Venom Proteomic Analysis of Two Medically Important Nigerian Viper (Echis Ocellatus and Bitis Arietans) Snake Species

Emeka John Dingwoke (dinhimself@yahoo.com)
Ahmadu Bello University

Fatima Amin Adamude
Federal University

Aliyu Salihu
Ahmadu Bello University

Mujitaba Suleiman Abubakar
Ahmadu Bello University

Gadija Mohamed
Infrutec-Nietvoorbij

Ashwil Klein
University of Western Cape

Abdullahi Balarabe Sallau
Ahmadu Bello University

Research Article

Keywords: Snakebite, neglected tropical disease, viperidae, Bitis arietans, Echis ocellatus, snake venom, proteomic, mass spectrometry.

DOI: https://doi.org/10.21203/rs.3.rs-381219/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract
Snakebite envenoming remains a neglected tropical disease, which poses severe health hazard, especially for the rural inhabitants in Africa. In Nigeria, vipers are the most toxin-producing snakes that cause the highest number of deaths. Hydrophilic interaction liquid chromatography coupled with LC-MS/MS was used to analyze the crude venom extracts of Echis ocellatus (Carpet viper) and Bitis arietans (Puff adder) in order to understand their venom proteomic identities. Results obtained revealed that gel-free proteomic analysis of the crude venom extracts from E. ocellatus and B. arietans yielded the identification of 85 and 79 proteins, respectively. Seventy-nine (79) proteins were common between the two snake species with a 90.8% similarity score. The identified proteins belong to 12 protein families where serine proteases (22.31%) and metalloproteinases (21.06%) were the dominant proteins in the venom of B. arietans. Metalloproteinases (34.84%), phospholipase A₂s (25.69%) and serine proteases (17.25%) represents the major toxins in the E. ocellatus venom. Other protein families such as three-finger toxins and cysteine-rich venom proteins were also detected, albeit, in low proportions. This study represents the venom proteomic analysis of the two Nigerian viper species, which provides some valuable insights into the toxin families to be neutralized in case of envenomation. Data are available via ProteomeXchange with identifier PXD024638.

1. Introduction
Venomous snakes pose severe health problem especially for the rural dwellers in the tropical regions of developing countries\(^\text{1,2,3}\). Despite the remarkable efforts for effective management of envenomings, the World Health Organization (WHO) incorporated snakebite envenoming in the list of neglected tropical diseases\(^\text{4}\). Given the inclusion of snakebite envenoming as a neglected tropical disease, better efforts are required to address the life-threatening situation posed by this disease. The scourge is predominant in the Savanna region of West Africa including other developing countries where over 95% of all reported cases occur\(^\text{3,4,5}\). In Nigeria, the viperidae, notably Bitis arietans (Puff adder) and Echi ocellatus (Carpet viper) are associated with the highest number of morbidity and mortality\(^\text{5,6,7,1}\), and are regarded as the most dangerous venomous snake species. The actual fatality index resulting from envenomings by these vipers remains unknown due to inaccurate epidemiological data\(^\text{1,2,8}\), as most of the victims do not have access to health care facilities and therefore resort to ethno-medicinal means for treatments. Reports from community-based surveys suggest a higher envenomation incidence compared to the hospital-based estimates\(^\text{9,8,2}\). As reported by the WHO, about 5.4 million snakebites occur annually, resulting in 2.7 million cases of envenomings, 138,000 deaths and 400,000 cases of disabilities\(^\text{10}\). In the sub-Saharan Africa, the incidence of snakebites was estimated at 314,000 cases, which resulted in over 6,000 amputations and 7,300 deaths\(^\text{4}\). In Nigeria, the incidence of snakebites leads to 10,000 deaths annually with envenomation by E. ocellatus responsible for 66% of total cases\(^\text{7}\). Snakebite occurs accidentally by infiltration of venom components, which are a mixture of toxic secretions into humans, through the fangs of the snake\(^\text{6}\). The incidents are high during the rainy season when farming activities coincide with the snakes’ breeding season\(^\text{2}\). The victims are predominantly young males that are engaged in farming, plantation, agricultural harvests and herding activities\(^\text{1,11,6,12}\). Snakebite envenoming inflicts severe damage to different organs of the body, depending on the nature of toxins in the venom. Envenomings by the viperidae snakes are characterized by life-threatening symptoms, primarily due to local tissue damage and bleeding\(^\text{1}\).

The evolution of venomics and antivenomics using the ‘-omics’ technologies has paved a way to obtain valuable insights into snake venom compositions, revealing the protein constituents and different types of toxic components\(^\text{13,14}\). In addition, the ‘-omics’ approach has led to the rapid examination of the immune reactivity of antivenoms against the toxins in snake venoms\(^\text{2,15}\). It is widely inferred that the pathological complications associated with snake envenomation is influenced by the geographical location of the snake\(^\text{16,17,18,19}\), diet type\(^\text{20}\), age\(^\text{21}\) and mutations in the venom-related genes. This in turn hinders progressive treatment of envenomed patients. Hence, analyzing venom proteomes to gain insight into snake venom variability can help in understanding their biological and pathological effects\(^\text{22}\), especially the viperids B. arietans and E. ocellatus. It will also help in making appropriate choice of antivenom to use in treatment. The venom gland transcriptomic of Nigerian B. arietans and E. ocellatus was reported previously\(^\text{23,24}\). In the current study, we analyzed the venom proteomes of B. arietans and E. ocellatus captured from different regions of Nigeria with the view to understanding their toxin profile and protein identities. This contributes in enhancing the effectiveness of the treatment for envenoming by these Nigerian viper snakes.

2. Materials And Method
2.1. Bitis arietans and Echi ocellatus venom samples

All protocols for snake handling were observed and carried out in accordance with the Ahmadu Bello University Committee on Animal Use and Care with approval number ABUCAUC/2021/028, and in compliance with the revised ARRIVE guidelines 2.0. Adult snakes *B. arietans* and *E. ocellatus* were randomly captured from different regions of the Southern (Niger-Delta, South-East and South-West) and Northern (North-East, North-West and North-Central) Nigeria, during the raining season. Six (6) snakes (comprising of 3 snakes from each of the specie) were captured in each of the geo-political areas listed, making a total of 36 snakes. They were maintained at the serpentarium of the Department of Veterinary Pharmacology and Toxicology. After 3 days, their venoms were manually extracted as described by Hill and Mackessy. Venoms from snakes of the same species were combined. They were frozen at -80°C, lyophilized using a freeze-dryer, and stored at -20°C.

2.2. Chemicals and reagents

Magnetic bead-based hydrophilic interaction liquid chromatography (HILIC) particles were purchased from Sigma Aldrich (USA), whereas the protein standard marker and analytical-grade trypsin were obtained from Agilent Technologies (Santa Clara, CA, USA).

2.3. Venom protein extraction and pellet solubilization

The venom proteins were extracted as described by Adamude et al. Two milligrams (dry-weight) of the lyophilized crude venom (from each species) were solubilized in 50 µL 1X PBS (pH 7.4), vortexed for 10 min, and centrifuged at 15,700 × g for 5 min at 4°C. Acetone precipitation was performed on the supernatant by the addition of 600 µL cold acetone, and the samples were incubated at -20°C for 15 min. Precipitated samples were centrifuged at 15,700 × g for 15 min at 4°C. Protein pellets were air-dried and resuspended in 100 µL 1X PBS buffer (pH 7.4). Protein concentration of each sample was quantified using the RC DC Protein Assay Kit 11 (Bio-Rad Laboratories) and confirmed with the microvolume protein concentration determination method. Data was acquired on a Nanodrop Spectrophotometer 2000c (Thermo Fisher Scientific, USA).

2.4. One-dimensional SDS-PAGE

Aliquots of each sample (15 µg) were subjected to 12% SDS-PAGE under non-reducing conditions and ran for 90 min at 100 V. The gels were stained with Coomassie Brilliant Blue (G-250), and de-stained in 10% glacial acetic acid containing 1% glycerol. The resolved protein bands were visualized using a Molecular Imager PharosFX Plus System (Bio-Rad, California, USA).

2.5. Hydrophilic Interaction Liquid Chromatography (HILIC) and Trypsin digestion

HILIC was carried out as described. Fifty micrograms (50 µg) of protein from each venom sample was suspended in a final concentration of 50 mM triethylammonium bicarbonate (TEAB; Sigma). The proteins were reduced with a final concentration of 10 mM Dithiothreitol (DTT; Sigma) in 50 mM TEAB for 40 min at 56°C. Samples were cooled to room temperature and alkylated with a final concentration of 30 mM iodoacetamide (Sigma) in 50 mM TEAB at 25°C and kept in the dark for 30 min. After alkylation, two-fold dilutions of the samples were made with binding buffer (100 mM ammonium acetate, 30% acetonitrile, pH 4.5). The protein solution was added to pre-equilibrated MagResyn® HILIC magnetic particles (Resyn Biosciences) prepared according to the manufacturer’s instructions and incubated overnight at 4°C. After binding, the supernatant was removed and magnetic particles washed twice with 95% acetonitrile for 1 min. For digestion, the magnetic particles were suspended in 50 mM ammonium formate (pH 8.0) containing trypsin (Promega) to a final enzyme:protein ratio of 1:10 and incubated overnight at 27°C with constant shaking. Following digestion, peptides were recovered with 1% trifluoroacetic acid (TFA) and incubated at room temperature for 3 min and analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

2.6. Characterization of venom proteins

Liquid chromatography was performed as described previously with slight modification using a Ultimate™ 3000 RSLCnano System (Thermo Fisher Scientific, USA) equipped with a C18 trap column (5 mm × 300 µm; Thermo Fisher Scientific) and a CSH C18 analytical column (1.7 µm, 25 cm x 75 µm; Waters), with a linear gradient of 0.1% trifluoroacetic acid in water (solution A) and acetonitrile (solution B). The samples were loaded on the trap column and the flow rate was set at 250 nL/min and the gradient was generated as follows: 5–35% solution B for 60 min and 35–50% solution B for 60–75 min. Chromatography was performed at 40°C, and the outflow was delivered to the mass spectrometer through a stainless-steel nano-bore emitter. LC-MS analysis was performed on a
Fusion mass spectrometer (Thermo Scientific, San Jose, CA) equipped with a nanospray ion source (Nanospray Flex™ Ion Sources, Thermo Fisher Scientific) coupled to a Dionex Ultimate 3000 RSLC nano-HPLC system. Peptides recovered from the on-bead HILIC digestion for each venom sample was introduced through a stainless-steel emitter. Data were collected in positive mode with a spray voltage set to 1.8 kV and ion transfer capillary set to 280°C. Spectra were internally calibrated using polysiloxane ions at m/z 445.12003 and 371.10024. The first MS scan was performed using the orbitrap detector set to a resolution of 120,000 resolutions over an m/z range of 350–1650 with an AGC target at 3E5 and maximum injection time of 40 milliseconds. Data were acquired in profile mode. The second MS scan was performed using monoisotopic precursor selection for the ion with charges of +2 to +7 with error tolerance set at ±10 ppm. Precursor ions were excluded for 60 seconds after fragmentation. Precursor ions were selected for fragmentation in the HCD mode using the quadrupole mass analyzer with HCD energy set to 30%. Fragment ions were detected in the orbitrap mass analyzer at a resolution of 30000. The AGC target was set to 5E4 and the maximum injection time was set to 80 milliseconds. Data were acquired in centroid mode.

2.7. Data analysis

Data was analyzed following the protocol described previously. The mass spectra obtained were subjected to Proteome Discoverer v1.4 software (Thermo Fisher Scientific, USA) and processed using the Sequest and Amanda algorithms. Database interrogation was performed against a concatenated database created by concatenating all ‘snake protein’ entries in the Uniprot-Serpentes database with the cRAP contaminant database (https://www.thegpm.org/crap/). The precursor mass tolerance was set to 10 ppm and fragment mass tolerance was set to 0.02 Da. Peptide validation was performed using the Target-Decoy PSM validator node. The search results were subjected to Scaffold Q+ version 4.10.0 for further validation (www.proteomesoftware.com). Peptide identification, by peptide-spectrum match approach against Uniprot-Serpentes database, and subsequent assembly, matching, and identification of protein sequences were performed using X! Tandem and Sequest search engines. The search engines were incorporated into Scaffold Q+, version 4.10.0 at 99% protein threshold, 95% peptide threshold, and two-peptide minimum criterion. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE with identifier PXD024638.

3. Results And Discussion

3.1. One-dimensional SDS-PAGE of the crude snake venoms

A fraction (15 µg) of each crude venom extract was size fractionated on a one dimensional SDS polyacrylamide gel and visualized using Coomassie Brilliant Blue R-250 dye (Fig. 1). The protein banding patterns observed for the two venoms extract were distinctly different and clearly separated in a molecular weight range of 7 kDa, 22 kDa and 100 kDa (Fig. 1). The distinct banding pattern on the 1D gel correlated with the LC-MS/MS analysis which showed that *B. arietans* had serine proteases and metalloproteinases as the major venom proteins (Fig. 3), while the venom of *E. ocellatus* was composed of three major proteins; phospholipase A2s, serine proteases and metalloproteinases (Fig. 4). In Nigeria, the highest number of mortality is associated with envenoming by *E. ocellatus*.

Our findings indicated that *E. ocellatus* produced more toxins due to the high amount of phospholipase A2s compared to the amount found in the venom of *B. arietans*, this could be a possible reason for the higher mortality rates associated with envenoming by *E. ocellatus*. In related reports, high rates of lethality are caused by viper envenoming.

3.2. Snake venom proteomic characterization using LC-MS/MS

A total of 79 proteins were detected in the venom of *B. arietans* whereas 85 proteins were detected in the venom of *E. ocellatus* (Table 1) when the tryptic digested crude venom extract was analysed using LC-MS/MS. A total of 79 proteins were common in the venom of the two snake species (Fig. 2). In addition, only one uncommon protein was identified in the venom of *B. arietans* whereas 7 uncommon proteins were identified in the venom of *E. ocellatus* (Fig. 2), with a protein similarity index of 90.8%. All the proteins detected (in the molecular mass range of 4–93 kDa) (Table 2) belonged to 12 protein families (Table 3). The most abundant proteins in the venom of *B. arietans* were serine proteases (22%) and metalloproteases (21%) (Fig. 3), followed by C-type lectins/ sxnacles (10.65%), phospholipase A2 (10.6%), vascular endothelial growth factors (10.3%) and L-amino acid oxidases (8.72%). Three-finger toxins (4.32%), disintegrins (3.4%), 5'-nucleotidases (2.5%), and cysteine-rich venom proteins (2.05%) were distributed in much lower abundance. Kunitz-type peptides and phospholipase B were the least abundant protein families (<1%) identified in *B. arietans* (Fig. 3). On the other hand, the most abundant proteins identified in the venom extract of *E. ocellatus* include metalloproteinases (34.84%),
phospholipase A\textsubscript{2} (25.69\%), and serine proteases (17.25\%) (Fig. 4). C-type lectins/ snaclecs (3.95\%), three-finger toxins (3.73\%), vascular endothelial growth factors (3.3\%) and cysteine-rich venom proteins (2.9\%) were found in much lower abundance. Disintegrins, 5'-nucleotidases, L-amino acid oxidases, and Kunitz-type peptides families were very low in abundance (< 3 \%), while phospholipase B was virtually absent (0.6\%) as shown in Fig. 4.
| S/no. | Protein/peptide detected                                      | Originating specie | Originating specie | Originating specie |
|-------|----------------------------------------------------------------|-------------------|-------------------|-------------------|
| 1     | Acidic phospholipase A2                                         | *Echis ocellatus*  | Acidic phospholipase A2 | *Echis ocellatus*  |
| 2     | Acidic phospholipase A2                                         | *Naja mossambica* | Acidic phospholipase A2 | *Naja mossambica* |
| 3     | Alpha-fibrinogenase                                             | *Macrovipera lebetina* | Alpha-fibrinogenase | *Macrovipera lebetina* |
| 4     | Alpha-fibrinogenase-like                                       | *Daboia siamensis* | Alpha-fibrinogenase-like | *Daboia siamensis* |
| 5     | Beta-fibrinogenase brevinase                                    | *Gloydius blomhoffii* | Beta-fibrinogenase brevinase | *Gloydius blomhoffii* |
| 6     | C-type lectin 1                                               | *Bitis gabonica*  | C-type lectin 1 | *Bitis gabonica*  |
| 7     | Cationic trypsin                                              | *Bos taurus*      | Cationic trypsin | *Bos taurus*      |
| 8     | Coagulation factor X-activating enzyme heavy chain             | *Daboia siamensis* | Coagulation factor X-activating enzyme heavy chain | *Daboia siamensis* |
| 9     | Coagulation factor X-activating enzyme heavy chain             | *Macrovipera lebetina* | Coagulation factor X-activating enzyme heavy chain | *Macrovipera lebetina* |
| 10    | Cysteine-rich venom protein                                    | *Trimeresurus stejnegeri* | Cysteine-rich venom protein | *Trimeresurus stejnegeri* |
| 11    | Cytotoxin 1                                                    | *Naja mossambica* | Cytotoxin 1 | *Naja mossambica* |
| 12    | Disintegrin EO5A                                              | *Echis ocellatus*  | Disintegrin EO5A | *Echis ocellatus*  |
| 13    | Disintegrin bitistatin                                        | *Bitis arietans*  | Disintegrin bitistatin | *Bitis arietans*  |
| 14    | Glutaminyl-peptide cyclotransferase                            | *Boiga dendrophila* | Kunitz-type serine protease inhibitor bitisilin-1 | *Bitis gabonica* |
| 15    | Hemoglobin subunit beta                                        | *Erythrolamprus miliaris* | Kunitz-type serine protease inhibitor bitisilin-3 | *Bitis gabonica* |
| 16    | Kunitz-type serine protease inhibitor bitisilin-1             | *Bitis gabonica*  | L-amino-acid oxidase | *Bitis gabonica* |
| 17    | Kunitz-type serine protease inhibitor bitisilin-3             | *Bitis gabonica*  | L-amino-acid oxidase | *Echis ocellatus* |
| 18    | L-amino-acid oxidase                                          | *Bitis gabonica*  | L-amino-acid oxidase | *Pseudechis australis* |
| 19    | L-amino-acid oxidase                                          | *Echis ocellatus*  | Long neurotoxin 1 | *Naja anchietae* |
| 20    | L-amino-acid oxidase                                          | *Pseudechis australis* | Peroxiredoxin-4 | *Crotalus atrox* |
| 21    | Long neurotoxin 1                                              | *Naja anchietae*  | Phospholipase A2 homolog | *Echis ocellatus* |
| 22    | Peroxiredoxin-4                                               | *Crotalus atrox*  | Phospholipase-B 81 | *Drysdalia coronoides* |
| 23    | Phospholipase A2 homolog                                      | *Echis ocellatus*  | Serine protease harobin | *Hydrophis hardwickii* |
| 24    | Phospholipase A2 inhibitor 1                                   | *Protobothrops flavoviridis* | Serine protease sp-Eoc49 | *Echis ocellatus* |
| 25    | Phospholipase-B 81                                             | *Drysdalia coronoides* | Short neurotoxin 1 | *Naja nivea* |
| 26    | Secretory phospholipase A2 receptor                            | *Pongo abelii*     | Snaclce 2 | *Bitis gabonica* |

*Table 1: Protein identification in the venoms of *B. arietans* and *E. ocellatus*
| No. | Protein/peptide detected                                      | Species (Genus) | Source (Subunit) | Animal (Genus) |
|-----|-------------------------------------------------------------|-----------------|-----------------|---------------|
| 27  | Serine protease harobin                                     | Hydrophis       | Snaclec 3       | Bitis gabonica |
| 28  | Serine protease sp-Eoc49                                    | Echis ocellatus | Snaclec agglucetin subunit beta-1 | Deinagkistrodon acutus |
| 29  | Short neurotoxin 1                                          | Naja nivea      | Snaclec bitiscetin subunit alpha | Bitis arietans |
| 30  | Snaclec 2                                                    | Bitis gabonica  | Snaclec bitiscetin subunit beta | Bitis arietans |
| 31  | Snaclec 3                                                    | Bitis gabonica  | Snaclec clone 2100755 | Deinagkistrodon acutus |
| 32  | Snaclec CTL-Eoc124                                          | Echis ocellatus | Snake venom 5'-nucleotidase | Crotalus adamanteus |
| 33  | Snaclec CTL-Eoc125                                          | Echis ocellatus | Snake venom metalloproteinase ACLH | Agkistrodon contortrix laticinctus |
| 34  | Snaclec agglucetin subunit beta-1                           | Deinagkistrodon acutus | Snake venom metalloproteinase kistomin | Calloselasma rhodostoma |
| 35  | Snaclec bitiscetin subunit alpha                            | Bitis arietans  | Snake venom metalloproteinase lebetase-4 | Macrovia lebetina |
| 36  | Snaclec bitiscetin subunit beta                             | Bitis arietans  | Snake venom metalloproteinase-disintegrin-like mocularhagin | Naja mossambica |
| 37  | Snaclec clone 2100755                                       | Deinagkistrodon acutus | Snake venom serine protease BthaTL | Bothrops alternatus |
| 38  | Snaclec convulxin subunit beta                              | Crotalus durissus terrificus | Snake venom serine protease HS114 | Bothrops jararaca |
| 39  | Snake venom 5'-nucleotidase                                  | Crotalus adamanteus | Snake venom serine protease NaSP | Naja atra |
| 40  | Snake venom metalloproteinase ACLH                          | Agkistrodon contortrix laticinctus | Snake venom serine protease | Philodryas ofersii |
| 41  | Snake venom metalloproteinase kistomin                      | Calloselasma rhodostoma | Snake venom vascular endothelial growth factor toxin barietin | Bitis arietans |
| 42  | Snake venom metalloproteinase lebetase-4                    | Macrovia lebetina | Thrombin-like enzyme cerastocytin | Cerastes cerastes |
| 43  | Snake venom metalloproteinase-disintegrin-like mocularhagin  | Naja mossambica | Vascular endothelial growth factor A | Bitis gabonica |
| 44  | Snake venom serine protease BthaTL                          | Bothrops alternatus | Venom nerve growth factor 2 | Naja sputatrix |
| 45  | Snake venom serine protease HS114                           | Bothrops jararaca | Weak neurotoxin 7 | Naja naja |
| 46  | Snake venom serine protease NaSP                            | Naja atra       | Weak toxin CM-2a | Naja annulifera |
| 47  | Snake venom serine protease Philodryas ofersii              | Zinc metalloproteinase homolog-disintegrin albolatin | Trimeresurus albolabris |
| 48  | Snake venom vascular endothelial growth factor toxin barietin | Bitis arietans  | Zinc metalloproteinase leucurolysin-B | Bothrops leucurus |
| 49  | Thrombin-like enzyme cerastocytin                            | Cerastes cerastes | Zinc metalloproteinase-disintegrin BA-5A | Bitis arietans |
| 50  | Venom nerve growth factor 2                                  | Naja sputatrix  | Zinc metalloproteinase-disintegrin Blath1 | Bothriechis lateralis |
| Protein/peptide detected | Source | Zinc metalloproteinase-disintegrin-like | Source |
|--------------------------|--------|----------------------------------------|--------|
| 51 Weak neurotoxin 7     | Naja naja | Zinc metalloproteinase-disintegrin bilitoxin-1 | Agkistrodon bilineatus |
| 52 Weak toxin CM-2a      | Naja annulifera | Zinc metalloproteinase-disintegrin-like BFMP | Bungarus fasciatus |
| 53 Zinc metalloproteinase homolog-disintegrin albolatin | Trimeresurus albolabris | Zinc metalloproteinase-disintegrin-like EoMP06 | Echis ocellatus |
| 54 Zinc metalloproteinase leucurolysin-B | Bothrops leucurus | Zinc metalloproteinase-disintegrin-like EoVMP2 | Echis ocellatus |
| 55 Zinc metalloproteinase-disintegrin BA-5A | Bitis arietans | Zinc metalloproteinase-disintegrin-like Eoc1 | Echis ocellatus |
| 56 Zinc metalloproteinase-disintegrin BlatH1 | Bothriechis lateralis | Zinc metalloproteinase-disintegrin-like HF3 | Bothrops jararaca |
| 57 Zinc metalloproteinase-disintegrin bilitoxin-1 | Agkistrodon bilineatus | Zinc metalloproteinase-disintegrin-like MTP9 | Drysdalia coronoides |
| 58 Zinc metalloproteinase-disintegrin-like BFMP | Bungarus fasciatus | Zinc metalloproteinase-disintegrin-like NaMP | Naja atra |
| 59 Zinc metalloproteinase-disintegrin-like EoMP06 | Echis ocellatus | Zinc metalloproteinase-disintegrin-like | Cerberus rynchops |
| 60 Zinc metalloproteinase-disintegrin-like EoVMP2 | Echis ocellatus | Zinc metalloproteinase-disintegrin-like TSV-DM | Trimeresurus stejnegeri |
| 61 Zinc metalloproteinase-disintegrin-like Eoc1 | Echis ocellatus | Zinc metalloproteinase-disintegrin-like VLAIP-A | Macrovia lebetina |
| 62 Zinc metalloproteinase-disintegrin-like HF3 | Bothrops jararaca | Zinc metalloproteinase-disintegrin-like VLAIP-B | Macrovia lebetina |
| 63 Zinc metalloproteinase-disintegrin-like MTP9 | Drysdalia coronoides | Zinc metalloproteinase-disintegrin-like acurhagin | Deinagkistrodon acutus |
| 64 Zinc metalloproteinase-disintegrin-like NaMP | Naja atra | Zinc metalloproteinase-disintegrin-like atrolysin-A | Crotalus atrox |
| 65 Zinc metalloproteinase-disintegrin-like | Cerberus rynchops | Zinc metalloproteinase-disintegrin-like batroxstatin-1 | Bothrops atrox |
| 66 Zinc metalloproteinase-disintegrin-like TSV-DM | Trimeresurus stejnegeri | Zinc metalloproteinase-disintegrin-like batroxstatin-3 | Bothrops atrox |
| 67 Zinc metalloproteinase-disintegrin-like VLAIP-A | Macrovia lebetina | Zinc metalloproteinase-disintegrin-like berythracitavase | Bothrops erythromelas |
| 68 Zinc metalloproteinase-disintegrin-like VLAIP-B | Macrovia lebetina | Zinc metalloproteinase-disintegrin-like bothrojarin-2 | Bothrops jararaca |
| 69 Zinc metalloproteinase-disintegrin-like acurhagin | Deinagkistrodon acutus | Zinc metalloproteinase-disintegrin-like brevlyisin H2a | Gloydius brevicaudus |
| 70 Zinc metalloproteinase-disintegrin-like atrolysin-A | Crotalus atrox | Zinc metalloproteinase-disintegrin-like cobrin | Naja kaouthia |
| 71 Zinc metalloproteinase-disintegrin-like batroxstatin-1 | Bothrops atrox | Zinc metalloproteinase-disintegrin-like daborhagin-K | Daboia russelii |
| 72 Zinc metalloproteinase-disintegrin-like batroxstatin-3 | Bothrops atrox | Zinc metalloproteinase-disintegrin-like stejnihagin-A | Trimeresurus stejnegeri |
| 73 Zinc metalloproteinase-disintegrin-like berythracitavase | Bothrops erythromelas | Zinc metalloproteinase-disintegrin-like stejnihagin-B | Trimeresurus stejnegeri |
| 74 Zinc metalloproteinase-disintegrin-like bothrojarin-2 | Bothrops jararaca | Zinc metalloproteinase/disintegrin | Protobothrops mucrosquamatus |
| No. | Protein/peptide detected                                      | Organism                  | Metalloproteinase/disintegrin               | Organism                  |
|-----|-------------------------------------------------------------|----------------------------|--------------------------------------------|----------------------------|
| 75  | Zinc metalloproteinase-disintegrin-like brevilysin H2a      | *Gloydius brevicaudus*    | Zinc metalloproteinase/disintegrin         | *Trimeresurus gramineus*    |
| 76  | Zinc metalloproteinase-disintegrin-like cobrin              | *Naja kaouthia*           | Zinc metalloproteinase/disintegrin         | *Calloselasma rhodostoma*   |
| 77  | Zinc metalloproteinase-disintegrin-like daborhagin-K        | *Daboia russeli*          | metalloproteinase/disintegrin              | *Echis ocellatus*           |
| 78  | Zinc metalloproteinase-disintegrin-like stejnihagin-A       | *Trimeresurus stejnegeri* | Zinc metalloproteinase/disintegrin         | *Macrovipera lebetina*      |
| 79  | Zinc metalloproteinase-disintegrin-like stejnihagin-B       | *Trimeresurus stejnegeri* | Zinc metalloproteinase/disintegrin PMMP-1  | *Protobothrops mucrosquamatus* |
| 80  | Zinc metalloproteinase/disintegrin                          | *Protobothrops mucrosquamatus* |
| 81  | Zinc metalloproteinase/disintegrin                          | *Trimeresurus gramineus*  |
| 82  | Zinc metalloproteinase/disintegrin                          | *Calloselasma rhodostoma* |
| 83  | metalloproteinase/disintegrin                               | *Echis ocellatus*         |
| 84  | Zinc metalloproteinase/disintegrin                          | *Macrovipera lebetina*    |
| 85  | Zinc metalloproteinase/disintegrin PMMP-1                   | *Protobothrops mucrosquamatus* |

Proteins were identified using Multi-Dimensional Protein Identification Technology (MuDPIT) incorporated on Scaffold Proteome software version 4.10.0 at 99% protein threshold, 95% peptide threshold, 0.5% false discovery rate (FDR), and two-peptide minimum criterion. Peptides and proteins were searched against the UniprotKB- Serpentes-database.
Table 2
Characterization of proteins identified in the venom extracts of *B. arietans* and *E. ocellatus*

| S/No. | Protein/Toxin detected                          | Protein accession number | B. arietans | E. ocellatus |
|-------|------------------------------------------------|--------------------------|-------------|-------------|
|       |                                                 | Mol. mass (kDa) | AA (%) | NET | TUSC | EUP | AA (%) | NET | TUSC | EUP |
| 1     | Acidic phospholipase A2                         | PA2A5_ECHOC             | 16        | 83  | 32  | 12  | 8      | 61  | 439  | 79  | 38  |
| 2     | Acidic phospholipase A2                         | PA2A1_NAJMO             | 13        | 39  | 19  | 1   | 1      | 39  | 9    | 1   | 1   |
| 3     | Alpha-fibrinogenase                             | VSPA_MACLB              | 29        | 8.5 | 29  | 0   | 0      | 8.1 | 20   | 0   | 0   |
| 4     | Alpha-fibrinogenase-like                        | VSPAF_DABSI             | 28        | 3.5 | 28  | 0   | 0      | 0.8 | 20   | 1   | 1   |
| 5     | Beta-fibrinogenase brevinase                    | VSPB_GLOBL              | 26        | 8.6 | 54  | 0   | 0      | 16  | 76   | 2   | 1   |
| 6     | C-type lectin 1                                 | LEC1_BITGA              | 19        | 16  | 4   | 2   | 2      | 8.2 | 2    | 1   | 1   |
| 7     | Cationic trypsin                                | TRY1_BOVIN              | 26        | 16  | 5   | 3   | 3      | 8.5 | 2    | 1   | 1   |
| 8     | Coagulation factor X-activating enzyme heavy chain | VM3CX_DABSI            | 70        | 8.1 | 33  | 0   | 0      | 21  | 43   | 9   | 4   |
| 9     | Coagulation factor X-activating enzyme heavy chain | VM3CX_MACLB            | 69        | 8.5 | 14  | 0   | 0      | 18  | 54   | 4   | 3   |
| 10    | Cysteine-rich venom protein                      | CRVP_TRIST              | 26        | 4.3 | 1   | 0   | 0      | 13  | 14   | 0   | 0   |
| 11    | Cytotoxin 1                                     | 3SA1_NAJMO              | 7         | 47  | 9   | 0   | 0      | 33  | 30   | 0   | 0   |
| 12    | Disintegrin E05A                                | DDISA_ECHOC             | 12        | 9.6 | 7   | 0   | 0      | 36  | 31   | 0   | 0   |
| 13    | Disintegrin bitistatin                          | VM2_BITAR               | 9         | 88  | 80  | 11  | 6      | 27  | 6    | 12  | 6   |
| 14    | Glutaminyl-peptide cyclotransferase             | QPCT_BOIDE              | 42        | 0.0 | 0   | 0   | 0      | 17  | 12   | 1   | 1   |
| 15    | Hemoglobin subunit beta                         | HBB_ERYML               | 16        | 0.0 | 0   | 0   | 0      | 6.2 | 3    | 1   | 1   |
| 16    | Kunitz-type serine protease inhibitor bitisilin-1 | VKT1_BITGA            | 10        | 18  | 5   | 2   | 2      | 10  | 2    | 2   | 2   |
| 17    | Kunitz-type serine protease inhibitor bitisilin-3 | VKT3_BITGA            | 17        | 42  | 12  | 5   | 4      | 17  | 7    | 2   | 2   |
| 18    | L-amino-acid oxidase                            | OXLA_BITGA              | 7         | 12  | 4   | 0   | 0      | 20  | 7    | 1   | 1   |
| 19    | L-amino-acid oxidase                            | OXLA_ECHOC              | 57        | 34  | 58  | 1   | 1      | 63  | 109  | 1   | 1   |
| 20    | L-amino-acid oxidase                            | OXLA_PSEAU              | 59        | 1.4 | 2   | 0   | 0      | 6.2 | 8    | 0   | 0   |
| 21    | Long neurotoxin 1                               | 3L21_NAJAC              | 8         | 90  | 60  | 6   | 2      | 93  | 64   | 11  | 5   |
| 22    | Peroxiredoxin-4                                 | PRDX4_CROAT             | 4         | 58  | 4   | 2   | 2      | 31  | 2    | 1   | 1   |
| 23    | Phospholipase A2 homolog                        | PA2HS_ECHOC             | 16        | 60  | 42  | 19  | 13     | 78  | 312  | 75  | 46  |
| 24    | Phospholipase A2 inhibitor 1                    | PLI1_PROFL              | 22        | 0.0 | 0   | 0   | 0      | 8.5 | 20   | 1   | 1   |
| 25    | Phospholipase-B 81                              | PLB_DRYCN               | 64        | 19  | 121 | 14  | 11     | 20  | 73   | 14  | 11  |
| 26    | Secretory phospholipase A2 receptor             | PLA2R_PONAB             | 18        | 0.0 | 0   | 0   | 0      | 1.2 | 3    | 1   | 1   |
| 27    | Serine protease harobin                        | VSPHA_HYDHA             | 29        | 7.9 | 21  | 3   | 1      | 7.9 | 21   | 4   | 3   |
| 28    | Serine protease sp-Eoc49                       | VSP_ECHOC               | 28        | 24  | 27  | 4   | 4      | 49  | 51   | 18  | 13  |
|    | Protein Name                                  | Species  | B. arientans | E. ocellatus |
|----|------------------------------------------------|----------|--------------|--------------|
| 29 | Short neurotoxin 1                            | 3S11_NAJNI | 7            | 61           | 8  | 0  | 0  | 66 | 17 | 2  | 2  |
| 30 | Snaclec 2                                     | SL2_BITGA | 18           | 35           | 26 | 6  | 5  | 31 | 18 | 7  | 6  |
| 31 | Snaclec 3                                     | SL3_BITGA | 18           | 18           | 7  | 3  | 2  | 24 | 9  | 4  | 2  |
| 32 | Snaclec CTL-Eoc124                            | SL124_ECHOC | 17         | 0.0          | 0  | 0  | 0  | 65 | 33 | 14 | 9  |
| 33 | Snaclec CTL-Eoc125                            | SL125_ECHOC | 18         | 0.0          | 0  | 0  | 0  | 47 | 11 | 6  | 6  |
| 34 | Snaclec agglucetin subunit beta-1             | SLB1_DEIAC | 17         | 9.6          | 2  | 0  | 0  | 9.6| 4  | 0  | 0  |
| 35 | Snaclec bitiscetin subunit alpha              | SLA_BITAR  | 15          | 96           | 124| 0  | 0  | 91 | 82 | 0  | 0  |
| 36 | Snaclec bitiscetin subunit beta               | SLB_BITAR  | 15          | 60           | 71 | 27 | 13 | 50 | 60 | 18 | 7  |
| 37 | Snaclec clone 2100755                         | SL_DEIAC   | 18          | 4.5          | 4  | 27 | 13 | 14 | 5  | 18 | 7  |
| 38 | Snaclec convulxin subunit beta                | SLB_CRODU  | 17          | 0.0          | 0  | 0  | 0  | 11 | 4  | 0  | 0  |
| 39 | Snake venom 5'-nucleotidase                    | V5NTD_CROAD | 65         | 39           | 43 | 11 | 5  | 30 | 52 | 5  | 3  |
| 40 | Snake venom metalloproteinase ACLH             | VM1AH_AGKCL | 46        | 5.2          | 3  | 0  | 0  | 7.1| 7  | 0  | 0  |
| 41 | Snake venom metalloproteinase kistomin        | VM1K_CALRH | 47          | 5.3          | 10 | 0  | 0  | 2.9| 4  | 0  | 0  |
| 42 | Snake venom metalloproteinase lebetase-4      | VM1L4_MACLB | 24        | 12           | 4  | 0  | 0  | 25 | 17 | 1  | 1  |
| 43 | Snake venom metalloproteinase-                 | VM3M1_NAJMO | 68        | 2.0          | 30 | 4  | 2  | 4.4| 18 | 5  | 2  |
|    | disintegrin-like mocarhagin                   |           |              |              |    |    |    |    |    |    |    |
| 44 | Snake venom serine protease BthaTL            | VSPTL_BOTAL | 26        | 5.6          | 7  | 0  | 0  | 5.6| 6  | 0  | 0  |
| 45 | Snake venom serine protease HS114             | VSP14_BOTJA | 28         | 5.4          | 1  | 0  | 0  | 5.4| 3  | 0  | 0  |
| 46 | Snake venom serine protease NaSP              | VSP1_NAJAT | 31          | 7.4          | 3  | 0  | 0  | 7.4| 2  | 0  | 0  |
| 47 | Snake venom serine protease VSP PHIOL         | VSP_PHIOL  | 28          | 15           | 6  | 1  | 1  | 14 | 3  | 1  | 1  |
| 48 | Snake venom vascular endothelial growth factor | TXVE_BITAR   | 17         | 55           | 302| 30 | 15 | 59 | 107| 24 | 13 |
|    | toxin barietin                                |           |              |              |    |    |    |    |    |    |    |
| 49 | Thrombin-like enzyme cerastocytin             | VSPP_CERCE | 28         | 13           | 67 | 4  | 3  | 13 | 48 | 3  | 3  |
| 50 | Vascular endothelial growth factor A          | VEGFA_BITGA | 22         | 9.9          | 3  | 2  | 2  | 0.0| 0  | 0  | 0  |
| 51 | Venom nerve growth factor 2                   | NGFV2_NAJSP | 27       | 13           | 10 | 0  | 0  | 18 | 10 | 0  | 0  |
| 52 | Weak neurotoxin 7                             | 3NO27_NAJNA | 8          | 5            | 2  | 1  | 1  | 12 | 9  | 0  | 0  |
| 53 | Weak toxin CM-2a                              | 3SOK2_NAJHA | 7          | 46           | 1  | 2  | 1  | 46 | 3  | 3  | 2  |
| 54 | Zinc metalloproteinase homolog-disintegrin    | VM2AL_TRIAB | 54         | 5.4          | 13 | 2  | 1  | 12 | 13 | 1  | 1  |
|    | albolatin                                     |           |              |              |    |    |    |    |    |    |    |
| 55 | Zinc metalloproteinase leucurolysin-B         | VM3LB_BOTLC | 36         | 13           | 8  | 1  | 1  | 18 | 26 | 1  | 1  |
|   | Zinc metalloproteinase-disintegrin |   | B. arientans | E. ocellatus |
|---|----------------------------------|---|-------------|-------------|
| 56 | VM25A_BITAR | 59 | 13 | 10 | 6 | 5 | 31 | 96 | 28 | 21 |
| 57 | VM2H1_BOTLA | 54 | 4.1 | 10 | 0 | 0 | 2.5 | 4 | 0 | 0 |
| 58 | VM2B1_AGKBI | 32 | 13 | 8 | 3 | 2 | 10 | 6 | 3 | 2 |
| 59 | VM3_BUNFA | 68 | 1.3 | 4 | 0 | 0 | 3.8 | 7 | 0 | 0 |
| 60 | VM3E6_ECHOC | 58 | 17 | 21 | 6 | 4 | 92 | 165 | 28 | 17 |
| 61 | VM3E2_ECHOC | 69 | 19 | 20 | 6 | 5 | 55 | 241 | 117 | 66 |
| 62 | VM3E1_ECHOC | 69 | 22 | 59 | 6 | 8 | 57 | 201 | 68 | 36 |
| 63 | VM3H3_BOTJA | 68 | 9.6 | 14 | 0 | 0 | 13 | 30 | 0 | 0 |
| 64 | VM39_DRYCN | 68 | 1.3 | 4 | 0 | 0 | 5.4 | 8 | 0 | 0 |
| 65 | VM3_NAJAT | 69 | 3 | 5 | 1 | 1 | 1.6 | 2 | 1 | 1 |
| 66 | VM3_CERRY | 69 | 5.7 | 9 | 0 | 0 | 6.2 | 4 | 0 | 0 |
| 67 | VM3TM_TRIST | 69 | 8.2 | 12 | 0 | 0 | 6.8 | 7 | 0 | 0 |
| 68 | VM3VA_MACLB | 69 | 19 | 58 | 7 | 6 | 22 | 92 | 10 | 7 |
| 69 | VM3VB_MACLB | 69 | 14 | 37 | 2 | 2 | 11 | 38 | 2 | 1 |
| 70 | VM3AH_DEIAC | 69 | 9.7 | 14 | 1 | 1 | 5.4 | 9 | 1 | 1 |
| 71 | VM3AA_CROAT | 47 | 4.3 | 4 | 1 | 1 | 7.6 | 12 | 3 | 3 |
| 72 | VM31_BOTAT | 46 | 1.9 | 4 | 0 | 0 | 5.7 | 3 | 1 | 1 |
| 73 | VM33_BOTAT | 69 | 8 | 7 | 0 | 0 | 4.3 | 11 | 1 | 1 |
| 74 | VM3BE_BOTER | 69 | 4.9 | 9 | 0 | 0 | 9 | 14 | 3 | 1 |
| 75 | VM3B2_BOTJA | 24 | 7.8 | 2 | 0 | 0 | 14 | 20 | 4 | 4 |
| 76 | VM32A_GLOBR | 47 | 6.4 | 6 | 0 | 0 | 11 | 29 | 0 | 0 |
| 77 | VM3_NAJKA | 68 | 4.7 | 2 | 1 | 1 | 8.2 | 5 | 2 | 2 |
| 78 | VM3DK_DABRR | 70 | 7.3 | 5 | 2 | 2 | 15 | 34 | 5 | 5 |
|    | Description                                                                 | Accession | B. arientans | E. ocellatus |
|----|-----------------------------------------------------------------------------|-----------|--------------|--------------|
| 79 | Zinc metalloproteinase-disintegrin-like stejnhagin-A                         | VM3SA_TRIST | 68           | 7.7          | 13           | 0           | 0           | 7.2          | 11           | 2           | 1           |
| 80 | Zinc metalloproteinase-disintegrin-like stejnhagin-B                         | VM3SB_TRIST | 68           | 7           | 11           | 0           | 0           | 8.3          | 13           | 0           | 0           |
| 81 | Zinc metalloproteinase/disintegrin                                         | VM2P3_PROMU | 46           | 9.2         | 8            | 0           | 0           | 2.9          | 1            | 0           | 0           |
| 82 | Zinc metalloproteinase/disintegrin                                         | VM3G1_TRIGA | 48           | 2.1         | 3            | 0           | 0           | 7.4          | 22           | 0           | 0           |
| 83 | Zinc metalloproteinase/disintegrin                                         | VM2RH_CALRH | 54           | 12          | 16           | 0           | 0           | 6.3          | 13           | 2           | 1           |
| 84 | Metalloproteinase/disintegrin                                              | VM2OC_ECHOC | 55           | 11          | 22           | 10          | 4           | 39           | 216          | 45          | 29          |
| 85 | Zinc metalloproteinase/disintegrin                                         | VM2L2_MACLB | 53           | 13          | 17           | 0           | 0           | 17           | 2            | 4           | 2           |
| 86 | Zinc metalloproteinase/disintegrin PMMP-1                                   | VM2P1_PROMU | 53           | 7.9         | 13           | 0           | 0           | 9.8          | 11           | 1           | 1           |

The characterization of proteins was achieved using Sequest and X!Tandem incorporated Scaffold Proteome Software 4.10.0: 2019/100

Abbreviation: Mol. mass: molecular mass, %AA: percentage of amino acids, NET: Number of estimated enzymatic cleavage, EUP: Exclusive unique Peptide, TUPC: Total Unique Protein Count.
| S/No. | Protein detected                                      | Protein families                              |
|-------|-------------------------------------------------------|----------------------------------------------|
| 1     | Acidic phospholipase A<sub>2</sub>                    | Phospholipase A<sub>2</sub>s                  |
| 2     | Acidic phospholipase A<sub>2</sub>                    |                                              |
| 3     | Phospholipase A<sub>2</sub> homolog                   |                                              |
| 4     | Phospholipase A<sub>2</sub> inhibitor 1               |                                              |
| 5     | Secretory phospholipase A2 receptor                   |                                              |
| 6     | Phospholipase-B 81                                    | Phospholipase B                               |
| 7     | Alpha-fibrinogenase                                   | Snake venom Serine proteases                 |
| 8     | Alpha-fibrinogenase-like                              |                                              |
| 9     | Beta-fibrinogenase brevinase                          |                                              |
| 10    | Cationic trypsin                                      |                                              |
| 11    | Coagulation factor X-activating enzyme heavy chain    |                                              |
| 12    | Coagulation factor X-activating enzyme heavy chain    |                                              |
| 13    | Serine protease harobin                               |                                              |
| 14    | Serine protease sp-Eoc49                              |                                              |
| 15    | Snake venom serine protease BthaTL                    |                                              |
| 16    | Snake venom serine protease HS114                     |                                              |
| 17    | Snake venom serine protease NaSP                      |                                              |
| 18    | Snake venom serine protease                           |                                              |
| 19    | Thrombin-like enzyme cerastocytin                     |                                              |
| 20    | Glutaminyl-peptide cyclotransferase                   |                                              |
| 21    | Hemoglobin subunit beta                               |                                              |
| 22    | Kunitz-type serine protease inhibitor bitisilin-1     | Kunitz peptides                              |
| 23    | Kunitz-type serine protease inhibitor bitisilin-3     |                                              |
| 24    | Snake venom metalloproteinase ACLH                    | Snake venom Metalloproteinases               |
| 25    | Snake venom metalloproteinase kistomin                |                                              |
| 26    | Snake venom metalloproteinase lebetase-4              |                                              |
| 27    | Snake venom metalloproteinase-disintegrin-like mocarhagin |                                 |
| 28    | Zinc metalloproteinase homolog-disintegrin albolatin  |                                              |
| 29    | Zinc metalloproteinase leucurolysin-B                 |                                              |
| 30    | Zinc metalloproteinase-disintegrin BA-5A              |                                              |
| 31    | Zinc metalloproteinase-disintegrin BlatH1             |                                              |
| 32    | Zinc metalloproteinase-disintegrin bilitoxin-1        |                                              |
| 33    | Zinc metalloproteinase-disintegrin-like BfMP          |                                              |
| 34    | Zinc metalloproteinase-disintegrin-like EoMP06        |                                              |
| S/No. | Protein detected                          | Protein families          |
|-------|------------------------------------------|---------------------------|
| 35    | Zinc metalloproteinase-disintegrin-like EoVMP2 |                           |
| 36    | Zinc metalloproteinase-disintegrin-like Eoc1   |                           |
| 37    | Zinc metalloproteinase-disintegrin-like HF3    |                           |
| 38    | Zinc metalloproteinase-disintegrin-like MTP9   |                           |
| 39    | Zinc metalloproteinase-disintegrin-like NaMP  |                           |
| 40    | Zinc metalloproteinase-disintegrin-like       |                           |
| 41    | Zinc metalloproteinase-disintegrin-like TSV-DM |                           |
| 42    | Zinc metalloproteinase-disintegrin-like VLAIP-A |                           |
| 43    | Zinc metalloproteinase-disintegrin-like VLAIP-B |                           |
| 44    | Zinc metalloproteinase-disintegrin-like acurhagin |                       |
| 45    | Zinc metalloproteinase-disintegrin-like atrolysin-A |             |
| 46    | Zinc metalloproteinase-disintegrin-like batrostatin-1 |       |
| 47    | Zinc metalloproteinase-disintegrin-like batrostatin-3 |       |
| 48    | Zinc metalloproteinase-disintegrin-like berythracitivase |       |
| 49    | Zinc metalloproteinase-disintegrin-like bothrojarin-2 |             |
| 50    | Zinc metalloproteinase-disintegrin-like brevilysin |                       |
| 51    | Zinc metalloproteinase-disintegrin-like cobrin |                          |
| 52    | Zinc metalloproteinase-disintegrin-like daborhagin-K |                     |
| 53    | Zinc metalloproteinase-disintegrin-like stejnihagin-A |                 |
| 54    | Zinc metalloproteinase-disintegrin-like stejnihagin-B |                |
| 55    | Zinc metalloproteinase/disintegrin           |                           |
| 56    | Zinc metalloproteinase/disintegrin           |                           |
| 57    | Zinc metalloproteinase/disintegrin           |                           |
| 58    | Metalloproteinase/disintegrin                |                           |
| 59    | Zinc metalloproteinase/disintegrin           |                           |
| 60    | Zinc metalloproteinase/disintegrin PMMP-1    |                           |
| 61    | Cysteine-rich venom protein                  | Cysteine-rich venom protein |
| 62    | Disintegrin EO5A                            | Disintegrins              |
| 63    | Disintegrin bitistatin                      |                           |
| 64    | L-amino-acid oxidase                        | L-amino acid oxidases     |
| 65    | L-amino-acid oxidase                        |                           |
| 66    | L-amino-acid oxidase                        |                           |
| 67    | Long neurotoxin 1                           | Three-finger toxins       |
| 68    | Short neurotoxin 1                          |                           |
| 69    | Weak toxin CM-2a                            |                           |
| 70    | Peroxiredoxin-4                            |                           |
3.3. Relative distribution of the protein families

In principle, the composition of the analyzed venoms indicated that metalloproteinases (SVMPs), serine proteinases (SVSPs), and phospholipases A₂ (PLA₂s) constituted the major proportions of the venom proteomes, which is consistent with the report of previous studies of viper venomics\(^\text{17,29,30}\). However, a deep insight into the proportion of these major protein families revealed a significant variation among species from different regions of the world; the protein composition of the venoms from vipers indigenous to Gaboon in West Africa showed that the percentages of PLA₂s, SVSPs and SVMPs in \textit{B. arietans} were 4.3\%, 19.5\% and 38.5\% respectively\(^\text{30}\). More so, PLA₂s, and SVMPs were the major toxin families, with varied proportions in the venoms of \textit{Echis} species\(^\text{31}\). In the venoms of these true vipers, indigenous to Nigeria, the percentages are higher with PLA₂s, SVSPs and SVMPs having 10.6\%, 22.31\%, and 21.06\% respectively in \textit{B. arietans} while in \textit{E. ocellatus}, 25.69\%, 17.25\% and 34.84\% were recorded in PLA₂s, SVSPs and SVMPs respectively (Figs. 3 and 4). These variations are attributed to factors such as snake's gender, age\(^\text{21}\), geographical origin\(^\text{18,19}\) and diet\(^\text{20}\). It is believed that mutations in the venom-related genes may impact the venom composition\(^\text{32}\). Nine secondary protein families were detected, namely, kunitz-type peptides, cysteine-rich venom proteins, three-finger toxins, disintegrins, L-amino acid oxidases, C-type lectins/ Snaclecs, 5’nucleotidases, vascular endothelial growth factors and phospholipase B with a distribution range between 1–4\% (Figs. 3 and 4).

In a previous study, venom gland transcriptomic analysis showed that snake venom metalloproteinases and serine proteases represent majority of the toxin genes transcribed in Nigerian \textit{B. arietans}, and they are also the most abundant toxin family secreted in the venom\(^\text{23}\). This is in tandem with our findings that SVSPs and SVMPs were the dominant proteins in the venom of \textit{B. arietans}. Similarly, snake venom metalloproteinases, phospholipase A₂ and serine proteases constituted the most abundant toxin genes transcribed in the venom glands of \textit{E. ocellatus} from Nigeria\(^\text{24}\). The high abundance of these 3 toxin families (PLA₂s, SVSPs and SVMPs) in the venom gland transcriptomic analyses correlates with this present report that these toxins represent the highest in proteomic abundance. In a parallel comparison, transcriptomic analyses revealed that C-type lectins and L-amino acid oxidases were also present in high abundances but they are present in low abundances 3.95\% and 2.24\% respectively in the venom proteome (Fig. 4). This disparity is due to the fact that toxins are known to be transcribed at the highest level in the venom gland\(^\text{23}\).

3.4. Pathological mechanisms of the toxin families
Snake venom toxins are thought to act synergistically in exerting a wide range of biochemical and toxicological effects\(^{33,34}\). Envenomation by the viper primarily gives rise to local effects and bleeding\(^{35}\). Snake venom metalloproteinases (SVMPs) are zinc-dependent proteinase toxins that form the major component of viper venom\(^{17}\). They are the most lethal venom protein of snakes from the viper family\(^{29,36,37}\) which play a key role in coagulopathies commonly associated with viper envenoming\(^{38,29,36}\). Similarly, SVMPs provoke a manifold of clinical manifestations, including hemorrhagic, pro-coagulant, anticoagulant, fibrinolytic, apoptotic, and antiplatelet activities\(^{39,40}\). Thus, SVMPs are believed to evolve from ADAM (a Disintegrin and Metalloproteinase) proteins, precisely ADAM28, characterized by metalloproteinase, disintegrin-like and cysteine-rich domains\(^{41}\), which induce hemorrhagic activity by rupturing the capillary vessels, resulting to incoagulable blood in envenomed victims\(^{42}\). This occurs through cleaving of the basement membrane and adhesion proteins of the endothelial cells-matrix, causing the endothelial cells to detach and become thin, leading to the obstruction of the capillary walls and blood effusion\(^{29,43}\). More so, SVMPs alters homeostasis by disrupting coagulation, through modulation of fibrinogenase and fibrilase that mediate the coagulation cascade which aids in eliciting their hemorrhagic role\(^{44,45}\).

Snake venom serine proteinases (SVSPs) are hemotoxic affecting hemostatic system by provoking edema and hyperalgesia, which result in alteration of blood coagulation processes (pro-coagulant or anti-coagulant), fibrinolysis, platelet aggregation and lethal blood pressure\(^{31,33,46,45}\).

Along with SVMPs and SVSPs as the major toxin families in venom of the vipers analyzed in this study, the venom of *E. ocellatus* were found to be dominated by Phospholipases A\(_2\) (PLA\(_2\)S) which constituted \(\approx 26\%\) of the venom proteome. Our data correlates with the previous studies in which PLA\(_2\)S were described as one of the commonest toxin in the venom of the front-fanged snakes\(^{47,48,17}\). Although PLA\(_2\)S were present in both venoms, it was in lower abundance (10.6\%) in the venom of *B. arietans* (Fig. 3). These toxins had molecular masses of 13–22 kDa (Table 2) and are important causes of neurotoxic and myotoxic effects of viper envenomation\(^{49,50,51}\). The myotoxic effect often leads to severe necrosis\(^{51}\), while respiratory paralysis seen in victims is caused by the neurotoxins\(^2\). PLA\(_2\)S elicits its toxicity through the alteration of pre-synaptic terminals and the sensory nerve endings\(^{52,50,53,54}\).

Other toxin families identified in the venom of these vipers were three-finger toxins, kunitz-type peptides, cysteine-rich secretory proteins, disintegrins, L-amino acid oxidases, C-type lectins, 5-nucleotidase, vascular endothelial growth factors and phospholipase B. These secondary toxins were detected, albeit, at lower abundances in the venom of *B. arietans* (Fig. 3) compared to that of *B. ocellatus* (Fig. 4). The toxin 5’-nucleotidases inhibit the aggregation of platelet through the release of adenosine from guanosine monophosphate and adenosine monophosphate which binds to receptors on the platelets\(^{55}\), this shows that they act in synergy with hemorrhagic toxins to elicit anticoagulant effect observed in envenoming\(^{56,57}\).

L-amino acid oxidases (LAAOs) in snake venoms catalyze oxidative deamination of amino acids, leading to the release of hydrogen peroxide\(^{58}\), which provokes toxicity by impacting on platelet aggregation and inducing hemorrhage that ultimately leads to apoptosis of vascular endothelial cells\(^{59,60}\).

Additionally, the cysteine-rich venom proteins (CRISPs) are toxin families whose exact function in snake venoms is not known, they are however, potential blockers of Ca\(^{2+}\) and cyclic nucleotide-gated channels\(^{61}\). The C-type lectins/ snaclecs (CTLs) are calcium-dependent non-enzymatic proteins that bind carbohydrates, mainly galactose\(^{34}\) and play important roles in the agglutination of erythrocytes and aggregation of platelet\(^{62}\).

Also, three-finger toxins (3FTXs) are non-enzymatic three-finger fold structure that are neurotoxic in nature\(^{63}\) and are commonly described in the venoms from snakes of the elapid family\(^{17}\). However, their relative proportion was found to be 4\% and 3\% in the venoms of *B. arietans* (Fig. 3) and *E. ocellatus* (Fig. 4) respectively. 3FTXs bind at the postsynaptic neuromuscular junctions, thereby inducing flaccid paralysis in victims\(^{64}\) as well as respiratory paralysis\(^2\).

In the case of kunitz-type peptides, a very low percentages (1\%) in the venom of these vipers (Figs. 3 and 4) was obtained in the low molecular mass range of 10-17kDa (Table 2). This toxin family is reported to possess antifibrolytic activity\(^{65}\), and elicits toxicity by obstructing the ion-channels, induction of inflammation and interfering with blood coagulation processes\(^{66}\).
Disintegrins are venom toxins that were first detected in viper snakes\textsuperscript{67}, and are potent inhibitor of platelet aggregation, hence, antagonize the clotting of blood\textsuperscript{68}, thereby contributing to lethality in envenomed victims.

Moreover, snake venom vascular endothelial growth factors were distributed in minute quantities notably in the venom of \textit{E. ocellatus}. They possess vasculotoxin-like activity which makes snakebite site liable to rapid infiltration of venom into victims\textsuperscript{69}. Phospholipase B (PLB) was the least abundant protein with 1.8\% (Fig. 3) and 0.6\% (Fig. 4) in the venoms of \textit{B. arietans} and \textit{E. ocellatus} respectively. In snake venomics analysis, much interest has not been given to PLB probably because the enzyme is not commonly identified in snake venoms\textsuperscript{70}, therefore, there are limited data on the enzyme, and hence, the exact pathological function of PLB in snake venom has not been explained. Several studies reported the presence of PLB in some viper venoms\textsuperscript{71,72,73}, which is in accord with our findings.

4. Conclusion

This report has provided baseline information describing the venom proteome of \textit{E. ocellatus} and \textit{B. arietans} indigenous to Nigeria, with \textit{E. ocellatus} producing more of the toxins. In both snake venoms, there was 90.8\% similarity in protein profile. SVMPs, PLA\textsubscript{2}s and SVSPs were the toxin families. \textit{E. ocellatus} had 34.84\%, 25.69\% and 17.25\% respectively of these venom protein families, and for \textit{B. arietans}, 22.31\%, 21.06\% and 10.6\% were recorded for SVSPs, SVMPs and PLA\textsubscript{2}s respectively. Going by these findings, one can say that although the envenomation by these snakes may present some common pathogenic signs due to some similarities in the venom composition, their venoms are very much different when the toxins are taken into consideration and as such, this should guide in the use and design of a potential antivenin.

Declarations

Acknowledgements

Authors gratefully acknowledge Em. Professor Jose Maria Gutiérrez of the Instituto Clodomiro Picado, University of Costa Rica, whose support, contributed tremendously to the success of this work. We thank Dr. Peter Ofemile of the Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for his assistance in sourcing and milking of the snakes. This work did not receive any specific grants from public and private sectors.

Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE\textsuperscript{26} partner repository with the dataset identifier PXD024638 and 10.6019/PXD024638, at https://www.ebi.ac.uk/pride/archive/projects/PXD024638.

Reviewer account details:

Username: reviewer_pxd024638@ebi.ac.uk

Password: jbhLaCfk

Author contributions

FAA, EJD, ABS: Conceptualization. ABS, AS, MSA: Supervision. EJD, FAA, GM, AK: Investigation. FAA, EJD: Data analysis. EJD: Writing original draft preparation. All authors critically reviewed and edited the final manuscript version.

Funding

This work received no external funding.

Competing interests

The authors declare no competing interests.

References
1. Gutierrez, J. M., Theakston, R. D. & Warrell, D. A. Confronting the neglected problem of 607 snakebite envenoming: the need for a global partnership. *PLoS. Med.* **3**, e150 (2006).

2. Gutiérrez, J. M. *et al.* Snakebite envenoming. *Nat. Rev. Dis. Primers.* **3**, 17063 https://doi.org/10.1038/nrdp.2017.63 (2017).

3. Chippaux, J. P. Snakebite envenomation turns again into a neglected tropical disease. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **23**, 38 (2017).

4. Chippaux, J. P. Estimate of the burden of snakebites in sub-Saharan Africa: a meta-analytic approach. *Toxicon.* **57**, 586–599 (2011).

5. Warrell, D. A. *et al.* Poisoning by bites of the saw-scaled or carpet viper (Echis carinatus) in Nigeria. *Q. J. Med.* **46**, 33–62 (1977).

6. Habib, A. G., Gebi, U. I. & Onyemelukwe, G. C. Snake bite in Nigeria. *Afr. J. Med. Med. Sci.* **30**, 171–178 (2001).

7. Habib, A. G. Venomous snakes and snake envenomations in Nigeria. *Toxinol.* **2**, 275–298 (2013).

8. Ghosh, R., Mana, K., Gantait, K. & Sarkhel, S. A retrospective study of clinico–epidemiological prole of snakebite related deaths at a tertiary care hospital in Midnapore, West Bengal, India. *Toxicol. Rep.* **5**, 1–5 (2018).

9. Mohapatra, B. *et al.* Snakebite mortality in India: a nationally representative mortality survey. *PLoS Negl. Trop. Dis.* **5**, e1018 (2011).

10. WHO. Snakebite envenoming: Member States provide WHO with clear mandate for global action. 25th May, 2018 Geneva. Printed at https://www.who.int/news/item/25-05-2018-snakebite-envenoming-member-states-provide-who-with-clear-mandate-for-global-action. (2020).

11. Abubakar, S. B. *et al.* Nigeria-UK EchiTab Study Group, Pre-clinical and preliminary dose-finding and safety studies to identify candidate antivenoms for treatment of envenomings by saw-scaled or carpet vipers (Echis ocellatus) in northern Nigeria. *Toxicon.* **55**, 719–723 (2010).

12. Pla, D., Gutiérrez, J. M. & Calvete, J. J. Second generation snake antivenomics: comparing immunoanity and immunodepletion protocols. *Toxicon.* **60**, 688–699 (2012).

13. Fry, B. G. *et al.* Evolution of arsenal: structural and functional diversification of the venom system in advanced snakes (Caenophidia). *Mol. Cell Proteom.* **7**, 215–246 (2008).

14. Kasoulis, T. & Isbister, G. K. A review and database of snake venom proteomes. *Toxins.* **9**, 673290 (2017).

15. Dias, G. S. *et al.* Individual variability in the venom proteome of juvenile Bothrops jararaca specimens. *J. Proteome Res.* **12**, 4585–4598 (2013).

16. Kunalan, S., Othman, I., Hassan, S. S. & Hodgson, W. C. Proteomic Characterization of Two Medically Important Malaysian Snake Venoms, Calloselasma rhodostoma (Malayan Pit Viper) and Ophiophagus hannah (King Cobra). *Toxins.* **10**, https://doi.org/10.3390/toxins10110434 (2018).

17. Casewell, N. R. *et al.* Postgenomic processes dictate venom variation. *Proc. Natl. Acad. Sci.* **111**, 9205–9210 (2014).

18. Barlow, A., Pook, C. E., Harrison, R. A. & Wuster, W. Coevolution of diet and prey-specific venom activity supports the role of selection in the evolution of snake venom. *Proc. Biol. Sci.* **276**, 2443–2449(2009).

19. Díaz, G. S. *et al.* Proteomic Characterization of Two Medically Important Malaysian Snake Venoms, Calloselasma rhodostoma (Malayan Pit Viper) and Ophiophagus hannah (King Cobra). *Toxins.* **10**, https://doi.org/10.3390/toxins10110434 (2018).

20. Barlow, A., Pook, C. E., Harrison, R. A. & Wuster, W. Coevolution of diet and prey-specific venom activity supports the role of selection in the evolution of snake venom. *Proc. Biol. Sci.* **276**, 2443–2449(2009).

21. Dias, G. S. *et al.* Individual variability in the venom proteome of juvenile Bothrops jararaca specimens. *J. Proteome Res.* **12**, 4585–4598 (2013).

22. Kunalan, S., Othman, I., Hassan, S. S. & Hodgson, W. C. Proteomic Characterization of Two Medically Important Malaysian Snake Venoms, Calloselasma rhodostoma (Malayan Pit Viper) and Ophiophagus hannah (King Cobra). *Toxins.* **10**, https://doi.org/10.3390/toxins10110434 (2018).

23. Casewell, N. R. *et al.* Postgenomic processes dictate venom variation. *Proc. Natl. Acad. Sci.* **111**, 9205–9210 (2014).

24. Wagstaff, S. C. & Harrison, R. A. Venom gland EST analysis of the saw-scaled viper, Echis ocellatus, reveals novel alpha9beta1 integrin-binding motifs in venom metalloproteinases and a new group of putative toxins, renin-like aspartic proteases. *Gene.* **377**, 37–42 (2018).
21–32 (2006).
25. Hill, R. E. & Mackessy, S. P. Venom yields from several species of colubrid snakes and differential effects of ketamine. Toxicon. 35, 671–678 (1997).
26. Perez-Riverol, Y. et al. The PRIDE database and related tools and resources in 2019: improving support for quantification data. Nucleic acids Res. 47 (D1), D442–D450 (2019).
27. Sousa, L. F. et al. Comparison of Phylogeny, Venom Composition and Neutralization by Antivenom in Diverse Species of Bothrops Complex. PLoS Negl. Trop. Dis. 7, e2442 (2013).
28. Calvete, J. J. Antivenomics and venom phenotyping: A marriage of convenience to address the performance and range of clinical use of antivenoms. Toxicon. 56, 1284–1291 (2010).
29. Gutierrez, J. M., Rucavado, A., Escalante, T. & Dias, C. Hemorrhage induced by snake venom metalloproteinases: Biochemical and biophysical mechanisms involved in microvessel damage. Toxicon. 45, 997–1011 (2005).
30. Calvete, J. J., Juarez, P. & Sanz, L. Snake venomics. Strategy and applications. J. Mass Spectrom. 42, 1405–1414 (2007).
31. Casewell, N. R., Harrison, R. A., Wüster, W. & Wagstaff, S. C. Comparative venom gland transcriptome surveys of the saw-scaled vipers (Viperidae: Echis) reveal substantial intra-family gene diversity and novel venom transcripts. BMC Genom. 10, 564 https://doi.org/10.1186/1471-2164-10-564 (2009).
32. Adamude, F. A. et al. Proteomic analysis of three medically important Nigerian Naja (Naja haje, Naja katiensis and Naja nigricollis) snake venoms. Toxicon. 26, https://doi.org/10.1016/j.toxicon.2021.03.014 (2021).
33. Kang, T. S. et al. Enzymatic toxins from snake venoms: Structural characterization and mechanism of catalysis. FEBS J. 278, 4544–4576 (2011).
34. Sajevic, T., Leonardi, A. & Krizaj, I. Haemostatically active proteins in snake venoms. Toxicon. 57, 627–645 (2011).
35. Warrell, D. A. Snake bite. Lancet. 375, 77–88 (2010).
36. Moura-da-Silva, A. M. et al. Processing of Snake Venom Metalloproteinases: Generation of Toxin Diversity and Enzyme Inactivation. Toxins. 8, 183 https://doi.org/10.3390/toxins8060183 (2016).
37. Kohlhoff, M. et al. Exploring the proteomes of the venoms of the Peruvian pit vipers. J. Proteomics. 75, 2181–2195 (2012).
38. White, J. Snake venom and coagulopathy. Toxicon. 45, 951–967 (2005).
39. Casewell, N. R., Wüster, W., Vonk, F. J., Harrison, R. A. & Fry, B. G. Complex cocktails: The evolutionary novelty of venoms. Trends Ecol. Evol. 28, 219–229 (2013).
40. Calvete, J. J. & Venomics Integrative venom proteomics and beyond. Biochem. J. 474, 611–634 (2017).
41. Casewell, N. R. On the ancestral recruitment of metalloproteinases into the venom of snakes. Toxicon. 60, 449–454 (2012).
42. Markland-Jnr, F. S. & Swenson, S. Snake venom metalloproteinases. Toxicon. 62, 3–18 (2013).
43. Escalante, T., Rucavado, A., Fox, J. W. & Gutierrez, J. M. Key events in microvascular damage induced by snake venom hemorrhagic metalloproteinases. J. Proteomics. 74, 1781–1794 (2011).
44. Takeda, S., Takeya, H. & Iwanaga, S. Snake venom metalloproteinases: Structure, function and relevance to the mammalian ADAM/ADAMTS family proteins. Biochim. Biophys. Acta. 1824, 164–176 (2012).
45. Slagboom, J., Kool, J., Harrison, R. A. & Casewell, N. R. Haemotoxic snake venoms: their functional activity, impact on snakebite victims and pharmaceutical promise. Br. J. Haematol. 177, 947–959 (2017).
46. Serrano, S. M. T. The long road of research on snake venom serine proteinases. Toxicon. 62, 19–26 (2013).
47. Bharati, K. et al. Molecular cloning of phospholipases A2 from venom glands of Echis carpet vipers. Toxicon. 41, 941–947 (2003).
48. Jiménez-Charris, E. et al. Proteomic and functional analyses of the venom of Porthidium lansbergii lansbergii (Lansberg’s hognose viper) from the Atlantic Department of Colombia. J. Proteomics. 114, 287–299 (2015).
49. Harris, J. B., Grubb, B. D., Maltin, C. A. & Dixon, R. The neurotoxicity of the venom phospholipases A2, notexin and taipoxin. Exp. Neurol. 161, 517–526 (2000).
50. Harris, J. B., Scott-Davey, T. & Secreted phospholipases A2 of snake venoms: Effects on the peripheral neuromuscular system with comments on the role of phospholipases A2 in disorders of the CNS and their uses in industry. Toxins. 5, 2533–2571 (2013).
51. Gutierrez, J. M. & Ownby, C. L. Skeletal muscle degeneration induced by venom phospholipases A2: insights into the mechanisms of local and systemic myotoxicity. *Toxicon.* **42**, 915–931 (2003).

52. Camara, P. R. et al. Inflammatory oedema induced by phospholipases A2 isolated from Crotalus durissus sp. in the rat dorsal skin: a role for mast cells and sensory C-fibers. *Toxicon.* **41**, 823–829 (2003).

53. Sribar, J., Oberckal, J. & Krizaj, I. Understanding the molecular mechanism underlying the presynaptic toxicity of secreted phospholipases A2: an update. *Toxicon.* **89**, 9–16 (2014).

54. Zhang, C. C., Medzihradsky, K. F., Sanchez, E. E., Basbaum, A. I. & Julius, D. Lys49 myotoxin from the Brazilian lancehead pit viper elicits pain through regulated ATP release. *Proc. Natl. Acad. Sci. U. S. A.* **114**, E2524-E2532 (2017).

55. Trummal, K. et al. 5’-Nucleotidase from Vipera lebetina venom. *Toxicon.* **93**, 155–163 (2015).

56. Aird, S. D. Ophidian envenomation strategies and the role of purines. *Toxicon.* **40**, 335–393 (2002).

57. Dhananjaya, B. L. & D’Souza, C. J. M. The pharmacological role of nucleotidases in snake venoms. *Cell Biochem. Funct.* **28**, 171–177 (2010).

58. Du, X. Y. & Clemetson, K. J. Snake venom L-amino acid oxidases. *Toxicon.* **40**, 659–665 (2002).

59. Torii, S., Naito, M. & Tsuruo, T. Apoxin, a novel apoptosis-inducing factor with L-amino acid oxidase activity purified from Western diamondback rattlesnake venom. *J. Biol. Chem.* **272**, 9539–9542 (1997).

60. Fox, J. W. & Serrano, S. M. Insights into and speculations about snake venom metalloproteinase (SVMP) synthesis, folding and disulide bond formation and their contribution to venom complexity. *FEBS J.* **275**, 3016–3030 (2008).

61. Komori, Y., Nikai, T., Tohkai, T. & Sugihara, H. Primary structure and biological activity of snake venom lectin (APL) from Agkistrodon. p. piscivorus (Eastern cottonmouth). *Toxicon.* **37**, 1053–1064 (1999).

62. Ozeki, Y. et al. C-type galactoside-binding lectin from Bothrops jararaca venom: Comparison of its structure and function with those of botrocetin. *Arch. Biochem. Biophys.* **308**, 306–310 (1994).

63. Kessler, P., Marchot, P., Silva, M. & Servent, D. The three-finger toxin fold: a multifunctional structural scaffold able to modulate cholinergic functions. *J. Neurochem.* **142**, 7–18 (2017).

64. Barber, C. M., Isbister, G. K. & Hodgson, W. C. Alpha neurotoxins. *Toxicon.* **66**, 47–58 (2013).

65. Qiu, Y. et al. Molecular cloning and antibrinolytic activity of a serine protease inhibitor from bumblebee (Bombus terrestris) venom. *Toxicon.* **63**, 1–6 (2013).

66. Earl, S. T. H. et al. Identification and characterisation of Kunitz-type plasma kallikrein inhibitors unique to Oxyuranus sp. snake venoms. *Biochimie.* **94**, 365–373 (2012).

67. Chakrabarty, D. & Chanda, C. Snake Venom Disintegrins. In: Gopalakrishnakone P., Inagaki H., Vogel, C. W., Mukherjee, A. & Rahmy, T. (eds) Snake Venoms. Toxinol.Springer, Dordrecht. https://doi.org/10.1007/978-94-007-6410-1_14 (2017).

68. Niewiarowski, S., MacLane, M. A., Klocsewiak, M. & Stewart, G. J. Disintegrins and other naturally occurring antagonists of platelet fibrinogen receptor. *Semin. Hematol.* **31**, 289–300 (1994).

69. Kostiza, T. & Meier, J. Nerve growth factors from snake venoms: Chemical properties, mode of action and biological significance. *Toxicon.* **34**, 787–806 (1996).

70. Tan, N. H. et al. Functional venomics of the Sri Lankan Russell’s viper (Daboia russelii) and its toxicological correlations. *J. Proteomics.* **128**, 403–423 (2015).

71. Ziganshin, R. H. et al. Quantitative proteomic analysis of Vietnamese krait venoms: Neurotoxins are the major components in Bungarus multicinctus and phospholipases A2 in Bungarus fasciatus. *Toxicon.* **107**, 197–209 (2015).

72. Kovalchuk, S. I., Ziganshin, R. H., Starkov, V. G., Tsetlin, V. I. & Utkin, Y. N. Quantitative proteomic analysis of venoms from Russian vipers of Pelias group: Phospholipases A2 are the main venom components. *Toxins.* **8**, 105 https://doi.org/10.3390/toxins8040105 (2016).

73. Abidin, S. A. Z. et al. Proteomic characterization and comparison of malaysian Tropidolaemus wagleri and Cryptelytrops purpureomaculatus venom using shotgun-proteomics. *Toxins.* **8**, 299 https://doi.org/10.3390/toxins8100299 (2016).
Figure 1

One-dimensional SDS-PAGE profile of B. arietans and E. ocellatus venoms

Figure 2
Venn diagram presenting the common and exclusive proteins in the analyzed venom of B. arietans and E. ocellatus as revealed by mass spectrometry

Figure 3

Relative distribution of protein families in the venom proteome of B. arietans

- **Phospholipase A2s**: 10.6%
- **Snake venom metalloproteinases**: 21.06%
- **Cysteine-rich venom proteins**: 2.05%
- **Disintegrins**: 3.4%
- **C-type lectins/snaclecs**: 10.65%
- **Vascular endothelial growth factors**: 10.3%
- **Snake venom serine proteases**: 22.31%
- **Kunitz-type peptides**: 1.1%
- **Three-finger toxins**: 4.32%
- **L-amino acid oxidases**: 8.72%
- **5’ Nucleotidases**: 2.5%
- **Phospholipase B**: 1.8%
Figure 4

Relative distribution of protein families in the venom proteome of E. ocellatus

- Phospholipase A2s: 25.69%
- Snake venom metalloproteinases: 34.84%
- Cysteine-rich venom proteins: 2.9%
- Disintegrins: 1.98%
- C-type lectins/snaclecs: 3.95%
- Vascular endothelial growth factors: 3.3%
- Snake venom serine proteases: 17.25%
- Kunitz-type peptides: 1.5%
- Three-finger toxins: 3.37%
- L-amino acid oxidases: 2.24%
- 5' Nucleotidases: 2%
- Phospholipase B: 0.6%