CATALOGING OF TOXIN REPERTOIRE AND FOLLOWING MUTATIONAL PATTERN IN *BITIS ARIETANS* OF MOSTLY PREVALENT SNAKES IN NIGERIA

A Project Submitted in Partial Fulfillment of the Requirements for the Award of the

Degree of Bachelor of Science in Biochemistry

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DECLARATION

I hereby declare that this work is the product of research efforts undertaken under the supervision of Dr. Susanta Pahari and has not been presented and will not be presented elsewhere for the award of a degree or certificate. All the sources have been duly acknowledged.

_____ _____

Fatima Muhammad Bulama Date

(1195)

CERTIFICATION

This is to certify that this study was carried out by Fatima Muhammad Bulama (1195) in the Department of Biochemistry, School of Science and Information Technology, Skyline University Nigeria, under my supervision.

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APPROVAL

The panel of examiners recommends the candidate, Fatima Muhammad Bulama (1195) for the award of the Degree of Bachelor of Science in Biochemistry subject to effecting all the corrections pointed out during the oral examination.

_____ _____

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_____ _____

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DEDICATION

I dedicate this work to my wonderful parents, Alhaji Shettima Mohammed Bulama and Hajiya Maryam Bulama for their endless support and prayers. Thank you for always being there for me and may Allah (S.W.T) reward and protect you. I pray that I never let you down.

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My utmost thanks to Almighty Allah for his blessings and unexplainable favours in my life for helping me start and finish this work. My profound gratitude goes to my beloved family for their moral support and also, their love, advices and prayers. May Allah reward them.

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ABSTRACT

Animal venoms have evolved over millions of years for prey capture and defence from predators and rivals. Snake venoms, in particular, have evolved a wide diversity of peptides and protein that induce harmful inflammatory and neurotoxic effects including severe pain and paralysis, hemotoxic effects such as haemorrhage and coagulopathy, and cytotoxic/ myotoxic effects, such as inflammation and necrosis. Variety in toxin structure is a universal peculiarity in snakes and happens both interspecifically and interspecifically. However, there are about hundreds of toxins available in snake venom gland, but not all the toxins are available in equal proportion for a particular snake. In this work, what kind of family of toxins are available in one of the prevalent snake in Nigeria, *Bitis arietans*. *Bitis arietans* has been catalogued from NCBI database, those collected sequences have been aligned. Then the pattern of variation has been observed, and analyzed. It has been observed that not all of the toxins mutate with equal rate, some are highly mutating and others are quite slow. However, mutations being the driver of changes of toxin sequences.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Snake are elongated, limbless, carnivorous reptiles of the suborder Serpentes. Snakes are classified in the phylum Chordata, subphylum Vertebrata, class *Reptilia*, order Squamata, suborder Serpentes. There are 14 families, but Colubridae, Elapidae, Hydrophidae, Viperidae, Crotalinae, and Viperinae are the families and subfamilies of poisonous snakes). Snake, (suborder Serpentes), additionally called snake, any of in excess of 3,400 types of reptiles recognized by their limbless condition and significantly prolonged body and tail. Grouped with reptiles in the request Squamata, snakes address a reptile that, throughout development, has gone through primary decrease, rearrangements, and misfortune as well as specialization. All snakes need outside appendages, however not all legless reptiles are snakes.

Classification of Snake

All snakes are members of the suborder Serpentes, a monophyletic clade that is deeply rooted in the Squamata phylogeny (lizards). More than 3500 different species of snakes exist. Their head and dental morphology, which first emerge in the fossil record during the Middle Jurassic-Lower Cretaceous periods, set them apart from other legless lizards. The loss of limbs is thought to have been preceded by alterations to the skull, and the earliest snakes were probably short-bodied lizards with limbs [1].

Changes are made to the grouping consistently. The Colubridae family, which contains both harmful (venomous) and nonpoisonous snakes, is home to the greatest gathering of snakes. Venom glands are located in the upper jaw of poisonous snakes. The venom is released from the venom glands through hollow fangs and varies depending on the species, season, and age.

Some species produce oral poisonous secretions instead of separate venom glands. The Elapidae family includes well-known venomous snakes like cobras and mambas (Dendroaspis). Some Elapidae have the ability to spray their venom as an aerosol into their victim's face and eyes at a distance of more than 2 meters. The zigzag lines on the back, the big triangular head, and the big fangs are characteristics of members of the viper family (Viperidae and Crotalidae).

Snakes are classified in the phylum Chordata, subphylum Vertebrata, class Reptilia, order Squamata, suborder Serpentes. There are 14 families, but Colubridae, Elapidae, Hydrophidae, Viperidae, Crotalinae, and Viperinae are the families and subfamilies of poisonous snakes [2].

- **Colubridae:** Nearly 1400 species, or 75% of all snake genera and 78% of all snake species in the world, are included in this family. 400 of these Colubridae species have larger solid teeth at the back of the maxilla or short, immovable fangs. A third or more of the Colubrid species have rear teeth that release poisonous saliva when they chew. Except for Australia, all continents have a predominance of colubrid snake species. Examples include the mountain racer, rat snakes, wandering garter snakes, parrot snakes, Western and Eastern hognose snakes, etc [2].

- **Atractaspididae:** These snakes, also known as tunnelling asps, mole snakes, adders, false snakes, and side-cutting snakes, are found in Africa and the Middle East. They have exceptionally long front fangs that they use to side-swipe their victim, immobilizing it. These teeth often protrude from the side of the partially closed mouth.

- **Elapidae:** These snakes have moderately short, fixed front (proteroglyph) teeth, which anyway may reach out up to 10 mm long). They are fixed set up in the forward portion of the maxilla**.**

- Examples include the following—

  a. Cobras (*Naja*)

  b. Kraits (*Bungarus*)

  c. coral snakes (*Calliophis, Maticora, Micrurus*)

  d. Mambas (*Dendroaspis*)

- **Viperidae:** These snakes have extremely developed long curved, hinged, front fangs, which are channeled in the form of a hypodermic needle.

  There are two subfamilies**. Viperinae or true vipers:** Vipers and adders

- **Hydrophidae:** This family contains sea snakes, which have short fixed fangs as on account of the elapids.

- **Pythonidae**: The household Pythonidae includes some from the entire world 's longest snakes, which consists of the Southeast Asian reticulated python. The snakes from this family happen to be collectively referred to as "pythons", though there are many specific species occurring in many parts of the world

1.2 Statement of Problem

Venomous snakes pose severe health problems especially for the rural dwellers in the tropical regions of developing countries [3,4,5]. Despite the remarkable efforts for effective management of envenomings, the World Health Organization (WHO) incorporated snakebite in the list of neglected tropical diseases [6]. Thus, further and better efforts are required to

address the life-threatening situation posed by this disease. Snakebites are a common health hazard in the Savanna region of West Africa [4,5]. In Nigeria, the Viperidae, notably *Bitis arietans* (Puff adder) is associated with the highest incidence of morbidity and mortality [3,4,7] and is regarded as one of the most dangerous venomous snake species. The actual fatality index resulting from envenomings by this viper remains unknown due to inaccurate epidemiological data [8], as most of the victims do not have access to health care facilities and therefore resort to ethno-medicinal means for treatments. The WHO reported about 5.4 million incidences of snakebites annually, with 2.7 million cases of envenoming, 138,000 deaths and 400,000 cases of disabilities [9]. In Nigeria, the incidence of snakebites leads to 10,000 deaths annually. Snakebite inflicts severe damage to different organs of the body, and envenomings by the Viperidae are characterized by life-threatening symptoms, primarily due to local tissue damage and bleeding [4].

1.3 Aim and Objectives

The aim of this study is to establish the catalogue of toxin repertoire of mostly prevalent snakes (Carpet viper, Puff adder, Gaboon viper, night adder, black-necked spitting cobra, Egyptian cobra, Mali cobra, mole viper, stiletto snakes and burrowing asps) in Nigeria.

Objectives of the Study

1. To retrieve toxin sequences from Databases
2. To align gene sequences of the selected venomous snakes in Nigeria.
3. To follow mutational pattern and intensity of available toxins in puff Adder (*Bitis Arietans*)

1.4 Significance of the Study

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 [10,11]. In addition, the '-omics' approach has led to the rapid examination of the immune reactivity of antivenoms against the toxins found in snake venoms [12-14]. Due to the complex nature of the protein mixture in snake venoms, envenomation is characterized by varied pathological manifestations [10]. It is reported that geographical inhabitation of snake [12,13,14,15], diet type [16] and age [17] contribute to the variability of the venom composition [18,19]. Hence, analyzing venom proteomes to gain an insight into snake venom compositions can help in understanding their biological and pathological effects [20]. It will also help in making appropriate choice of antivenom to use in treatment. The venom gland transcriptomic of Nigerian *B. arietans* was reported previously [21,22]. In the current study, the cataloging of toxin repertoire and mutational pattern of *B. arietans* from Nigeria were analyzed with the view to understanding their toxin profile and protein identities.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 Snake venom

Snake venom is a highly toxic saliva [23] containing zootoxins that facilitates in the immobilization and digestion of prey. Additionally, it offers defense against dangers. During a bite, a snake's distinctive fangs inject venom; however, certain species can also spit venom. [24]. Other vertebrates have modified parotid salivary glands called zootoxin-producing glands. They are normally located below and behind the eyes on each side of the head and are covered by a muscular sheath. Prior to being delivered by a duct to the base of channeled or tubular fangs, where it is ejected, the venom is first stored in enormous glands called alveoli. [25,26].

Venom contains more than 20 different compounds, which are mostly proteins and polypeptides [25]. The complex mixture of proteins, enzymes, and various other substances has toxic and lethal properties [24]. Venom serves to immobilize prey [27]. Enzymes in venom plays a very important role in the digestion of prey[26] and many other substances are responsible for important but non-lethal biological effects [24]. Some of the proteins in snake venom have very specific effects on various biological functions, including, blood pressure regulation, blood coagulation and transmission of muscle impulses or nerves. These venoms have been developed for use as diagnostic tools or pharmacological and also drugs [24].

2.2 Types of Snake Venom

Different animals have different types of venom, which might vary depending on the animal's species, habitat, climate, geographic location, age, and other factors. According to their effects, venom comes in three different forms. cytotoxic, neurotoxic, and hematologic [28].

Every type of venom targets a certain organ or system of the organism. Every kind has the potential to be lethal or very harmful. In addition to its more particular effects, all venom is thought to contain proteolytic processes, which in fact cause the initial damage to the bite or injection site of the venom by killing the tissue there.

2.2.1 Neurotoxic Venom

The word neurotoxic comes from its effects on the nervous system. In essence, neurotoxic venom poisons the neurological system. To convey information from the brain to our bodies, the nervous system uses neurotransmitters, which are chemical signals, and neurotransmitter receptors, which are sites where neurotransmitters connect. Neurotoxic venom enters the body and immediately starts to produce issues. Neurotoxic venom can drastically impede the functioning of the nervous system by either reducing neurotransmitter production or completely blocking it. These disruptions have the potential to basically paralyze the breathing muscles, which can lead to respiratory failure and make it impossible for bite patients to breathe. Neurotoxic venom can occasionally cause neurotransmitters to be overstimulated, which can result in fast muscle twitching or convulsions. Other than scarring near the bite site, snake bite survivors (with neurotoxic venom) typically do not experience any long-lasting symptoms [28].

Fasciculins

By eliminating acetylcholinesterase, these poisons harm cholinergic neurons (neuron types that use ACh as a transmitter) (AChE). ACh remains in the receptor as a result since it cannot be broken down. Tetany (involuntary muscle contraction) is brought on by this, and it can be fatal. The toxins are known as fasciculins because they elicit severe, widespread, and long-lasting (5-7 h) fasciculations in mice after injection (rapid muscle contractions). Snake

example: found mostly in the venom of some rattlesnakes (*Crotalus* spp) and of mambas (*Dendroaspis* spp).

Dendrotoxins

Dendrotoxins disrupt neurotransmissions and the transmission of nerve impulses, which paralyzes the neurons by stopping the interchange of positive and negative ions across the neuronal membrane.

α-neurotoxins

Over 100 postsynaptic neurotoxins have been identified and sequenced, making alpha-neurotoxins a sizable category [30]. The Nicotinic acetylcholine receptors on cholinergic neurons are the target of neurotoxins. Because of their shape, they fit into the receptors; they obstruct acetylcholine flow, causing numbness and paralysis.

Snake examples: king cobra (Ophiophagus Hannah) (known as hannahtoxin containing α-neurotoxins) [31] sea snakes (Hydrophiinae) (known as erabutoxin), many-banded krait (Bungarus multicinctus) (known as α-bungarotoxin), and cobras (Naja spp.) (known as cobratoxin) [28].

2.2.2 Cytotoxic Venom

Cytotoxic venom impacts the cells that make up our body's tissues, organs, and muscles. The bodily cells are immediately killed and damaged by cytotoxic venom. Those who are bitten by snakes that have cytotoxic venom start feeling the consequences practically right away. Cytotoxic bites result in necrosis, which kills the body's tissues [28]. When a snake bites a victim, the tissues around the bite site may be similarly affected and become liquified, just as cytotoxins aid in the digestion and breakdown of prey before it is consumed. Necrosis can

also result in the skin developing blisters, swelling to extremes, and/or turning black as tissue cells perish. Necrosis might eventually move from the bite site to other areas of the body. If the damaged area of the body is not treated straight away, necrosis may spread and the patient may require an amputation. Lasting effects of a bite from a cytotoxic venom often include permanent tissue damage.

Phospholipases

Phospholipase is an enzyme that changes phospholipid atoms into lysophospholipids (Soap), which pulls in and binds fats and breaks down molecular films. One particular variety of phospholipase found in snake toxins is phospholipase A2. Snake example: Okinawan habu (*Trimeresurus flavoviridis*) [29].

Cardio toxins / Cytotoxins

Cardio toxins are components that are specifically toxic to the heart. They bind to particular sites on the surface of muscle cells and cause depolarisation → the toxin prevents muscle contraction. These toxins may cause death or causes the heart to beat irregularly or stop beating. A major is the three-fingered cardio toxin III from Chinese cobra, an example of the short three-fingered family. Snake example: some Naja species and mambas.

2.2.3 Hemotoxic Venom

The circulatory system or bloodstream are both poisoned by hemotoxic venom. As soon as hemotoxic venom enters the bloodstream, it begins to target and damage red blood cells. The body's normal blood coagulation process is virtually stopped when red blood cells rupture. Internal bleeding is caused by blood cells bursting, which prevents the blood from clotting. The damaged red blood cells could prevent the kidneys from working properly if they start to stack up or collect. Blood artery obstructions brought on by hemotoxic venom may

potentially cause heart failure [28]. Compared to neurotoxic or cytotoxic venom, hemotoxic venom often takes longer to have an effect on the victim. The best chance of survival after a venomous snake bite is to seek medical attention as soon as possible.

2.3 Puff Adder

In sub-Saharan Africa, where it is native, the puff adder (*Bitis arietans*), a highly lethal venomous snake, is to blame for the majority of venomous snakebites. In North America, puff adder bites are caused by captive snakes. Although puff adder venom contains thrombolytic enzymes, severe coagulopathy has not yet been linked to a proven puff adder envenomation [30]. With the exception of the Sahara and rainforest zones, the puff snake (*Bitis arietans*), a toxic snake animal groups, can be found in savannah and prairies from Morocco and western Arabia into Africa [31]. Because of various factors, including its far reaching range, continuous presence in thickly populated regions, and forceful nature, it is at fault for most of snakebite fatalities in Africa [32,33]. There are presently two subspecies perceived, including the assign subspecies remembered for this article [34]. Puff-adders are powerful and about a meter long. On their back, they display a conspicuous "V" or "U" plan. They display forceful way of behaving, murmuring noisily, and body expansion when upset. The puff-viper is an exceptionally dangerous snake with long teeth and a great deal areas of strength for of. It normally dwells in areas that are exceptionally packed. This species is responsible for a huge part of the serious nibbles that happen all through the whole African savannah. The essential issue is serious confined edema, which could advance to remember the whole appendage and result for hypovolemic shock [35].

2.3.1 Description of Puff Adder

Usually measuring around 1.0 m (39.3 in) in length, the snake is quite huge (body and tail). Large specimens with girths of 40 cm (16 in), overall lengths of 190 cm (75 in), and weights

of more than 6.0 kg have been reported (13.2 lb). Generally speaking, males tend to be larger than females and have longer tails [36]. Two different black bands can be visible on the head: one on the crown and the other between the eyes. The color pattern varies locally. On either side of the skull, two oblique, black bands or bars go from the eye to the supralabials. The head has intermittent dark patterns and is paler than yellow. Iris colors range from gold to silver-gray.

The ground colour varies dorsally and might be anything from straw yellow to light brown to orange or reddish brown. A pattern of 18 to 22 backward-directed, dark brown to black bars that go down the back and tail is placed on top of this [32,33].

2.3.2 Distribution and Habitat

The most prevalent and widespread snake in Africa most likely belongs to this species [32]. It is found in most of sub-Saharan Africa south to the Cape of Good Hope, including southern Morocco, Mauritania, It also occurs on the Arabian Peninsula, where it is found in southwestern Saudi Arabia and Yemen. Except for genuine deserts, rainforests, and (tropical) alpine settings, it can be found anywhere. It is frequently related to rocky grasslands [34]. It is absent from locations that are covered in rainforests, such as the coasts of West Africa and Central Africa (i.e., the central DR Congo), and it is also not present in North Africa's Mediterranean coastline zone [33].

2.3.3 Behaviour of Puff Adder

The puff adder, a species that is often slow moving, uses camouflage to stay safe [36,38]. These snakes are primarily terrestrial, but they can swim and climb easily. They frequently hang out in low bushes to warm up. One species was discovered in a heavily branched tree 4.6 meters above the ground [36].

They adopt a tightly coiled defensive posture with the forepart of their body held in a taut "S" form and hiss loudly and continuously if disturbed. They might simultaneously try to retreat away from the danger and toward cover. Before rapidly resuming their defensive posture and preparing to strike again, they may strike suddenly and quickly, to the side as well as forward. The impact during a strike is so powerful, and the long fangs pierce so deeply, that prey items frequently die from the physical stress alone. It appears that the fangs can pierce soft leather [36,38].

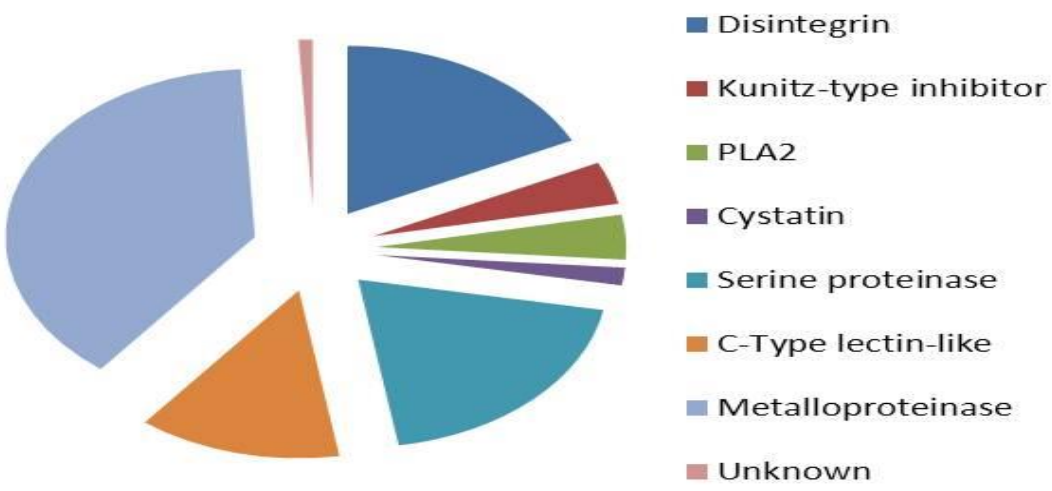
Rarely do these snakes retain their prey; instead, they swiftly release to resume their striking posture. Juveniles can hurl their entire bodies forwards while striking, but they can only reach a distance of roughly one-third of their body length [36].

2.3.4 Reproduction of Puff Adder

Males are drawn to females by a pheromone, which causes them to perform neck-wrestling battle dances. Seven men arrived in Malindi after a female [36]. They have huge litters; litter sizes of over 80 have been recorded, while litter sizes of 50–60 are common. Newborns range in length from 12.5 to 17.5 cm [38].

2.4 Venom Composition of *Bitis arietans*

Composition of *B. arietans* venom, studied at the proteomic level. This toxin contains the accompanying families: disintegrin, kunitz-type inhibitor (toxin Kunitz-type), PLA2 (phospholipase A2), cystatin, serine proteinase (peptidase S1), C-type lectin-like (snaclec), toxin metalloproteinase.



2.4.1 Phospholipases (PLA2s)

When it comes to the neurotoxic and myotoxic effects of snakebite, phospholipases A2 play a significant part. These proteins, which are organized in groups I and II and have atomic weights of 1315 kDa, are each recognized as important components of Viperidae poisons [38]. Many PLA2 toxins also promote inflammation by inducing edema, cytokine production, and leukocyte recruitment, pain by inducing thermal allodynia and mechanical hyperalgesia, paralysis by blocking neuromuscular transmission, and increase discharge. Several PLA2 have strong myotoxic effects that frequently result in severe necrosis. via preventing coagulation [39]. They are enzymes that cleave the fatty acid at position two of the phospholipids by hydrolyzing the bond between the second fatty acid's "tail" and the glycerol molecule [40]. PLA2 enzymes can be present in mammalian tissues as well as in arachnid, insect, and snake venom [40].

2.4.2 Snake Venom Metalloproteinases (Svmps)

According to their structural domains, snake venom metalloproteinases (SVMPs), which range in size from 20 to 110 kDa, are divided into three groups: PI, PII, and PIII [41]. These poisons are key parts of viper venom and contribute significantly to its toxicity. Toxin

SVMPs (a disintegrin and a metalloproteinase) have evolved from ADAM proteins, specifically ADAM28, with PIII being the basic variant that incorporates metalloproteinase, disintegrin, and cysteine-rich spaces [42]. The hemorrhagic and coagulopathic poison shedding that occurs after a snake bite is mostly attributed to SVMPs. The range of SVMP isoforms present in their toxin likely has synergistic effects, operating simultaneously during various stages of blood coagulation, for instance [43].

A metalloproteinase, frequently alluded to as a metalloprotease, is a protease protein with a metal reactant system. Most metalloproteases require zinc, albeit some additionally require cobalt. Three ligands are utilized to append the metal particle to the protein.

2.4.3 Snake Venom Serine Proteinases (Svsps)

Snake Venom Serine Proteinases (SVSPs) belong to the S1 category of serine proteinases and contain two distinct underlying spaces as well as sub-atomic masses ranging from 26 to 67 kDa. When hydrophobic or strongly charged amino corrosive buildups are attached to polypeptide chains on the C-terminal side, SVSPs catalyze the cleavage [42,43]. Although they are often just abundant in snake toxins and significantly less common in the toxins of elapid and "colubrid" snakes, SVSPs have been depicted in the toxin of a wide variety of snake families, similar to SVMPs [44,45]. While SVMPs are renowned for their ability to rupture thin blood vessels, SVSPs carry out their primary injury by altering the haemostatic arrangement of their victims and causing edema hyperalgesia through mechanisms that are currently poorly understood. Hemotoxic effects caused by SVSPs include problems with blood coagulation (supportive of or hostile to coagulant), fibrinolysis, platelet collection, and pulse, with potentially dangerous repercussions for victims of snakebite [46,47]. The inflammatory reactions and hyperalgesia that SVSPs cause are poorly understood. According to studies, SVMPs and PLA2s play a major role in the inflammation and torment brought on

by snake toxins, whereas SVSPs play a moderate role in torment and a major role in inflammation [48,49,50,51].

2.4.4 Three-Finger Toxins (3ftxs)

Three-finger poisons (3FTXs) are non-enzymatic neurotoxins with 58–81 residues that have a disulfide-balanced three-finger crease structure. The majority of them are present in the poisons of elapid and colubrid snakes, and they restrict postsynaptically at the neuromuscular intersections to induce limp loss of mobility in snakebite victims [52]. While long-chain 3FTXs, which contain neurotoxins, hannalgesin, and neurotoxins, have 66-74 buildups and five disulfide spans, short-chain 3FTXs, which include neurotoxins, cardiotoxins, cytotoxins, fasciculins, and mambalgins, have 57–62 deposits and four disulfide spans.

2.4.5 C-type lectins

A lectin is a category of protein domain that binds to carbohydrates and goes by the name C-type lectin (CLEC) [53]. The C-type designation alludes to the fact that they require calcium to bind. Among the many functions carried out by proteins with C-type lectin domains are cell-cell adhesion, the immune system's reaction to pathogens, and apoptosis [54,55,56].

CHAPTER THREE

3.0 METHODOLOGY

3.1 Retrieval of *Bitis arietians* Toxin sequence

National Center for Biotechnology Information (NCBI)

The National Center for Biotechnology Information (NCBI) is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health (NIH). The NCBI is a valuable source for bioinformatics tools and services and is home to a number of databases pertinent to biotechnology and biomedicine. GenBank, a database of DNA sequences, and PubMed, a bibliographic database of biomedical literature, are both significant databases.

Phospholipase

>AAX86638.1 PLA2-18, partial [*Bitis arietans*]

MRTLWIVAVWLMGVEGNLYQFGKMIKNKTGKPATFSYSAYGCYCGWGGQGKPQD
PSDRCRFMHDCCYTRV

NNCSPKMTLYSYRFENGDIICGDNDPCRKAVCECDREAAICLGENVNTYDEKYRFYS
SSYCTEEESEKC

C-type lectin

>BBN67125.1 bitiscetin-3 subunit alpha [*Bitis arietans*]

MGRFIFLSSGLLVVFLSLRGTGADEGCLPDWSSRVEHCYKVFKERKTWEDAEKFCV
ENSGHLASIEGKEE

ADFVAQLLSQALKKSKYDYNVWIGLRDESKTQQCSPQWTDGSLTFYENLDEPTKCF

GLGEHTGYRTWTDL

PCGQKNPFICKSRLPH

3.2 Search of Toxin Sequence Similarity using BLAST Program

BLAST (Basic local alignment search tool) A program and technique for comparing primary biological sequence data, such as the amino acid sequences of proteins or the nucleotides of DNA and/or RNA sequences. A BLAST search allows a researcher to compare a topic protein or nucleotide sequence (referred to as a query) with a library or database of sequences and find database sequences that match the query sequence more frequently than a predetermined threshold. It runs the query sequence against NCBI databases and servers, and then posts the findings in the preferred format back to the user's browser. Most input sequences to BLAST are in GenBank or FASTA format [56].

1. ***Echis carinatus***

   >AJA90797_ *Echis carinatus*

   MRTLWIVAVWLMGVEGNLYQFGKMIKNKTGKPAMFSYSAYGCYCGWGGQ

   GKPQDPSDRCCFVHDCCYTRV

   NNCGPKMTLYSYRFENGDIICGDNDPCRKAVCECDREAAICLGENVNTYDEK

   YRFYSSSYCTEKETEQC

2. ***Vipera renardi***

   >sp|F8QN51_ *Vipera renardi*

   MRTLWIVAVCLIGVEGNLFQFGKMIKYKTGKSALLSYSAYGCYCGWGGQGK

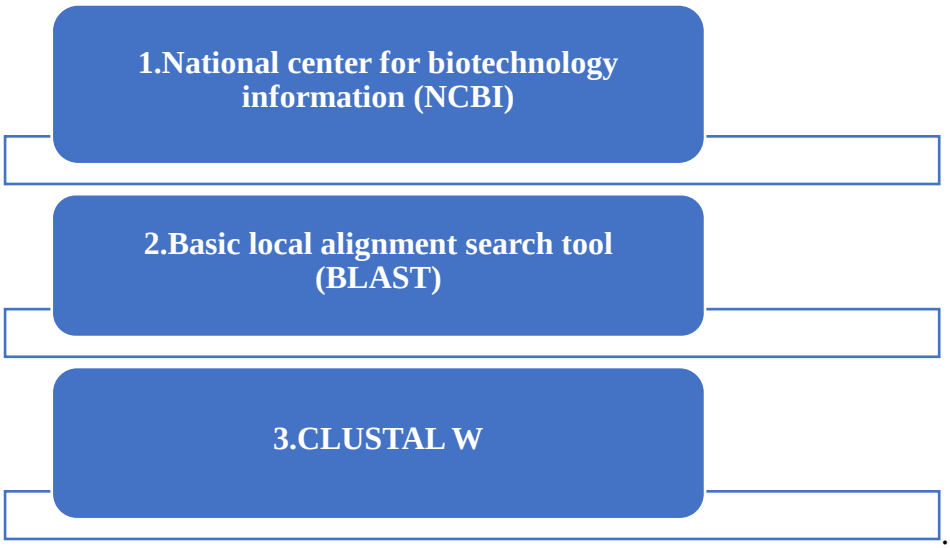
   PQDPTDRCCFVHDCCYGRV

NGCNPKMDTYSYSFLNGDIVCGDDDPCLRAICECDRAAAICFGENVNTYDKK

YKYYSSSHCTETEQC

3.3 Alignment of Sequences

Clustal W is a series of commonly used software tools for multiple sequence alignment in the bioinformatics field. Clustal W is a program for worldwide multiple sequence alignment [57]. It aligns DNA or amino acid sequences using a progressive alignment technique with affine gap penalties and a guide tree based on sequence similarity. Using a heuristic that creates a multiple sequence alignment from a succession of pairwise alignments, Clustal software variants align sequences [58]



CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Amino Acid Constituents in Snake Venoms

Table 4.1 shows the amino acid constituents of the snake venoms used in the study. From the study, the percentages of conserved amino acids were higher compared to the percentage of non-conserved amino acids of the various snake venoms. In addition, six snake species were observed to have phospholipase A2, five snake species had C-type lectin while six had metalloprotease.

The result shows that the snake species have the same number (264) of conserved amino acid residues in the phospholipase A2 constituent of the snake venom. The snake species had varying number of non-conserved amino acid residues of *Causus rhombeatus* (78), *Cerastes cerastes* (66), *Daboia siamensis* (67), *Vipera renardi* (66), *Echis carinatus* (68) and *Bitis arietans* (68) (Figure 4.1).

Figure 4.2 show that the snake species have the same number (64) of conserved amino acid residues in the C-type lectin constituent of the snake venom. However, *Ovophis okinavensis, Bitis arietans, Pseudocerastes urarachnoides, Vipera transcaucasiana* and *Macrovipera lebetina* have 93, 92, 90, 89 and 92 number of non-conserved amino acid residues respectively in the C-type lectin proteome.

Table 4.1: Total Number of Amino Acid

Snake Venoms	Conserved Amino Acid	Non-Conserved Amino Acid
Phospholipase A2		
Causus rhombeatus: 149	71(47.7%)	78(52.3%)
Cerastes cerastes: 137	71(51.8%)	66(48.2%)
Daboia siamensis: 138	71(51.4%)	67(48.6%)
Vipera renardi: 137	71(51.8%)	66(48.2%)
Echis carinatus: 139	71(51.1%)	68(48.9%)
Bitis arietans: 139	71(51.1%)	68(48.9%)
C-type Lectins		
Ovophis okinavensis: 157	64(40.7%)	93(59.3%)
Bitis arietans: 156	64(41.0%)	92(59.0%)
Pseudocerastes urarachnoides: 154	64(41.5%)	90(58.5%)
Vipera transcaucasiana: 153	64(41.8%)	89(58.2%)
Macrovipera lebetina: 156	64(41.0%)	92(59.0%)
Metalloprotease		
Vipera ammodytes ammodytes: 537	264(49.2%)	273(50.8%)
Bothrops jararaca: 536	264(49.3%)	272(50.7%)
Protobothrops mucrosquamatus: 537	264(49.2%)	273(50.8%)
Pseudocerastes urarachnoides: 540	264(48.9%)	276(51.1%)
Echis carinatus sochureki: 540	264(48.9%)	276(51.1%)
Bitis arietans: 515	264(51.3%)	251(48.7%)

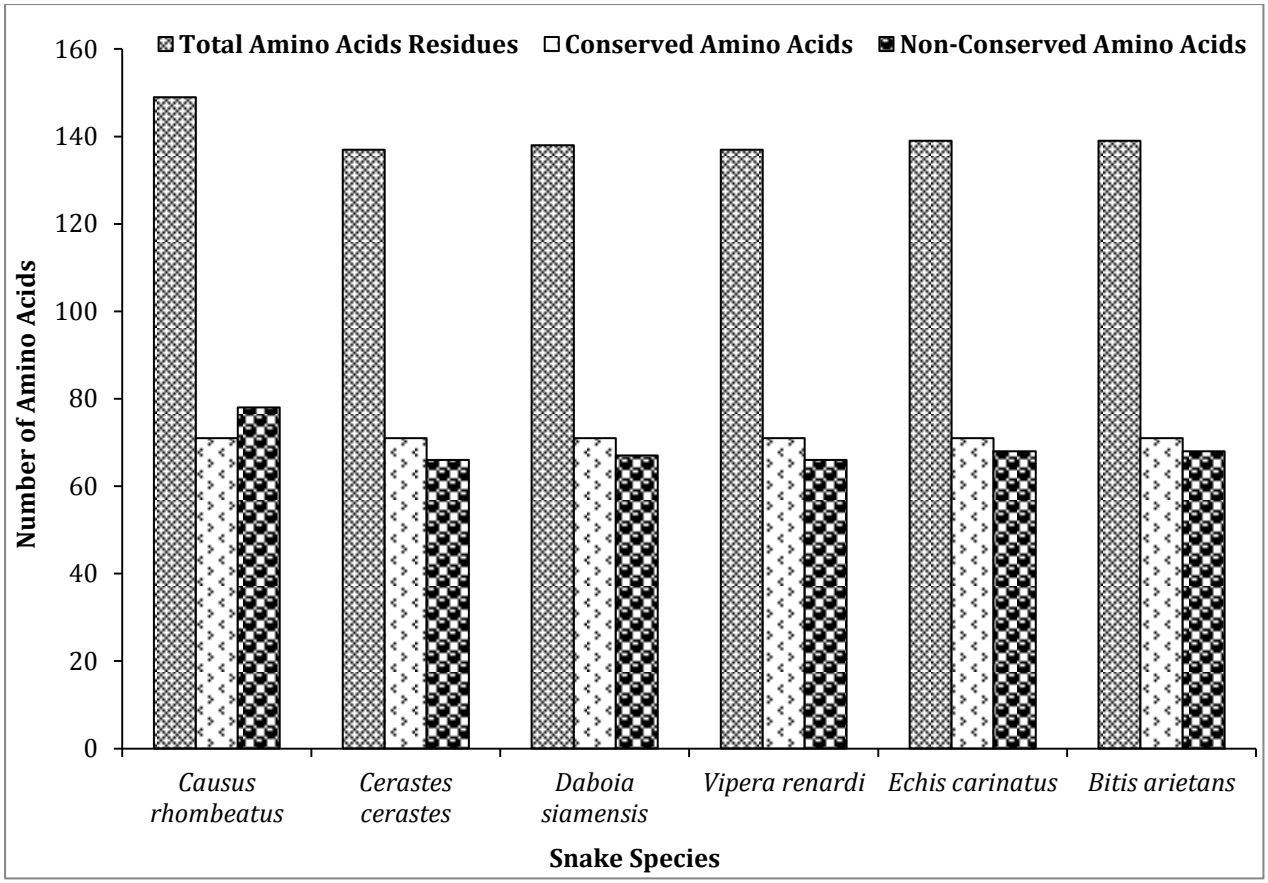


Figure 4.1: Phospholipase A2 Amino Acid Residues in the Snake Species

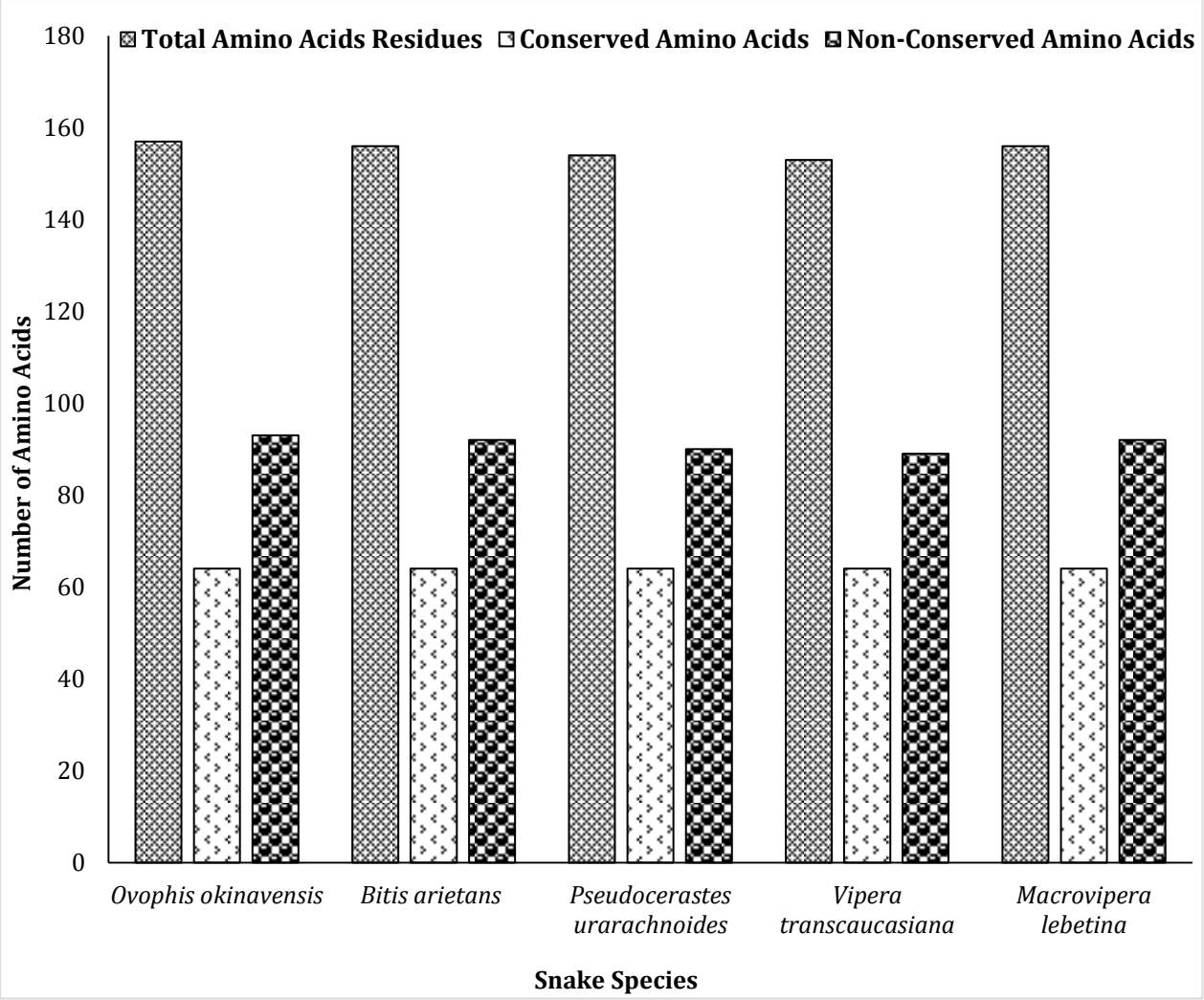


Figure 4.2: C-type lectin Amino Acid Residues in the Snake Species

The result shows that the snake species have the same number (264) of conserved amino acid residues with varying number of non-conserved amino acid residues in the metalloprotease constituent of the snake venom (Figure 4.3). The number of non-conserved amino acid residues in the snake species are *Vipera ammodytes ammodytes* (273), *Bothrops jararaca* (272), *Protobothrops mucrosquamatus* (273), *Pseudocerastes urarachnoides* (276), *Echis carinatus sochureki* (276) and *Bitis arietans* (251).

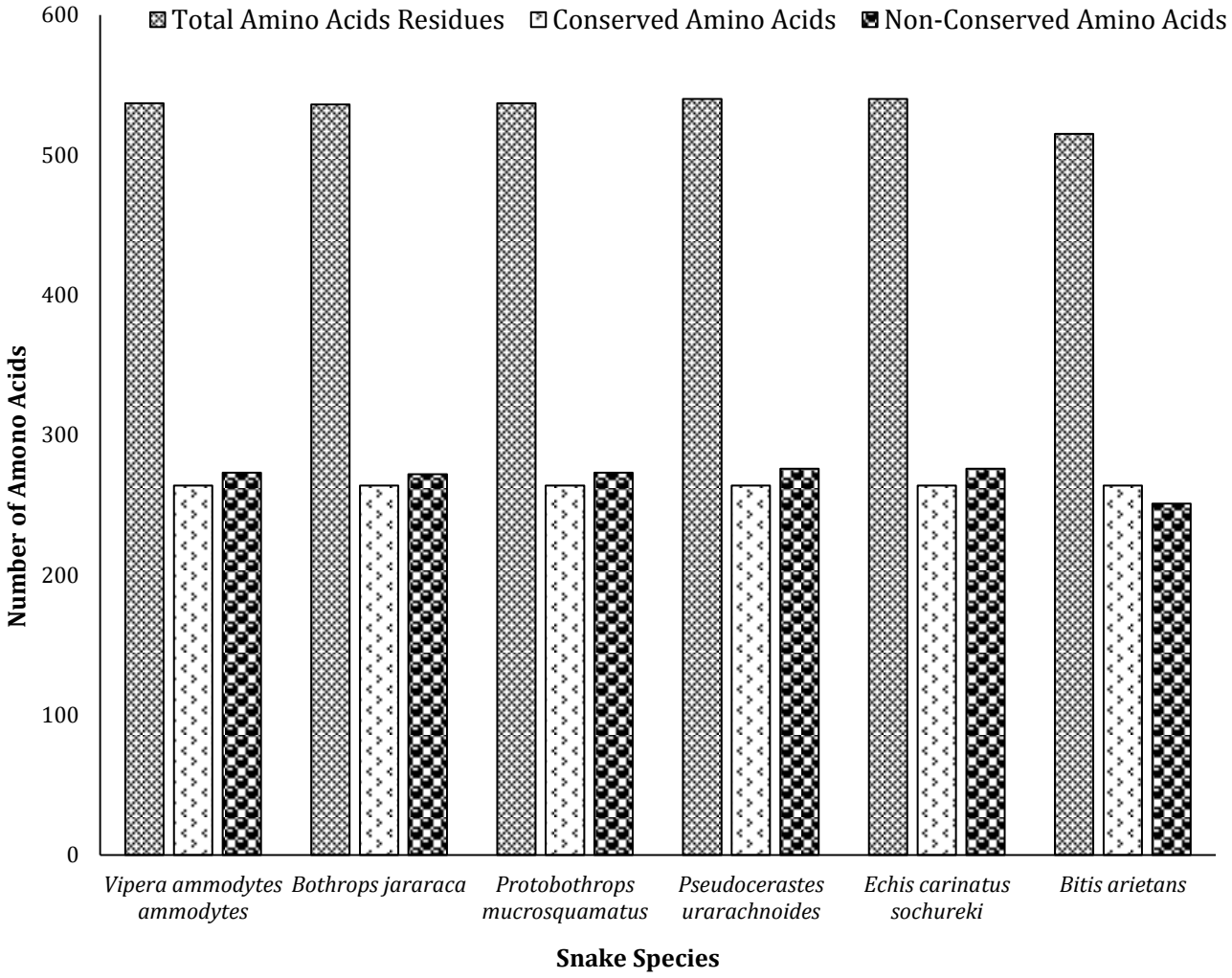


Figure 4.3: Metalloprotease Amino Acid Residues in the Snake Species

4.1.2 Conserved Amino Acid Residues

The result shows the number of conserved amino acid residues in the snake proteomes (Figure 4.4). From the result, metalloprotease proteome had the highest number (1584) of conserved amino acids followed by phospholipase A2 (426) and C-type lectin (320).

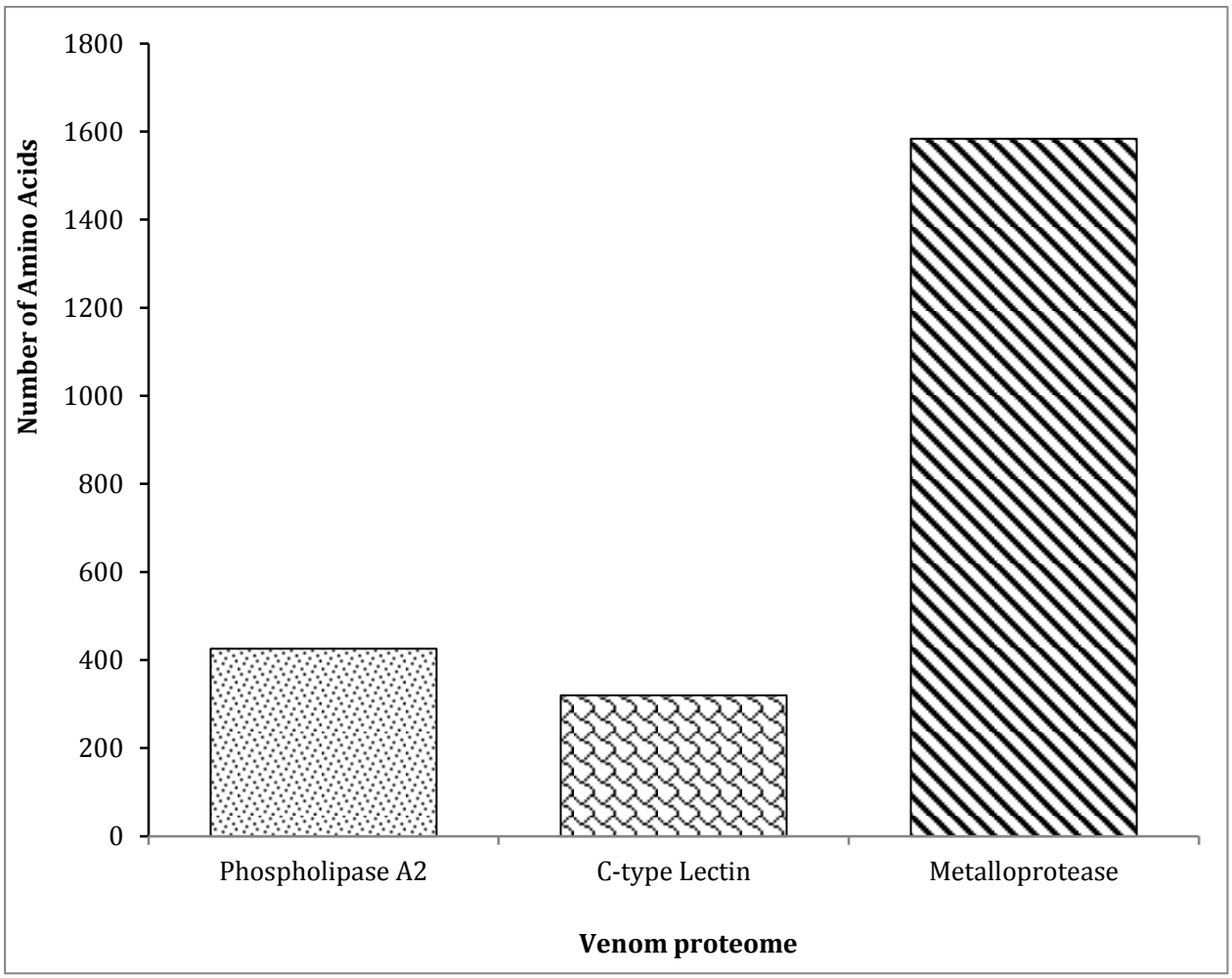


Figure 4.4: Number of Conserved Amino Acids

4.1.3 Non-Conserved Amino Acid Residues

Figure 4.5 shows the number of non-conserved amino acid residues in the snake proteomes. Metalloprotease proteome had the highest number (1621) of non-conserved amino acids followed by C-type lectin (456) and phospholipase A2 (413).

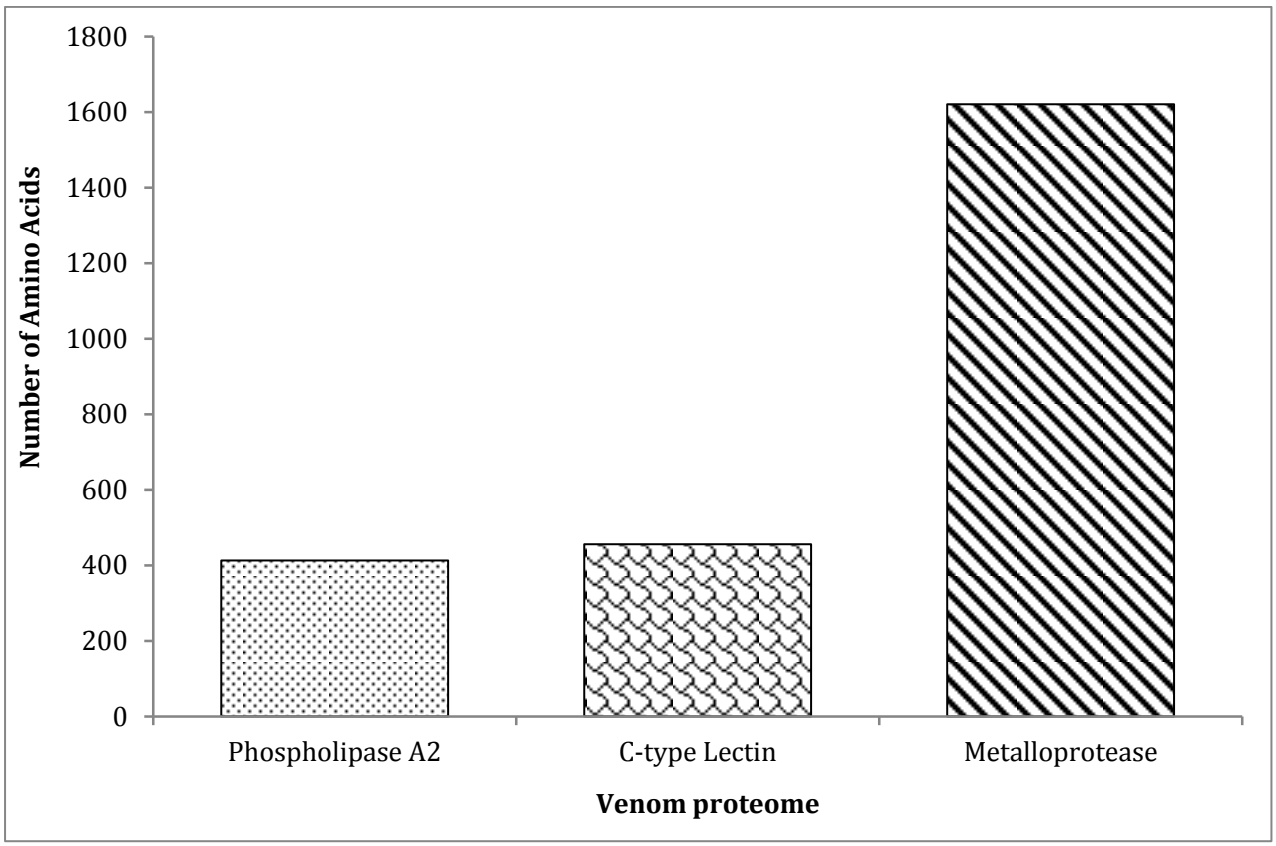


Figure 4.5: Number of Non-Conserved Amino Acids

4.2 Discussion

The transcriptomic variability survey of three most abundant venom proteins, phospholipase A2, C-type lectin and metalloprotease in Bitis arietans were compared to similar proteins in some prevalent snakes in Nigeria. The finding corresponded to the transcriptomic and proteomic finding of Dingwoke et al. [59] who identified these proteins to be among the most abundant 79 proteins they isolated in their study. Casewell et al. [60] showed a transcriptomic analysis of venom gland where snake venom metalloproteinases and serine proteases were the major toxin genes transcribed in Nigerian B. arietans, and were also found to be the most abundant toxin family secreted in the venom [60]. In a comparison, the transcriptomic analyses showed that C-type lectins was present in high abundances but in this study, the protein was present in low abundances compared to phospholipase A2 and metalloprotease in the venom proteome (Table 4.1). This disparity may be due to the fact that toxins are known to be transcribed at the highest level in the venom gland [60].

The finding of the present study showed that *B. arietans* had same number of conserved amino acid residues in the venom proteins of the selected prevalent snakes in Nigeria (Table 4.1). However, transcriptomic comparison between *B. arietans* and the selected snakes, some variability were observed in the number of non-conserved amino acids of the venom proteins. These variations could be attributed to factors such as snake's gender, age [61], geographical origin [62, 63] and diet [16]. It is believed that mutations in the venom-related genes may affect the venom composition [64].

Qualitative discrepancies in the compositional agreement of venom acquired using transcriptomic approach evident from the current study have been reported for other species, i.e. *Lachesis muta* [65] and *Bitis gabonica gabonica* [66]. Venom composition may be influenced by a number of different factors, most notably the potential for genetic drift on

account of (i) gender based variation such as those reported for the venom proteomes of *Bothrops jararaca* siblings [67] and (ii) ontogenetic variations reported in the venom proteomes of other snakes i.e. *Crotalus viridis viridis*, *C.v. oreganus*, *Bothrox atrox*, and *Bothrops asper* [68, 69], and perhaps other species. The discrepancies in venom gland transcriptomic specifically in the non-conserved region will have an impact upon the design and selection of immunogens to generate toxin-specific antivenom for *Bitis arietans* and the selected prevalent snakes in Nigeria.

In this study, the venom of *Bitis arietans* was found to be dominated by metalloproteinase in the venom proteome. This finding contradicts previous studies in which phospholipase A2 were described as one of the commonest toxins in the venom of the front-fanged snakes [70, 71].

Snake venom toxins are thought to act synergistically in exerting a wide range of biochemical and toxicological effects [72, 73]. Envenomation by the viper primarily gives rise to local effects and bleeding [74, 75]. Snake venom metalloproteinases are zinc-dependent proteinase toxins that are responsible for many of the generally known pathological phenotypes in envenomation by the viper snake species [76, 77] and play a key role in coagulopathies commonly associated with viper envenoming [77, 78]. Similarly, metalloproteinases provoke a manifold of clinical manifestations, including hemorrhagic, pro-coagulant, anticoagulant, fibrinolytic, apoptotic, and antiplatelet activities [79, 80]. Thus, metalloproteinases are believed to evolve from ADAM (a Disintegrin and Metalloproteinase) proteins, precisely ADAM28, characterized by metalloproteinase, disintegrin-like and cysteine-rich domains [81], which induce hemorrhagic activity by rupturing the capillary vessels, resulting to extravasation in envenomed victims [82]. This occurs through cleavage 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 [83]. More so, metalloproteinases alters homeostasis by disrupting coagulation, through modulation of fibrinogenase and fibrolase that mediate the coagulation cascade which aids in eliciting their hemorrhagic role [84, 85].

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In the present study, venom proteins phospholipase A2, metalloprotease and C-type lectins were detected in both the conserve and non-conserve regions. In the former, metalloprotease had the highest percentage occurrence of 61% followed by phospholipase A2 (50%) and C-type lectins (41%). However, in the non-conserved region phospholipase A2 had the highest occurrence of (29%) followed by metalloprotease (20%) and C-type lectins (26%). This study has provided a baseline information on the venom proteome of *B. arietans* indigenous to Nigeria. Going by these findings, the venom composition could be taken into consideration and as such, this can serve as a guide in the use and design of a potential anti-venom.

5.2 Recommendations

The following recommendations have been made based on the finding of the present study:

1. Cautious 'over-engineering' of the immunogen design is necessary to overcome the proteomic–transcriptomic discrepancies.

2. Further extensive studies should be carried out to confirm the design of antibodies to the conserved motifs in the transcriptome which would likely bind to and negate the function of the venom-only isoforms.

REFERENCES

1. Caldwell, M.W., Nydam, R.L., Palci, A., Apesteguía, S., 2015. The oldest known snakes from the middle jurassic-lower cretaceous provide insights on snake evolution. Nat. Commun. 6, 5996

2. Pillay, V.V. (2013). *Modern Medical Toxicology*, 4th Edition. Jaypee Brothers Medical Publishers, 137-145.

3. Habib, A.G., Gebi, U.I. and Onyemelukwe, G.C. (2001). *Snake bite in Nigeria*. *Afr J Med Med Sci*. 30(3):171-8.

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

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

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

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

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

9. WHO (2018). *Snakebite envenoming*: member States provide WHO with clear mandate for global action, Geneva, https://www.who.int/news/item/25-05-2018-snakebite -envenoming-member-states-provide-who-with-clear-mandate-for-global-action, 25th May, 2018. (Accessed July 2022).

10. Calvete, J.J. (2014). Next-generation snake venomics: protein-locus resolution through venom proteome decomplexation. *Expert Rev. Proteomics* 11:315–329.

11. Lomonte, B., Tsai, W.C., Urena-Díaz, J.M., Sanz, L., Mora-Obando, D., S´anchez, E.E., Fry, B.G., Guti´errez, J.M., Gibbs, H.L., Calvete, J.J. (2014). *Venomics of New*

World pit vipers: genus-wide comparisons of venom proteomes across Agkistrodon. *J. Proteom.* 96: 103–116.

12. Fry, B.G., Scheib, H., Van DerWeerd, L., Young, B., McNaughtan, J., Ramjan, S.F., Vidal, N., Poelmann, R.E., Norman, J.A. (2008). Evolution of arsenal: structural and functional diversification of the venom system in advanced snakes (Caenophidia). *Mol. Cell. Proteomics* 7:215–246.

13. Tasoulis, T. and Isbister, G.K. (2017). A review and database of snake venom proteomes. *Toxins* 9:673. E290.

14. Durban, J. Sanz, L., Trevisan-Silva, D., Neri-Castro, E., Alagon, A., Calvete, J.J. (2017). Integrated venomics and venom gland transcriptome analysis of juvenile and adult Mexican rattlesnakes *Crotalus simus*, *C. tzabcan*, and *C. culminatus* revealed miRNA-modulated ontogenetic shifts. *J. Proteome Res*. 16:3370–3390.

15. L. Goncalves-Machado, D. Pla, L. Sanz, R.J.B. Jorge, M. Leitao-De-Araujo, M. L. Alves, D.J. Alvares, J. De Miranda, J. Nowatzki, K. De Morais-Zani, W. Fernades, A.M. Tanaka- Azevedo, J. Fernandez, R.B. Zingali, J.M. Gutierrez, C. Correa-Netto, J.J. Calvete, Combined venomics, venom gland transcriptomics, bioactivities, and antivenomics of two Bothrops jararaca populations from geographically isolated regions within the Brazilian Atlantic rainforest, *J. Proteomics* 135 (2016) 73–89.

16. Barlow, A., Pook, C.E., Harrison, R.A., Wuster, W. (2009). 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.

17. Dias, G.S., Kitano, E.S. Pagotto, A.H., Sant'anna, S.S., Rocha, M.M., Zelanis, A., Serrano, S. M. (2013). Individual variability in the venom proteome of juvenile Bothrops jararaca specimens, *J. Proteome Res.* 12 4585–4598.

18. J.M. Guti´errez, J.J. Calvete, A.G. Habib, R.A. Harrison, D.J. Williams, D.A. Warrell, (2017). *Snakebite envenoming, Nat. Rev. Dis. Primers 3* (2017) 17063,

19. D. Pla, J.M. Guti´errez, J.J. Calvete, Second generation snake antivenomics: comparing immunoaffinity and immunodepletion protocols, *Toxicon 60* (2012) 688–699.

20. [21] S. Kunalan, I. Othman, S.S. Hassan, W.C. Hodgson, Proteomic characterization of two medically important Malaysian snake venoms, Calloselasma rhodostoma (Malayan pit viper) and Ophiophagus hannah (king Cobra), *Toxins 10* (2018), https://doi.org/10.3390/toxins10110434.

21. [22] N.R. Casewell, S.C. Wagstaff, W. Wüster, D.A.N. Cook, F.M.S. Bolton, S.I. King, D. Pla, L. Sanz, J.J. Calvete, R.A. Harrison, *Postgenomic processes dictate venom variation, Proc. Natl. Acad. Sci. Unit. States Am. 111* (2014) 9205–9210.

22. [23] S.C. Wagstaff, R.A. Harrison, 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 (2006) 21–32.

23. "*Reptile Venom Research*". Australian Reptile Park. Archived from the original on 2 February 2010. Retrieved 21 December 2010

24. Bauchot R (1994). Snakes: A Natural History. New York City, NY, USA: *Sterling Publishing Co*., Inc. pp. 194–209. ISBN 978-1-4027-3181-5.

25. Halliday A, Kraig T, eds. (2002). Firefly Encyclopedia of Reptiles and Amphibians. Toronto, Canada: *Firefly Books Ltd. pp*. 202–203. ISBN 978-1-55297-613-5.

26. Bottrall JL, Madaras F, Biven CD, Venning MG, Mirtschin PJ (September 2010). "Proteolytic activity of Elapid and Viperid Snake venoms and its implication to digestion". *Journal of Venom Research. 1* (3): 18–28. PMC 3086185. PMID 21544178.

27. Mattison C (2007). *The New Encyclopedia of Snakes*. New Jersey, USA (first published in the UK): Princeton University Press (Princeton and Oxford) first published in Blandford. p. 117. ISBN 978-0-691-13295-2.

28. Samantha, S. (2022). The 3 Types of Snake Venom (Explained). *Wildlife Informer*. [Assessed on 8 April, 2022 from https://wildlifeinformer.com/types-of-snake-venom/]

29. Lynch VJ (2007). Inventing an arsenal: adaptive evolution and neofunctionalization of snake venom phospholipase A2 genes. *BMC Evolutionary Biology*. 7 (2): 2.

30. Eric J Lavonas 1, Christian A Tomaszewski, Marsha D Ford, Anna M Rouse, William P Kerns 2ndPMID: 12507061 DOI: 10.1081/clt-120016963

31. U.S. Navy. 1991. Venomous Snakes of the World. US Govt. New York: *Dover Publications Inc.* 203 pp. ISBN 0-486-26629-X.

32. Mallow D, Ludwig D, Nilson G. 2003. True Vipers: Natural History and Toxinology of Old World Vipers. *Krieger Publishing Company, Malabar, Florida*. 359 pp. ISBN 0-89464-877-2.

33. Spawls S, Howell K, Drewes R, Ashe J. 2004. *A Field Guide to the Reptiles Of East Africa. A & C Black Publishers Ltd., London*. 543 pp. ISBN 0-7136-6817-2.

34. "Bitis arietans". *Integrated Taxonomic Information System*. Retrieved 24 July 2006

35. Gregor Pollach, in Clinical Cases in Tropical Medicine, 2015

36. Mallow D, Ludwig D, Nilson G. 2003. True Vipers: *Natural History and Toxinology of Old World Vipers. Krieger Publishing Company, Malabar, Florida*. 359 pp. ISBN 0-89464-877-2.

37. Spawls S, Branch B. 1995. The Dangerous Snakes of Africa. Ralph Curtis Books. *Dubai: Oriental Press*. 192 pp. ISBN 0-88359-029-8.

38. Mehrtens JM. 1987. *Living Snakes of the World in Color. New York: Sterling Publishers.* 480 pp. ISBN 0-8069-6460-X.

39. Harris, J. B., and Scott-Davey, T. (2013). 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. doi: 10.3390/toxins5122533

40. Six, D. A., and Dennis, E. A. (2000). The expanding superfamily of phospholipase A2 enzymes: classification and characterization. *Biochim. Biophys*. Acta 1488, 1–19. doi: 10.1016/S1388-1981(00)00105-0

41. Hite, L. A., Jia, L. G., Bjarnason, J. B., and Fox, J. W. (1994). cDNA sequences for 4 snake-venom metalloproteinases–structure, classification, and their relationshiptomammalianreproductiveproteins.Arch. *Biochem. Biophys*.308, 182–191. doi: 10.1006/abbi.1994.1026

42. Page, M. J., and Di Cera, E. (2008). Serine peptidases: classification, structure and function. *Cell Mol. Life Sci*. 65, 1220–1236. doi: 10.1007/s00018-008-7565-9

43. Jia, L. G., Shimokawa, K., Bjarnason, J. B., and Fox, J. W. (1996). Snake venom metalloproteinases: structure, function and relationship to the ADAMs family of proteins. *Toxicon 34*, 1269–1276. doi: 10.1016/S0041-0101(96)00108-0

44. Serrano, S. M. T. (2013). The long road of research on snake venom serine proteinases. *Toxicon 62*, 19–26. doi: 10.1016/j.toxicon.2012.09.003

45. Tasoulis, T., and Isbister, G. K. (2017). A review and database of snake venom proteomes. *Toxins 9*:E290. doi: 10.3390/toxins9090290

46. Modahl, C. M., Frietze, S., and Mackessy, S. P. (2018a). Transcriptome-facilitated proteomic characterization of rear-fanged snake venoms reveal abundant metalloproteinases with enhanced activity. *J. Proteomics 187*, 223–234. doi: 10.1016/j.jprot.2018.08.004

47. Murakami, M. T., and Arni, R. K. (2005). Thrombomodulin-independent activation of protein C and specificity of hemostatically active snake venom serine proteinases: crystal structures of native and inhibited Agkistrodon contortrix contortrix protein C activator. *J. Biol. Chem*. 280, 39309–39315. doi: 10.1074/jbc.M508502200

48. Kang, T. S., Georgieva, D., Genov, N., Murakami, M. T., Sinha, M., Kumar, R. P., *et al*. (2011). Enzymatic toxins from snake venom: structural characterization and mechanism of catalysis. *Febs. J*. 278, 4544–4576. doi: 10.1111/j.1742-4658.2011.08115.x

49. Zychar, B. C., Dale, C. S., Demarchi, D. S., and Goncalves, L. R. (2010). Contribution of metalloproteases, serine proteases and phospholipases A2 to the inflammatory reaction induced by Bothrops jararaca crude venom in mice. *Toxicon* 55, 227–234. doi: 10.1016/j.*toxicon*.2009.

50. Menaldo, D. L., Bernardes, C. P., Pereira, J. C., Silveira, D. S., Mamede, C. C., Stanziola, L., *et al*. (2013*).* Effects of two serine proteases from Bothrops pirajai snake venom on the complement system and the inflammatory response. Int. Immunopharmacol. 15, 764–771. doi: 10.1016/j.intimp

51. Osipov,A.V.,andUtki,Y.N.(2015).“*Snakevenomtoxinstargetedatthenervous system*,” in Snake Venoms, eds H. I. P. Gopalakrishnakone, A. K. Mukherjee, T. R. Rahmy, C.-W. Vogel, (*Springer Science*) 1–21. doi: 10.1007/978-94-007-6648-8_23-1

52. Kessler, P., Marchot, P., Silva, M., and Servent, D. (2017). *The three-finger toxin fold*: a multifunctional structural scaffold able to modulate cholinergic functions. J. *Neurochem. 142(Suppl 2),* 7–18. doi: 10.1111/jnc.13975

53. Barber, C. M., Isbister, G. K., and Hodgson, W. C. (2013). Alpha neurotoxins. *Toxicon* 66, 47–58. doi: 10.1016/j.*toxicon*.2013.01.019

54. Zelensky AN, Gready JE (December 2005). "*The C-type lectin-like domain superfamily*". FEBS J. 272 (24): 6179–217. doi:10.1111/j.1742-4658.2005.05031.x. PMID 16336259. S2CID 7084402.

55. Drickamer K (October 1999). "*C-type lectin-like domains*". Curr. Opin. *Struct. Biol. 9* (5): 585–90. doi:10.1016/S0959-440X(99)00009-3. PMID 10508765

56. Cambi A, Figdor C (May 2009). "Necrosis: *C-type lectins sense cell death*". *Curr. Biol*. 19 (9): R375–8. doi:10.1016/j.cub.2009.03.032. PMID 19439262. S2CID 17409821.

57. Altschul Stephen; Gish Warren; Miller Webb; Myers Eugene; Lipman David (1990). "Basic local alignment search tool". *Journal of Molecular Biology*. 215 (3): 403–410.

58. Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (July 2003). "Multiple sequence alignment with the Clustal series of programs". *Nucleic Acids Research. 31* (13): 3497–500.

59. Dingwoke, E.J., Adamude, F.A., Mohamed, G., Klein, A., Salihu, A., Abubakar, M.S., Sallau, A.B. (2021). Venom proteomic analysis of medically important Nigerian viper *Echis ocellatus* and *Bitis arietans* snake species. *Biochemistry and Biophysics Reports* 28(101164):1-12

60. Casewell, N.R., Wagstaff, S.C., Wüster, W., Cook, D.A.N., Bolton, F.M.S., King, S.I., Pla, D., Sanz, L., Calvete, J.J., Harrison, R.A. (2014). Postgenomic processes dictate venom variation. *Proc. Natl. Acad. Sci. Unit. States Am*. 111: 9205–9210

61. Durban, J., Sanz, L., Trevisan-Silva, D., Neri-Castro, E., Alagon, A., Calvete, J.J. (2017). Integrated venomics and venom gland transcriptome analysis of juvenile and adult Mexican rattlesnakes Crotalus simus, C.

tzabcan, and C. culminatus revealed miRNA-modulated ontogenetic shifts. *J. Proteome Res.* 16:3370–3390.

62. Goncalves-Machado, L., Pla, D., Sanz, L., Jorge, R.J.B., Leitao-De-Araujo, M., Alves, L.M., Alvares, D.J. et al. Combined venomics, venom gland transcriptomics, bioactivities, and antivenomics of two Bothrops jararaca populations from geographically isolated regions within the Brazilian Atlantic rainforest, J. Proteomics 135 (2016) 73–89.

63. Tasoulis, T. and Isbister, G.K. (2017). A review and database of snake venom proteomes. *Toxins* 9:673. E290.

64. Adamude, F.A., Dingwoke, E.J., Abubakar, M.S., Ibrahim, S., Mohamed, G., Klein, A., Sallau, A.B. (2021). Proteomic analysis of three medically important Nigerian Naja (Naja haje, Naja katiensis and Naja nigricollis) snake venoms. *Toxicon* 197:24–32

65. Sanz L, Escolano J, Ferretti M, Biscoglio MJ, Rivera E, Crescenti EJ, et al. (2008). Snake venomics of the South and Central American Bushmasters. Comparison of the toxin composition of *Lachesis muta* gathered from proteomic versus transcriptomic analysis. *J Proteomics* 71:46–60.

66. Calvete JJ, Marcinkiewicz C, Sanz L. (2007). Snake venomics of *Bitis gabonica gabonica*. Protein family composition, subunit organization of venom toxins, and characterization of dimeric disintegrins bitisgabonin-1 and bitisgabonin-2. *J Proteome Res* 6:326–36.

67. Menezes MC, Furtado MF, Travaglia-Cardoso SR, Camargo AC, Serrano SM. (2005). Sex-based individual variation of snake venom proteome among eighteen Bothrops jararaca siblings. *Toxicon* 47:304–12.

68. Guércio RAP, Shevchenko A, Shevchenko A, López-Lozano JL, Paba J, Sousa MV, et al. (2006). Ontogenetic variations in the venom proteome of the Amazonian snake *Bothrops atrox*. *Proteome Sci* 4:11.

69. Alape-Girón A, Sanz L, Escolano J, Flores-Díaz M, Madrigal M, Sasa M, et al. (2008). Snake venomics of the lancehead pitviper *Bothrops asper*: geographic, individual, and

ontogenetic variations. *J Proteome Res* 7:3556–71.

70. Bharati, K., Hasson, S.S., Oliver, J., Laing, G.D., Theakston, R.D.G., Harrison, R.A. (2003). Molecular cloning of phospholipases A_2 from venom glands of *Echis* carpet vipers. *Toxicon* 41:941–947.

71. Jiménez-Charris, E., Montealegre-Sanchez, L., Solano-Redondo, L., Mora-Obando, D., Camacho, E. et al. (2015). Proteomic and functional analyses of the venom of *Porthidium lansbergii lansbergii* (*Lansberg's hognose* viper) from the Atlantic Department of Colombia. *J. Proteom*. 114:287–299.

72. Kang, T.S., Georgieva, D., Genov, N., Murakami, M.T., Sinha, M., Kumar, R.P. et al. (2011). Enzymatic toxins from snake venoms: structural characterization and mechanism of catalysis. *FEBS J*. 278:4544–4576.

73. Sajevic, T., Leonardi, A., Krizaj, I. (2011). Haemostatically active proteins in snake venoms. *Toxicon* 57:627–645.

74. Warrell, D.A. (2010). Snake bite. *Lancet* 375:77–88.

75. Waiddyanatha, S., Silva, A., Siribaddana, S., Isbister, G.K. (2019). Long-term effects of snake envenoming. *Toxins* 11:193.

76. Kohlhoff, M., Borges, M.H., Yarleque, A., Cabezas, C., Richardson, M., Sanchez, E.F. (2012). Exploring the proteomes of the venoms of the Peruvian pit vipers. *J. Proteom*. 75:2181–2195.

77. Moura-da-Silva, A.M., Almeida, M.T., Portes-Junior, J.A., Nicolau, C.A., Gomes- Neto, F., Valente, R.H. (2016). Processing of snake venom metalloproteinases: generation of toxin diversity and enzyme inactivation. *Toxins* 8:183, https://doi.org/ 10.3390/toxins8060183.

78. White, J. (2005). Snake venom and coagulopathy. *Toxicon* 45:951–967.

79. Casewell, N.R., Wüster, W., Vonk, F.J., Harrison, R.A., Fry, B.G. (2013). Complex cocktails: the evolutionary novelty of venoms. *Trends Ecol. Evol*. 28:219–229.

80. Calvete, J.J. (2017). Venomics: integrative venom proteomics and beyond. *Biochem. J.* 474: 611–634.

81. Casewell, N.R. (2012). On the ancestral recruitment of metalloproteinases into the venom of snakes. *Toxicon* 60:449–454.

82. Markland-Jnr, F.S. and Swenson, S. (2013). Snake venom metalloproteinases. *Toxicon* 62: 3–18.

83. Escalante, T., Rucavado, A., Fox, J.W., Gutierrez, J.M. (2011). Key events in microvascular damage induced by snake venom hemorrhagic metalloproteinases. *J. Proteom*. 74: 1781–1794.

84. Takeda, S., Takeya, H., Iwanaga, S. (2012). Snake venom metalloproteinases: structure, function and relevance to the mammalian ADAM/ADAMTS family proteins. *Biochim. Biophys. Acta* 1824:164–176.

85. Slagboom, J., Kool, J., Harrison, R.A., Casewell, N.R. (2017). Haemotoxic snake venoms: their functional activity, impact on snakebite victims and pharmaceutical promise. *Br. J. Haematol*. 177:947–959