDNA sampling of a small fraction of *Micrurus* species diversity. Even more surprising is that despite the explosion in phylogeographic studies of Neotropical species, no phylogeographic studies of any *Micrurus* species has yet been produced. Within the genus, *Micrurus surinamensis* and *Micrurus lemniscatus* are excellent subjects for phylogeographic studies due to their known intraspecific variation throughout an extensive area in South America. The morphological variation found in these two species fueled in the last 20 years an intense taxonomic debate that resulted in the formal recognition of several subspecies, some of them posteriorly erected to the species level. *Micrurus lemniscatus* contains three morphologically distinguishable populations that are presently recognized at the subspecific level (*M. l. lemniscatus*, *M. l. carvalhoi*, and *M. l. helleri*), occurring in open and forested habitats. On the other hand, some previously recognized subspecies (e.g., *M. l. frontifasciatus*) were posteriorly considered as invalid, indicating that further studies are necessary to evaluate the evolutionary status of these populations. We intend to test the status of these populations using Next Generation Sequencing data (3RADseq), and compare the results with the morphological evidence at hand, and used to define the various subspecies of *M. lemniscatus*. Similarly, we explore both phylogeographic patterns and evolutionary history of *Micrurus surinamensis* using 3RADseq data. *Micrurus surinamensis* is one of the largest species of coralsnakes, and occurs throughout northern South America, with morphologically distinct populations. In a recent revision of the species, morphological characters were used to split this species (with two subspecies) into two taxa: *M. surinamensis* (occurring in the Amazonas drainage) and *M. nattereri* (occurring in the Orinoco river system). Notably, this semi-aquatic species feeds primarily on fish, having several anatomical specializations related to a freshwater environment. For that reason we hypothesize that rivers of the region will strongly influence genetic structure within this species. We here explore the validity of the newly described taxonomic units and the role of river drainages in structuring populations of this species. The method of Next Generation

Sequencing we use here is based on double-digest RADseq and has several advantages over previous methodologies, in particular lower quantities of DNA starting material, multiple enzyme compatibility of the adapters, and lower cost. For our study we included 67 samples of *M. lemniscatus* and 44 samples of *M. surinamensis* covering most of the geographic distribution of both species.

Key words: phylogeography; mimicry; biogeography; coralsnake; RAD; next generation sequencing.

THE TAXONOMIC STATUS OF THE AMAZONIAN CORAL SNAKE (*MICRURUS SPIXII*)

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The *Micrurus* species with triadal color pattern comprises a clade with about 35 taxa restricted to South America and beyond design pattern and coloration share hemipenis short and slightly bilobed. The group of species have large overlap of meristic characters, morphometric, coloring, as well as cases of sympatric between taxa, which makes the definition and identification of the same, creating a taxonomic instability for various complex. Four subspecies are recognized in *M. spixii*: *M. s. spixii*, *M. s. martiusi*, *M. s. obscurus* and *M. s. princeps* with predominantly Amazon distribution. In order to review the taxonomy of the complex *M. spixii*, we analyze the type-series of all taxa involved in the group as well as 357 species along the geographical distribution. *Micrurus s. martiusi* is synonymized *M. s. spixi*i because there is absence of significant differences in the staining pattern, hemipenis and skull. *M. s. princeps* is synonymized *M. s. obscurus* mainly by color pattern indistinct. Differences in morphology of hemipenis, skull and color pattern support the existence of only two different taxa, *M. spixii* and *M. obscurus* (elevated to ranking of species). Morphometric and meristic characters showed no significant differences between the two species, however, *M. spixii* distinguished from *M. obscurus* to present the following set of characters: cephalic hood defined and black head (vs. absent cephalic hood, red parietal region); body of hemipenis with thorns distributed randomly in the face asulcate (vs. body spines distributed in rows in face asulcate); clear capitated condition and basal bag (vs. absence of capitated condition); narrow parietal bone and jaw strongly inclined towards the dorsal part of the cranium (vs. parietal bone with no angular edges and jaw bone contacting the prefrontal in the anterior portion thereof). Sexual dimorphism was observed in snout-vent length in *M. spixii* and snout-vent length and length head *M. obscurus*. According to the new taxonomic arrangement, we restricted *M. spixii* to Brazil, in the southern and northern rails of the Amazon River (in Amazonas and Pará states) and east of the Rio Madeira (in Rondônia and Mato Grosso states), registered in the states of Tocantins and Maranhão; while *M. obscurus* presents with a wider distribution, occurs in the forests east of the Andes, with records in southeastern Colombia, northeast and southeast of Peru to central Bolivia and northern Brazil (in Amazonas, Acre and Rondônia states).

Key words: *Micrurus*; morphology; taxonomy; coralsnake.

A FIRST INSIGHT INTO THE SYSTEMATICS AND SPATIO-TEMPORAL EVOLUTION OF OLD WORLD CORALSNAKES (GENERA *CALLIOPHIS* AND *SINOMICRURUS*)

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Usually small, shy and fossorial, Old World Coralsnakes (genera *Calliophis* and *Sinomicrurus*) are amongst the rarest Asian elapids and their systematics and biogeography have remained elusive. The first half of this talk will present the current understanding of the phylogenetic relationships within the widespread genus *Calliophis* based on our molecular systematic studies. The second half will discuss the molecular systematics, species boundaries and biogeography of *Sinomicrurus* species. In addition to recovering a strongly resolved tree of intergeneric relationships within *Sinomicrurus*, our analyses delimit four cryptic species in this genus. Biogeographic hypothesis testing reveals the eastern margin of the Chinese continent to be the center of origin for these snakes. Contrary to previously proposed hypotheses for the drivers of faunal divergence in the Taiwan-Ryukyu Archipelago, our divergence-time analysis shows that the majority of cladogenetic events in the genus significantly pre-date the outbreak of Quaternary diversification. Geological upheavals during the Middle Miocene and subsequent geographic changes may have provided potential dispersal routes and opportunities for isolation between the continent and the Taiwan-Ryukyu Archipelago. However, given the lack of temporal correspondence to known historical events, we propose that speciation in *Sinomicrurus* was most likely driven by ancient, unidentified vicariant events or adaptive speciation.

Key words: biogeography; coralsnakes; molecular systematics; *Sinomicrurus*; Taiwan-Ryukyu Archipelago.

NEW PHARMACOLOGICAL TARGET OF THE *MICRURUS LEMNISCATUS* SNAKE VENOM: THE L-GLUTAMATE RELEASE IS REGULATED BY IONOTROPIC GLUTAMATERGIC RECEPTORS AND COULD BE INVOLVED IN CENTRAL NEUROTOXICITY OF THE ENVENOMING

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Accidents with snakes are a serious public health problem and were included as "neglected tropical diseases" by the World Health Organization (WHO). *Micrurus sp* is responsible for only 0.5% of the snake accidents. Clinical manifestations of envenoming by this snake consist primarily of the neurotoxic activity in the peripheral nervous system, mainly by the action of the three-finger toxins (3Ftx) (6-8 KDa), present in the venom, that cause progressive blockade of nAChR, at the motor endplate. Previous studies showed that β-neurotoxins (phospholipase A_2) isolated from elapidic venoms, including coralsnakes, induce cortical neurons death and promote glutamatergic excitotoxicity, causing neuronal degeneration. In pathological conditions as epilepsy and brain ischemia, there is a massive release of Lglutamate, leading to neurotoxicity. Although some symptoms associated to disorders in the CNS related with 3Ftx toxins have been described, little is known about their mechanisms of neurotoxicity. In the present study, we proposed to investigate the possible pharmacological targets contributed to central neurotoxic effects of *M. lemniscatus* crude venom (CV) and those components were involved in neurotoxicity. Lyophilized crude venom of *Micrurus lemniscatus* was fractionated by RPC-HPLC. The isolated fractions were analyzed by MALDI-TOF mass spectrometry and used on the neurochemistry and toxicity assays. We used hippocampal neurons in culture treated at different concentrations and times. Our results indicated that neurotoxicity was observed after 3h and that exposure NMDA and nACh receptors were involved in the toxic effects. Moreover, the isolated fraction (F22) increased L-glutamate release in rat cortical brain synaptosomes. The contributions of calcium channels, ionotropic glutamatergic receptors as well as the external and internal calcium concentration were also evaluated. The results showed that calcium influx is involved in the L-glutamate release promoted by F22, and that this effect was abolished in the presence of glutamate ionotropic antagonists. These studies could lead to a better understanding of the neurotoxic effects of *M. lemniscatus* venom on the CNS and showed for the first time the pre-synaptic activity of 3FTx toxins.

Key words: L-glutamate release; three-finger toxins; *Micrurus lemniscatus*; ionotropic glutamate receptors.

19 OCTOBER 2016 (WEDNESDAY)

MICRURUS LEMNISCATUS SNAKE VENOM: BIOPROSPECTING OF NEW MOLECULES WITH ANTITUMOR ACTIVITY IN GLIOMAS CELLS

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Cancer is a serious public health problem in the world. According to the estimated by World Health Organization in 2012 were reported approximately 14 million new cases and 8.2 million cancer related deaths. Gliomas are the most frequent tumors occurring in the central nervous system (CNS) and are responsible for 80% of all malignancies of the brain and CNS. These tumors are categorized into four grades named I, II (astrocytoma, oligodendroglioma or oligoastrocytoma), III (anaplastic-astrocytoma/oligodendroglioma) and IV (glioblastoma). Compared to other types of tumors, gliomas are more challenging to treat because of the shield of the blood–brain barrier (BBB). In this context, new opportunities for efficient drug across the BBB are urgently needed. Many proteins and peptides possess biological activities that mark them as potential therapeutics or powerful pharmacological tools, in particular, for neurological disorders of the CNS. Snake venoms are complex mixtures of proteins, nucleotides and inorganic ions. The main toxins from *Micrurus* spp. venom are neurotoxins, three-fingers toxins (MW= 6-8 KDa) and PLA_2 toxins with specific activities in different pathways, mainly ionic channels and receptors. The goal of the present study was the prospection of the toxins isolated from *Micrurus lemniscatus* venom searching for antitumor activity on human gliomas, human neuroblastoma and human epidermoid carcinoma cell lines. Lyophilized crude venom of *Micrurus lemniscatus* was fractionated by RPC-HPLC. The isolated fractions were analyzed by MALDI-TOF mass spectrometry and used on the toxicity assays. Human glioma U87 (ATCC_HTB-14), U373, neuroblastoma SH-SY5Y (ATCC_2266), human epidermoid carcinoma A431NS (ATCC_2592) and HEK293 (ATCC_1573) control cells ($1x10^4$cells/well) were treated with different concentrations of toxins ($5x10^{-2}$, $5x10^{-1}$ and 5 μg/mL) at 24h. Cell survival was quantified by MTT method. Our preliminary results showed toxic effects of *M. lemniscatus* crude venom and its fractions on mice neuroblastoma cell line (Neuro-2A), after 24 h. Moreover, the crude venom (dose-dependent manner) and most of its fractions caused toxicity (40-80%) on cells. The F2, F9, F11, F22 and F24/25 fractions promoted significant cell death (60-80%) on Glioblastoma U87 cells (grade IV) and F3, F4, F24/25 and F26 caused toxicity (40-90%) on Glioblastomaastrocytoma cells (grade III). However, the SH-SY5Y, A431 and HEK293 cell lines were not susceptible to death. We observed that the isolated fractions tested and involved in cell death contained mainly toxins in the range of 6 to 8 kDa. These studies suggest a specific toxicity of some fractions to human glioma cells seem to be an evidence to bioprospecting of selective and promising antitumor drugs.

Key words: *Micrurus lemniscatus*; toxins; human glioma; antitumor**.**

NEW METHODOLOGY FOR MICRURUS ALTIROSTRIS (COPE, 1960) MAINTENANCE IN CAPTIVITY

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The maintenance of captive snakes is necessary to support researches related to behavior, proteomics and pharmacology, involving high investment in infrastructure and qualified professionals. For some species this doesn't guarantee success, given the difficulty of adapting the animals, requiring some improvement of the husbandry techniques. The genus *Micrurus* produces low amount of venom and has a short survival time in captivity, which is mainly related to feeding difficulties, since most species have a specialized diet composed of amphibians and snake-like reptiles, which have an unfeasible production in captivity. The *Micrurus altirostris* occurs in southern Brazil, Argentina and Uruguay and has a diet such as the most species of the genus. Its venom, experimentally, has a lethal toxic action, and isn't neutralized by the antivenom produced in Brazil from the venom of *M. frontalis* and *M. corallinus*, requiring reevaluation of the immunization pool composition. The necessity for greater amount of *M. altirostris* venom to researches led the Núcleo Regional de Ofiologia de Porto Alegre (NOPA) to seek for new forms of maintenance methods in captivity. Aiming to increase survival rates, a new option to assist in periods of a lower prey availability was developed. To test it, was compared the lifetime (in days) of two groups: A, with 149 individuals held in NOPA between 1994 and 2010, fed according to the availability and sporadic sounding; and B, with 16 specimens received since 2011. The group B is fed fortnightly with a ration that consists of eggs, chicken liver, vitamin supplements, H_2O and NaCl, administered orally by an urethral probe nº 8 in the final portion of the esophagus in an amount equivalent to 20% of the weight of the animal after it being subjected to inhalation of CO_2. After the ingestion, the animal is supported upright to recover muscle tone and return to regular breathing. Serving environmental enrichment and given its semi-fossorial habit, it was employed vermiculite as substrate. Among the 16 animals of group B, seven remain alive, considering the lifetime until 06.01.2016. The captive survival rate ranged from 60 to 690 days (mean = 204.9 ± 114.9 days) in group A and 90-1640 days (mean = 980.3 ± 631.8 days) in group B, with an average lifetime significantly higher in group B (Mann-Whitney U, $P < 0.001$). No individual died during the sounding and within 48 hours after the procedure. An autopsy was performed in one animal of group B and showed no evidence of lesions in the digestive tract where the probe was inserted, or caused by inhalation of CO2. Researches on the longevity of *M. altirostris* showed maximum survival in captivity for 397 days. The results showed that the new method is effective to prolong the survival rates of *M. altirostris* in captivity and probably other species of the genus, contributing to the increase in venom production.

Key words: Elapidae; snakes; sounding; survival.

DAILY ACTIVITY IN *MICRURUS FRONTALIS* AND *MICRURUS LEMNISCATUS*

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Daily activity is a relevant aspect concerning to the natural history of the species. It enables the animals to synchronize their behavior and physiology with the environmental conditions. Although it has been studied in several taxa, there are few data comprising snakes. For fossorial or cryptozoic snakes, such as those belonging to the genus *Micrurus*, most data arise from scarce data on field observations. This topic has also taken part in the hypothesis of mimicry involving coral snakes, since its validation would rely on the models being diurnal, and, therefore, subjected to visual predators. *Micrurus frontalis* and *M. lemniscatus* belong to the triad-group of South American coralsnakes. Both species occur in Atlantic Forest areas, from Paraguay to Central and Southeastern Brazil, although *M. frontalis* may also be present in Cerrado areas. In order to investigate the daily activity in these species, specimens of *M. lemniscatus* (n = 3) and *M. frontalis* (n = 5) were observed using closed-circuit television system during ten consecutive days. During the experiment, the animals were not fed, water was offered *ad libitum*, and they were kept in terraria, individually, maintained in a room at around 25°C. All records were made between January 14th and 23rd, 2015, at Fundação Ezequiel Dias (FUNED), Belo Horizonte, Minas Gerais State. Both species showed to be diurnal (*M. frontalis*: x^2 = 115.925, p<0,001; *M. lemniscatus*: x^2 = 49.209, p<0,001). *Micrurus frontalis* showed bimodal activity, with one peak in the morning and another peak in the afternoon. On the other hand, *M. lemniscatus* showed unimodal activity, with peak in the early morning. Data from observations in the field recorded in literature report diurnal and nocturnal activity for both species from November to February. In the present study, animals were also observed in activity during the day (with reduction of activity around noon, which is supposedly the hottest period of the day), and the night. However, registers of nocturnal activity were less frequent, and restricted to the early evening (*M. frontalis*: 19.8%; *M. lemniscatus*: 23.3%). Accordingly, activity data obtained herein also support that *M. lemniscatus* and *M. frontalis* may be exposed to visual predators, therefore, meeting one of the premises of posing as models in mimetic complexes. It is noteworthy that, despite belonging to groups which are phylogenetic closed, and being subjected to the same environmental conditions during the experiments, *M. frontalis* and *M. lemniscatus* showed different activity patterns. In this sense, such differences are likewise to be intrinsic of the species themselves. Ongoing studies including a higher number of specimens may help elucidating these variations, as well as the influence of the sex and ontogeny on the daily activity.

Key words: daily activity; natural history; animal behavior; coralsnakes.

CAN THE FEMALE CORAL SNAKE STORE SPERM? A STUDY OF REPRODUCTIVE STRATEGIES IN *MICRURUS CORALLINUS* AND *MICRURUS FRONTALIS*

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The reproductive cycle of oviparous snakes comprehends a sequence of events such as oogenesis, vitellogenesis, mating, ovulation and oviposition. The period of these events may be maintained evolutively within the lineage or the species may have the plasticity to adapt the period of reproductive events according to the climate conditions in the environment. Within the group Squamata some species exhibit the reproductive strategy of sperm storage, and in some species this can happen in both sexes or only in one sex. In the case of females, this strategy allows the dissociation between the time of copulation and ovulation (fertilization). The purpose of this research was to investigate the existence of reproductive strategy of sperm storage by females, in two species of coralsnakes, *Micrurus corallinus e M. frontalis,* from two different phylogenetic lineages inside the genus, based on the color pattern black ring monad (BRM) and black ring triads (BRT). The analysis was based on the combination of body biometrics data and oviduct histology of 40 adult specimens of *M. corallinus* and 23 of *M. frontalis* from biological collections. According to the date of collection of the specimens, they were separated and grouped between the four seasons of the year. The specimens analyzed had biometric data such as snoutvent length (SVL) and circumference measured. In addition, tissue samples were collected from the posterior infundibulum, anterior and posterior oviduct for histology. As a result, tubules and sperm storage receptacles were found in *M. corallinus*, three of which were collected in the spring, one in summer, one in the fall and one in winter. In *M. frontalis,* 4 specimens showed storage, two in the fall, one in summer and one in the spring. The specimens presented sperm storage, SVL greater than 540 mm and circumference greater than 25 mm in both species. The regions with the highest incidence of storage was the posterior oviduct. The morphology of these regions displayed the simple columnar ciliated epithelium and surrounding the receptacles it was observed a higher concentration of capillaries. The *M. corallinus* showed greater storage efficiency compared to *M. frontalis* by being capable of storing sperm in the oviduct in all seasons. Possibly *M. corallinus* showed a higher number of specimens with storage in the spring because this is the period of copulation. The strategy of storing sperm can function as a guarantee mechanism of oocyte fertilization, if new copulations don't occur in the following cycles and can also serve as a sperm competition strategy in the event of new copulations. This is the first work that proves the existence of the reproductive strategy of sperm storage in female *Micrurus*.

Key words: Elapidae; Environmental conditions; Reproductive cycle; Dissociation cycle

EXPERIENCES IN MAINTAINING A LIVE CORAL SNAKE COLLECTION IN CAPTIVITY FOR VENOM EXTRACTION PURPOSE: THE CASE OF THE CLODOMIRO PICADO INSTITUTE

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The development of a colony of snakes for venom production purposes has been reported for many institutions worldwide. In captivity, maintenance of a long-term collection is affected by the quality of food provided, the overall conditions of maintenance and health protocols applied. Usually, coralsnakes feed on its natural prey, such as colubrid snakes belonging to the genera *Geophis* sp., and *Ninia* sp. Nevertheless, most coral snakes do not accept to be fed, and generally die few months after being captured. Although some herpetologists use a forced-feeding strategy, the survival rate of these specimens rarely exceeds one year. In addition, no controlled conditions, insufficient nutrients provided with the food and no control over the parasitic load in the animals contribute to such short-term expectancy lifetime in captivity. Moreover, unsuitable collections of these animals would lead to poor venom yield in the elaboration of antivenoms. Thus, the Serpentarium of the Clodomiro Picado Institute has made several improvements in the general conditions in which the coralsnakes are maintained, ensuring a long-term lifetime expectancy with a suitable quota of venom yield for the fabrication of antivenom. Improvements in feeding programs, diets, deworming programs and venom extraction process have been established to ensure good maintenance conditions and high amount and quality of venoms. Therefore, all coralsnakes are submitted to body condition and health assessments, in which length and weight of animals are used to determinate scale mass indexes. In addition, an alternative fish-based diet was used to feed the animals and high standards were met in order to extract venom manually. As a result, the fish-based diet and deworming programs against Acanthocephala, *Rhabdias* sp., Pentastomids, Cestoda, Coccidia, *Kalicephalus* sp., and *Strongyloides* sp., parasites; has helped to keep, in average, 90 *Micrurus* sp., in a good body condition when compared to wild-caught animals. In addition, life expectancy has increased to 3.5 ± 0.6 years in species such as *Micrurus nigrocinctus*. Furthermore, a particular case of longevity by one specimen of *M. nigrocinctus* was reported; living in captivity for 24 years, with changes in diet (snakes, mice, fish-based), and submitted 67 times to extraction venom processes through the whole lifetime in captivity. On the other hand, the venom yield of *Micrurus* species increased since the implementation of the alternative diet, health assesses, and deworming program. Thus, the main species (*M. nigrocinctus*) extracted to produce antivenom yielded 2.2 ± 0.7 mg of venom per year. Accordingly, to the extent of our knowledge, this coralsnake collection is one of the largest collections on *Micrurus* sp., snakes, longest lifetime expectancy and highest quota of venom yielded for antivenom production. Therefore, the implementation of body condition and health assessments, deworming programs, well-balanced alternative diets and good procedures in venom extraction process, guarantee a long life-time expectancy and high and quality venom yield for antivenom production.

Key words: coralsnakes; captivity; venom yield; alternative diet; deworming snakes.

21 OCTOBER 2016 (FRIDAY)

Papers presented as results of the Workshop of Niche Modelling and Distribution of Species of New World Coralsnakes held at the Pontifícia Universidade Católica de Goiás, from 27 June to 1 July 2016. Coordinators: Dr. José Alexandre Felizola Diniz-Filho, Dr. Levi Carina Terribile, and Dr. Guilherme de Oliveira.

IMPACTS OF CLIMATE CHANGE AND CONSERVATION OF THE SOUTH AMERICAN CORALSNAKE *MICRURUS ALTIROSTRIS* (COPE, 1860)

Márcio Borges-Martins, Alejandro Giraudo, Juan Andrés Sarquis, Júlio Cesar de Moura-Leite, Raúl Maneyro, Guilherme de Oliveira, Levi Carina Terribile & José Alexandre Felizola Diniz-Filho.

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MEASURING THE LONG-TERM EFFECTIVENESS OF PROTECTED AREAS BASED ON HABITAT SUITABILITY: THE CASE OF THE POOR DISPERSAL CORALSNAKE *MICRURUS BRASILIENSIS* (SERPENTES: ELAPIDAE)

Cléber Ten Caten, Matheus de Souza Lima-Ribeiro, Nelson Jorge da Silva Jr., Ana Karolina Moreno & Levi Carina Terribile.

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CLIMATE CHANGE AND GEOGRAPHIC CONSTRAINTS COULD THREATEN THE DISTRIBUTION OF THE CORALSNAKE *MICRURUS BALIOCORYPHUS* (COPE, 1862) IN THE FUTURE

Alejandro Giraudo, Nelson Jorge da Silva Jr., Juan Andrés Sarquis, Guilherme de Oliveira & José Alexandre Felizola Diniz-Filho & Levi Carina Terribile.

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CLIMATE CHANGE AND RANGE SHIFTS IN THE DISTRIBUTION OF THE CORALSNAKE *MICRURUS CORALLINUS* (MERREM, 1820)

Darlan Tavares Feitosa, Paula Carolina Rodrigues de Almeida, Alejandro Giraudo, Juan Andrés Sarquis, Raúl Maneyro, Francisco Luís Franco, Renato Silveira Bérnils, Levi Carina Terribile, José Alexandre Felizola Diniz-Filho & Guilherme de Oliveira.

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CLIMATE CHANGE VERSUS ALTITUDINAL RANGE FOR THE CORALSNAKE *MICRURUS DECORATUS* (JAN, 1858)

Renato Silveira Bérnils, Júlio Cesar de Moura-Leite, Guilherme de Oliveira, José Alexandre Felizola Diniz-Filho & Levi Carina Terribile.

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FORECASTING CLIMATE CHANGE EFFECTS ON THE BRAZILIAN CORAL SNAKE *MICRURUS FRONTALIS* DUMÉRIL, BIBRON & DUMÉRIL, 1854 DISTRIBUTION

Francisco Luís Franco, Guilherme de Oliveira, Levi Carina Terribile, José Alexandre Felizola Diniz-Filho & Nelson Jorge da Silva Jr.

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REPORTING LATITUDINAL EXPANSION IN THE DISTRIBUTION OF THE BRAZILIAN CORALSNAKE *MICRURUS IBIBOBOCA* (MERREM, 1820)

Lywouty Reymond de Souza Nascimento, Nelson Jorge da Silva Jr., Darlan Tavares Feitosa, Levi Carina Terribile, José Alexandre Felizola Diniz-Filho & Guilherme de Oliveira.

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REDUCING WALLACEAN SHORTFALLS FOR THE *MICRURUS LEMNISCATUS* (LINNAEUS, 1758) SPECIES' COMPLEX: PRESENT AND FUTURE DISTRIBUTIONS UNDER A CHANGING CLIMATE

Levi Carina Terribile, Darlan Tavares Feitosa, Matheus Godoy Pires, Paula Carolina Rodrigues de Almeida, Guilherme de Oliveira, José Alexandre Felizola Diniz-Filho & Nelson Jorge da Silva Jr.

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ANTICIPATING THE IMPACTS OF CLIMATE CHANGE ON THE DISTRIBUTION OF THE SOUTH AMERICAN CORALSNAKE *MICRURUS PYRRHOCRYPTUS* (COPE 1862): IMPLICATIONS FOR ITS CONSERVATION

Alejandro Giraudo, Vanesa Arzamendia, Gustavo Scrocchi, Juan Andrés Sarquis, Guilherme de Oliveira, José Alexandre Felizola Diniz-Filho & Levi Carina Terribile.

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RANGE EXPANSION THROUGH FRAGMENTED LANDSCAPES UNDER A CHANGING CLIMATE: IMPLICATIONS FOR CONSERVATION OF THE SOUTHAMERICAN CORALSNAKE *MICRURUS SILVIAE* (DI-BERNARDO, BORGES-MARTINS & SILVA JR., 2007)

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PREDICTING SHIFTS IN HABITAT SUITABILITY FOR THE AQUATIC CORALSNAKE *MICRURUS SURINAMENSIS* (CUVIER, 1817) THROUGH THE SOUTH AMERICAN HYDROGRAPHIC BASINS DUE TO CLIMATE CHANGE

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C. POSTER PRESENTATIONS

18 OCTOBER 2016 (TUESDAY)

STANDARDIZATION AND COMPARISON OF METHODS APPLIED TO EXTRACTION OF TOTAL RNA FROM SNAKES OF THE ELAPIDAE FAMILY

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Studies that analyze the transcripts present in tissues of snakes allowed great advances in the areas of genome and transcriptome. The extraction of total RNA from venom glands of coral snakes of the genus *Micrurus* is essential to analyze the genetic profile of the toxins produced by these animals. High quality RNA molecules are essential to generate sequence data of high scientific value. In this study, adaptations were introduced in the process of RNA extraction by the method of Trizol, resulting in two modified protocols, which highlight the importance of each step in obtaining genetic material with high integrity. The objective of this study was to standardize and evaluate the methodology used for the extraction of total RNA obtained from snakes venom glands. Total RNA was extracted from venom glands harvested by 18 *Micrurus surinamensis* snakes preserved in RNAlater® (Thermo Fisher Scientific), by using the Trizol method (Thermo Fisher Scientific). Two protocols have been tested in this procedure. Protocol 1 used a Tissue Ruptor (Qiagen), keeping the samples on ice during the whole processing, and Protocol 2 followed the original method proposed by the manufacture specifications, excluding the homogenization step. RNA samples obtained by the two protocols were submitted to quantitative analysis by using the NanoDrop spectrophotometer® (Thermo Fisher Scientific) and to qualitative analysis in non-denaturant agarose gel. The obtained data were tabulated and analyzed by descriptive and comparative statistics. By comparing the same amount of venom gland tissue, samples submitted to Protocol 1 extraction resulted in an average amount higher than that obtained by the Protocol 2, with averages of 1560 ng (±366ng) and 132ng (±31, 5ng) of RNA, respectively (p= 0.0005). It is important to note that, despite the modifications introduced in the protocol, the purity of the RNA product obtained from the two groups was equivalent, with the ratios of 1.94 (0, 022ng±) and 1.96 (±0, 013ng), respectively (p= 0.55). It is concluded that the Protocol 1, which uses the homogenization step, should allow more contact of the tissue with Trizol, ensuring better tissue proteins denaturation, while low temperatures should stop the enzymatic activity of RNases, allowing higher concentrations of RNA and preserving the quality of the extracted material.

Key words: total RNA extraction; *Micrurus;* venom gland; Trizol.

PHYLOGEOGRAPHY AND DEMOGRAPHICAL HISTORY OF *MICRURUS LEMNISCATUS* (ELAPIDAE)

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Micrurus lemniscatus is a South American coralsnake species, popularly known as "coral verdadeira". It is widely distributed in seasonally dry tropical forests (SDTF), riparian forests and rainforests. The Tertiary events and the climatic oscillations of the Quaternary affected the distribution of these ecosystems changing, in turn, the distribution of animals associated to these habitats. We hypothesize that the cycles of forest expansion and contraction caused with climate fluctuations may have influenced the current distribution and genetic structure of *M. lemniscatus*. In this work, we studied the evolutionary relationships and patterns of divergence among *M. lemniscatus* lineages and infer the historical biogeographic events that influenced the distribution and genetic variation. Twenty-nine individuals of *M. lemniscatus* were sampled from 16 localities in the states of Tocantins, Bahia, Goiás, Alagoas, Mato Grosso, Maranhão, Pará and Amazônia. Three mitochondrial regions (COI, 16S and ND4L) were sequenced, and together generated a fragment of 1595 bp and 23 different haplotypes. The analyses showed an ancient lineage divergence *c.* 4.5 Ma and high genetic differentiation among populations (FST = 0.932; $p<0.01$). Bayesian Skyline Plot showed constant population size through time. Phylogenetic tree showed a spatial pattern in divergence among lineages, with a gradient of differentiation from Eastern towards the Western. However, haplotype sharing among very distant populations suggest a wider range distribution in the past and high connectivity.

Key words: Elapidae; Tertiary; Quaternary climatic changes; phylogeography; genetic divergence.

CURRENT REPRESENTATION OF CORALSNAKES (*MICRURUS*) MUSEUM VOUCHERS IN THE STATE OF GOIÁS

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Among the 33 species of coralsnakes known to occur in Brazil, four are found in the State of Goiás. We conducted a preliminary search and confirmation of the current distribution based on museum vouchers of the following herpetological collections: Centro de Estudos e Pesquisas Biológicas (CEPB), Museu Nacional do Rio de Janeiro (MNRJ), Museu de Zoologia da Universidade de São Paulo (MZUSP), and Universidade de Brasília (UnB). A total of 227 specimens were found and are related to the following species: *Micrurus brasiliensis*, *Micrurus frontalis*, *Micrurus lemniscatus carvalhoi*, and *Micrurus surinamensis*. Their distribution was plotted in maps by municipality because of the poor quality of the collecting data, which have been changing only in the last decade. An interesting result is the decline of findings of *M. brasiliensis* in the field, which might be related to land use and deforestation within its geographic range. The records of *Micrurus surinamensis* on the gallery forests of the Rio Araguaia and tributaries are also a reminder of the habitat use by this species. Not much we can say about *Micrurus frontalis* and *Micrurus lemniscatus carvalhoi* owing to their extremely wide geographical distribution within the Cerrado biome.

Key words: coralsnakes; Goiás; herpetological collections; museum.

ACTIVITY OF *MICRURUS CORALLINUS* IN CAPTIVITY

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The Biological Museum of Instituto Butantan has the mission to develop scientific dissemination, environmental education and research by means of the permanent exhibit of live animals, mainly of the Brazilian herpetological fauna. The animals are kept in terrariums based on the natural habits of each species in order to provide a suitable environment for the animals and a nice visual to the visitors. Among the species kept in captivity, independently of the intended purpose (research, exhibition, or venom production), snakes of the genus *Micrurus* present greater difficulties in adaptation. We analyzed the activity of a female *Micrurus corallinus* (SVL 790mm; tail length 75mm; mass 59g) in exhibition in Biological Museum for 18 months. The terrarium measuring 1,2 x 0,7 x 0,9m has as substrate earth, sterilized dry leaves and Pinus bark, with a water tank (40 x 30 cm; 10 cm deep) and a heating stone. Tree branches and tree trunks serving as shelter, mosses and natural plants complement the environmental enrichment. The terrarium ornamentation allows the snake to move through different substrates, in addition to providing shelter diversity and gradients of moisture and temperature. For the observations, we applied the focal animal sampling method (Delclaro, 2004) for 55 consecutive weekdays. In each day, 5 observations were done in pre-determined period (7h00-9h00; 9h00-11h00; 11h00-13h00; 13h00-15h00; 15h00-17h00), resulting 275 observations. The snake was considered hidden (under the substrate) or exposed (resting or in mobility in the terrarium). When exposed, we considered: (a) resting; (b) in mobility on the substrate; (c) in mobility in water; (d) on the higher tree trunks (resting or in mobility).Out of the 275 observations, in 88% the snake was hidden under the substrate and in 12% it was exposed allowing observations of its activity. Concerning the period of the day, the snake was more active in the early morning between 7h00-9h00 (70%). In relation to activity, in 49% of the observations the snake was in mobility on the substrate, 24% resting on the substrate, 21% in mobility in water, and 6% on the higher tree branches of the terrarium (always in the early morning). During these observations, the *M. corallinus* spent most of time hidden under the substrate. In relation to activity, the snake presented more intense mobility in the early morning, corroborating information of literature. We observed that during activity, the snake was predominantly in mobility on the substrate, occasionally exploits the water tank and, although terricolous and fossorial, it was also found on the higher tree branches of the terrarium. In continuation of this study, we will extend the observation period, analyze the influence of temperature and humidity of the terrarium on the activity of the snake, and then evaluate possible variations in activity according to the environmental enrichment interventions already scheduled for *M. corallinus* terrarium.

Key words: behavior; activity; captivity; *Micrurus corallinus*.

FEEDING PREFERENCES OF *MICRURUS CORALLINUS* IN CAPTIVITY

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The Biological Museum of Instituto Butantan has the mission to develop scientific dissemination, environmental education and research by means of the exhibit of live animals. The animals are kept in terrariums based on natural habits of each species in order to provide a suitable environment for the animals and a nice visual to visitors. Among the species kept in captivity, snakes of the genus *Micrurus* present greater difficulty in adaptation and their natural food is not easily obtained. Feeding records of two specimens of *Micrurus corallinus*, one female (SVL 790mm; tail length 75mm; mass 59g) and one male (SVL 565mm; tail length 85mm; mass 52g), kept in the Biological Museum. As food, they received snakes of the animal reception sector of Instituto Butantan. *M. corallinus* female: During 18 months, 67 snakes were given as food, with 64.2% of acceptance and 35.8% of rejection. In all rejection cases, the female subject was in ecdysis period. We witnessed successful predations on 8 dipsadid species. We did not observe preferences according either to prey's dentition, ingesting both aglyphous and opistoglyphous snakes, or to prey's habits, ingesting both terricolous and fossorial snakes. The only prey rejected by the female subject out of the ecdysis period was the exotic species *Pantherophis guttatus,* offered in 5 opportunities; in one day a *P. guttatus* was offered and rejected but when one *Oxyrhopus guibei* and one *Sibynomorphus mikaniii* were offered both were promptly taken. *M. corallinus* male: During 14 months, 46 snakes were given as food, with 69.6% of acceptance and 30.4% of rejection. We did not find relation between food rejection and ecdysis periods. Just as the female subject, the male took as food various dipsadid species, but it rejected opisthoglyphous snakes in six feeding attempts with *Philodryas patagoniensis*. Male subject also rejected the exotic species *Pantherophis guttatus* whenever offered. Opisthoglyphous snakes given as food to *Micrurus* defend themselves biting and possibly inoculating secretions of Duvernoy glands, which turns the predation longer and with greater energy expenditure by the predator. We observed that the female subject takes opisthoglyphous snakes as food, in most cases being bitten at the beginning of the predatory behavior. We also observed that the prey rapidly ceases the defensive behavior probably due to venom inoculation by the *Micrurus* during the capture. The male subject has until now rejected opisthoglyphous snakes, taken only aglyphous snakes which do not display defensive behavior during ingestion. We observed both male and female subjects rejecting *Pantherophis guttatus*, aglyphous exotic species, maybe because *M. corallinus* did not recognize it as food. On the other hand, a new coming *M. corallinus* has eaten this exotic species, so that it may have individual variation concerning the refusal of some species. We will continue this study in order to shed light on the feeding preferences of *M. corallinus* aiming to expand knowledge on the biology of the species and to improve the captive snake welfare in the Biological Museum.

Key words: diet; feeding preferences; captivity; *Micrurus corallinus*.

COLLECTION PROTOCOL AND SEMINAL EVALUATION OF CORAL SNAKE, *MICRURUS CORALLINUS* (SNAKES: ELAPIDAE)

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The assisted reproduction is a useful method for the conservation of endangered species, as well as for the expansion squad of animal breeding in captivity, like *Micrurus corallinus*, a commom coralsnake used in venom extract for serum production at Butantan Institute. The collection and evaluation of semen are the main steps for any program of assisted reproduction, because they allow the selection of the best breeding males as the preservation of genetic material. Thus, this work was aimed to test a protocol for collection and performing evaluation of seminal quality in *Micrurus corallinus*. The collection was made in an adult specimen, held in captivity in the Biologic Museum Biotery of Butantan Institute (SISBIO 25650-1), in February. First, the animal was physically restrained and submitted to a local anesthesia to relax the cloaca region (Lidocaine 1% 15mg/kg) Then digital massages were performed in the third ventral posterior of the animal for the semen to be expelled. The seminal material was collected directly from the urogenital papilla with microhematocrit capillary and macroscopically to analyze its volume, aspect and coloration. After, part of the semen was diluted in culture means of HAM-F10 cells, for motility and progressive motility evaluation. The other part was diluted in formal saline solution for concentration and morphology evaluation of the cell. The semen of *Micrurus corallinus* shows whitish color and thick consistency. Its volume was 3,0 μL with 85% of mobility and 70% of progressive mobility. The sperm of *M. corallinus* has thread-like format and elongated, slim and pointed head, following the morphological pattern described to other Squamata. The semen concentration was $0,33x10^9$ sptz/mL. These results demonstrate the applicability of the technique of digital massage for viable sample collection of *M. corallinus* semen and for artificial insemination programs and seminal preservation of this specie.

Key words: semen analysis; reptiles; snakes; andrology; spermatozoa.

SEXUAL MATURITY IN AMAZONIAN SNAKES OF THE GENUS *MICRURUS:* WEARING THE SNAKE'S SHOES

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Sexual maturity in snakes preserved on scientific collections is characterized in males by the presence of sperm in convoluted vas deferens and in females by presence of follicles in secondary vitellogenesis, eggs or embryo in the oviduct or by the presence of distended uterus which indicates post parturition or oviposition. However these parameters are not always consistent with the real reproductive condition of the individual, which can lead to misinterpretations. Thus, this work shows morphological and histological criteria to assess sexual maturity of Amazonian coral snakes *Micrurus hemprichii, Micrurus lemniscatus lemniscatus* and *Micrurus lemniscatus diutius*. For this, data were collected from specimens of scientific collections in Northern Brazil, from which the snout-vent length (SVL) and the length of the largest follicle in secondary vitellogenesis were measured. Small fragments (approximately 5 mm) were collected from testis, vas deferens, kidneys, ovarian follicles and oviduct for the preparation of histological slides in HE. The males considered mature had at least one of the following parameters: testicular activity, kidney sexual hypertrophied segment or presence of sperm in the vas deferens. The females considered mature had follicles in secondary vitellogenesis and/or presence of ovidutal secretion. For *M. hemprichii* the smallest mature male had 328 mm SVL (median 479 mm; range 328–679 mm; N = 30) and the smallest female 439 mm SVL (median 460 mm; range 439–510 mm; N = 9). For *M. l. lemniscatus* the smallest mature male had 461 mm SVL (median 766 mm; range 261–1388 mm; N = 44) and the smallest female 468 mm SVL (median 690 mm; range 342–1001 mm; N = 23). For *M. l. diutius* the smallest mature male had 486 SVL (median 788 mm; range 409–1051 mm; N = 13) and the smallest female 551 mm SVL (median 735 mm; range 551–1036 mm; N = 11). The females of *Micrurus* in this study showed that sexual maturity was achieved later than males and reached higher body length, which allows generating more descendants; however, the greater length in males allows dominance and victory in male-male ritual combats. The species with relatively larger SVL take longer to reach sexual maturity and the dimorphism may be connected to two evolutionarily stable reproductive strategies.

Key words: reptiles; snakes; testes; follicles.

DAILY ACTIVITY IN *MICRURUS CORALLINUS*

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Micrurus corallinus is endemic to the Brazilian Atlantic Forest, being widely distributed through this biome. Even though some aspects of the natural history of this species have already been studied, such as reproduction, ecology, and seasonal activity, data about its daily activity are still lacking, since most information come from punctual observations in the field. In the current study, individuals of *M. corallinus* were observed using closed-circuit television system during ten consecutive days. Juveniles from a same brood (n=5) and adults (n=5) were video-recorded. During the experiments, the animals were not fed, water was offered *ad libitum*, and they were kept in terraria, individually, maintained in an isolated room at Instituto Butantan. The experiments took place during the spring and summer in 2012, 2013 and 2014. Both adults and juveniles *M. corallinus* showed bimodal activity, with one peak in the morning and another peak in the afternoon, and reduction of the activity around noon. The only exception was one of the juveniles, which showed unimodal pattern, being active from early morning to early evening. Nevertheless, juveniles showed greater activity than adults (x^2 = 102.123; p<0,001), and 92.2% of this activity was concentrated in the diurnal period, against 74.8% of adults' activity. Two out of the five adults observed were postpartum females, and one of them was also observed during pregnancy. During this period, there was a displacement on the activity peaks from dawn to early morning, and early evening. This is noteworthy, since during these periods the risk of being predated by visual predators could be reduced, and it is even more adaptive considering that, because of the pregnancy, the female's locomotion could be compromised. The general pattern of activity obtained herein is in accordance with those previously reported, from observations in the field. Despite the fact that *M. corallinus* and *M. frontalis* belong to different groups (monad-group and triad-group, respectively), they present a similar pattern of daily activity, according to other studies also conducted by our research group. Up to now, no studies comparing differences due to reproduction or ontogeny upon daily activity pattern in *M. corallinus* had been made. However, since the temperature was not maintained constant during the experiments, and the animals were not recorded at the same period, it cannot be affirmed that the differences in activity of adults and juveniles arise exclusively from ontogeny. Nevertheless, the different pattern shown by one of the juveniles is noteworthy, especially considering that all of them were littermates. Future studies with individuals recorded in controlled conditions of temperature could help elucidating better these questions.

Key words: daily activity, natural history, animal behavior, coralsnakes.

EVALUATION OF THE CORAL SNAKES SURVIVAL MAINTAINED INTO A NEW EXPERIMENTAL VIVARIUM BASED ON ISOLATED SYSTEM

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The maintenance of many coral snakes species on captivity is a difficult task due the challenges to supply the biological conditions required for their survival. *Micrurus corallinus* is the most coral snake specie found at state of Rio de Janeiro, Brazil. This specie is small in size, fragile and suffers from high mortality after few months kept in traditional captive (closed plastic boxes with small holes). For change this scenario we developed an experimental vivarium at Vital Brazil Institute to set the basic conditions to increase longevity of coral snakes maintained in captivity. For this purpose we manufactured isolated systems adapting a ventilated rack and cages designed for mouse's micro-isolation. The cages are interconnected and docked on a rack to keep an environment controlled via regulation of airflow, air clearance via filtration, temperature (22°C) via an air conditioner inside the room and humidity (high and low) via variation the ratio of water/substrate into the cage. The feed was done every 15 days via gastric probe feeding the animals with a soft food made by liver, eggs and vitamins complex (Hemolitan®). We measured the time of survival, weight variation and venom production of 39 coral snakes keeping in this system. The species analysed was M. *corallinus* (N=32), *M. decoratus* (N=1), *M. lemniscatus* (N=3) and *M. frontalis* (N=2). The amount of food was calculated by the weight of the snake. For *M. corallinus* and *M. decoratus* the amount of food was based on 25% of animal's weight while 20% for *M. frontalis* and *M. lemniscatus*. The animals were separated in three groups; *M. corallinus* (A<15g>B) and triadal specimens (C = *M. frontalis*, *M. lemniscatus* and *M. decoratus*). The mortality rate of total coral snakes was 55,5%. Nevertheless, the A group (N=11) was 88,8% while B group (N=21) was 47%. The mortality rate of triadal specimens was 14,2% (N=7). In *M. corallinus*, the groups A and B showed the weight variation near 15% and 17%, respectively. The group C reached 22% with a trend toward gain of weight. The average of survival rate was 8,3; 13,5 and 8,2 months in A, B e C, respectively. Regarding the survival utmost time, six specimens reached 24 months alive in this system and four of them keeping alive. Our results suggest a better adaptation for triadal coral snakes than *M. corallinus* groups. In *M. corallinus*, the group B showed the highest survival rate and maximum time of life in captivity. The animals below 15g showed a high rate of mortality arguing the necessity for adjusting in this system for maintenance juveniles coral snakes. However, the group B, which includes the specimens used in the venom production, showed higher survival rate than traditional procedures. The elongation of lifetime in the heaviest specimens of *M. corallinus* in captivity allowed us the production, in one year, of 45,8mg of its venom.

Key words: *Micrurus;* biology; handling snakes; venom production.

THE NEUROTOXICITY OF *MICRURUS LEMNISCATUS LEMNISCATUS* (SOUTH AMERICAN CORAL SNAKE) VENOM

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The South American coralsnake (*Micrurus lemniscatus lemniscatus*) occurs in French Guyana, Guyana, Suriname and the northern Brazilian Amazon states of Amapá, Maranhão and Pará; this species can cause envenomation in humans. In this work, we used myographic and electrophysiological methods to examine the neuromuscular activity of *M. l. lemniscatus* venom in chick biventer cervicis (BC) and mouse phrenic nerve-diaphragm (PND) preparations. PND preparations suspended in Tyrode solution and BC preparations suspended in Krebs solution were incubated with venom (0.1-30 μg/ml, n=4) or physiological solution alone (n=4). Contractile responses to exogenous acetylcholine (ACh, 1 mM), carbachol (CCh, 20 μM) and KCl (40 mM) were obtained in BC preparations. This work was approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 3477-1). Venom (0.1-30 μg/ml) caused concentration-dependent neuromuscular blockade in BC preparations that was maximal at 10 μg/ml (times for blockade with 1 and 10 μg/ml: 50% – 21.7±4.2 min and 6.5±0.6 min; 90% – 42.1±9 min and 13.1±0.6 min; n=4); muscle contractures to exogenous acetylcholine and carbachol were completely abolished by concentrations ≥0.3 μg/ml, but there was no significant alteration in contractures to KCl. In PND preparations, venom caused irreversible time- and concentration-dependent neuromuscular blockade in normal Ca^{2+} solution, with complete blockade at >10 μg/ml within 60 min (times for blockade with 3 and 30 μg of venom/ml: 50% – 32.2±7.6 min and 8±1 min; 90% – 54.1±5.4 min and 16.2±1.6 min; n=4). In low Ca^{2+} solution, the neuromuscular blockade by 10 μg/ml occurred in ~10 min and was reversible by washing; the addition of Ca^{2+} immediately after blockade temporarily restored the twitches but did not prevent the progression to irreversible blockade in ~30 min. *M. l. lemniscatus* venom (3 μg/ml) had a biphasic effect on the frequency of spontaneous miniature end-plate potentials (MEPPs)/min (from 32.6 ± 2.2 (t_0) to 49.3 ± 4.4 (t_{15}) and 19.9 ± 5.2 (t_{60}); $p<0.05$, n=5) and reduced the decay-time of the potentials (from 6.31±0.69 ms (t_0) to 3.40±0.62 ms (t_{60}), $p<0.05$, n=5); venom (10 μg/ml) did not alter the diaphragm muscle resting membrane potential (DRMP) (-85.8 ± 2.7 mV vs. -82 ± 0.8 mV after 60 min; n=3) and did not affect the compound action potential (CAP) of mouse sciatic nerve, except for a slight decrease in amplitude (from 10.7 ± 1.2 mV (t_0) to 8.8 ± 1.3 mV (t_{30}), n=4). The venom had PLA_2 activity that was Ca^{2+}-dependent (activity, expressed as nmol of HCl formed per min: 28.6±0.51 and 2.3±0.0 in normal and low Ca^{2+} buffer, respectively, for 100 mg of venom/ml at 37 ºC, $p<0.05$, n=5). *M. l. lemniscatus* venom produced potent neuromuscular blockade in both vertebrate preparations, with BC being more sensitive. The biphasic effect on the frequency

of spontaneous MEPPs and the irreversible Ca^{2+}-dependent blockade in PND preparations suggested the presence of presynaptic toxins in this venom. The lack of effect on CAPs and DRMP indicated that the neurotoxicity of *M. l. lemniscatus* venom was restricted to motor end-plates.

Key words: electrophysiology; neurotoxicity; neuromuscular junction; PLA_2; presynaptic; postsynaptic.

19 OCTOBER 2016 (WEDNESDAY)

BEING A BRIGHT SNAKE: TESTING APOSEMATISM AND MIMICRY IN A NEOTROPICAL FOREST IN SOUTH AMERICA

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Polymorphisms provide an excellent experimental approach for investigating whether there is a selective advantage to certain morphs in putative mimics. Based on phenotypic and behavioral similarities, it has been suggested that the highly venomous coralsnake *Micrurus corallinus* (Elapidae) may act as a model to two polymorphic species, *Erythrolamprus aesculapii* (Dipsadidae) and *Micrurus decoratus* (Elapidae). Plasticine replicas adult and juvenile-sized were used to investigate the warning coloration of such coralsnake patterns in two Atlantic Forest localities at Serra do Mar, Juquitiba and Santa Virgínia. The results demonstrate that, besides aposematism, the importance of the multiple effects of the coralsnake pattern concerning protection against predation must be considered. The coral pattern showed to be aposematic, when compared to a brown control. However, the existence of the hypothesized mimetic complexes themselves is not completely supported. *Micrurus corallinus* pattern do, indeed, confer higher protection against predation than that of *Erythrolamprus aesculapii* with dyads in Juquitiba, albeit this protection is restricted to adult-sized replicas. Nevertheless, in Santa Virgínia, *Micrurus corallinus* replicas are attacked more frequently by mammals, showing no support for this species to act as a model in a mimetic complex at this specific locality. Most importantly, the data demonstrate that the protectiveness provided by the pattern may vary according to the predators considered and the body size of the snakes, and, therefore, these aspects should always be considered when investigating mimicry in coralsnakes.

Key words: Atlantic Forest; colour pattern; defence; *Erythrolamprus aesculapii*; *Micrurus corallinus*; *Micrurus decoratus*.

MAINTENANCE OF CORALSNAKES (*MICRURUS*) IN CAPTIVITY AT THE FUNDAÇÃO EZEQUIEL DIAS – MINAS GERAIS, BRAZIL

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The Fundação Ezequiel (FUNED), located in Belo Horizonte, Minas Gerais, is linked to the Department of Health of Minas Gerais, as an institution that provides services to health, science and technology for the state and the country. This institution performs functions such as the production of antivenom, antiviral and antitoxic hyperimmune serum; laboratory support for activities of epidemiological and health surveillance; development, encouragement and participation in scientific research of social interest, aimed at the production of biotechnological, prophylactic and therapeutic products and the formation and training of human resources in the field of public health. The Poisonous Animals Service is responsible for the production of antigens and poisons for the production of antivenom sera including the bivalent antielapidic of *Micrurus frontalis* and *Micrurus corallinus*. Animals received by donation are subjected to screening (species, biometrics, and medical condition) and sent to quarantine where they undergo a period of adaptation, clinical and sanitary control and microchipping. If approved, the animal goes to permanent captivity, which will be integrated into the venom extraction program and where are also observed all animal welfare standards with air-conditioned and monitored environment. Animals that die are sent to the scientific collection of snakes. We end up with healthy, active, able to reproduce and long-lived animals, and with high venom production capacity production of venom, with the appropriate traceability. Vivarium in closed rearing system, is controlled by IBAMA, IEF, CONCEA and CRMV standards.

Key words: antivenom; epidemiological; environment; venom.

MIMETIC RELATIONSHIPS BETWEEN *ATRACTUS LATIFRONS* (GÜNTHER, 1868) (SERPENTES: DIPSADIDAE) AND TRUE CORALSNAKES IN THE AMAZON

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Atractus latifrons is a species of false coralsnake, presenting different color patterns, similar to the color pattern of true coralsnakes of the genus *Micrurus*, which co-occur throughout its distribution in the Amazon biome. In the current study the morphological similarities and consistent geographic distribution of these species were tested mimetic existence of relationships between the color patterns of *A. latifrons* (bicolor in monads, tricolor in dyads and tricolor in tetrads) and color patterns of species of *Micrurus* with Amazon occurrence (*Micrurus albicinctus*, *M. averyi*, *M. diutius*, *M. filiformis*, *M. langsdorffi* and *M. lemniscatus*). Based on co-occurrence test, we tested the hypothesis that the distribution models mimics and may or not be explained at random. To estimate the potential geographic distribution of species of coral snake and each chromatic pattern *A. latifrons*, 12 algorithms of ecological niche modelling were used, including the simplest methods of presence (eg. BIOCLIM) and more complex methods of presence and absence based on artificial intelligence (eg. artificial neural networks). Climate variables were obtained from five climate models of global circulation. For the modelling, the trigger points were randomly divided into two groups: 75% for training and 25% for the test with 50 repetitions. Pseudo-absences were randomly selected in modelling in proportion to the number of attendance for each species (50% prevalence of presence data and pseudo-absence). From 60 distribution models (12 x 5 algorithms climate models) generated for each type of *Micrurus* and for each chromatic pattern *A. latifrons*, the estimated potential distributions by the ensemble forecasting approach. The environmental suitability maps of each species were used in the co-occurrence analysis. From the co-occurrence test were recognized six species of true-coral-snakes mimetic capable of serving as templates for *A. latifrons*, among these, the existence of mimetic relationships was confirmed only in two species, *M. diutius* and *Micrurus lemniscatus*.

Key words: Amazon; coralsnakes; ecological niche modelling; mimicry.

VENOMICS OF CORALSNAKES OF CLINICAL IMPORTANCE FOR COLOMBIA

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In Colombia, about 2.8% of 4200 snakebites reported annually are caused by snakes of the genus *Micrurus*, known as coral. *Micrurus mipartitus* species and *M. dumerilii* have a wide distribution in the country and probably are main responsible of these accidents. The aim of this work was to determine the composition and biological activities of these venoms. The methods included RP-HPLC, SDS-PAGE and MS/MS MALDI or ESI combined to evaluate the proteomic profile. The results showed similarities in the venoms especially in the most abundant proteins, the three finger toxins (3FTx), phospholipase A_2 (PLA_2), L-amino acid oxidases, Metalloproteinases P-III, Kunitz inhibitors, serine proteases and lectins type-C were in both venoms. However, it was the amount of two of them showed differences related to abundance, thus 3FTx were in greater proportion in the venom of *M. mipartitus* (60%), than for *M. dumerilli* (30%); on the contrary in the *M. dumerilii* predominated PLA_2 (52.0%). These profiles showed similitude to recently proposed *Micrurus* venoms phenotype whose venom *M. dumerilii* corresponds to "Rich in PLA_2"and *M. mipartitus* to "Rich in 3FTX ". Additionally, in the venom of *M. dumerilii* low amounts of other protein families (hyaluronidases, B phospholipases, phosphodiesterases and 5'-nucleotidase) were found. *M. mipartitus* venom showed greater toxicity with an LD_{50} of 0.47μg / g that of *M. dumerilii* of 1.18μg / g, and one of their toxins had a LD_{50} less than 0.10μg / g. However *M. dumerilii* demonstrated a conspicuous myotoxicity, cytotoxicity and anticoagulant effect, but low edematous and proteolytic activity. An equine antivenom produced with the venom of *M. nigrocinctus* of Central America, did not demonstrate cross reaction with the components of the venom of *M. mipartitus*. By the contrary the antivenom inmunorecognized all proteins of M. *dumerilii* venom, which would explain the neutralization ability of their lethality; suggesting that this antivenom can be used for the treatment of a envenomation caused by *M. dumerilii*, but not in *M. mipartitus* cases. Consequently, this study showed the relevance of including both venoms in immunizing mixture or use a combination of the main toxins in the production of a polyspecific antivenom to Colombia.

Key words: coralsnakes; *Micrurus*; venom; snakebite.

COMPARATIVE NEUROTOXICITY AND CYTOTOXICITY OF SOME BRAZILIAN CORALSNAKE VENOMS

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Neurotoxicity is the hallmark of coralsnake (*Micrurus* spp.) venoms and is mediated by presynaptic (PLA_2) and postsynaptic (three-finger toxins; 3-FTx) neurotoxins. In this work, we compared the neuromuscular activity of four *Micrurus* venoms (*M. surinamensis*, *M. frontalis*, *M. brasiliensis* and *M. spixii*) in chick biventer cervicis (BC) and mouse phrenic nerve-diaphragm (PND) preparations and examined their cytotoxicity (and that of *M. lemniscatus lemniscatus*) in SH-SY5Y neuroblastoma cells. PND preparations suspended in Tyrode solution and BC preparations suspended in Krebs solution were incubated with venom (0.1-30 μg/ml, n=3) or physiological solution alone (n=6). Contractile responses to exogenous acetylcholine (ACh, 1 mM), carbachol (CCh, 20 μM) and KCl (40 mM) were obtained in BC preparations. The animal experiments were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 3477-1). For cytotoxicity, adherent SH-5HSY cells in 96-well plates were incubated with medium alone (negative control), 1% Triton-X 100 (positive control) or venom (0.1-30 μg/ml) for 24 h prior to the neutral red assay. The PLA_2 activity of the venoms was assayed colorimetrically based on phosphatidylcholine hydrolysis. All venoms produced concentration-dependent neuromuscular blockade in both preparations. The minimum concentrations for partial blockade in PND preparations after 60 min were 3 μg/ml (*M. surinamensis* - 44±7%) and 10 μg/ml (*M. brasiliensis* - 88±3%; *M. frontalis* - 32±2%; *M. spixii* - 58±3%), while the times for 90% blockade (t_{90}) with 30 mg/ml were 11.5±0.8 min (*M. surinamensis*), 16.1±1.3 min (*M. brasiliensis*), 30.1±3.8 min (*M. frontalis*) and 53.9±6.9 min (*M. spixii*). In BC preparations, the minimum concentrations for partial blockade were 0.3 μg/ml (*M. brasiliensis* - 26±7%; *M. surinamensis* - 53±11%) and 1 μg/ml (*M. frontalis* - 53±22%; *M. spixii* - 26±7%), while the t_{90} with 30 mg/ml were 7.6±1.4 min (*M. surinamensis*), 10.0±1.5 min (*M. brasiliensis*), 11.7±2.0 min (*M. frontalis*) and 18.5±5.5 min (*M. spixii*). At 10 μg/ml, the neuromuscular blockade produced by *M. spixii*, *M. brasiliensis* and *M. frontalis* in PND was partially reversed by washing; at 30 μg/ml, only blockade by *M. spixii* was irreversible. *M. surinamensis* was the only venom to produce blockade totally reversible by washing. None of the venoms reduced the contractures to exogenous KCl, but those to ACh and CCh were inhibited by 33-100% at venom concentrations of 0.3-3 mg/ml; in all cases, concentrations >3 mg/ml caused complete inhibition. Incubation of SH-5HSY neuroblastoma cells with *Micrurus* venoms (0.1-30 mg/ml) resulted in a concentration-dependent decrease in cell viability after 24 h. The minimum cytotoxic concentrations for the venoms were 0.3 mg/ml (*M. brasiliensis*), 1 mg/ml (*M. l. lemniscatus*), 3 mg/ml (*M. spixii*), 10 mg/ml (*M. surinamensis*)

and 30 mg/ml (*M. frontalis*). For 30 mg/ml, viability was 32±2% (*M. brasiliensis*), 31±4% (*M. l. lemniscatus*), 17±3% (*M. spixii*), 13±2% (*M. frontalis*) and 9.8±2.3% (*M. surinamensis*) ($p<0.05$ in relation to negative control group; n=5). All venoms except for *M. surinamensis* showed PLA_2 activity, yet *M. surinamensis* was the most cytotoxic venom at 30 μg/ml. All of the venoms tested were neurotoxic and cytotoxic. BC preparations were the most sensitive to blockade and *M. surinamensis* venom was the most active. None of the venoms impaired KCl-induced contractures, indicating the absence of major muscle damage. Inhibition of the responses to ACh and CCh was suggestive of nicotinic receptor blockade by 3-FTx. The greater neurotoxicity and cytotoxicity of *M. surinamensis* venom is noteworthy as this species differs from other *Micrurus* spp. in its venom composition and in being aquatic and feeding primarily on fish.

Key words: cytotoxicity; neurotoxicity; neuromuscular junction; presynaptic; postsynaptic.

RECOGNITION CAPACITITY OF ANTI-VENOM PRODUCED IN BRAZIL AGAINST *MICRURUS* VENOMS AND PROTEOMIC PROFILES OF VENOM FROM *NAJA KAOUTHIA* AND SIX SPECIES OF *MICRURUS* (SERPENTES, ELAPIDAE)

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Anti-venom production in Brazil has improved through the implementation of new processes and good manufacturing practices over the last 15 years. For anti-elapidic therapies, mixtures of *M. frontalis* and *M. corallinus* venoms are used for horse immunization that includes venom from coralsnakes, which are responsible for most bites in South and Southeastern Brazil. Related to the effective against snake bites in these regions, some studies suggest that the anti-elapid sera produced in Brazil show more variable capacity to neutralize snake bite effects in other regions of Brazil. Through a critical examination of the clinical specificity, safety and efficacy of anti-venoms, further advancements can be achieved in anti-venom performance. A comparative analysis of the immuno-recognition profile of anti-elapidic serum manufactured by I. Butantan and Funed from Brazil against venoms from six species, whose habitats cover most regions of Brazil, of the genus *Micrurus* (*M. brasiliensis, M. frontalis, M. lemniscatus, M. spixii, M. surinamensis, M. corallinus*) along with venom from *Naja kaouthi* found in southeast of Asia and housed in herpetariums worldwide. Venoms (~25 μg) from each species were fractionated by SDS-PAGE (15%) and visualized by coomasie blue or silver stain. The recognition of proteins in venoms by anti-elapidic hyperimmune sera produced by I. BUTANTAN and FUNED was determined by western blotting using a 1:1000 dilution of the sera followed by detection with mouse anti-horse IgG-HRP using an immunoblotting kit (Bio-Rad®). The banding pattern of proteins in the seven venoms separated by SDS-PAGE showed prominent clusters at MWs of 6-8 kDa (3FTx) and 12-14 kDa (PLA2). By western blotting, the anti-elapid sera recognized proteins in all venoms from the *Micrurus* species in the 12-14 kDa region. The venom from *N. kaouthi* also showed proteins in this size range, but no recognition by the anti-venom was observed. Recognition was also not detectable for the 31 kDa protein in *M. corallinus* venom or the abundant 45 kDa fraction *M. surinamensis* venom as well as the same proteins in the venom from *M. lemniscatus*. The results are consistent with the reported in vivo neutralization assays that have shown the national anti-elapidic sera is not capable to fully neutralizing the venoms of *M. lemniscatus*. Anti-elapidic sera produced by both I. BUTANTAN and FUNED have similar performance in recognizing, or not, the various proteinaceous components of the studied elapid venoms. Recognition and non-recognition correlate with their efficacy or inefficacy, respectively, to complete neutralization the effects of snake bites by different species. The absence of fully neutralization of venoms from species

whose venom is not included in the horse immunization mixtures strongly demonstrates the uniqueness of the protein profile in the venom of a species. Generation of effective antivenoms against an individual species most likely requires the inclusion of its venom in the mixture used for immunization.

Key words: elapid venom; western blotting; protein profile; antielapidic serum.

IDENTIFICATION AND CHARACTERIZATION OF SIMPLE SEQUENCE REPEATS (SSR) IN GENOME OF *MICRURUS SURINAMENSIS* (SERPENTES: ELAPIDAE)

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Micrurus surinamensis is a species of coralsnake that has aquatic and semi-aquatic habits, feeding preferably on fish. It is found in flooded forests and streams (main forest area) of the Amazon regions of Bolivia, Colombia, Ecuador, Guyana, Peru, Venezuela and Brazil, it occurs in the states of Acre, Amapá, Amazonas, Goiás, Maranhão, Mato Grosso , Pará, Rondônia, Roraima and Tocantins. *Micrurus surinamensis* has a highly toxic poison, leading to a wide spectrum of pharmacological activities and for these reasons, it is very important for the medical field. Next generation sequencing platforms are excellent tools for genomic studies and in last years have been widely used for the new sequencing fauna species. This study aimed at identifying and characterizing simple sequence repeats (SSR) in the genome of *Micrurus surinamensis*. The DNA sample was extracted from tissue of an individual *M. surinamensis* coming from Altamira (PA). Genomic libraries were prepared by following the protocol Nextera and sequenced using a illumina Sequencer. The quality of the *reads* was evaluated and the assembly of genomic sequences was performed with the software Newbler version 2.3. The simple sequence repeats (SSR) were identified using the QDD software. The minimum number of repeats for each motif size was 35 for di-nucleotide, 18 to tetra-nucleotide and 14 to penta-nucleotide. Tri- and hexa-nucleotides were not considered in this analysis because the likelihood of being in gene context. The draft assembly genome of *M. surinamensis* comprised 1,473,694 scaffolds with N50 of 12,700 bp. The size of the largest and the smallest scaffolds, as well as the total size of the scaffolds, were 2164465761 bp, 200 bp and 169,236 bp, respectively. In total, 67,348 SSR regions were found, of which the most common motif were the penta-nucleotides (56.08%), followed by tetra-nucleotides (38.45%) and di-nucleotides (5.46%). The most frequently repeat motifs were GAATA (6.5%) and TATTC (6.4%). The number of times the simple motif is repeated varied between 14 and 100 times. Simple sequence repeats (SSR) identified may be used for future development of microsatellite markers may be useful in population genetic studies, phylogeography and phylogeny of the genus *Micrurus*.

Key words: coralsnake; genomic; next generation sequencing; SSR.

ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN MICE TISSUES IN RESPONSE TO THE ENVENOMING CAUSED BY *MICRURUS CORALLINUS*

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Micrurus corallinus is a coralsnake widely distributed in Central and South America. Although, the reported cases of bites by *M. corallinus* are not numerous, envenoming could be fatal due to respiratory arrest, leading to the death of the victims. The effects observed are elicited by potent neurotoxins present in the venom, acting pre- and post-synaptically in neuromuscular junction. Differential gene expression by RNA-Seq analysis becomes a powerful strategy for studying the molecular mechanisms underlying pathological effect of toxins, being a suitable approach for the study of snake envenoming, *in vitro*, as well as *in vivo*. The goal of this study is to evaluate the differential gene expression profiles of different tissues in animals treated with a sublethal doses of *Micrurus corallinus* venoms, in order to investigate the molecular pathways affected by the envenomation. Male Swiss mice (30-35g) were injected into the right gastrocnemius with *M. corallinus* venom, at a concentration of 50% of the DL_{50}, previously determined. Control mice were treated similarly with a sterile saline solution. The animals were euthanized after 8 and 24 hours of the treatment and the brain, kidneys, liver and gastrocnemius muscles were rapidly removed, frozen in liquid nitrogen and stored at -80°C until processed. Total RNA was isolated from the organs and tissues using Trizol reagent. Afterwards, the samples were assessed for quality, by electrophoresis (Bioanalyzer), quantified by spectrofluorimetry (kit Quant-iTTMRiboGreen) and submitted to sequencing library construction following the protocol for TruSeq® Stranded mRNA Sample Preparation Guide from Illumina. Sequencing was performed in an Illumina HiSeq1500 instrument, at a 2 × 93bp paired-end resolution. The raw sequencing reads from sixty libraries were pre-processed by an "in house" pipeline to trim and remove reads with low-complexity and homopolymer enriched regions, poly-A/T/N tails, the adapter sequences and low-quality bases with the software fastq-mcf 1.04.662 and bowtie2 2.2.5. Sequenced reads were mapped to the mouse reference genome using TopHat2. 25,000,000 reads per sample were randomly selected for downstream analysis. To determine the differential gene expression profile in the five samples, two statistical tools were applied: DESeq and edgeR. For a four-fold change cutoff, a total of 2,281 genes were found to be differential expressed using edgeR, whereas DESeq identified 1,895 genes, being 406 genes unique for the former and 20 for the latter. Genes involved in inflammation process, such as Saa1, Saa2 (serum amyloid A1 and 2) and Serpinb2 (serine or cysteine) peptidase inhibitor, clade B, member 2) were found highly differentially expressed. Accordingly, preliminary data from an enrichment analysis carried out for liver and gastrocnemius tissues samples, suggest that the main pathways involved are related to acute immune response. In recent years, NGS strategies are being used in toxicology studies, coining the term "Toxicogenomis". By employing a high-throughput technology and a

comprehensive systems biology analysis (MetaCore), we intent to demonstrate the feasibility of toxigenomic studies to investigate the organism response to envenomation and to gain a deeper knowledge in the mode of action of *M. corallinus* venom.

Key words: differential gene expression; *Micrurus corallinus;* RNA-Seq; snake envenoming.

ENVENOMATION BY BRAZILIAN CORALSNAKE, *MICRURUS DECORATUS* (JAN, 1858) (SERPENTES: ELAPIDAE): A CASE REPORT

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Coralsnake (Elapidae) bites are uncommon in Brazil, accounting for only 1% of all officially reported cases in 2014. Accidents with corals display neurotoxic, myotoxic, nephrotoxic, hemorrhagic, edematogenic and, sometimes, hemolytic activities. The most evident symptoms in human envenomation involves systemic neuromuscular blockage, causing an acute myasthenic syndrome with variable clinical spectrum, which can evolve to acute respiratory failure and death. Despite the high diversity and wide distribution of elapid snakes in Brazil (40 taxa related to the genera *Micrurus* and *Leptomicrurus*), the existing information about coralsnake bites is only restricted to a quarter of the species/subspecies recorded in Brazil. We present here the first report of human envenomation caused by *Micrurus decoratus*. This report refers to a male patient, 27 years old, healthy, bitten on the right ankle by inadvertently stepping on the animal while walking on his sandals on a grassy area in Tijucas do Sul municipality, metropolitan region of Curitiba, Paraná, Brazil. The patient sought treatment immediately after the bite, bringing the snake with him. The animal was later incorporated into the herpetological collection of Museu de História Natural Capão da Imbuia, Curitiba (MHNCI 16537, young male, 284 mm total length). The patient reported intense local pain immediately after the bite, which spread on the calf, accompanied by paresthesia. The symptoms progressed, reaching within minutes the left leg to the thigh root, and the right upper limb, hand to shoulder. Within thirty minutes after the bite, dyspnea, tachycardia and a feeling of heavy eyelids were recorded, although the patient denied the presence of blurred vision, diplopia, or pronounced palpebral ptosis. The patient was lead to an emergency care unit, arriving within four hours of evolution in his clinical condition. Upon arriving, there were reports of pain and paresthesia in right side upper and lower limbs. In addition, discrete marks of the bite were noted, without active bleeding or perilesional edema. It was recorded heart rate of 154 bpm, blood pressure 118X84 mmHg, and Eupnea (saturating at 96% in ambient air), normal heart and lung auscultation. Ten vials of divalent antielapidic serum were administered IV (immunoglobulins for venoms of *Micrurus corallinus* and *M. frontalis*), preceded by ranitidine (1ml / 25 mg), promethazine (50 mg / 2ml) and hydrocortisone (500 mg). After the administration of antivenom therapy, the patient developed hypotension (BP 90 x 40 mmHg), reversed with volume administration (saline 0.9%, 1000 ml). The patient remained under observation for 24 hours, recovering completely of paresthesia and showing no other myasthenic or paralytic symptoms. He was later discharged with mild pain at the bite

location. A slight increase in total CK (392 U / L - reference value RV <200 U / L) was recorded, although not accompanied by myalgia. Arterial blood gas analysis showed mild compensated respiratory acidosis. The lack of previous data on human accidents with *M. decoratus* should be related to the rarity of encounters with this species, which inhabits elevated (above 400m a.s.l.) and forested areas of the Atlantic Rainforest in South and Southeastern Brazil.

Key words: snakebites; elapids; Southern Brazil; Paraná State.

ENVENOMATION BY *MICRURUS CORALLINUS* (CORALSNAKE) ATTENDED IN TERESÓPOLIS CLINICAL HOSPITAL CONSTANTINE OTTAVIANO-RJ: CASE REPORT

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Snakebites envenoming is a serious public health problem in tropical countries by the frequency with which they occur and the mortality they cause. Accidents caused by snakes of the genus *Micrurus* (coralsnake) represent less than 1% of cases reported in Brazil, a number extremely low compared to the other groups of poisonous snakes in the country, such as the genus *Bothrops* and *Crotalus* which summed come to represent approximately 96% of accidents. However the severity of the accidents with coralsnakes require adequate and immediate medical attention as these poisonings often go on to develop progressive muscle weakness and respiratory failure that can lead to the death of the victim in a short period of time. The venom of snakes is a complex of proteins and peptides with important pharmacological properties different among the various families and variable within species of the same genus, and may have different pharmacological actions, the most common being the myotoxic, edema, neurotoxic (pre- and postsynaptic) and cardiotoxic anticoagulant. The *Micrurus* genus has a wide geographic distribution in Brazil, small snakes to medium sized with proteroglyphous dentition and striking aposematic color (red, black and white/yellow rings). Their habits, morphology and little aggressive behavior are determining factors for the low representation of accidents. We reported a case of accident by *Micrurus corallinus*; male patient, 28 years old, white, single, sailor, born and resident of Cachoeiras de Macacu - RJ, bitten in his right hand between the first and second finger, around 1:00 p.m. of 11/01/2015. He was sent to the Teresópolis Clinical Hospital Constantine Ottaviano, in the city of Teresópolis - RJ, already had neurological disorders, classic symptoms of elapidic poisoning, such as ptosis, sore throat, tongue paresthesia, dysphagia, blurred vision and difficulty inmaintaining upright posture. He received 10 ampoules of antielapidic serum (NCS) and was referred to the Treatment Center Intensive Care Unit (ICU) for cardiovascular and respiratory monitoring. After serum therapy, he had an uneventful recovery and was discharged cured on 11/05/2015. It should be noted that the administration of antivenom in time coupled with the appropriate procedure is an effective treatment to prevent complications, sequels and adverse reactions, along with a qualified medical staff and adequate hospital facilities for the treatment of these poisonings, and that manipulation of these animals should only be done by trained professionals, yet with special attention.

Key words: Micrurus; accident; poisoning; case report.

ISOLATION AND CHARACTERIZATION OF MICROSATELLITE MARKERS FOR *MICRURUS SURINAMENSIS* (SERPENTES: ELAPIDAE) USING NEXT GENERATION SEQUENCING

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Micrurus surinamensis (Cuvier, 1817) is an aquatic coralsnake that occurs in the Amazon valley and upper Negro and Orinoco rivers, being part of the South American triadal group. The distribution comprises the countries Brazil, Ecuador, Colombia, Guianas, Peru and Venezuela. It may reach lengths of 1,2 to 1,6 m for both males and females and by far is the species with the highest venom yield among other congeners. Human envenomations caused by coralsnake bites may not be of significance on the bite site, but it has the potential to cause significant general (systemic) effects, such as muscle paralysis, and respiratory failure. In this context, our goal was to develop a battery of polymorphic microsatellite markers for *Micrurus surinamensis* using the Illumina high throughput platform to generate data from population genetic approach. Genomic DNA was extracted from tissues of specimens of *M. surinamensis* from Altamira (Pará) and used to prepare the genomic libraries using the TruSeq LT PCR free kit by Illumina kit. A *de novo* assembly was obtained using DISCOVAR. The sequences were submitted to the QDD software to find microsatellite regions and primers design. To design the microsatellite primers we selected only sequences composed of tetra- and pentanucleotide repeats with at least 60 bp. DNA of three individuals was used to test different PCR conditions (annealing temperature) were used to optimize amplification of microsatellites primers. The PCR products were analyzed by electrophoresis on 1.5% agarose gel and 6.0% denaturing polyacrylamide gels and silver stained and alleles were sized using a 10bp ladder. We selected 30 microsatellite regions with AAGG/TTCC; AGAT/TCTA; ATCC/TAGG; AATAG/TTATC; AAGAG/TTCTC; AGGAT/TCCTA repeat motifs. This set includes microsatellites with 26 tetranucleotide and 4 pentanucleotide repeat motifs. Considering the 30 tested primers, 24 had good amplification pattern, with no artifacts and unspecific amplifications. For the three individuals, 23 microsatellite marker were polymorphic with sizes ranging from 136 to 290 bp. The use of this approach for microsatellite marker development is promising for species with limited genomic information, such as *M. surinamensis*. This methodology allowed the rapid isolation of informative microsatellite markers in *M. surinamensis* for further population genetic and kinship studies. To our knowledge, this is the first set of microsatellite markers developed for this important species.

Key words: genomic library, NGS, Illumina, polymorphism, primer design.

ISOLATION AND CHARACTERIZATION OF MICROSATELLITE MARKERS FOR MICRURUS SURINAMENSIS (SERPENTES: ELAPIDAE) USING NEXT GENERATION SEQUENCING

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Micrurus surinamensis (Cuvier, 1817) is an aquatic coralsnake that occurs in the Amazon valley and upper Negro and Orinoco rivers, being part of the South American triadal group. The distribution comprises the countries Brazil, Ecuador, Colombia, Guianas, Peru and Venezuela. It may reach lengths of 1,2 to 1,6 m for both males and females and by far is the species with the highest venom yield among other congeners. Human envenomations caused by coralsnake bites may not be of significance on the bite site, but it has the potential to cause significant general (systemic) effects, such as muscle paralysis, and respiratory failure. In this context, our goal was to develop a battery of polymorphic microsatellite markers for *Micrurus surinamensis* using the Illumina high throughput platform to generate data from population genetic approach. Genomic DNA was extracted from tissues of specimens of *M. surinamensis* from Altamira (Pará) and used to prepare the genomic libraries using the TruSeq LT PCR free kit by Illumina kit. A *de novo* assembly was obtained using DISCOVAR. The sequences were submitted to the QDD software to find microsatellite regions and primers design. To design the microsatellite primers we selected only sequences composed of tetra- and pentanucleotide repeats with at least 60 bp. DNA of three individuals was used to test different PCR conditions (annealing temperature) were used to optimize amplification of microsatellites primers. The PCR products were analyzed by electrophoresis on 1.5% agarose gel and 6.0% denaturing polyacrylamide gels and silver stained and alleles were sized using a 10bp ladder. We selected 30 microsatellite regions with AAGG/TTCC; AGAT/TCTA; ATCC/TAGG; AATAG/TTATC; AAGAG/TTCTC; AGGAT/TCCTA repeat motifs. This set includes microsatellites with 26 tetranucleotide and 4 pentanucleotide repeat motifs. Considering the 30 tested primers, 24 had good amplification pattern, with no artifacts and unspecific amplifications. For the three individuals, 23 microsatellite marker were polymorphic with sizes ranging from 136 to 290 bp. The use of this approach for microsatellite marker development is promising for species with limited genomic information, such as *M. surinamensis*. This methodology allowed the rapid isolation of informative microsatellite markers in *M. surinamensis* for further population genetic and kinship studies. To our knowledge, this is the first set of microsatellite markers developed for this important species.

Key words: genomic library, NGS, Illumina, polymorphism, primer design.

FACTORS THAT INFLUENCE THE SURVIVAL OF *MICRURUS CORALLINUS* (SERPENTES: ELAPIDEA) IN CAPTIVITY.

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The genus Micrurus represents the largest radiation of the family Elapidae in the Americas being present in most habitats of this continent. The Laboratory of Herpetology at Instituto Butantan (LHIB) maintains coral snakes in captivity since the 90s, aiming venom extraction for elapid antivenin production and immunobiological researches. Snakes of the species *Micrurus corallinus* are difficult animals to maintain in captivity. Their natural diet consists mainly of elongated prey such as amphisbaenids, some lizards (especially legless ones), caecilians and some species of snakes. This specific diet usually represents a challenge in the maintenance of *M. corallinus* in captivity, given the fact that these preys are not kept in large scale as live food. Besides, *M. corallinus* is easily stressed when the conditions offered are sub-optimal, which is readily reflected in loss of appetite, diseases manifestations and even death. Aware of these difficulties, the LHIB over the years has given special attention to the maintenance of this species. In 25 years important changes were made in order to improve their captive husbandry. The objective of this study was to verify if the management changes done over the years affected significantly the survival rate of *Micrurus corallinus* in captivity. Data gathered from 289 coral snakes from the animal facilities of LHIB were divided in three different groups according to the prophylactic and nutritional management used: Group I (from 1997 to 1999; n = 210): snakes were kept in individual boxes with *Sphagnum* as substrate. Food was offered weekly, consisting of living preys including: snakes (Viperidae, Colubridae, Dipsadidae), amphisbaenas (*Amphisbaena sp, Leposternon sp*) and lizards (*Ophiodes sp*). In this period, food items from the wild were offered to coral snakes without any prior prophylactic treatment. The animals that didn't eat voluntarily were force-fed by gavage with a chelonian commercial food (Reptomin®) softened in saline; Group II (2010; n = 26): snakes were kept in individual boxes with Sphagnum as substrate. Coral snakes were fed every week with preys obtained from the wild (mostly Colubridae) and also from the breeding program of LHIB (pitvipers, rattlesnakes and cornsnakes), which were euthanized and frozen before being offered. Coral snakes that did not eat voluntarily were force-fed by gavage with a recipe developed by us; and Group III (from 2011 to 2013; n = 53): in this period, the substrate was changed to tree bark, and all the changes previously implemented in Group II. The survival rates increased significantly, mainly in the last two groups. The most important factors for the increase in the survival rates were the nutritional adequacy that included freezing the food items before offering them to the coral snakes and the new diet developed by LHIB to force-feed them. Another important factor was the shift of the cage substrate from *Sphagnum* to tree bark in 2010, since then no other cases of blister disease were identified. The junction of these factors seemed to have been crucial in the success of maintaining *Micrurus corallines* in captivity.

Key Words: Elapidae, Micrurus corallinus, Husbandry

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