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Assessment of venom variation and phylogenetic relationships of *Micrurus dumerilii* from three different regions of Colombia

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ABSTRACT

In the Americas, the genus *Micrurus* (coral snakes) includes the highest number of snake species, and Colombia is the second country with the greatest species diversity. *Micrurus dumerilii* has wide distribution and clinical importance in the country. The variability of its venom has not been extensively studied, and this could have implications for the neutralization by antivenoms. In this study, we explored the phylogenetic relationships between specimens from three regions of Colombia (Antioquia, Chocó, and Santander) and the variation in their venoms using proteomics, *in vitro* and *in vivo* assays, and assessment of antigenic recognition by the anticoral-INS antivenom. Phylogenetic analyses using *nd4*, *Cyt b*, and 16S rRNA gene fragments showed a close relationship between *M. dumerilii* from Ecuador and Chocó (Colombia), and within the *M. dumerilii* clade, a particularly close relationship between specimens from Antioquia and Santander. The venoms of *M. dumerilii* showed high overall similarity in their chromatographic profiles, with peaks corresponding to the three-finger toxin (3FTx) and phospholipase A_2 (PLA_2) protein families being predominant. Some differences were observed in the number of protein families identified in each venom, but the main fraction responsible for lethality in the venoms from Antioquia, Chocó, and Santander was preserved. The commercial antivenom available in Colombia recognizes venom from all three regions. These general antigenic similarities between samples suggest that it may not be necessary to include *M. dumerilii* venoms from different geographic areas as immunogens for the production of antivenom against this species.

1. Introduction

New World coral snakes are represented by two genera, *Micrurus* and *Micruroides*, of which the former is by far the most diverse, with 93 species currently described [1]. Snakes of the genus *Micrurus* are terrestrial in habit and are distributed from the southern United States, through Mexico and Central America, to the countries of South America, except Chile [2]. Their extensive radiation possibly results from their high adaptive capacity, allowing them to occupy various important ecoregions throughout their distribution [2,3]. Thus, they occupy several habitats ranging from lowland tropical humid forests, dry forests, and deserts to highland cloud forests [2,3].

This adaptive capacity is reflected at the intraspecific level, especially in species with a wide distribution, such as *M. dumerilii*, one of Colombia's 31 species of coral snakes [1]. This species has a broad ecological distribution, occupying the ecoregions of the Inter-Andean, Pacific, Caribbean, and Andean valleys of the country [4,5]. It can be found in an altitudinal range from 0 to 2600 masl, occupying a wide variety of habitats, including humid forests, dry forests, and montane forests [3,6].

Although *M. dumerilii* is included in the group of tricolored

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snakes within the monadal-pattern clade [3,7], there is a striking variation in the hue and width of the black rings and the color and intensity of the white or yellow rings, adding to its visual diversity [3,6]. Adults of *M. dumerilii* measure, on average, between 50 and 70 cm, although some specimens have reached up to 95 cm [8].

Due to its size, wide distribution and abundance, and potent toxicity of its venom, *M. dumerilii* is one of Colombia's most medically important coral snake species [9], which altogether account for 1–3 % of the more than 6000 cases of snakebite envenomations in the country registered annually [10,11].

The venom of *M. dumerilii* from Antioquia has been characterized and contains proteins that belong to at least eleven families [12]. Although this venom is very rich in phospholipases A_2 (PLA_2), its most abundant PLA_2 does not seem to have a lethal effect in mice [13]. In contrast, one of the venom fractions obtained by reverse-phase high-performance liquid chromatography (RP-HPLC) which contains a mixture of PLA_2 and 3FTx proteins shows high lethality [14]. Despite these findings, little is known about the composition of the venom of other populations of this widely distributed species.

Snake venom, used to capture prey and defend against predators, shows wide variation in its composition at interspecific and intraspecific levels. This variation is related to adaptive traits associated with local environments, variable diets, and association with prey in coevolution and may also be related to phylogenetic structures, among other factors [15,16].

The intraspecific diversity in venom composition could have profound clinical implications [17], since variations may lead to differences in toxicity or pathogenicity, and may affect the effectiveness of treatments. Given that antivenoms are formulated using venoms from species in specific regions, their effectiveness is directly linked to the composition of the venoms used as immunogens. Therefore, it is crucial to understand the venom composition of clinically relevant species in each country or region, and examine the intraspecific variation at the geographic level [17,18]. By considering the geographic variation of venoms when evaluating antivenoms, we can significantly enhance their effectiveness, thereby making a substantial impact on public health by identifying the most suitable venoms for immunization protocols.

Given the widespread distribution of *M. dumerilii* and its significance in coral snake bites in Colombia, this study aims to investigate the potential variability of its venom composition across different regions. We also determine whether differences exist in the immunorecognition of venoms from different locations by a commercial antivenom. Furthermore, we aim to explore the phylogenetic relationships between specimens from various regions and compare them with other *Micrurus* species.

2. Material and methods

2.1. Specimens

Micrurus dumerilii individuals were collected in the departments of Chocó (western Andes), Antioquia (central Andes) and Santander (eastern Andes) (Fig. 1). These specimens, kept in the serpentarium of the University of Antioquia, were used to obtain venom and DNA from shedding skin, blood or buccal swabs. After extraction, the venoms were pooled, lyophilized, and stored at −20 °C.

2.2. Molecular analysis

Genomic DNA was extracted from shed skin, blood, or buccal swabs of specimens from different regions, Antioquia (Mutatá n = 3), Santander (San Vicente Chucurí n = 1) and Chocó (Bahia Solano n = 1), using E.Z.N.A.® Omega Tissue DNA Kit (Cat. D3396-01), and following the manufacturer's protocols. The primers, listed in supporting information (Supplementary S1), allowed us to obtain sequences for three mitochondrial genes (16S, *cyt b,* and *nd4*). PCR reactions were set up to a final volume of 25 μL, using 1 μL genomic DNA (2 ng/μL), 0.5 μL of each primer (0.2 μM), 2.5 μL of 10X PCR buffer, 0.5 μL total dNTPs (0.2 mM), 0.75 μL of $MgCl_2$ (1.5 mM), 0.1 μL of Platinum® Taq DNA Polymerase (1 U), and 19.15 μL of H_2O. Typical amplification conditions involved initial denaturation at 94 °C for 5 min, followed by 35 cycles with a denaturation step at 95 °C for 45 s, an annealing stage at 55 °C for 45 s, an extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. Amplicons were separated by electrophoresis on 1.5 % agarose gels in 1X TAE buffer, dyed with GelRed™ Nucleic Acid Gel Stain (Biotium, Inc.) and visualized under UV light. We performed Sanger sequencing in a capillary automated ABI3500 sequencer (Applied Biosystems®), at the AUSTRAL omics (Santiago, Chile). The DNA sequences were edited (Trim Ends and de Novo Assemble) and aligned in Geneious Prime v2025.0.3 [19]. For *nd4* and *cyt-b* genes, the nucleotide sequences were translated into proteins to evaluate the reading frame and ensure the absence of premature stop codons or other nonsense mutations in GeneDoc [20]. Novel sequences were deposited in GeneBank (Supplementary S2).

2.3. Phylogenetic analysis

Data from a total of 49 coral snakes (Supplementary S2) were used in the phylogenetic analysis, including *Micruroides euryxanthus* as an outgroup, according to the phylogenetic analysis performed by Jowers et al. [21]. PhyloSuite v 1.2.3 [22] was used for data standardization and concatenation. The CDS genes were aligned using the MACSE algorithm [23] with the vertebrate mitochondrial genetic code and standard code for nuclear genes. 16S rRNA was aligned using MAFFT the G–INS–I strategy. As most of the species used to reconstruct the phylogenetic relationships of *Micrurus dumerilii* from Colombia have the *nd4* gene sequenced, we performed a phylogenetic reconstruction using the maximum likelihood and Bayesian inference algorithms with the *nd4* gene (54 sequences). Additionally, we concatenated the *cytb*, *nd4*, and 16S genes for species with at least two genes sequenced. The maximum likelihood (ML) tree was reconstructed using IQ-TREE v.1.6.8 [24] and the substitution models were estimated using ModelFinder [25] implemented in the IQ-TREE package with the Bayesian information criterion (BIC). Branch support was estimated using 10,000 replicates of the ultrafast bootstrap. Bayesian inference (BI) trees were reconstructed using MrBayes 3.2.7 [26] and fitting substitution models were determined in MEGA v 11.0.13 [27] with the BIC criterion. BI analysis using the default settings by four simultaneous Markov chains was run for five million generations in two independent runs, with sampling every 1000 generations, and the initial 25 % of samples were discarded as burn-in. Posterior probabilities were calculated from the consensus of the remaining trees. The confidence of the Bayesian sampling was verified for the free parameters using the effective sample size statistic (ESS) implemented in the software Tracer v. 1.5 [28]. All parameters showed ESS greater than 300 and the analyses converged asymptotically, indicating reliable performance. Mass spectrometry data were deposited to the ProteomeXchange Consortium via the MassIVE repository with the dataset identifier PXD063157.

2.4. Chromatographic and electrophoretic analysis of M. dumerilii venoms

To explore whether there is geographical variability in *M. dumerilii* venoms, individual samples (two specimens for each

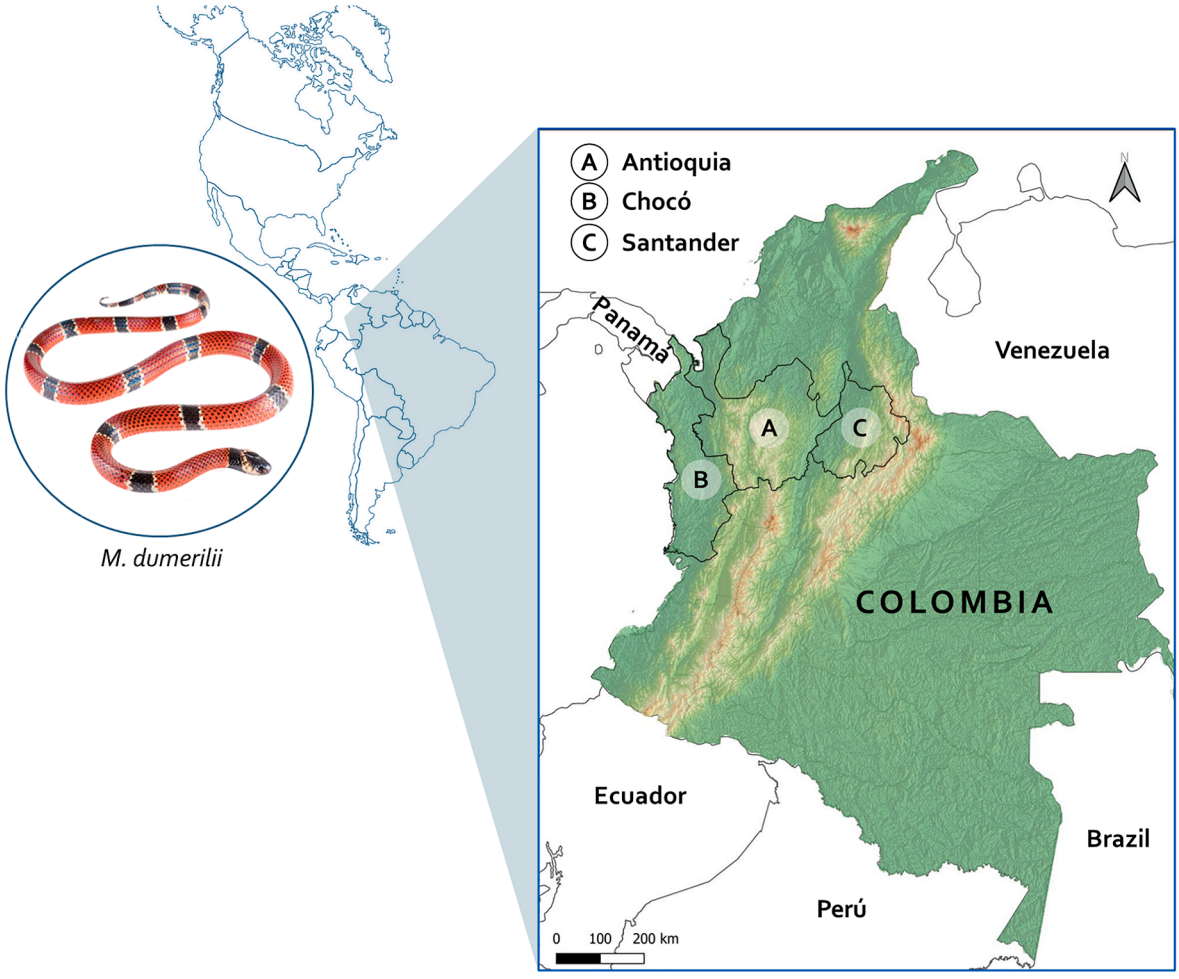

Fig. 1. Departments from Colombia where specimens of *M. dumerilii* were recollected.

venom), and pools from Chocó (two specimens), Antioquia (four specimens), and Santander (three specimens) were analyzed by RP-HPLC. Venom samples of 2.0 mg from each region were dissolved in 200 μL of 0.1 % trifluoroacetic acid (Solution A; TFA), centrifuged at 1250×*g* for 5 min and fractionated on a C_{18} column (250 × 4.6 mm, 5 μm particle size) using a Shimadzu Prominance-20A chromatographic equipment (Alvert-Hahn, Disburg, Germany) with monitoring at 215 nm. Elution was performed at a flow rate of 1 mL/min, applying the following gradient towards solution B (acetonitrile, containing 0.1 % TFA): 5 % B for 5 min, 5–15 % B for 10 min, 15–45 % B for 60 min, and 45–70 % B for 12 min [29]. The fractions were collected manually and then dried in a Vacufuge plus® (Eppendorf) centrifuge, redissolved in distilled water, and their protein concentrations were determined by measuring absorbance at 280 nm in a NanoDrop 2000 (Thermo Scientific; Waltham, MA, USA).

In addition, 20 μg of venoms from each region were evaluated by SDS-PAGE [30] under non-reduced conditions using a 15 % gel. Electrophoresis was performed in a Bio-Rad Mini-PROTEAN Tetra Cell electrophoresis equipment (Hercules, Ca, USA), at 150 V. Coomassie blue R-250 dye was used to visualize the proteins.

2.5. Shotgun proteomic profiling

Venom samples of 15 μg from the three different regions were reduced with 10 mM dithiothreitol for 30 min at 56 °C, alkylated with 50 mM iodoacetamide for 20 min in the dark, and digested with sequencing grade trypsin (1:50 enzyme:protein w:w ratio) at 37 °C overnight, in a total volume of 40 μL. After the addition of 0.5 μL of formic acid, the resulting tryptic peptide mixtures were centrifuged and separated by RP-HPLC on a nano-Easy 1200 chromatograph (Thermo) in-line with a mass spectrometer (Thermo). Ten μL of peptide mixture, containing 0.7 μg, were loaded on a C_{18} trap column (75 μm × 2 cm, 3 μm particle; PepMap, Thermo), washed with 0.1 % formic acid (solution A), and separated at 200 nL/min on a C_{18} Easyspray® column (75 μm × 15 cm, 3 μm particle; PepMap, Thermo). A gradient toward solution B (80 % acetonitrile, 0.1 % formic acid) was developed in a total of 120 min (1–5 % B in 1 min, 5–26 % B in 84 min, 26–80 % B in 30 min, 80–99 % B in 1 min, and 99 % B for 4 min). MS spectra were acquired in positive mode at 1.9 kV, with a capillary temperature of 200 °C, using 1 μ scan in the range 400–1600 *m/z*, maximum injection time of 50 msec, AGC target of 1×10^6, and resolution of 70,000. The top 10 ions with 2–5 positive charges were fragmented with AGC target of 3×10^6, minimum AGC 2×10^3, maximum injection time 110 msec, dynamic exclusion time 5 s, and resolution 17,500. MS/MS spectra were processed against protein sequences contained in the UniProt/SwissProt database (https://www.uniprot.org/blast, Serpentes, 2022; 2760 entries) using Peaks X® (Bioinformatics Solutions) and matches were assigned to known protein families by similarity. Cysteine carbamidomethylation was set as a fixed modification, while deamidation of asparagine or glutamine, and

methionine oxidation were set as variable modifications, allowing up to 2 missed cleavages by trypsin. Parameters for match acceptance against the database were set to FDR <0.1 % both for peptide and protein levels, detection of at least 1 unique peptide, and −10lgP protein score ≥30 [31].

2.6. Comparison of the biological activities of whole venoms

The PLA_2 enzymatic activity of venoms was evaluated on the monodisperse synthetic substrate 4-nitro-3-octanoyloxy-benzoic acid (4-NOBA), as previously described [13]. This assay is used to quantify the hydrolytic activity of the PLA_2s present in the whole venoms. Five μg of venom from each region were dissolved in 25 μL of 10 mM Tris, 0.1 M NaCl, 10 mM $CaCl_2$ buffer (pH 8.0) and added to 200 μL of the same buffer and 25 μL of 4-NOBA (1 mg/mL acetonitrile). After incubation at 37 °C for 60 min the reaction product was quantified at 425 nm. Wells containing the same reagents without venom were used as controls, the absorbance value of these wells was subtracted from the absorbance values of the venom samples. All samples were tested in triplicate.

The myotoxic activity was evaluated by injecting 5 μg (in 100 μL of saline) of venom of each region into the gastrocnemius muscle in a group of three mice (18–20 g body weight). A control group was injected with 100 μL of saline solution. After 3 h, blood was collected from the tail vein, and creatine kinase (CK) activity was determined using a Kinetic UV assay kit (Weiner Lab, CK-NAC UV-AA) (Riobamba, Rosario-Argentina). All mouse (Swiss-Webster; 18–20 g) experiments were performed in accordance with the guidelines of License No. 110 of 2017 and License No. 160 of 2024, issued by the Institutional Committee for the Care and Use of Laboratory Animals (CICUA) from the University of Antioquia. In all cases, isoflurane was used as an anesthetic agent, and carbon dioxide overexposure was used as the method of euthanasia.

2.7. Toxicity screening and biological activities of the lethal venom fractions

Toxicity screening was performed for the most abundant chromatographic venom fractions. For this, the median lethal dose (LD_{50}) previously determined by Rey-Suárez et al., [12] for the venom of Antioquia was used as a reference. To this end, 24 μg (in 300 μL of saline) of each fraction was injected intraperitoneally into groups of two mice. Deaths were recorded within 24 h. A control group was injected only with 300 μL of saline solution. Fractions showing lethal activity at this dose were evaluated by SDS-PAGE [30] under non-reduced conditions using a 15 % gel as described above.

The PLA_2 activity of the lethal fractions from each venom was evaluated as described above using 10 μg of each fraction.

2.8. Antigenic recognition by commercial antivenom

To evaluate the antigenic recognition by the anticoral-INS antivenom (Instituto Nacional de Salud, Colombia; batch 23AMP01, expiry date November 2025) of whole venoms of *M. dumerilii* from each region, an enzyme-linked immunosorbent assay (ELISA) was performed. The antibody titers of the antivenom were assessed against the whole *M. dumerilii* venoms. For this, each well of the microplates was coated with 0.1 μg of the complete venom diluted in 100 μL of coating buffer (0.1 M Tris, 0.15 M NaCl, pH 9.0) and incubated overnight at room temperature. The wells were then washed with BSA-PBS and blocked with 100 μL of 1 % bovine serum albumin in phosphate buffer (0.04 M phosphates, 0.12 M NaCl, pH 7.2) for 90 min. Serial dilutions of the antivenom, or a non-immune equine serum as a control (1:500 to 1:256,000), were added and incubated for 90 min at room temperature. The plate was washed and a peroxidase labeled anti-horse IgG conjugate (Sigma-Aldrich) was added as a second antibody and incubated for 1 h and 30 min at room temperature. After washing, 100 μL of peroxidase substrate (2 mg/mL OPD diluted in 0.1 M sodium citrate, pH 5.0; 4 μL of 30 % H_2O_2 per 10 mL of final solution) was added. Finally, the absorbance was measured at 492 nm using a Multiskan sky spectrophotometer (Thermo Scientific; Waltham, MA, USA).

In addition, an ELISA was performed to evaluate the cross-recognition of antibodies obtained against the lethal fraction of *M. dumerilii* venom from Antioquia ((IgG anti-F9) obtained in a previous study [14] when confronted with the whole venoms of each region. For this, IgG anti-F9 were used as the first antibody and peroxidase labeled anti-rabbit IgG conjugate (Sigma-Aldrich) as the second antibody. The assay was carried out under the same conditions described above, and normal rabbit serum assayed in parallel was included as a negative control.

3. Results

3.1. Phylogenetic relationships

The final alignment of the *nd4* dataset consisted of 627 bp and exhibited significant variation, with 316 variable sites, 249 of which were parsimoniously informative. The phylogenetic trees obtained by both phylogenetic algorithms (ML and BI) were congruent in their topology, with some variations in node supports. Moreover, this result is consistent with previous phylogenetic hypotheses proposing two sister clades of *Micrurus*: a monadal clade and a triadal clade [21,32]. Our phylogenetic tree places *Micrurus dumerilii* within the monadal clade in a separate group from the one containing the sympatric *Micrurus nigrocinctus* from Colombia and Panama. Furthermore, our phylogenetic reconstruction reveals that *M. dumerilii* forms a well-supported clade that includes *M. dumerilii* from Ecuador and several localities in Colombia. This clade is sister to *Micrurus circinalis* from Trinidad and Tobago and *Micrurus psyches* from Guyana. Within the *M. dumerilii* clade, we see a particularly close relationship between those from Ecuador and those from Chocó, highlighting regional variations. Meanwhile, those from Antioquia and Santander are close to each other (Fig. 2).

Regarding the concatenated dataset of the *16S*, *cytb*, and *nd4* genes (1696 bp, 634 variable sites, and 464 parsimoniously informative sites), it was congruent with the *nd4* tree, with some variations due to the inclusion of fewer sequences (n = 33 species) (Fig. 3). Notably, *M. dumerilii* from Ecuador could not be included as it only has *nd4* sequences available. In this context, the tree shows *M. dumerilii* from Colombia forming a well-supported clade, with a closer phylogenetic relationship observed between specimens from Santander and Antioquia, and *M. circinalis* as the sister group.

3.1.1. Venom analysis

The chromatographic fractionation of *M. dumerilii* venoms from different geographical regions showed the typical profile previously reported by Rey-Suárez et al., [12] and Gómez-Robles et al., [14]. Differences were observed in the abundance of peaks eluting between 40 and 58 min among the three venom pools (Fig. 4). On the other hand, only slight changes in the chromatographic profiles of the individuals from the same region were observed (Fig. 5). In the eluting region of the lethal fraction described by Gómez-Robles et al., [14], variations were observed in the intensity and elution times of the peaks. An additional peak at approximately 28 min was observed in the venom from Santander, and another peak, exclusive to the venom from Chocó, eluted at 82 min (Fig. 4).

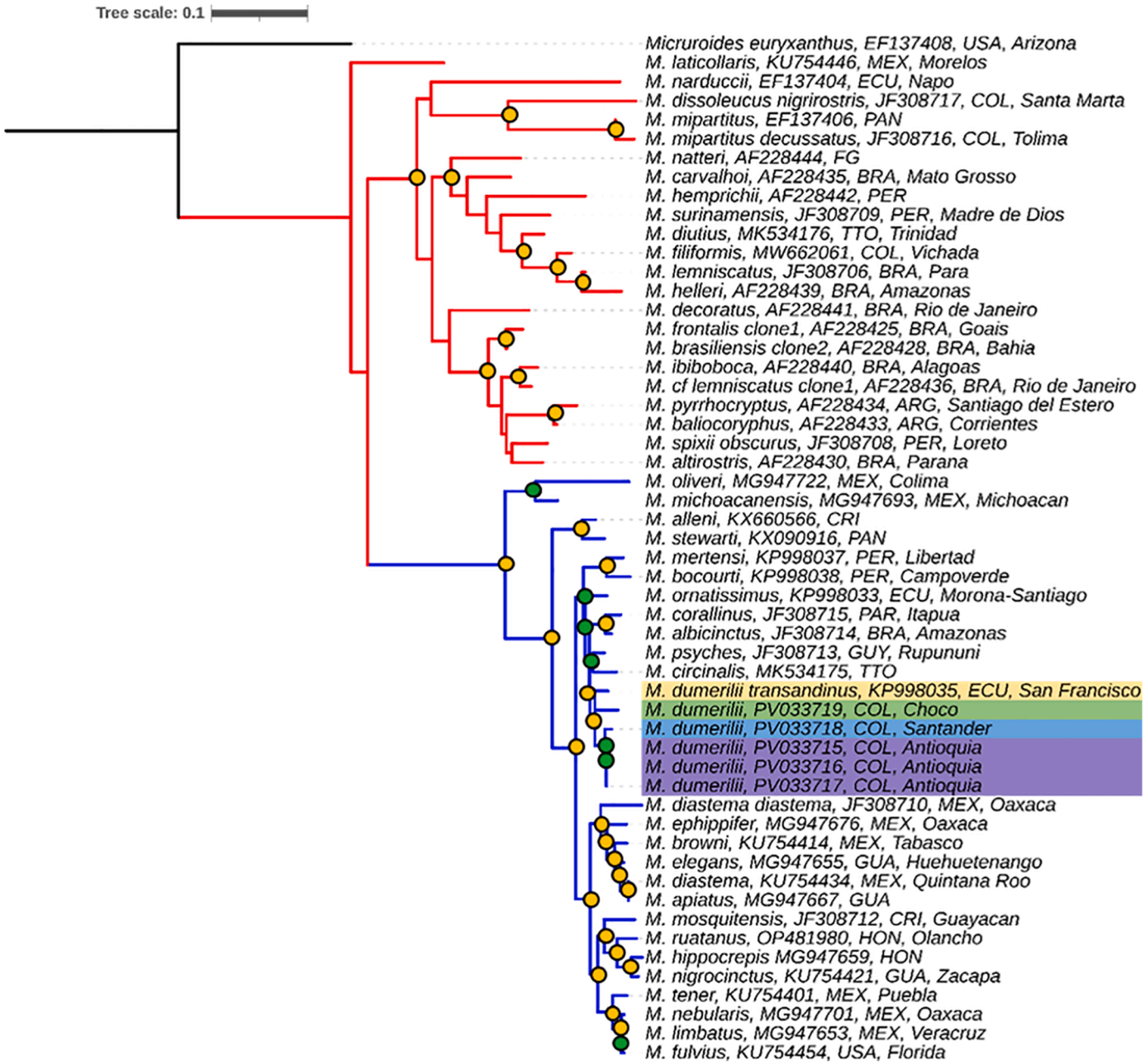

Fig. 2. Phylogenetic tree resulting from Maximum Likelihood and Bayesian inference of nd4 fragments from 54 coral snake sequences. The Monadal clade is shown in blue, and the Triadal clade in red. The yellow circle indicates ML bootstrap support (>0.80) and Bayesian posterior probability (BPP) > 0.90, while the green circle indicates ML bootstrap support (>0.80) only.

The electrophoretic profiles of *M. dumerilii* venoms were dominated by two intense low molecular mass bands between 10 and 15 kDa; a faint band near 25 kDa, and two thin intense bands between 50 and 75 kDa (Fig. 6). The variation in the venoms from the three regions was restricted to a single band near 20 kDa, present in the venoms from Antioquia and Santander, but absent in the venom from Chocó.

The shotgun proteomic profiling of the venoms detected 43 distinct proteins in the venom from Antioquia, 36 in the venom from Chocó, and 33 in the venom from Santander. These proteins belonged to 11, 11, and 9 protein families, respectively. In the venom from Santander, phospholipase B (PLB) and Vascular endothelial growth factor (VEGF) were not detected, and in the venom from Chocó, natriuretic peptides (NatP) were not detected. In contrast, hyaluronidases were only detected in the venom from Chocó. Finally, none of the three venoms presented the serine proteinase family proteins. The 3FTx and PLA_2 protein families showed the highest number of protein variants (Fig. 7 and Supplementary S3).

The PLA_2 activities showed significant differences between the venoms from Antioquia and Santander compared to Chocó, which had lowest activity (Fig. 8A). In contrast, the myotoxic activity of the venoms in mice, as judged by the plasma CK levels was similar for the three venoms (Fig. 8B).

The fraction responsible for the lethality in the venom from the Antioquia, Chocó and Santander was conserved, corresponding to

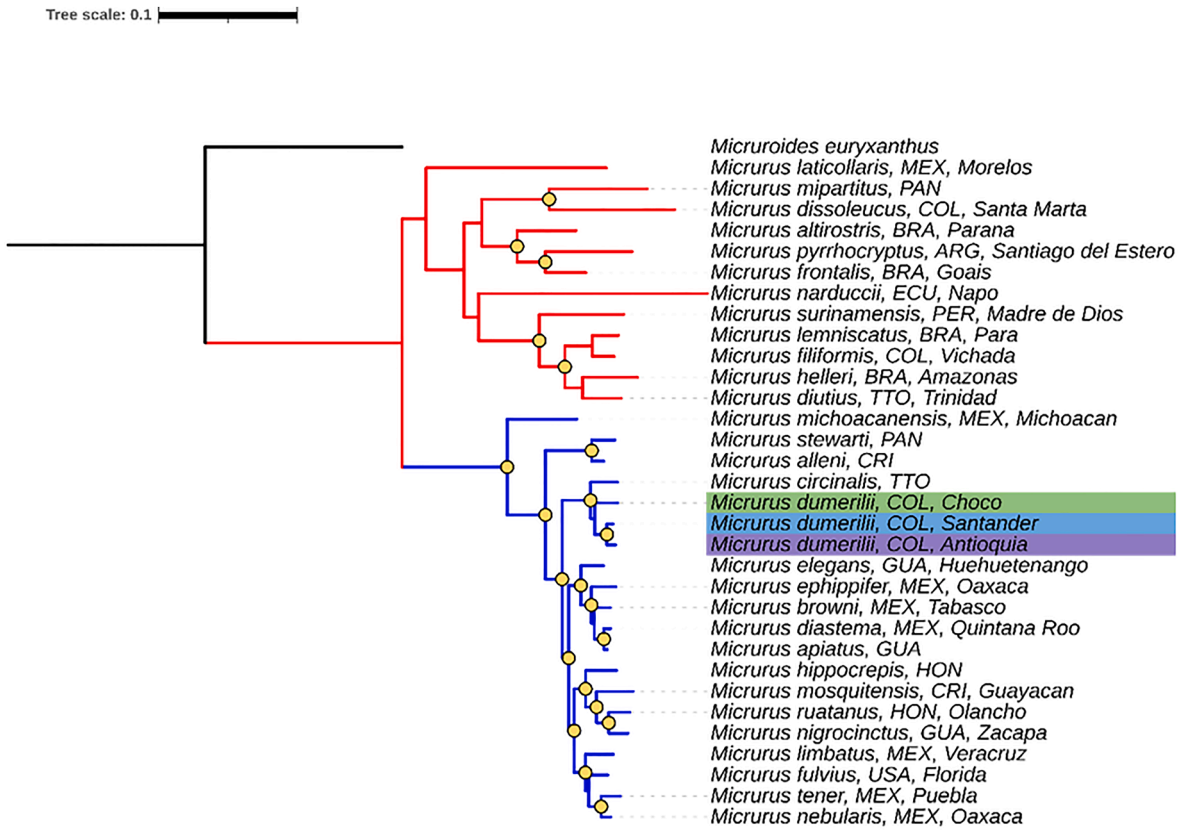

Fig. 3. Phylogenetic tree resulting from Bayesian inference of *nd4*, *Cyt b*, and *16S rRNA* gene fragments from 30 *Micrurus* species. The monadal clade is shown in blue, while the triadal clade is shown in red. Posterior probabilities (>0.90) are indicated by yellow circles.

the same retention time ≈46 min (marked fraction in Fig. 4). Additionally, it showed PLA_2 activity on the NOBA substrate (Fig. 9). The variation in the venoms from the three regions was mainly restricted to a single band near 20 kDa, present in the venoms from Antioquia and Santander, but absent in the venom from Chocó (Fig. 6A).

The commercial anti-coral antivenom recognized the venoms of *M. dumerilii* from all three regions equally when antibody titers were evaluated by ELISA (Fig. 10A). Antibodies anti-F9 (the lethal fraction of *M. dumerilii* venom) were also tested for their capacity to cross-recognize all the venoms from the three regions, showing similar binding against the three venoms ($p > 0.05$) (Fig. 10B).

4. Discussion

Colombia has a unique geography, with the northern end of the Andes divided into three mountain ranges, creating distinctive relief, climate, and soil conditions in each of its six ecoregions. This diverse landscape is a key factor in promoting its vast biodiversity [33], as also seen in the impressive number of coral snakes found in this country, representing around 35 % of all species described in the continent. Some of these species have a wide distribution in the territory and occupy several ecoregions [4,34]. Such is the case of *M. dumerilii*, which extends its distribution to the Andean region, including the departments of Antioquia and Santander, and to the Chocó biogeographic region. With such a wide distribution, it is not surprising that this species exhibits significant morphological variation at the geographic level, which has led some authors to recognize up to six subspecies: *M. d. trasandinus, M. d. antioquensis, M. d. dumerilii, M. d. carinicauda, M. d. colombianus* and *M. d. venezuelensis* [3,6]. Our study included individuals from Chocó, Antioquia, and Santander, corresponding to *M. d. trasandinus, M. d. antioquensis*, and *M. d. carinicauda*, respectively. However, the use of these categories depends on the criteria and the taxonomic approach adopted, and their relevance is still under discussion in the context of modern evolutionary theories [35]. Therefore, subspecies nomenclature was not adopted in the present work.

Our phylogenetic analysis supports the general notion that the genus *Micrurus* is divided into two monophyletic clades: one that includes the monadal group and another that includes the triadal species [36]. *M. dumerilii* is part of the former. Notably, individuals of *M. dumerilii* from different regions formed a well-supported clade, demonstrating distinct intraspecific differences based on geographic distribution, showing molecular differentiation between individuals from Colombia and Ecuador.

In contrast, the venoms from our *M. dumerilii* samples showed a high overall similarity in their chromatographic profile, with peaks corresponding to 3FTx and PLA_2 protein families being predominant. Even so, we observed differences in the abundance of peaks that eluted between 40 and 58 min, and some variation in the proteomic profiles among the three venoms, as detected by a shotgun approach. *M. dumerilii* from Antioquia showed the highest number of proteins from the two dominant families, while the Santander venom showed the lowest. Additional differences were observed in the number of families identified: eleven families in Antioquia and Santander, while in *M. dumerilii* from Chocó, only nine of them. As limitations of this study, it is possible that some components present in very low amounts could go undetected by the whole venom shotgun strategy employed, since it

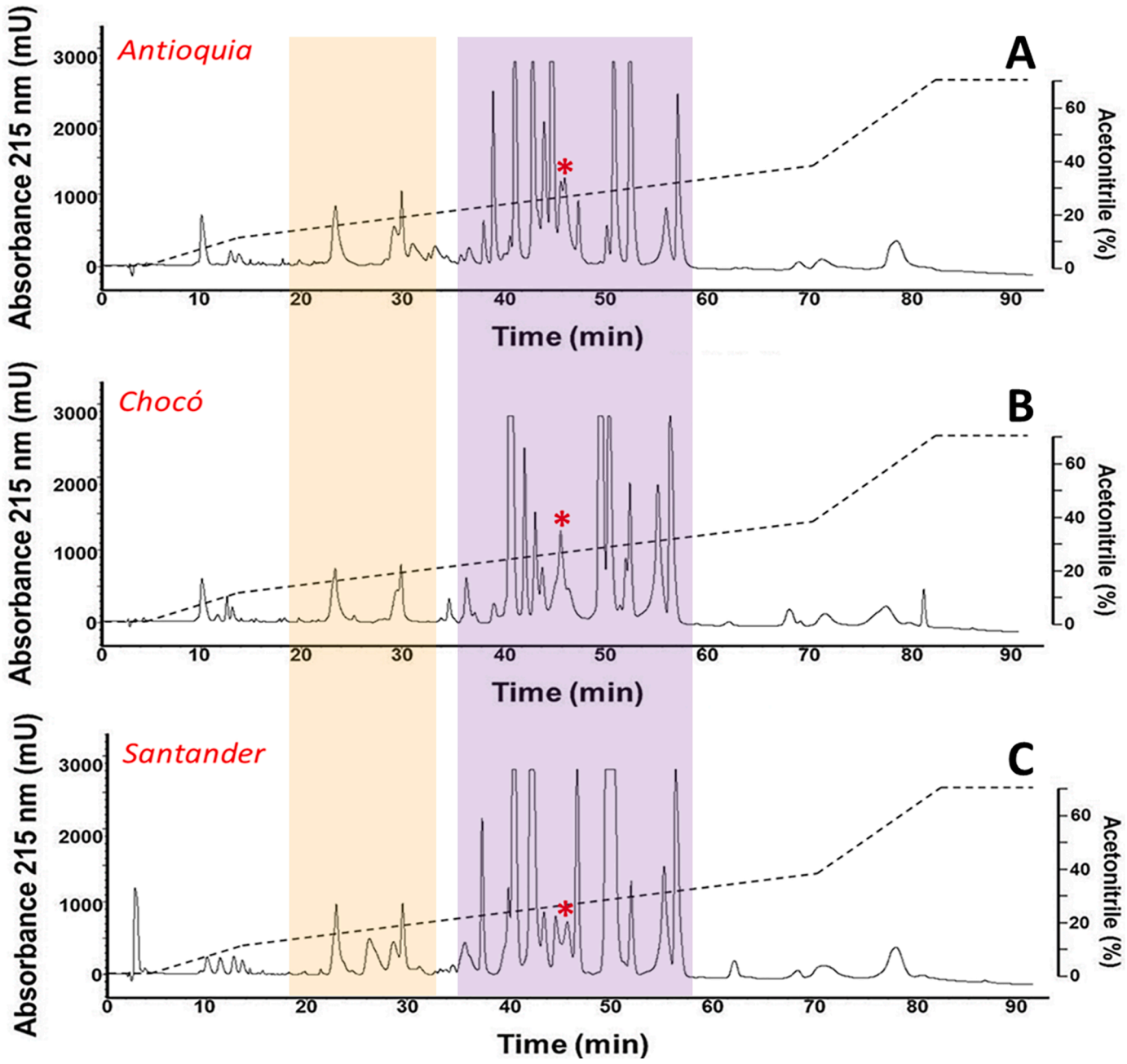

Fig. 4. RP-HPLC of the venoms of *M. dumerilii* from different regions (pool). Two mg of venom was fractionated on a C_{18} column (250 × 4.6 mm) eluted at 1 mL/min with an acetonitrile gradient (dotted line). **A)** venom from Antioquia, **B)** venom from Chocó, **C)** venom from Santander. The 3FTx (yellow) and PLA_2 (purple) regions were shaded according to proteomic analyses [12]. The red asterisk shows the lethal fraction of each venom (as reported by Gómez-Robles et al., [14]).

does not involve any decomplexation step. Furthermore, owing to constraints of analytical costs and limited availability of venom samples, we were not able to perform native intact mass (LC-MS) profiling. Neither PLB nor vascular endothelial growth factor (VEGF) were detected in the Santander venom. This component is related to hypotensive activity and the induction of vascular permeability and could contribute to the dispersion of toxins and enhancement of prey envenoming [37]. VEGF has been mainly identified and isolated in viper venoms, while in elapids it has been described as a minor component (~0.5 %) of the venoms of some cobras and New World coral snakes [38].

Another unexpected finding regards to the presence of NatP only in the venoms of Antioquia and Santander but not detected in the venom of Chocó. NatPs have been found in both elapid and viper venom [39,40], and their potential toxicity is associated with vasorelaxation and reduced myocardial contractility. Moreover, NatPs may have a role in prey immobilization, by contributing to the rapid diffusion of venom from the bite site [41]. This family of toxins has been cloned and expressed from the cDNA of the *M. corallinus* venom gland [42]. For the same species, Leão et al., [43] identified NatP precursors through a venom gland transcriptomic approach. Other transcriptomic studies have identified NatPs in other *Micrurus* species, such as *M. altirostris, M. lemniscatus carvalhoi, M. lemniscatus lemniscatus, M. spixii spixii*,

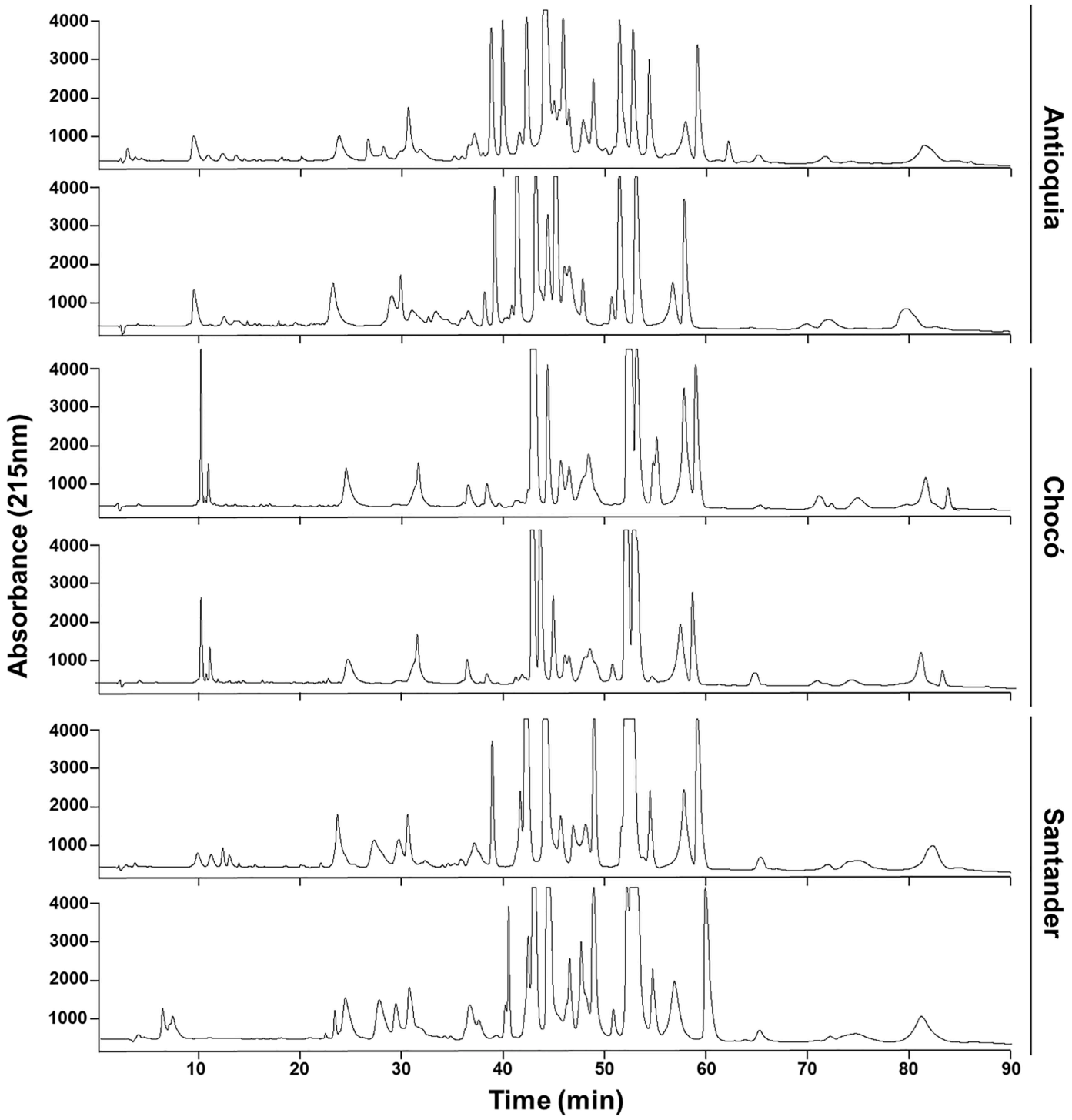

Fig. 5. RP-HPLC of the venoms of *M. dumerilii* (individual specimens). Two mg of venom was fractionated on a C_{18} column (250 × 4.6 mm) eluted at 1 mL/min, as described in Methods.

M. surinamensis, and *M. browni* [44–46]. However, only until recently evidence for expression of NatPs has been reported in the venom proteomes of *Micrurus*, such as those from four out of five species from Costa Rica [47]. This is the first report of its presence in the venom of *M. dumerilii*, expanding knowledge on the distribution of this protein family in the venoms of *Micrurus* species.

In spite of the slight compositional differences outlined above, the myotoxic effect and lethality of the *M. dumerilii* venoms here studied were similar. On the other hand, in line with the phylogenetic results, PLA_2 activity revealed an intriguing difference in the Chocó venom. It exhibited lower PLA_2 activity, as well as lower PLA_2 activity in the lethal fraction, and lacked a band near 20 kDa in the electrophoretic profile, setting it apart from the venoms from Antioquia and Santander. These features depict some degree of divergence of *M. dumerilii* from Chocó among the three geographical populations sampled in this study.

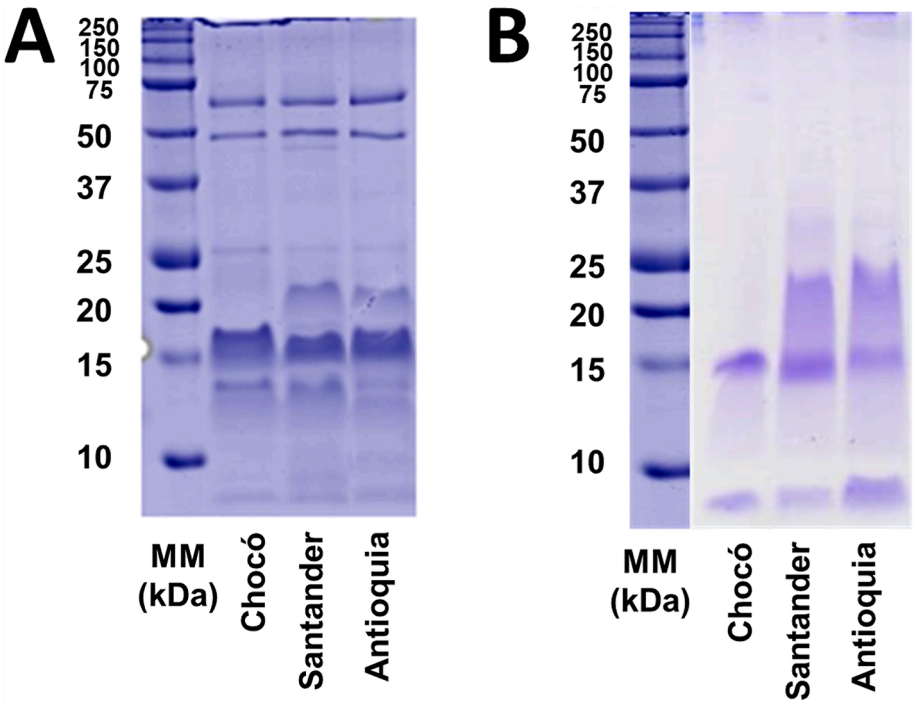

Fig. 6. SDS-PAGE under non-reducing conditions of (A) complete venom of *M. dumerilii* from different regions; and (B) the lethal fraction from Antioquia, Chocó and Santander venoms.

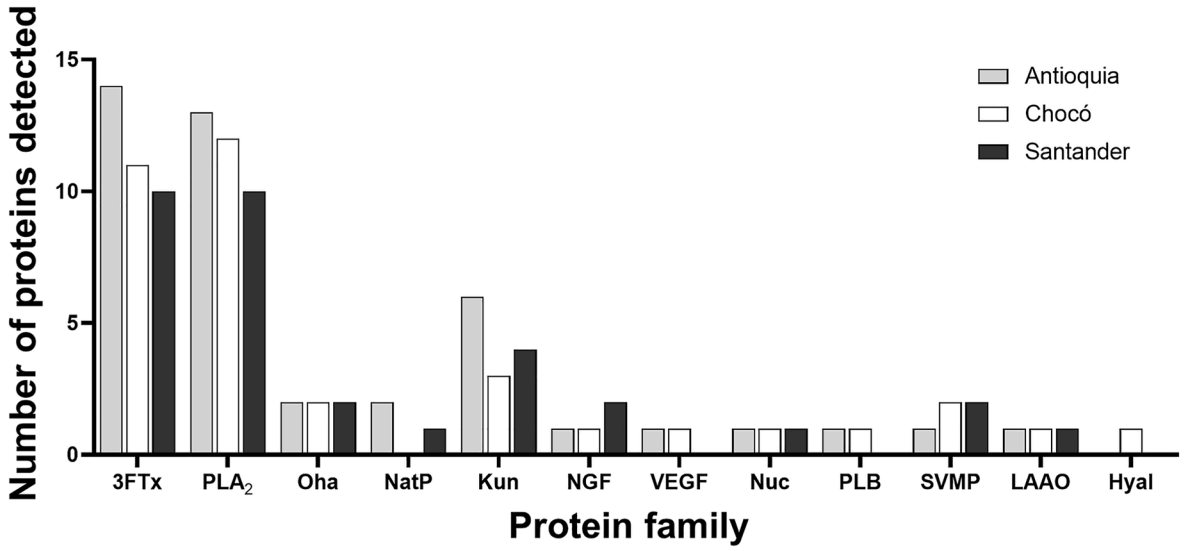

Fig. 7. Diversity of proteins identified by shotgun proteomics of *M. dumerilii* venoms. The number of proteins refers to the minimal number of protein groups defined by unique peptides, as identified in PEAKS X. **3FTx**: Three finger toxins; **PLA_2**: Phospholipase A_2; **Oha**: Ohanin; **NatP**: Natriuretic peptide; **Kun**: Kunitz-type Inhibitor; **NGF**: Nerve growth factor; **VEGF**: vascular endothelial growth factor; **Nuc:** Nucleotidase; **PLB:** Phospholipase B; **SVMP**: Snake Venom Metalloproteinase; **LAAO:** L-amino oxidase; **HYAL**: Hyaluronidase.

Intraspecific geographical variation in the composition of venoms has been often described in snakes, although most studies to date focus on vipers [16,48,49]. For example, the venom of *B. atrox* contains enzymes, such as phospholipase A_2, whose activity tends to vary depending on the origin. In contrast, other venoms remain constant or vary independently of the origin [15,50]. In the species *Crotalus scutulatus*, geographic differences in the composition and activity of Mojave toxin (neurotoxin) are associated with altitude: individuals with venom containing Mojave toxin were found in high, cold areas, and individuals without it were found in areas with low altitudes and warmer temperatures [51]. It is then possible that a similar situation exists in the venoms of coral snakes with a wide geographic distribution, such as *M. dumerilii*. Unfortunately, their secretive habits and the small amount of venom they produce have prevented similar analyses of venoms from this group of snakes.

The primary function of venom is to immobilize and/or digest prey. Since animals can vary in susceptibility to venom, geographic

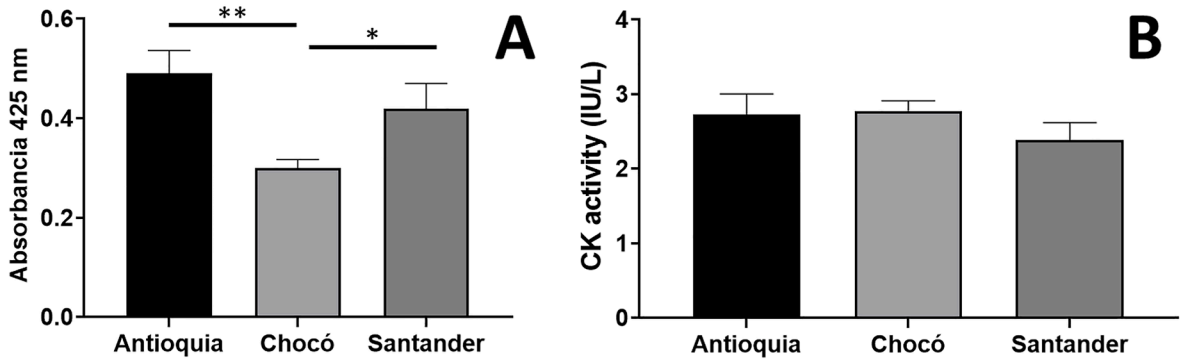

Fig. 8. Biological activities of the whole venoms of *M. dumerilii* from different regions. (**A)** Phospholipase A_2 activity on the 4-NOBA substrate; (**B)** Myotoxic activity in mice. The control group (PBS) showed a plasma CK activity of 0.250 IU/L. Bars represent mean ± SD (n = 3). Statistically significant differences are indicated by asterisks (**P = 0.001, *P=<0.05)".

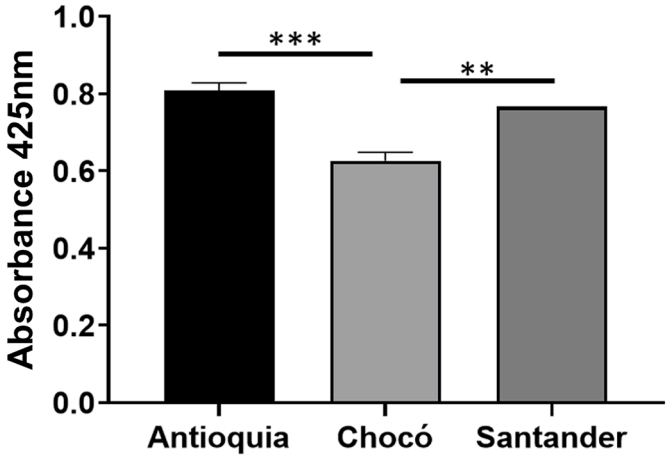

Fig. 9. Phospholipase A_2 activity on the 4-NOBA substrate of the lethal fraction from Antioquia, Chocó and Santander venoms. Bars represent mean ± SD (n = 3).

variation may reflect natural selection to feed on prey from specific regions [52]. Some studies have found a relationship between prey diversity and venom variation, and it is postulated that venom composition is driven primarily by local prey diversity rather than specific prey types [53]. However, although species within the genus *Micrurus* occupy a wide range of habitats in tropical and subtropical areas of America, natural history data for the genus generally show prey types associated with their fossorial habits [54]. Therefore, the type of prey in their diet seems to be limited to vertebrates available in their microhabitats and, therefore, the diversity of prey may be lower compared to other groups of snakes (e.g. viperids). Amphisbaenians have been commonly reported within the diet of *M. corallinus* [55], *M. hemprichii* [56] and *M. lemniscatus* [6,57]. Likewise, Cecilids, swamp eels, lizards and other fossorial snakes have been described within the diets of coral snakes [3,58–65]. In the case of *M. dumerilii*, swamp eels, snakes (*Tantilla supracincta*), lizards and caecilians have been recorded within its diet [58,66,67].

Despite being a species with a wide distribution in Colombia, with broad altitudinal range, and occupying a great variety of habitats [3,6], the fossorial habits and microhabitat use of *M. dumerilii* may lead to the type of prey being similar between its different populations and therefore, the variation between its venoms would be expected to be relatively minor, as here reported. There is a clear need for further research on the complex relationship between local variation in prey availability and its potential influence on venom compositional variation.

Alternatively, changes in venom could be attributed to variations between individuals of the same species, as described for *M. nigrocinctus*, where small changes in the intensity of chromatographic peaks were observed among six individuals [68]. Similarly, in *M. mipartitus*, changes in venom profiles were observed, with one individual not presenting the peak corresponding to mipartoxin-I [69], which is one of the main components responsible for the lethality of the venom of this species [70]. Recently,

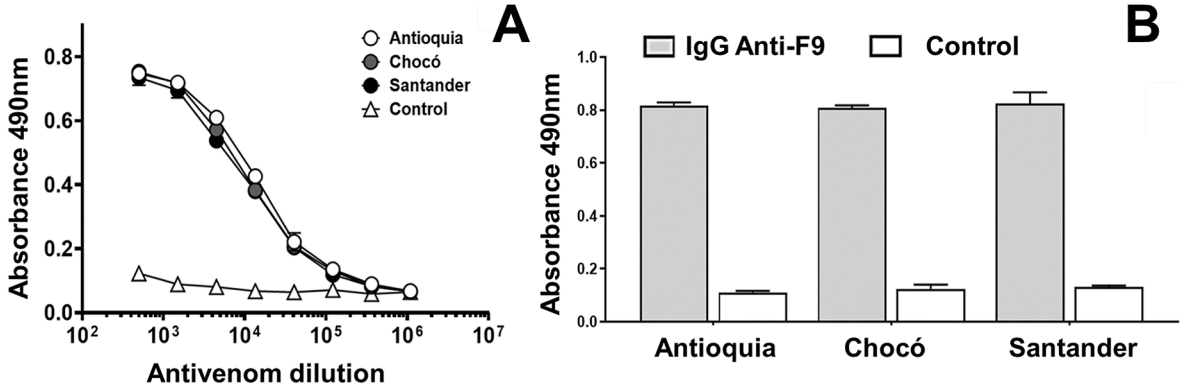

Fig. 10. Cross-recognition of *M. dumerilii* venoms from different regions: (**A)** recognition by the commercial equine anti-coral antivenom. Points represent mean ± SD of three replicate wells; Control: non-immunized horse serum. (**B)** recognition by rabbit IgG antibodies against the lethal fraction (F9) of *M. dumerilii* (Antioquia) against the three venoms. Control: non-immunized rabbit serum. Each bar represents mean ± SD of three replicate wells.

such changes were also observed in the venom of *M. yatesi* [71]. Slight variations were also observed in the chromatographic profiles obtained from *M. dumerilii* individuals.

Knowledge on the composition of medically important venoms is expected to lead to more efficient antivenom production [17]. Our results demonstrated that although *M. dumerilii* venoms from different origins present some differences, the polyvalent antivenom produced in Colombia (INS) recognized *M. dumerilii* venom from the three geographic areas similarly. In previous studies, this antivenom neutralized *M. dumerilii* venom from Antioquia [72] and from the Middle Magdalena Valley [73]. This could be related to the composition of the venom, which presents a higher proportion of PLA_2, since these toxins exhibit greater antigenic similarity between them, as previously observed in the evaluation of anti-PLA_2 antibodies of *M. dumerilii* [14]. In contrast, this antivenom has shown lower cross-recognition with 3FTx-rich *Micrurus* venoms [74]. The present results suggest that this antivenom could neutralize *M. dumerilii* venoms from different regions of Colombia; however, future confirmatory *in vivo* studies will be required.

CRediT authorship contribution statement

Paola Rey-Suárez: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Jeisson Gómez-Robles:** Writing – original draft, Methodology. **Julián Fernández:** Writing – review & editing, Methodology. **Bruno Lomonte:** Writing – review & editing, Methodology. **Mahmood Sasa:** Writing – review & editing, Methodology. **Mónica Saldarriaga-Cordoba:** Writing – review & editing, Writing – original draft, Formal analysis. **Jaime Andrés Pereañez:** Writing – review & editing. **Omayra Aguilera:** Methodology. **Vitelbina Núñez-Rangel:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Conceptualization.

Informed consent statement

Not applicable.

Institutional review board statement

The animal study protocol was approved by the institutional Committee for the Use and Care of Research Animals at the Universidad de Antioquia, license No 160. In addition, this study had an authorization from Ministry of Environment to allow the access genetic resources (RGE: 0156- 15; RGE: 0156–9).

Data availability statement

All data related to this study is provided within the manuscript and its attached Supplemental files. Raw mass spectrometry data files are available at the ProteomeXchange Consortium via the MassIVE repository, with the dataset identifier PXD063157.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biochi.2025.06.003.

References

[1] P. Uetz, J. Hošek (Eds.), The Reptile Database, 2022. http://www.reptiledatabase.org. (Accessed 10 May 2022).
[2] N.J. da Silva, M.A. Buonato, G.M. Pires, D. Tavares, New world coralsnake: on overview, in: Advances in Coralsnake Biology with an Emphasis on South America, Eagle Mountain Publishing, Eagle Mountain, UT, USA, 2021. ISBN 978-0972015462.
[3] J.A. Campbell, W.W. Lamar, The Venomous Reptiles of the Western Hemisphere, Comstock Pub. Associates, Ithaca [NY], 2004. Vol. 1, No. 2.
[4] J. Lynch, T. Angarita-Sierra, F. Ruiz-Gómez, Conservation National Program of the Colombian Snakes. Book. Publisher: Ministerio de Ambiente y Desarrollo Sostenible, Universidad Nacional de Colombia e Instituto Nacional de Salud, 2014. ISBN: 978-958-8901-18- 127.
[5] Y. Pitalua, J.T. Rengifo, L. Rivas, Contributions to the distribution of the genus *Micrurus* (Serpentes: elapidae) in the department of Chocó, Colombia, Rev Colombiana Cienc Anim 10 (2) (2018) 131–142, https://doi.org/10.24188/recia.v10.n2.2018.649. Julio-Diciembre.
[6] J.A. Roze, Coral Snakes of the Americas: Biology, Identification and Venoms, Krieger Publishing Company, Malabar, Florida, United States, 1996.
[7] J.B. Slowinski, A phylogenetic analysis of the new world coral snakes (Elapidae: *leptomicrurus, Micruroides*, and *Micrurus*) based on allozymic and morphological characters, J. Herpetol. 29 (3) (1995) 325–338.
[8] E. Meneses-Pelayo, J.R. Caicedo-Portilla, *Micrurus dumerilii* (Dumeril's cornsnake) maximum size, Herpetol. Rev. 46 (4) (2015) 647.
[9] R. Otero-Patiño, Snake bites in Colombia, in: P. Gopalakrishnakone, S. Faiz, C. Gnanathasan, A. Habib, R. Fernando, C.C. Yang (Eds.), Clinical Toxinology, Springer, Dordrecht, 2014, https://doi.org/10.1007/978-94-007-6288-6_41-2.
[10] L. León-Nuñez, Camero-Ramos, J.M. Gutiérrez, Epidemiology of snakebites in Colombia (2008-2016), Rev. Salud pública 22 (3) (2020), https://doi.org/10.15446/rsap.V22n3.87005.
[11] INS. Instituto Nacional de Salud, Protocolo de Vigilancia en Salud Pública de Accidente Ofídico, Versión 5 (2024), https://doi.org/10.33610/IMQO8860.
[12] P. Rey-Suárez, V. Núñez, J. Fernández, B. Lomonte, Integrative characterization of the venom of the coral snake *Micrurus dumerilii* (Elapidae) from Colombia: proteome, toxicity, and cross-neutralization by antivenom, J. Proteonomics 136 (2016) 262–273, https://doi.org/10.1016/j.jprot.2016.02.006.
[13] P. Rey-Suárez, V. Núñez, M. Saldarriaga-Córdoba, B. Lomonte, Primary structures and partial toxicological characterization of two phospholipases A_2 from *Micrurus mipartitus* and *Micrurus dumerilii* coral snake venoms, Biochimie 137 (2017) 88–98, https://doi.org/10.1016/j.biochi.2017.03.008.
[14] J. Gómez-Robles, P. Rey-Suárez, J.A. Pereañez, B. Lomonte, V. Núñez, Antibodies against a single fraction of *Micrurus dumerilii* venom neutralize the lethal effect of whole venom, Toxicol. Lett. 374 (2023) 77–84, https://doi.org/10.1016/j.toxlet.2022.12.005.
[15] D.R. Amazonas, J.A. Portes-Junior, M.Y. Nishiyama-Jr, C.A. Nicolau, H. M. Chalkidis, R.H.V. Mourão, F.G. Grazziotin, D.R. Rokyta, H.L. Gibbs, R. H. Valente, I.L.M. Junqueira-de-Azevedo, A.M. Moura-da-Silva, Molecular mechanisms underlying intraspecific variation in snake venom, J. Proteonomics 181 (2018 Jun 15) 60–72, https://doi.org/10.1016/j.jprot.2018.03.032. Epub 2018 Apr 3. PMID: 29621647.
[16] E.P. Hofmann, R.M. Rautsaw, J.L. Strickland, M.L. Holding, M.P. Hogan, A. J. Mason, D.R. Rokyta, C.L. Parkinson, Comparative venom-gland transcriptomics and venom proteomics of four Sidewinder Rattlesnake (*Crotalus cerastes*) lineages reveal little differential expression despite individual variation, Sci. Rep. 8 (1) (2018 Oct 19) 15534, https://doi.org/10.1038/s41598-018-33943-5.
[17] N.R. Casewell, T.N.W. Jackson, A.H. Laustsen, K. Sunagar, Causes and consequences of snake venom variation, Trends Pharmacol. Sci. 41 (8) (2020 Aug) 570–581, https://doi.org/10.1016/j.tips.2020.05.006.
[18] G. Zanetti, E. Duregotti, C.A. Locatelli, A. Giampreti, D. Lonati, O. Rossetto, M. Pirazzini, Variability in venom composition of European viper subspecies limits the cross-effectiveness of antivenoms, Sci. Rep. 8 (1) (2018 Jun 29) 9818, https://doi.org/10.1038/s41598-018-28135-0.
[19] M. Kearse, R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock,

S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, A. Drummond, Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data, Bioinformatics 28 (2012) 1647–1649.
[20] K.B. Nicholas, H.B. Nicholas Jr., D.W. Deerfield, GeneDoc: analysis and visualization of genetic variation, Embnewnews 4 (14) (1997).
[21] M.J. Jowers, U. Smart, S. Sánchez-Ramírez, J.C. Murphy, A. Gómez, R.J. Bosque, G.C. Sarker, B.P. Noonan, J.F. Faria, D.J. Harris, NJ Jr da Silva, A.L.C. Prudente, J. Weber, P.J.R. Kok, G.A. Rivas, R.C. Jadin, M. Sasa, A. Muñoz-Mérida, G. Moreno-Rueda, E.N. Smith, Unveiling underestimated species diversity within the Central American Coralsnake, a medically important complex of venomous taxa, Sci. Rep. 13 (1) (2023 Jul 19) 11674, https://doi.org/10.1038/s41598-023-37734-5. PMID: 37468518; PMCID: PMC10356846.
[22] D. Zhang, F. Gao, I. Jakovlić, H. Zou, J. Zhang, W.X. Li, G.T. Wang, PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies, Molecular Ecol. Res. 20 (1) (2020) 348–355, https://doi.org/10.1111/1755-0998.13096.
[23] V. Ranwez, E.J.P. Douzery, C. Cambon, N. Chantret, F. Delsuc, MACSE v2: toolkit for the alignment of coding sequences accounting for frameshifts and stop codons, Mol. Biol. Evol. 35 (2018) 2582–2584, https://doi.org/10.1093/molbev/msy159.
[24] L.T. Nguyen, H.A. Schmidt, A. Von Haeseler, B.Q. Minh, IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies, Mol. Biol. Evol. 32 (1) (2015) 268–274, https://doi.org/10.1093/molbev/msu300.
[25] S. Kalyaanamoorthy, B. Minh, T. Wong, et al., ModelFinder: fast model selection for accurate phylogenetic estimates, Nat. Methods 14 (2017) 587–589, https://doi.org/10.1038/nmeth.4285.
[26] F. Ronquist, J.P. Huelsenbeck, MrBayes 3: Bayesian phylogenetic inference under mixed models, Bioinformatics 19 (12) (2003) 1572–1574, https://doi.org/10.1093/bioinformatics/btg180.
[27] K. Tamura, G. Stecher, S. Kumar, MEGA11: molecular evolutionary genetics analysis version 11, Mol. Biol. Evol. 38 (7) (2021 Jun 25) 3022–3027, https://doi.org/10.1093/molbev/msab120. PMID: 33892491; PMCID: PMC8233496.
[28] A. Rambaut, A.J. Drummond, Tracer version 1.5. MCMC trace analysis package, Available from, http://tree.bio.ed.ac.uk/software/tracer/, 2009. (Accessed 1 January 2025).
[29] B. Lomonte, J.J. Calvete, Strategies in 'snake venomics' aiming at an integrative view of compositional, functional, and immunological characteristics of venoms, J. Venom. Anim. Toxins Incl. Trop. Dis. 23 (2017) 26, https://doi.org/10.1186/s40409-017-0117-8.
[30] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (5259) (1970 Aug 15) 680–685, https://doi.org/10.1038/227680a0.
[31] B. Lomonte, C. Díaz, F. Chaves, J. Fernández, M. Ruiz, M. Salas, A. Zavaleta, J. J. Calvete, M. Sasa, Comparative biochemical characterization of Viperidae snake venoms from Perú reveals two compositional patterns of phospholipase A_2 expression, Toxicon-X 7 (2020) 100044.
[32] M.J. Jowers, J.L. Garcia Mudarra, S.P. Charles, et al., Phylogeography of west indies coral snakes (*Micrurus*): island colonization and banding patterns, Zool Script 48 (3) (2019) 263–276, https://doi.org/10.1111/zsc.12346.
[33] G. Kattan, P. Franco, V. Rojas, G. Morales, Biological diversification in a complex region: a spatial analysis of faunistic diversity and biogeography of the Andes of Colombia, J. Biogeogr. 31 (11) (2004) 1829–1839, https://doi.org/10.1111/j.1365-2699.2004.01109.x.
[34] J. Lynch, El contexto de las serpientes de Colombia con un análisis de las amenazas en contra de su conservación, Rev. la Acad. Colomb. Ciencias Exactas Físicas y Nat. 36 (2012) 435–449.
[35] F.T. Burbrink, B.I. Crother, C.M. Murray, B.T. Smith, S. Ruane, E.A. Myers, R. A. Pyron, Empirical and philosophical problems with the subspecies rank, Ecol. Evol. 12 (7) (2022 Jul 10) e9069, https://doi.org/10.1002/ece3.9069.
[36] H. Zaher, F.G. Grazziotin, A.L. Prudente, A.B. Aguiar, V.C. Trevine, N.J. Da Silvia, in: Advances in Coralsnake Biology with an Emphasis on South America, Eagle Mountain Publishing, 2021. Eagle Mountain, UT, USA, 2021; ISBN 978-0972015462.
[37] C.S. Melincovici, A.B. Boşca, S. Şuşman, M. Mărginean, C. Mihu, M. Istrate, I. M. Moldovan, A.L. Roman, C.M. Mihu, Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis, Rom. J. Morphol. Embryol. 59 (2) (2018) 455–467.
[38] I.G. Ferreira, M.B. Pucca, I.S. Oliveira, F.A. Cerni, B.C.D.S. Jacob, E.C. Arantes, Snake venom vascular endothelial growth factors (svVEGFs): unravelling their molecular structure, functions, and research potential, Cytokine Growth Factor Rev. 60 (2021 Aug) 133–143, https://doi.org/10.1016/j.cytogfr.2021.05.003.
[39] Y. Zhang, J. Wu, G. Yu, Z. Chen, X. Zhou, S. Zhu, R. Li, Y. Zhang, Q. Lu, A novel natriuretic peptide from the cobra venom, Toxicon 57 (1) (2011 Jan) 134–140, https://doi.org/10.1016/j.toxicon.2010.10.014. Epub 2010 Nov 2.
[40] A.L. Oliveira, M.F. Viegas, S.L. da Silva, A.M. Soares, M.J. Ramos, P. A. Fernandes, The chemistry of snake venom and its medicinal potential, Nat. Rev. Chem 6 (2022) 451–469.
[41] W.F. Ang, C.Y. Koh, R.M. Kini, From snake venoms to therapeutics: a focus on natriuretic peptides, Pharmaceuticals 15 (9) (2022 Sep 16) 1153, https://doi.org/10.3390/ph15091153.
[42] P.L. Ho, M.B. Soares, T. Maack, I. Gimenez, G. Puorto, M.F. Furtado, I. Raw, Cloning of an unusual natriuretic peptide from the South American coral snake *Micrurus corallinus*, Eur. J. Biochem. 250 (1) (1997 Nov 15) 144–149, https://doi.org/10.1111/j.1432-1033.1997.00144.x. PMID: 9432002.
[43] L.I. Leão, P.L. Ho, Ide L. Junqueira-de-Azevedo, Transcriptomic basis for an antiserum against *Micrurus corallinus* (coral snake) venom, BMC Genom. 10 (2009) 112. Mar 16.
[44] C. Corrêa-Netto, L. Junqueira-de-Azevedo Ide, D.A. Silva, P.L. Ho, M. Leitão-de-Araújo, M.L. Alves, L. Sanz, D. Foguel, R.B. Zingali, J.J. Calvete, Snake venomics and venom gland transcriptomic analysis of Brazilian coral snakes, *Micrurus altirostris* and *M. corallinus*, J. Proteonomics 74 (9) (2011 Aug 24) 1795–1809.
[45] M. Bénard-Valle, E.E. Neri-Castro, M.F. Yañez-Mendoza, B. Lomonte, A. Olvera, F. Zamudio, R. Restano-Cassulini, L.D. Possani, E. Jiménez-Ferrer, A. Alagón, Functional, proteomic and transcriptomic characterization of the venom from *Micrurus browni browni*: identification of the first lethal multimeric neurotoxin in coral snake venom, J. Proteonomics 225 (2020) 103863, https://doi.org/10.1016/j.jprot.2020.103863.
[46] S.D. Aird, N.J. da Silva, L. Qiu, A. Villar-Briones, V.A. Saddi, M. Pires de Campos Telles, M.L. Grau, A.S. Mikheyev, Coralsnake venomics: analyses of venom gland transcriptomes and proteomes of six Brazilian taxa, Toxins (Basel). 9 (6) (2017 Jun 8) 187.
[47] J. Fernández, W. Chaves, D. Vargas-Díaz, D. Petras, B. Lomonte, Top-down proteomics of venoms from five *Micrurus* species from Costa Rica: comparative composition of phospholipase A_2-rich vs three-finger toxin-rich phenotypes, Toxicon 252 (2024) 108187.
[48] S. Bhatia, K. Vasudevan, Comparative proteomics of geographically distinct saw-scaled viper (*Echis carinatus*) venoms from India, Toxicon X 7 (2020 Jun 23) 100048, https://doi.org/10.1016/j.toxcx.2020.100048.
[49] A. Rodríguez-Vargas, N. Vega, E. Reyes-Montaño, G. Corzo, E. Neri-Castro, H. Clement, F. Ruiz-Gómez, Intraspecific differences in the venom of *Crotalus durissus cumanensis* from Colombia, Toxins (Basel) 14 (8) (2022 Aug 2) 532, https://doi.org/10.3390/toxins14080532.
[50] C. Ortiz, F. Lazo, C. Bellido, E. Gonzales, A. Yarlequé, Variation of the enzymatic activity of *Bothrops atrox* "Jergon" snake venom from three geographic regions, Peru Revista Peruana de Medicina Experimental y Salud Pública 29 (2) (2012) 198–205, https://doi.org/10.1590/S1726-46342012000200005.
[51] M. Borja, E. Neri-Castro, G. Castañeda-Gaytán, J.L. Strickland, C.L. Parkinson, J. Castañeda-Gaytán, R. Ponce-López, B. Lomonte, A. Olvera-Rodríguez, A. Alagón, R. Pérez-Morales, Biological and proteolytic variation in the venom of *Crotalus scutulatus scutulatus* from Mexico, Toxins (Basel). 10 (1) (2018 Jan 8) 35, https://doi.org/10.3390/toxins10010035.
[52] J.C. Daltry, W. Wüster, R.S. Thorpe, Diet and snake venom evolution, Nature 379 (6565) (1996 Feb 8) 537–540, https://doi.org/10.1038/379537a0. PMID: 8596631.
[53] E.L. Davies, K. Arbuckle, Coevolution of snake venom toxic activities and diet: evidence that ecological generalism favours toxicological diversity, Toxins (Basel). 11 (12) (2019 Dec 6) 711, https://doi.org/10.3390/toxins11120711.
[54] O.A. Vuolo, I. Sazima, The natural history of new world coralsnakes, in: Advances in Coralsnake Biology with an Emphasis on South America, Eagle Mountain Publishing, Eagle Mountain, UT, USA, 2021. ISBN 978-0972015462.
[55] O.A. Marques, Reproduction, seasonal activity and growth of the coral snake, *Micrurus corallinus* (Elapidae), in the southeastern Atlantic forest in Brazil, Amphibia-Reptilia 17 (3) (1996) 277–285.
[56] A. Baer, G. Mayer, Comparative anatomy of slime glands in Onychophora (velvet worms), Journal of Journal of morphology 273 (10) (2012) 1079–1088.
[57] M. Martins, M.E. Oliveira, Natural history of snakes in forests of the Manaus region, Central Amazonia, Brazil, Herpetol. Nat. Hist. 6 (2) (1998) 78–150.
[58] H.W. Greene, The Food Habits and Feeding Behavior of New World Coral Snakes, Unpublished M.A. thesis, The University Texas at Arlington, Arlington, Texas, United States, 1973.
[59] O.A. Marques, I. Sazima, Diet and feeding behavior of the coral snake, *Micrurus corallinus*, from the Atlantic forest of Brazil, Herpetol. Nat. Hist. 5 (1) (1997) 88–93.
[60] F. Maffei, G.R. Do Nascimento, D.G. Neto, Predationon the lizard *Ameiva ameiva* (sauria: teiidae) by a coral snake *Micrurus frontalis* (Serpentes: elapidae) in Brazil, Herpetol. Notes 2 (2009) 235–237.
[61] M.A. Arévalo, A.C. Montes-Correa, E. Rada, L.P. Saboyá-Acosta, J.M. Renjifo, Notes on the diet of pigmy coral snake (*Micrurus dissoleucus*) (Cope, 1860) (Elapidae) in northern Colombia, Herpetol. Notes 8 (2015) 39–41.
[62] L.E. Vera-Pérez, J.A. Zúñiga Baos, S. Ayerbe González, New records of length and diet of *Micrurus mipartitus* (duméril, bibron and duméril, 1854) (Serpentes: elapidae), Revista Novedades Colombianas 14 (1) (2019) 49–56, https://doi.org/10.47374/novcol.2019.v14.1434.
[63] J.D. Fernandez-Roldán, G.F. Medina-Rangel, Y.R. López-Perilla, Fist record of predation of *Micrurus mipartitus* (Serpentes: Elapidae) on *Oscaecilia polyzona* (Gymnophiona: Caeciliidae) in Colombia: Fernández et al. First record of *Micrurus mipartitus* feeding on *Oscaecilia polyzona*, Revista Latinoamericana de Herpetología 4 (1) (2021) 199–202.
[64] F. Barrera-Ocampo, T.R. Forrester, Primer registro de depredación sobre una cecilia, *Caecilia sp.* (Gymnophiona: Caeciliidae) por la poco conocida coral caucana, *Micrurus multiscutatus* (SQUAMATA: ELAPIDAE), Revista Latinoamericana De Herpetología 7 (1) (2024) e795, https://doi.org/10.22201/fc.25942158e.2024.1.795, 143 – 148.
[65] O.A.V. Marques, I. Sazima, The natural history of New World coralsnakes.

Advances in Coralsnake Biology: with an Emphasis in South America, Eagle Mountain Publishing, Utah, 2021, pp. 275–314.
[66] J.H. Valencia, K. Garzón-Tello, M.E. Barragán-Paladines, Serpientes venenosas del Ecuador: sistemática, taxonomía, historial natural, conservación, envenenamiento y aspectos antropológicos, Fundación Herpetológica Gustavo Orcés, Quito, 2016, p. 653.
[67] A. Arteaga, L. Bustamante, J. Vieira, Reptiles of Ecuador: Life in the Middle of the World, 1 Publisher, Khamai Foundation & Tropical Herping, Edition, 2024. ISBN: 978-9942-7249-0-810.47051/MNHT9360.
[68] J. Fernández, A. Alape-Girón, Y. Angulo, L. Sanz, J.M. Gutiérrez, J.J. Calvete, B. Lomonte, Venomic and antivenomic analyses of the Central American coral snake, *Micrurus nigrocinctus* (Elapidae), J. Proteome Res. 10 (4) (2011 Apr 1) 1816–1827, https://doi.org/10.1021/pr101091a.
[69] A.M. Henao Duque, V. Núñez, Maintenance of red-tail coral snake (*Micrurus mipartitus*) in captivity and evaluation of individual venom variability. Acta Biológica Colombiana, Acta Biol. Colomb. 21 (3) (2016) 593–600, https://doi.org/10.15446/abc.v21n3.51651.
[70] P. Rey-Suárez, R.S. Floriano, S. Rostelato-Ferreira, M. Saldarriaga-Córdoba, V. Núñez, L. Rodrigues-Simioni, B. Lomonte, Mipartoxin-I, a novel three-finger toxin, is the major neurotoxic component in the venom of the redtail coral snake *Micrurus mipartitus* (Elapidae), Toxicon 60 (5) (2012 Oct) 851–863, https://doi.org/10.1016/j.toxicon.2012.05.023.
[71] G. Mena, S. Chaves-Araya, J. Chacón, E. Török, F. Török, F. Bonilla, M. Sasa, J. M. Gutiérrez, B. Lomonte, J. Fernández, Proteomic and toxicological analysis of the venom of *Micrurus yatesi* and its neutralization by an antivenom, Toxicon X 13 (2022 Feb 12) 100097, https://doi.org/10.1016/j.toxcx.2022.100097.
[72] J.D. Piedrahita, A. Cardona-Ruda, J.A. Pereañez, P. Rey-Suárez, In-depth immunorecognition and neutralization analyses of *Micrurus mipartitus* and *M. dumerilii* venoms and toxins by a commercial antivenom, Biochimie 216 (2024) 120–125, https://doi.org/10.1016/j.biochi.2023.10.009.
[73] M.C. Castillo-Beltrán, J.P. Hurtado-Gómez, V. Corredor-Espinel, F.J. Ruiz-Gómez, A polyvalent coral snake antivenom with broad neutralization capacity, PLoS Negl Trop Dis 13 (3) (2019 Mar 11) e0007250, https://doi.org/10.1371/journal.pntd.0007250.
[74] P. Rey-Suárez, M. Saldarriaga-Córdoba, U. Torres, M. Marin-Villa, B. Lomonte, V. Núñez, Novel three-finger toxins from *Micrurus dumerilii* and *Micrurus mipartitus* coral snake venoms: phylogenetic relationships and characterization of Clarkitoxin-I-Mdum, Toxicon 170 (2019) 85–93, https://doi.org/10.1016/j.toxicon.2019.09.017.