DETERMINATION OF MOLECULAR TRACES OF VARIOUS SNAKE VENOMES USING PROTEOMICS AND GLYCOMIC APPROACHES BASED ON MASS SPECTROMETRY

KÜTLE SPEKTROMETRİSİ TEMELLİ PROTEOMİK VE GLİKOMİK YAKLAŞIMLAR KULLANILARAK ÇEŞİTLİ YILAN VENOMLARININ MOLEKÜLER İZLERİNİN BELİRLENMESİ

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ABSTRACT

Determination of Molecular Traces of Various Snake Venomes Using Proteomics and Glycomic Approaches Based on Mass Spectrometry

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Viperidae snake species are distributed in a wide geographical region in Turkey. Specific proteome and glycoproteome composition profiles provide comprehensive information to study the venom's biological function and taxonomical classification. In this context, we used proteomics, glycoproteomics, and glycomics strategies to characterize proteins present in the proteome and glycoproteome of five venoms belonging to the Viperidae family. The finding showed a distinct composition for each venom, particularly the glycoproteome profile. The overall mass spectrometry profiles identified 144 different proteins, 36 glycoproteins and 78 distinct *N*-glycan structures varying in composition across the five venoms. The glycoprotein composition data obtained from glycoproteomics aligns consistently with the findings from glycomics. Many the identified proteins across the five venoms belong to glycosylated protein families, snake venom serine protease (SVSP), snake venom metalloprotease (SVMP), and C-type lectins (CTL). The clustering and principal component analyses (PCA) illustrated the composition-based similarities and differences between venom proteome, glycoproteome and glycan profiles. Specifically, the *N*-glycan profiles of *M. xanthina (Mx)* and *V. a. ammodytes (Vaa)* venoms were identical and difficult to differentiate; in contrast, their proteome profiles were distinct. Clustering analysis enabled the classification of venom species into different groups presenting their taxonomical classification. Interestingly, the variety of the proteins across venom species highlights the impact of glycosylation on the diversity of glycosylated protein in venom proteome. This proposed high throughput approach provides accurate and comprehensive profiles of the composition and function of various Viperidae snake venoms.

ÖZET

Kütle Spektrometrisi Temelli Proteomik ve Glikomik Yaklaşımlar Kullanılarak Çeşitli Yılan Venomlarının Moleküler İzlerinin Belirlenmesi

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Viperidae zehrin türleri Türkiye'de geniş bir coğrafi bölgede yayılış göstermektedir. Spesifik proteom ve glikoproteom kompozisyon profilleri, zehrin biyolojik fonksiyonunu ve taksonomik sınıflandırmasını incelemek için kapsamlı bilgi sağlar. Bu bağlamda Viperidae familyasına ait beş zehirin proteomunda ve glikoproteomunda bulunan proteinleri karakterize etmek için proteomik, glikoproteomik ve glikomik stratejileri kullandık. Bulgu, her venom için, özellikle de glikoproteom profili için ayrı bir bileşim gösterdi. Genel kütle spektrometri profilleri, beş zehirin bileşiminde değişen 144 farklı protein, 36 glikoprotein ve 78 farklı N-glikan yapısını tanımladı. Glikoproteomiklerden elde edilen glikoprotein bileşimi verileri, glikomiklerden elde edilen bulgularla tutarlı bir şekilde uyumludur. Beş zehirde tanımlanan proteinlerin çoğu, glikosile edilmiş protein ailelerine, serin proteazına (SVSP), metaloproteazına (SVMP), C tipi lektinlere (CTL) aittir. Kümeleme ve temel bileşen analizleri (PCA), profileri proteomu, glikoproteom ve glikan profilleri arasındaki bileşime dayalı benzerlikleri ve farklılıkları gösterdi. Spesifik olarak M. xanthina (Mx) ve V. a.'nın N-glikan profilleri. Ammodytes (Vaa) zehirleri aynıydı ve ayırt edilmesi zordu; aksine proteom profilleri farklıydı. Kümeleme analizi, profileri türlerinin taksonomik sınıflandırmalarını sunarak farklı

gruplara sınıflandırılmasını sağlamıştır. İlginç bir şekilde, profileri türleri arasındaki protein çeşitliliği, glikozilasyonun, zehrin proteomunda glikosile edilmiş protein çeşitliliği üzerindeki etkisini vurgulamaktadır. Önerilen bu yüksek verimli yaklaşım, çeşitli Viperidae zehirlerinin bileşimi ve işlevine ilişkin doğru ve kapsamlı profile.

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LIST OF ABBREVIATIONS

MS: Mass Spectrometry.

MS/MS: Tandem mass spectrometry

MALDI: Matrix-Assisted Laser Desorption/Ionization

TOF: Time of Flight

ESI: Electrospray Ionization

CID: Collision Induced Dissociation

nLC: Nano Liquid Chromatography

TIMS-TOF: Trapped Ion Mobility Spectrometry Time of Flight

m/z: Mass to Charge ratio

LFQ: Label Free Quantitation

PCA: Principle Components Analysis

API: Atmospheric Pressure Ionization

2DE: Two-dimensional gel electrophoresis

2D-PAGE: Two-dimensional Polyacrylamide Gel Electrophoresis

SEC: Size-exclusion chromatography

MFP: Mass finger printing

BU: Bottom-up

TD: Top-down

Asp: Asparagine

Glu: Glutamine

Arg: Arginine

Lys: Lysine

Asp: Asparagine

Thr: Threonine

Ser: Seriene

1. INTRODUCTION

1.1. Venomous Snakes

Snakes are known as a subject of fascination, fear, treachery, and deathly folk stories owing to their lethality and physiological outcomes. Besides its dangerous side, snake venom contains a component can represent beneficial medical tools for the treatment of human diseases throughout history, making snake as a symbol of pharmacy and medicine (Mohamed Abd El-Aziz, Soares et al. 2019, Oliveira, Viegas et al. 2022).

Based on a regional estimation, globally, at least 421,000 envenoming and 20,000 deaths occur each year due to snakebite (Kasturiratne, Wickremasinghe et al. 2008). The only efficient treatment for snakebite is the administration with animal-derived antivenoms. It is a polyvalent antiserum prepared from animal-sera hyperimmunized with whole venom. It encompasses of several antibodies which limit specificity against the toxic target molecules and may result in adverse reactions to the envenomed victims. The production process is limited by its high cost, and long-term process, and therefore low affordability to those who need them (Maduwage and Isbister 2014). An essential challenge to the production of antivenoms is the variability in venom composition between snake families and even between species, which make it difficult to administrate a global treatment for envenomation caused by different species (Gutierrez, Calvete et al. 2017). The snake species is likely to be determined for appropriate treatment. It is difficult to define by its common morphology or clinical manifestation appears on the victim (Boldrini-França, Corrêa-Netto et al. 2010).

Snake venom proteins have a highly stable structure because of their richness with disulfide bonds. As well as having a specific and inherent bioactivity represented by their specific targeting, and versatile clinical manifestations across species. Based on these characteristics snake venom is employed as a diagnostic tool (Marsh 2001, Hillyer, Shaz et al. 2009, Schmidtko, Lötsch et al. 2010, Estevão-Costa, Sanz-Soler et al. 2018) and biological marker for understanding human physiology, and represents drug candidate that offer many paths towards developing new therapeutic drugs (Almeida, Resende et al. 2017, Bordon, Cologna et al. 2020, McDermott 2020, Modahl, Brahma et al. 2020, Oliveira, Viegas et al. 2022). Several drugs derived from snake venoms have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), Including Captopril and enalapril (Figure 1a), Triofiban (Figure 1b), and Eptifibatide (Figure 1c) (Mohamed Abd El-Aziz, Soares et al. 2019, Oliveira, Viegas et al. 2022).

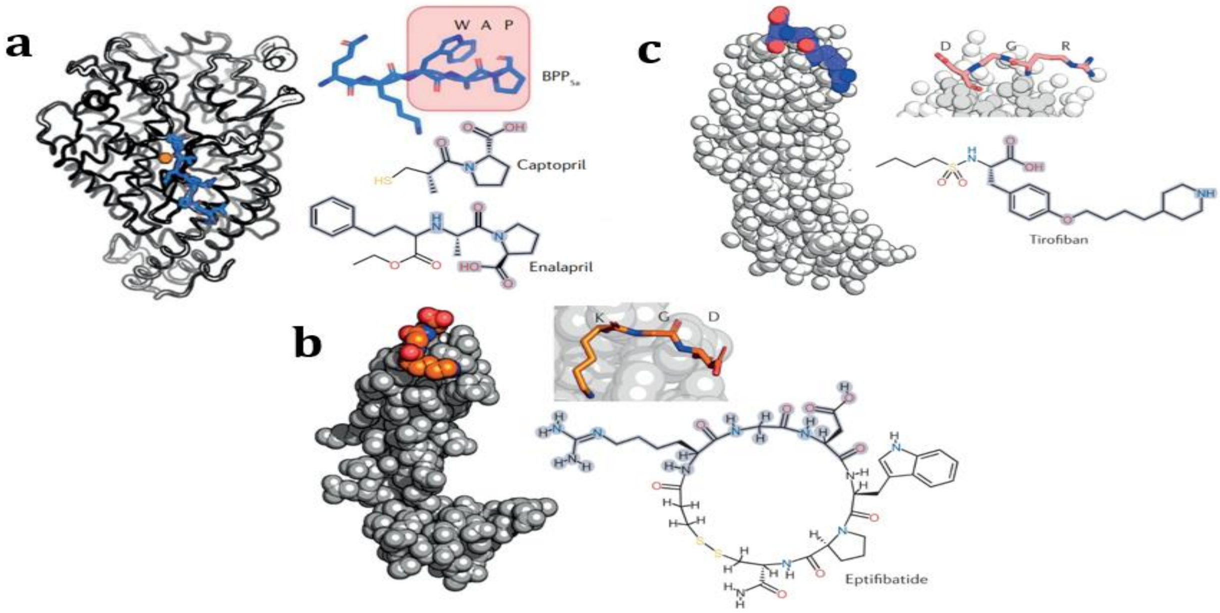


Figure 1: Approved drugs derived from snake venoms, a) Captopril and enalapril, are a designed antihypertension drug, and mimic of the Nine hypotensive bradykinin potentiating peptides (BPPs), that was isolated from the venom of the Jararaca Viper. b) Triofiban is designed to prevents platelet aggregation as a mimic for the disintigrin called echistatin found in the venom of the Saw-scaled Viper. C) Eptifibatide is a designed antiplatelet drug inspired by the disintigrin babourin purified from the venom of Barbour’s pygmy rattlesnake (Oliveira, Viegas et al. 2022)

1.2. Viperidae Snake Family

The most dangerous species are classified within the Viperidae and Elapidae families (Gutiérrez, Calvete et al. 2017). Viperidae and Elapidae snakes, which include almost all medically important snakes were intensively investigated as a diagnostic and taxonomic tool, and for envenomation treatment (Oliveira, Viegas et al. 2022). Venoms of Viperidae typically induce myotoxicity and haemotoxicity, cause local effects and enzymatic manifestation associated with bleeding, coagulopathies, and hypovolaemic shock (Warrell 2010, Gutierrez, Calvete et al. 2017, Oliveira, Viegas et al. 2022).

Viperidae family contains four genera (Daboia, Vipera, Macrovipera, and Montivipera), and it is the most prevalent family of venomous snakes distributed throughout Europe, Africa, and Asia (Damm, Hempel et al. 2021). In Turkey, a significant number of snakebite cases primarily involve species from the Viperidae family, such as M. lebetina, M. xanthin, V. ammodytes, and V. berus. The National Poison Information Center reported a total of 550 snakebite cases between 1995 and 2004 (Cesaretli and Ozkan 2010), commonly caused by Viperidae species and distributed in rural and agricultural areas, where people work or travel (Ertem 2004).

In this study, we have chosen to characterize the venom proteome component of five species from Viperidae snakes (M. lebetina obtusa, M. xanthin, V. ammodytes ammodytes, V. ammodytes montandoni, V. berus berus). The Vipera ammodytes ammodytes (Vaa) and Vipera ammodytes montandoni (Vam) are distributed in East Europe and are considered Europe's most dangerous venomous snake (Sket and Gubenšek 1976). V. berus berus (Vbb) the Adder(E) is common in Europe and East Asia, and is known to cause more snakebite incidents to other Vipera species (Reading 1996, Chippaux 2012). The West Asian Blunt-nosed Viper Macrovipera lebetina obtusa (Ml), and the Ottoman Viper Montivipera xanthina (Mx) both were suggested to be Eurasian vipers living in the Anatolia area in Turkey and Cyprus (Arıkan, Göçmen et al. 2005). Overall these species possess proteolytic, haemolytic, and cytotoxic properties, and are responsible for several disorders including local, systemic haemorrhage, and tissue damage, resulting from envenomation (Di Nicola, Pontara et al. 2021).

It is notable that due to their medical importance (Organization 2010), Viperidae species specifically Ml, Vaa, and Vbb have been frequently characterized using MS-based proteomics approach, pointing out the high number of these species protein sequences in the UniProt database. In contrast, Montivipera genus is one of the least investigated genera (Damm, Hempel et al. 2021, Di Nicola, Pontara et al. 2021).

1.3. Composition Diversity in Snake Venom Proteome

Snake venoms are composed of proteins and peptides that are used for immobilizing or killing prey and in defence against predators. (Warrell 2010, Gutierrez, Calvete et al. 2017, Oliveira, Viegas et al. 2022). Venom proteomes have evolved through single or different evolution processes, to produce homologous proteins, that share a significant structural feature. In accordance, these proteins can be grouped into protein families based on their shared sequence information (McCleary and Kini 2013), as well as based on compositional abundance and ubiquity (Tasoulis 2017). Proteins are key players in all biochemical processes, their de-regulation is often related to diseases. Consequently, knowledge of protein levels, function, interactions, localization and regulation is essential to expand our knowledge of all levels of biology (Tsiatsiani and Heck 2015) including protein evolution by post-translational modification (PTM).

In a recent review, the identified protein families that exist in 89 Viperinae venom proteomes, were sorted by their general abundance into major, secondary, minor, and rare families. Major families included phospholipase A_2 (PLA_2), snake venom serine protease (SVSP), snake venom metalloprotease (SVMP), and C-type lectins (CTL). Whereas secondary included disintegrins (DIS), L-amino acid oxidase (LAAO), cysteine-rich secretory protein (CRISP), Kunitz peptides (KUN), and Vascular endothelial growth factors (VEGF). Minor and rare families present in low abundance and in the proteome of a few snake species, included nerve growth factor (NGF), phosphodiesterase (PDE), and 82 natriuretic peptides (NP), SVMP inhibitor (SVMP-i), respectively (Damm, Hempel et al. 2021). and others. It is notable that some genus or species within the Viperidae family may possess unique protein families or protein subclasses (Tasoulis 2017, Tasoulis, Pukala et al. 2021).

It is well recognized that protein families exhibit variable existence and composition across Viperidae snake venom and even between species, resulting from the diversity of protein subclasses belongs to the same protein family, Besides some proteins have reserved their original structure as unique component for individual species (Casewell, Jackson et al. 2020). A considerable variation in the Viperidae venom species was reported in the major protein family's ratio between genera, while the secondary families were highly abundant in one genus or another (Damm et al., 2021).

It is believed that certain protein families have been recruited and utilized in a single event ,or become central components of the venom of a snake speceis, because of the evolutionary histories (up-regulation of expression and orthologous diversification), and direct selection on the ecological deployment of specific toxins against different types of prey (Casewell, Jackson et al. 2020, Tasoulis, Pukala et al. 2021, Oliveira, Viegas et al. 2022). Variation and diversity in Viperidae venom proteome composition and its implication on evolution processes and taxonomic studies is well documented by venomics and proteomics approach (Nawarak, Sinchaikul et al. 2003, Serrano, Shannon et al. 2005, Hempel, Damm et al. 2018, Hempel, Damm et al. 2020). For instance, the venom of Russell's viper (Daboia russelii) composition from different location is changing. The abundance of the three major protein families is changing in by 3: 9: 19, to PLA2, SVSP, and SVMP respectively. Another example is by comparing the abundance protein families present in Lancehead viper (Bothrops atrox), the venom of common lancehead viper from Venezuela is composed of 85% SVMPs, whereas in venom form Amazonian Peru, Colombia, and Para, it constitute of a lower amount of SVMPs, and shows an increase abundance of PLA2 (Sousa, Portes-Junior et al. 2017, Oliveira, Viegas et al. 2022).

1.4. Composition Diversity in Snake Venom Glycoproteome

Venoms from Viperidae species were reported to contain a high proportion of glycoproteins carrying the same or different N-linked glycan moieties and many of them belong to serine proteases group SVSP, and snake venom metalloproteinase SVMP (Soares and Oliveira 2009). They exhibit variable glycosylation levels and contribute to whole venom proteome composition and structural features.

In a venomic study applied to five closely related species belonging to Viperidae venom, Nawarak. J. et. al., revealed that many venom proteins are Post translationally modified by glycosylation and reported the variation in proteom and glycoproteome composition by variable lectin binding affinity in venom of related species. Result suggested the present of heterogeneous glycan structure in snake venom glycoproteome. They highlighted the essential role of glycosylation in the determination of protein affinity and interactions (Nawarak, Phutrakul et al. 2004).

In a comprehensive study on the venom of Bothrops snakes, they reported diversity and variation in proteome and glycoproteome composition among venoms. Furthermore, considering the high ratio of glycosylated SVSPs and SVMPs, glycomics were applied to uncover variable N-glycan moieties attached to the glycosite, as well as protein sequence similarities upon removal of N-glycan (Andrade-Silva, Zelanis et al. 2016, Andrade-Silva, Zelanis et al. 2021). Similarly, in an extensive glycomics study conducted on the venom of eight Bothrops species, they showed that the variable abundances of N-glycans reflect the variation in venom glycoproteome composition among Bothrops species, and consequently play essential role in biological function of venom proteome (Andrade-Silva, Ashline et al. 2018). The same latter study reported richness of Bothrops venom with complex N-glycans containing a high ratio of sialic acid and fucose residue. Nawarak. J. et. al. also, pointed to the significant amount of sialic acid in the venom of Viperidae species, verifying that the negatively charged sialic acid provides stability for proteins and decreases its enzymatic degradation (Nawarak, Phutrakul et al. 2004). The function of sialic acid was also evaluated in a recent study on nine Bothrops venoms, they revealed the effect on the proteolytic function of venom proteome and suggested that sialic acid has a role in protein-substrate interaction (Brás-Costa, Alencar Chaves et al. 2023).

1.5. Glycosylation Modification

Glycosylation is a protein post translational modification process and can be classified based on binding regions between specific acceptor residues in protein and glycoside residue occur on the extracellular membrane into two main types N-linked and O-linked glycosylation (Spiro, 2002)(Kayili, Ragoubi et al. 2022) as shown in Figure 2. The N-glycosylation process occurs at the sequence of Asn-X-Ser/Thr (often) and Asn-X-C (very rare), where X is any amino acid, except Proline of extracellular or secreted proteins (Andrade-Silva, Zelanis et al. 2016, Andrade-Silva, Ashline et al. 2018). N-glycans are large, flexible and hydrophilic can extend ~3 nm away from the glycoprotein (Soares and Oliveira 2009) and are covalently attached to protein at the first residue (Asn) of the sequon Asn-X-Ser/Thr by an N-glycosidic bond.

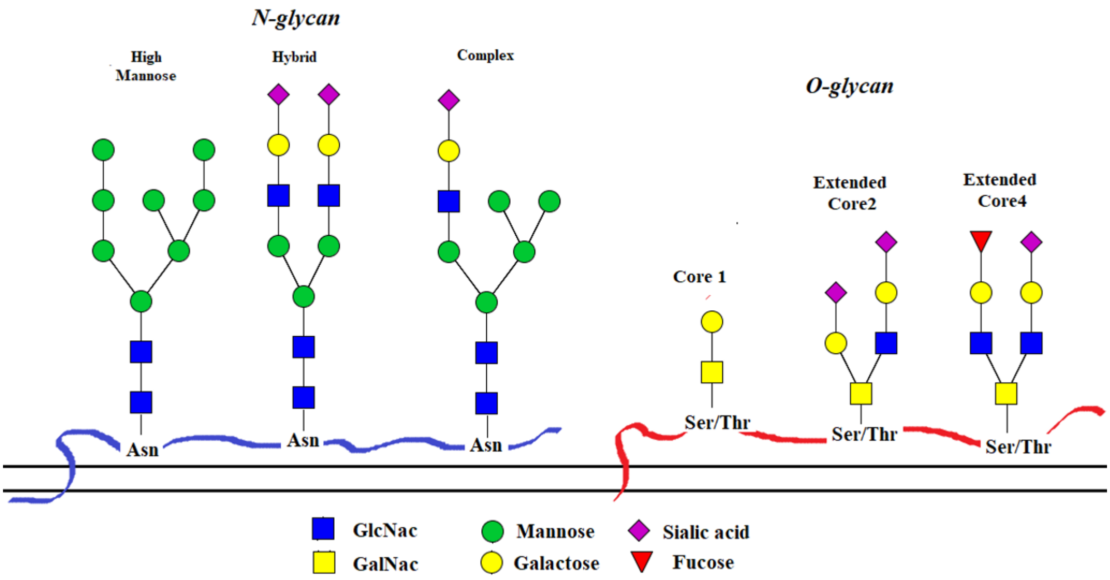


Figure 2: N-glycan are a N-acetylglucosamine (GlcNAc) linked to asparagine (N). O-linkage are a N-acetylgalacoseamine (GalNAc) linked to

Glycoproteins have a glucoside residue called glycan can bind to glycosite via multiple enzymatic processes, where Glycans are carbohydrates that is composed of varied monosaccharides. N-glycan classified into three types, including high-mannose, complex, and hybrid (Andrade-Silva, Ashline et al. 2018).In general, protein undergoes glycosylation process conserving their primary sequence, leading to variable glycoproteins structure with different glycan moiety attached to the glycosite of each protein (Nawarak, Phutrakul et al. 2004, Brás-Costa, Alencar Chaves et al. 2023). Glycosylation achieved on glycan by the removal or addition of sugar residue, such as mannose and glucose, leading to the formation of diverse glycoproteins structures with various level of branching and isomer structure. Glycosylation modification affects protein proteolytic activity as well as structural characteristics including folding, solubility, and stability providing sterically protection against proteolysis acted by other proteins

(Nawarak, Phutrakul et al. 2004, Soares and Oliveira 2009). Glycosylation can impact the glycan or glycoprotein abundance by increasing or decreasing their expression level. The change in the glycan level is used as biomarker for the early detection and diagnosis of diseases (Zelanis, Serrano et al. 2012, Andrade-Silva, Ashline et al. 2018, Andrade-Silva, Zelanis et al. 2021).

1.6. Protein Identification and Quantification

Protein identification and quantification is essential to characterize the venom proteome and glycoproteome composition and to investigate the expression and structural variations between venom species. Protein identified by their amino acid sequence and their amount quantified based on their intensities or peak areas.

Venom proteom have been commonly investigated based on Venomic approach, introduced by Calvete J.J. et al. (Gutiérrez, Calvete et al. 2017, Calvete 2018, Modahl, Brahma et al. 2020). Venomics workflow includes multiple fractionation and purification processes on digested peptides, using reverse-phase high-performance liquid chromatography (RP-HPLC), ion-exchange chromatography, 1D and 2D gel electrophoresis (1DE, 2DE) and/or size-exclusion chromatography (SEC) (Calvete, Juarez et al. 2007, Tran, Zamdborg et al. 2011, Eichberg, Sanz et al. 2015, Choudhury, McCleary et al. 2017, Modahl, Brahma et al. 2020, Tasoulis, Pukala et al. 2021). This is followed by cysteine mapping, N-terminal Edman degradation and de novo sequencing by on-line or off-line ESI-MS/MS analysis. Although, venomics is good at resolving proteins, it has drawbacks arising from the time-consuming multiple separation/purification and characterization processes used to differentiate individual proteins, this also require a milligram amount of samples (Escoubas, Quinton et al. 2008, Gutiérrez, Calvete et al. 2017, Calvete 2018, Abd El-Aziz, Soares et al. 2020, Damm, Hempel et al. 2021).

Tasoulis, T. et. al., (Tasoulis, Pukala et al. 2021) described the workflow of 71 studies investigated the venom proteins. They reported 50 studies that employed the multiple Bottom-Up approach, started with fractionation using reverse-phase high-performance liquid chromatography (RP-HPLC) or size-exclusion chromatography (SEC), while 33 studies applied on the crude venom directly without any fractionation. This was followed by isolation and purification using 1D and 2D gel electrophoresis (1DE, 2DE) and in-gel Trypsin digestion, or in solution Trypsin digestion, and then MS analysis. Abd El-Aziz, T. M. et al., (Abd El-Aziz, Soares et al. 2020) also reviewed different Venomics Bottom-up based approach and reported the use of MALDI-TOF-MS analysis of digested venom.

1.7. Mass spectrometry Based Proteomics

Mass spectrometry-based proteomics ‘shotgun’ approach without additional decomplexation steps before Mass spectrometry analysis MS has been successfully employed for analysis complex protein samples. MS- proteomics made it possible for identification and characterization protein sequences, including their posttranslational modifications (PTMs), and on the comprehensive quantification of the protein components. Proteomic can be performed at the protein level (Proteomic), peptide level (Peptidomic), glycan (Glycomic) and other metabolites (Metabolomics) (Woods, Sokolowska et al. 2019).

Compared to venomics, MS-based proteomics delivers more information, covering a larger amount of venom proteome content. MS-based Proteomic software facilitates accurate and rapid identification and quantification of a large number of proteins and glycans over a wide mass range, particularly the low abundant and the possible posttranslational modifications (PTM) and their sites. This enhances the robustness of composition-based variation studies applied to snake species to better understand the diverse composition features of snake species of a given venom and allows investigation of different evolutionary pathway for each venom (Damm, Hempel et al. 2021). (Damm, Hempel et al. 2021, Tasoulis, Pukala et al. 2021). In a recent extensive review, based on forty-one comparative proteomics data of 24 different Viperinae species. They reported the strong composition variation between closely related Vipera species and the correlation between venom composition and taxonomic level (Damm, Hempel et al. 2021). Andrade-Silva et al. employed proteomics to analyze proteomic and glycomic data derived from Bothrops snakes, aiming to categorize snake species according to their composition. Their findings indicated that each venom displayed a unique glycoproteomic composition, with the N-glycome showcasing a distinctive molecular signature for each venom. Notably, the venoms composition-based differentiation was in parallel with their taxonomical classification. (Andrade-Silva, Zelanis et al. 2016, Andrade-Silva, Ashline et al. 2018).

Proteomic profiling poses many challenges, including variability that arises from the proteomic profiling approach, experimental design, data processing, and sample complexity. Non-biosample related source of variation can be minimized through optimal experimental design. By controlling these sources of variability, researcher can focus on evaluating disease-related variability (White, Chan et al. 2004).

Tow common proteomics strategies are applied for protein characterization: Bottom-up (BU) and Top-down (TD) as domestrated in Figure 3. In Top-down proteomics, intact proteins are introduced directly into mass spectrometer MS, while in bottom-up proteomics, proteins are digested into peptides followed by separation or fractionation of peptides and the resulting mixture is analyzed with mass spectrometer MS techniques. For both strategies the acquired MS/MS data processed by proteomics data base search software to identify the peptides and parent proteins sequence and quantitation (Tasoulis, Pukala et al. 2021). A combination of both approaches can provide maximum protein identification and characterization (Woods, Sokolowska et al. 2019, Modahl, Brahma et al. 2020).

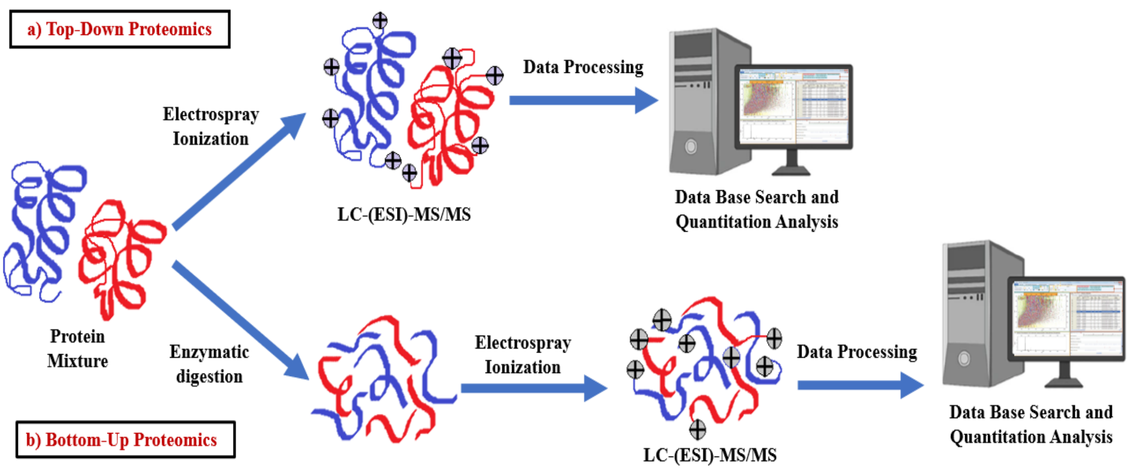


Figure 3. Top-Down Proteomics, Direct MS analysis of the crude samples (a) Bottom-up Proteomics, the protein sample are digested into peptide befor the MS

1.7.1. Bottom-up (BU) Proteomics

MS-based proteomics ‘shotgun’ approach without additional decomplexation steps before LC-(ESI)-MS proteomics has been successfully employed for identification and quantification of venom proteom components.

MS-based Bottom-up (BU) proteomic Figure (3b), first uses enzymatic digestion, commonly using Trypsin, then purification of peptides using fractionation techniquees, this is followed by speration of peptides by LC and introducing peptides into ESI-MS. BU has limitations rising mainly from the digestion process, where the digested peptide is part of the parent protein. The digested protein fragments might have a high molecular weight or low molecular weight for MS detection, or trypsin digestion might be blocked by glycosylated regions of the protein (Choudhury, McCleary et al. 2017, Modahl, Brahma et al. 2020). Moreover, denaturing fractionation destroys non-covalent protein-protein and protein-ligand interactions that contribute to biological activity, therefore some information about the whole protein sequence and the posttranslational modification sites might be lost through the analysis (Tasoulis, Pukala et al. 2021). Furthermore, BU targets the abundant proteins among a broad mass range of proteins, making it ineffective method for identifying low abundant proteins (Escoubas, Quinton et al. 2008, Gutiérrez, Calvete et al. 2017, Calvete 2018, Abd El-Aziz, Soares et al. 2020, Damm, Hempel et al. 2021).

1.7.2. Top-down (TD) Proteomics

Top-down proteomics TD Figure (3a) avoids protein enzymatic digestion to maintain the whole protein structure and allows the identification of the whole protein sequence, their fragments, and any potential PTMs. In TD MS/MS experiment, the intact protein is introduced into the ESI-MS directly, and ionized by an Electrospray ionization which allow the protein to be fragmented into smaller peptides keeping its intact form preserving a detailed sequence information including PTM and their sites (Calvete, Juarez et al. 2007, Woods, Sokolowska et al. 2019, Modahl, Brahma et al. 2020, Tasoulis, Pukala et al. 2021). Tasoulis, T. et. al. (Tasoulis, Pukala et al. 2021), also demonstrate some studies that applied a single Top-down approach, the MS analysis were performed directly on non-treated crude venom.

Compared to the BU proteomics, the obtained MS data from TD proteomics provides more MS information of the whole protein sequence, which allows a higher sequence coverage and full characterization of the protein, in addition to allow the identification of the PTM and their sites. TD proteomic is high throughput technique; direct, easy and faster to apply particularly with small proteins, on the other hand applying TD proteomics on large proteins produce MS data with low resolution, intensity and sensitivity restricting the analysis of protein of approximately 30 kDa (Choudhury, McCleary et al. 2017, Modahl, Frietze et al. 2018, Brown, Melby et al. 2020, Chanda and Mukherjee 2020, Tasoulis, Pukala et al. 2021).

1.8. Mass Spectrometry, Principle, and Instrumentation

Protein identification has become easier and less time consuming because of the advancement in mass spectrometry (MS) and tandem mass (MS/MS) techniques (Aebersold and Mann 2003, Thiede, Höhenwarter et al. 2005). Soft ionization methods, such as electrospray (Fenn, Mann et al. 1989) and matrix-assisted laser desorption ionization (MALDI) (Karas and Hillenkamp 1988), have made it possible to ionize peptides preserving the intact peptide form, which results with full sequence coverage, as well successfully eliminate the need for complicated purification, fractionation and derivatization applied on sample before the analysis (Voshol, Hoving et al. 2007, Woods, Sokolowska et al. 2019).

Basically, mass spectrometric measurements are carried out in the gas phase on ionized analytes. Mainly mass spectrometer measures the mass to charge ratio m/z and the intensities of the ionized analytes. Figure (4) illustrates the basic mass spectrometry experiment workflow sequence, which starts with sample introduction frequently using LC to regulate the flow of the peptide into the MS or it can be injected directly, the next step is generating peptides ions in the ion source, followed by their separation in a mass analyzer according to their mass to charge ratio m/z, reaching to the detector which records their molecular mass and intensities. Detectors generate an electric current according to the ions arrived at the detector amplify and transform it into intensity.

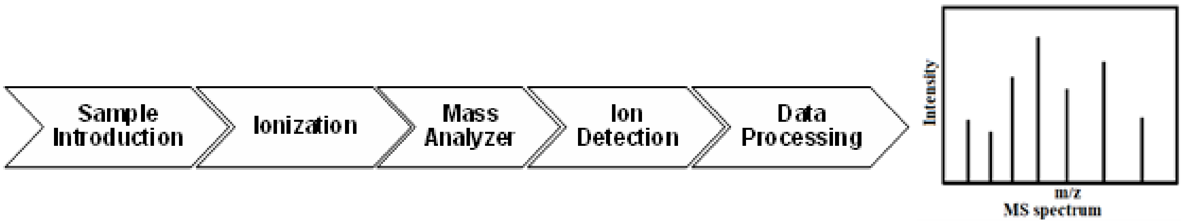


Figure 4: Mass spectrometry MS experiment workflow sequence. MS consists of three main components including Ion source, Mass analyzer, and Ion Detector.

Ionization refers to the production of gas phase ions to enable mass analysis. Electrospray ionization ESI and Matrix-Assisted Lazer Desorption/ Ionization MALDI are soft ionization method that allow to volatize and ionize the protein or peptides to be fragmented into smaller intact form, even peptides with poor stability and large molecular weight will ionize and not decompose to produce complex fragments.

1.8.1. Electrospray Ionization ESI

ESI is extensively used in Proteomics analysis. Peptides ionization is dependent on the electrical potential at the ion source and on the PH at which it is analyzed. In ESI-MS, proteins are ionized in the liquid phase, by an applied high voltage to produce a tiny droplet. At positive mode, under the influenced electrical field, positive ions coming out of the gas nebulizer accumulate at the surface leading to formation of Taylor cone and generation of positively charge droplets as shown in Figure 5A. Due to solvent evaporation the droplet shrinks while the surface charge increases. When the charge is sufficient to overcome the surface tension (Rayleigh limit) Coulomb explosion leads to the formation of smaller droplets which undergo further evaporation as shown in Figure 5B.

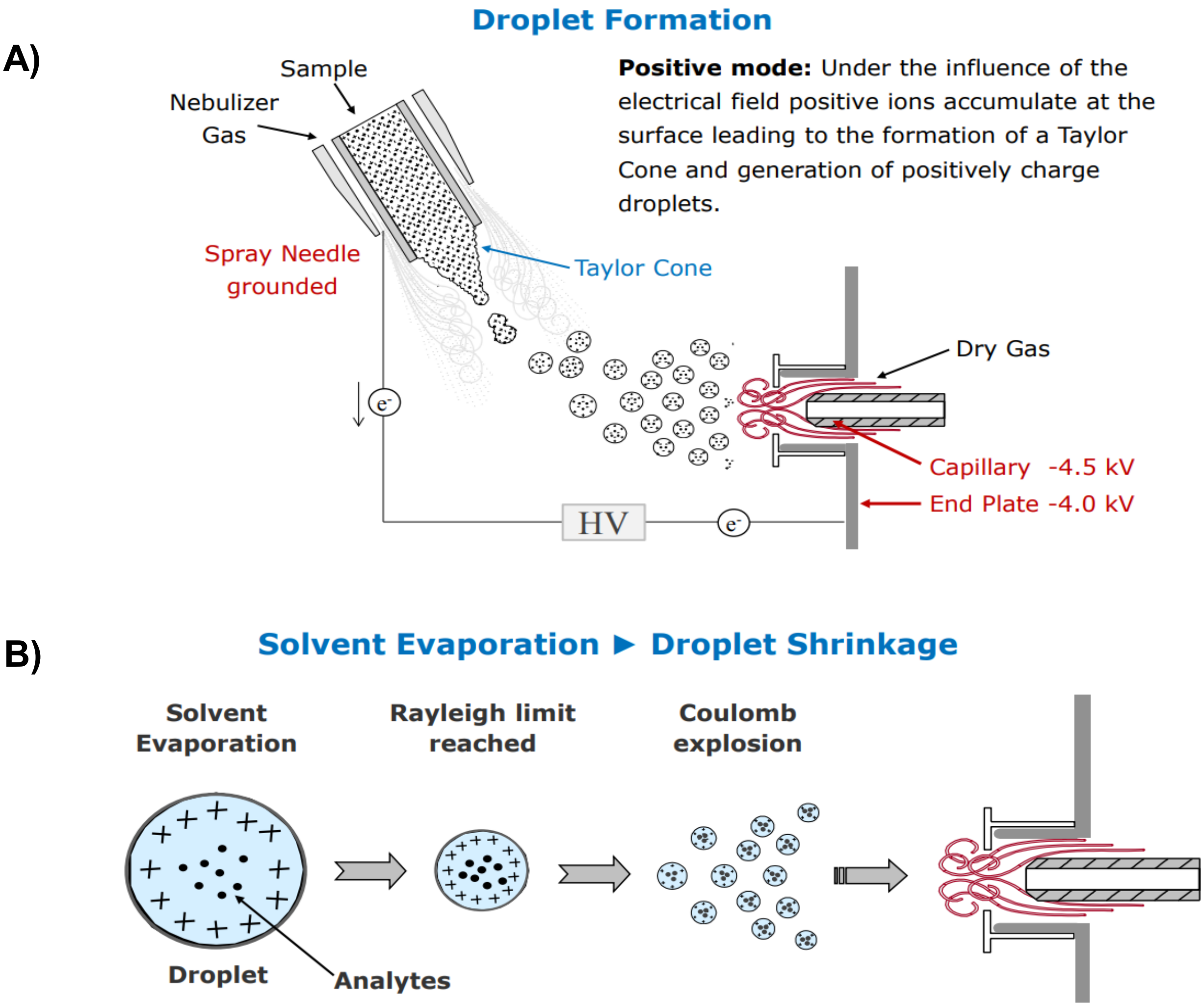
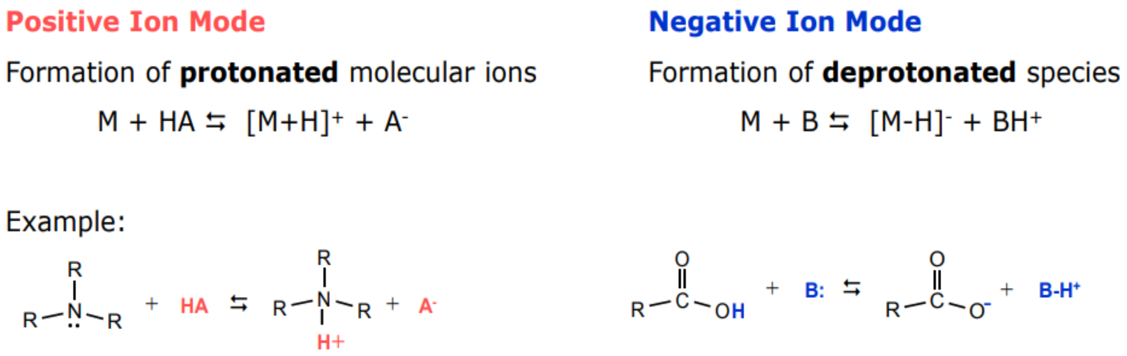


Figure5. Electrospray Ionization ESI. Droplet formation at Positive mode (A). Solvent evaporation to droplet shrinkage (B)

In the ESI proteins and peptides ionized in two types of mode: positive mode (electric potential is positive), when peptide with basic character analyzed at low pH, peptides are protonated through the amino-containing amino acids, at the C-terminus of Arginine Arg, Lysine Lys and Histidine His. While at negative mode (electric potential is negative) peptides with acidic character analyzed at high pH, and peptides are deprotonated through the carboxyl containing amino acids at the N-terminus of Asparagine Asp or Glutamine Glu, to produce a low intensity b and y ions fragment as shown in Figure 6. This explains common usage of trypsin in proteomics, which cleaves at C-terminus of Arg and Lys to produce a y product ion series by the collision dissociation fragmentation, these ions are at least doubly charged and are relatively stable species which allows identification of peptides sequencing (Aebersold and Mann 2003, Woods, Sokolowska et al.

2019). ESI is suitable for tandem mass spectrometry since it can couple to liquid phase chromatographic LC instruments. LC-ESI-MS is a popular technique in proteomic profiling workflows.



1.8.2. Mass Analyzer

Figure 6. Formation of protonated ions at positive ion mode in ESI (A). Formation of deprotonated ions at negative ion mode in ESI (B)

Ionized peptides are then filtered using mass analyzer. There are four basic types of mass analyzer currently used in proteomics research, ion trap, time-of-flight (TOF), quadrupole and Fourier transform ion cyclotron (FT-MS) analyzers, which offer a high sensitivity and resolution.

These ions are then transmitted into the mass analyzer and filtered according to their mass to charge m/z ratio. A high-quality MS spectrum require a mass analyzer with high mass accuracy and resolving performance, as well as the wide mass range limits. The most common type for proteomics is the time-of-flight (TOF) which disperse peptide ions of different m/z in time during their flight along a field-free drift region, where all ions accelerated to high kinetic energy KE at approximately the same time, the lighter ions will arrive earlier at the detector because of their different velocities. To increase the resolution and accuracy of the TOF reflectron is added to the end of the drift zone to repulse all the peptide ions with the same m/z to spend more time in the reflectron to compensate the slight difference in KE and allows the ions of low KE to catch the ions of high KE and subsequently, arrival of more peptides ion of the same m/z to detector at the same time as shown in Figure 7.

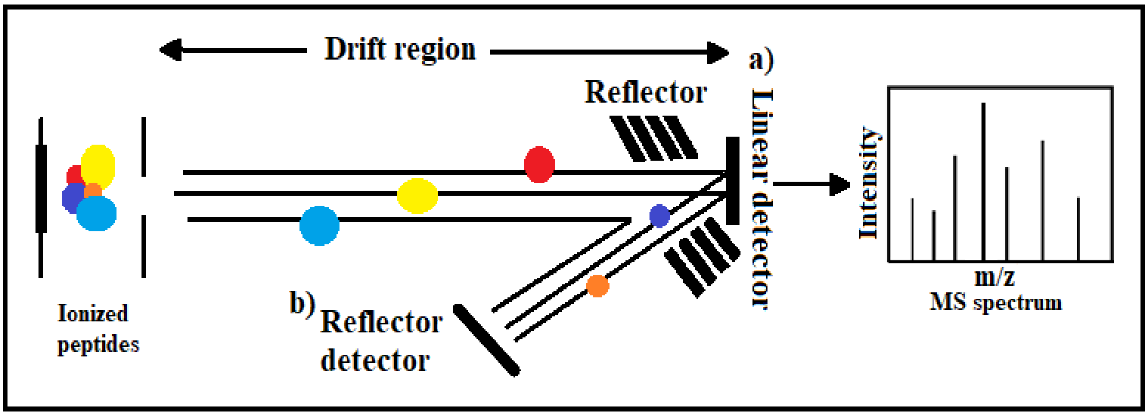


Figure 7. Time of Flight TOF Mass analyzer. a) Linear mode. b) Reflectron mode. The time taken by an ion of a particular m/z to reach the detector is then converted into a mass and plotted against intensity of the ions.

Quadrupole applies a time variable electric potential between the four rods to select and filter ions of a certain m/z ratio (voltages of the same amplitude and sign are applied to the rods of each pair, but the voltages applied to the different rod pairs have equal amplitude but opposite sign. The RF-to-DC-voltage ratios and their values determine the range of mass-to-charge ratio (m/z) ratios to be transmitted through the quadrupole mass filter). At the end of the quadrupole an applied fixed voltage allows filtration of only ions with a certain m/z ratio to pass to the detector. The ion trap, ions are captured for a certain time interval, where it lose their KE by collision with nitrogen gas, then transferred to the detector to transform their frequencies into m/z Ion trap is robust, sensitive and produce large mass data (Voshol, Hoving et al. 2007). Quadrupole can be combined with different mass analyzer such as TOF, and ion trap.

In Q Exactive Orbitrap-MS (Thermo-Fisher) as shown in Figure 8, the atmospheric pressure ionization (API) source can form gas phase sample ions and serve as the interface between liquid chromatography (LC) and the MS. Orbitrap-MS have a cylindrical ion trap that is considerably larger than the traditional ion trap, and allows a higher sensitivity, resolution, and mass accuracy. The ionized ions are focused in the stacked-ring ion guide (S-Lense). In a quadrupole mass filter, under a variable voltage ratio only ions with specific range of m/z ratio are maintained within bound oscillation as their velocity carries them through the mass filter. In the curved linear C-Trap, ions lose their kinetic energy by collision with nitrogen collision gas to prevent them from leaving the C-Trap. This is followed by fragmentation of the precursor ion (ejected from the C-Trap) in higher energy collisional dissociation (HCD) cell, and then transmitted into the Orbitrap mass analyzer where the ions oscillation frequency along the z-axis depends on theirs m/z. The detector transforms frequencies of the axial oscillation into m/z. The Q Exactive Orbitrap MS with HCD generates more fragmentation and higher quality mass spectra to improve identification compared to CID, which is accessible for proteomics research. The instrument provides robust and easy-to-use HR/AM capabilities for quantitative and qualitative proteomics applications.

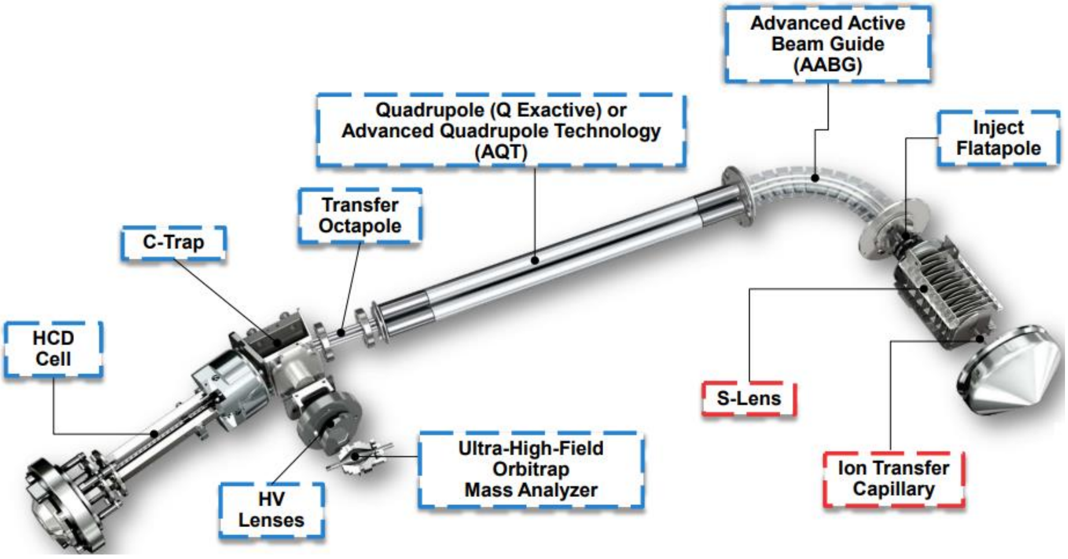


Figure 8. Q Exactive Orbitrap-MS (ThermoFisher)

In another MS instrument, TIMS-TOF-MS-Pro (Bruker) as shown in Figure 9, the ionized ions are filtered in the TIMS analyzer funnel where the ionized ions subjected to stationary buffer gas and electric field (same effect for same charge) as drag force, and filtered depends on the different individual collision cross section (CCS), then transmitted to the mass analyzer. The mass analyzer includes Hexapole, and Quadrupole mass filters which isolates ions according to their m/z ratio, followed by collision cell CID where the isolated precursor ions are fragmented and then detected by TOF orthogonal accelerator.

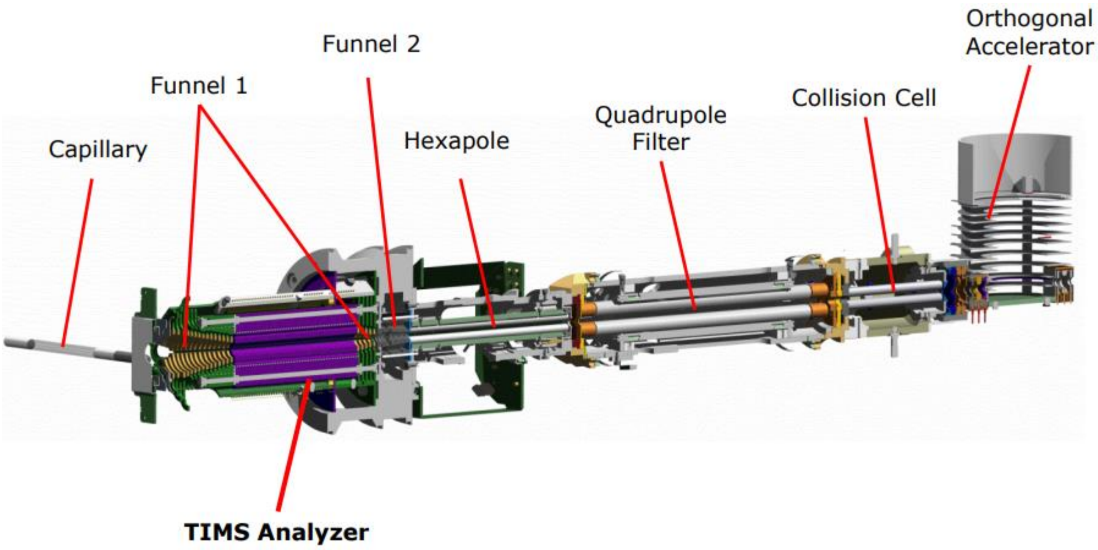


Figure 9. TIMS-TOF-MS-Pro (Bruker) mass analyzer spectrometer

MS/MS techniques combines different mass analyzers to take advantage of the strengths of each and to conduct multiple rounds of MS for fragment ions of the same precursor ion. It is distinguished by the fragmentation method, such as Collision induced dissociation CID, High energy collisional dissociation HCD, Electron capture dissociation ECD, and Electron transfer dissociation ETD. MS/MS experiment involves the selection of the high intense precursor ion from the primary MS spectrum, transmitting it into CID cell to generate multi charge fragments, finally obtain number of mass spectrum of the fragment ions of the precursor ion as given in Figure 10. which splits and evaporate during the process of dissociation and ionization into single or multiply charged precursor ion in the gas phase.

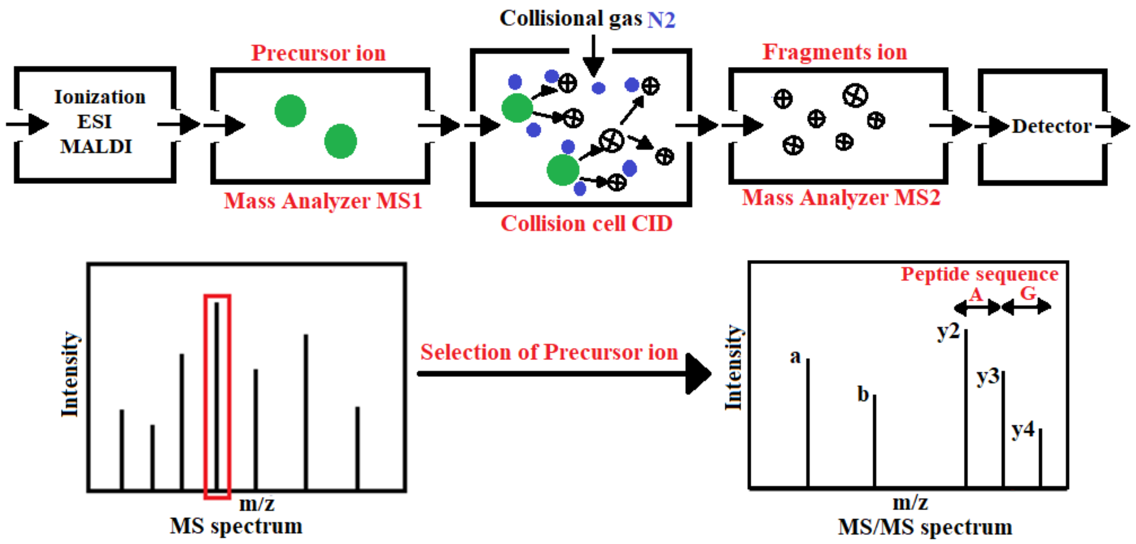


Figure 10: Tandem Mass spectrometry MS/MS experiment workflow sequence, including Collision Induced Dissociation Cell CID. The peptides precursor ion selected and then fragmented in CID cell using collisional gas to produce b and y ions, which result from cleavage of the peptide bond with the charged fragment occurring on the N- or C-terminal fragment, respectively.

The choice of the ionization method, fragmentation method, and mass analysis method, all are strongly dependent on the type of analyte targeted in the analysis, to obtain a high-quality spectrum that is accurate and resolved. However, it is important to not that different peptides respond differently to different ionization methods, which affect the result of experiments using MALDI- or electrospray-MS for the same sample (some peptides will be exclusively detected with only one of the two types of instruments) (Aebersold and Mann 2003, Voshol, Hoving et al. 2007, Woods, Sokolowska et al. 2019).

1.9. LC- (ESI)-MS Based Proteomics

ESI ionizes the analytes out of a solution and is therefore readily coupled to liquid-based (for example, chromatographic and electrophoretic) separation tools. To further enhance detection sensitivity and peptides separation and to reduce simultaneous ionization of the peptides, reversed phase (nanoflow)-liquid chromatography RP-(n)-LC is directly coupled to the ion source of mass spectrometer. LC separates peptides on a reversed phase column based on hydrophobicity, regulating the flow peptides into the MS/MS one by one at a time, this allows the selection and fragmentation of one protonated peptide in the

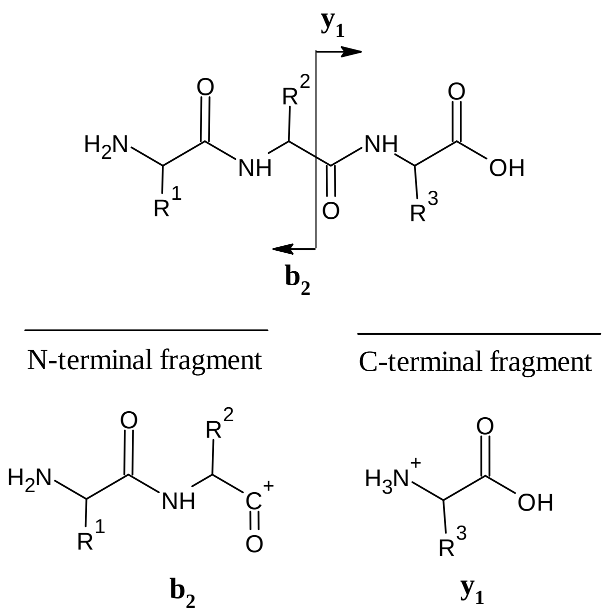
MS/MS experiment. Nano-LC takes place into capillary column containing selected stationary phase applying low flow rates, reduces sample dilution, lower sample, and mobile phase volumes. Moreover, allows to cover a higher amount of the sample proteom in a single n-LC-MS/MS run. In addition because of the acidic acetonitrile/water gradients used, RPLC can be directly coupled to electrospray MS, making it by far the preferred separation method used in shotgun proteomics (Tsiatsiani and Heck 2015).

LC-ESI-MS/MS have several advantages over other techniques, including the ability to generate multiple charged precursor ions that are easily fragmented to provide extensive mass information about the precursor ion sequence, but also more difficult to interpret than MALDI-MS which produce only singly charged peptide. LC -ESI-MS/MS also accurately detect the mass of all the possible precursor ions of large peptides in a complex mixture as well small peptides. In comparison MALDI-MS prefers to analyze relatively small peptides in a simple mixture. It also processed on a longer period, this allows the mass spectrometer to analyze more peptides, and to generate large amount of sequence data (Voshol, Hoving et al. 2007, Chapeaurouge, Silva et al. 2018, Woods, Sokolowska et al. 2019, Modahl, Brahma et al. 2020).

1.9.1. Trypsin Proteolytic Digestion, Purification and Enrichment of protein

Bottom-up proteomics workflow starts with sample digestion commonly using Trypsin. Trypsin is a very efficient and specific protease and has a relatively reasonable cost. Trypsin generates short peptides with a basic Arginine Arg or Lysin Lys at the C-terminus to produce a y product ion series by the collision dissociation fragmentation as shown in Figure 11. This allows peptides sequencing by digested peptides-based search algorithm. The classical strategy is the in-solution digestion, where digestion is performed in a homogeneous aqueous solution comprising a mixture of trypsin and the sample in 1: 50 enzymes to protein, followed by up to 24h incubation (Tsiatsiani and Heck 2015, Woods, Sokolowska et al. 2019).

A following step to the proteolytic digestion, the resulting tryptic peptides mixture needs to be purified and/or enriched prior to introduction into the MS. Moreover, to ensure the stability of LC-MS/MS system and to prevent column clogging by impurities and aggregates. Stage Tips technique can be used prior to LC-ESI-MS/MS analysis to clean the digested peptide mixture. Stage Tips can also be used to selectively enrich or pre-fractionate digested peptides. Stage Tips are ordinary pipette tips containing very small disks made of beads with reversed phase, cation-exchange or anion-exchange surfaces embedded in a Teflon mesh. Stage Tips are made by placing a small portion of Empore material (3M) in an ordinary pipette tip, it is simple, self-made, and extremely economical stop-and-go-extraction tips. Facilitate efficient retention and elution for peptides by preventing of formation of primary-flow channels, fast flow, high capacity and excellent recovery rates. In this study digested venom proteins were desalted using C18-containing stage tips, following a previously established protocol (Rappsilber, Mann et al. 2007).



R_2 = Lysin / Arginine

Figure 11: Peptide cleavage at the C-terminal of Arg and Lys to produce a y product ion using ESI at the positive ion mode, and at the N-terminus of Asparagine Asp or Glutamine Glu to produce series of b product ion using ESI at the positive ion mode

In this study the bottom-up proteomics started by the tryptic digestion and fractionation of peptides using C18 Stage-Tip as shown in Figure (12). The digested peptide injected into online nLC-Orbitrap-MS, firstly a primary MS were obtained includes the mass to charege ratio and intensities of the precusore ions, the high intense precursore ions selected from the MS scan is delivered into the tandem mass spectrometry MS/MS, and then fragmented at a time in the higher-energy C-trap dissociation HCD collision cell. The acquired raw data is then processed to identify the peptides sequence and quantitation of the peptides abundance using MS-based proteomic software and qunatitative analysis software. The recorded ESI-MS/MS peak list provide sequence information about a particular peptide and enable a complete or partial sequence identification of the whole protein using database search software (Chapeaurouge, Silva et al. 2018, Woods, Sokolowska et al. 2019, Modahl, Brahma et al. 2020).

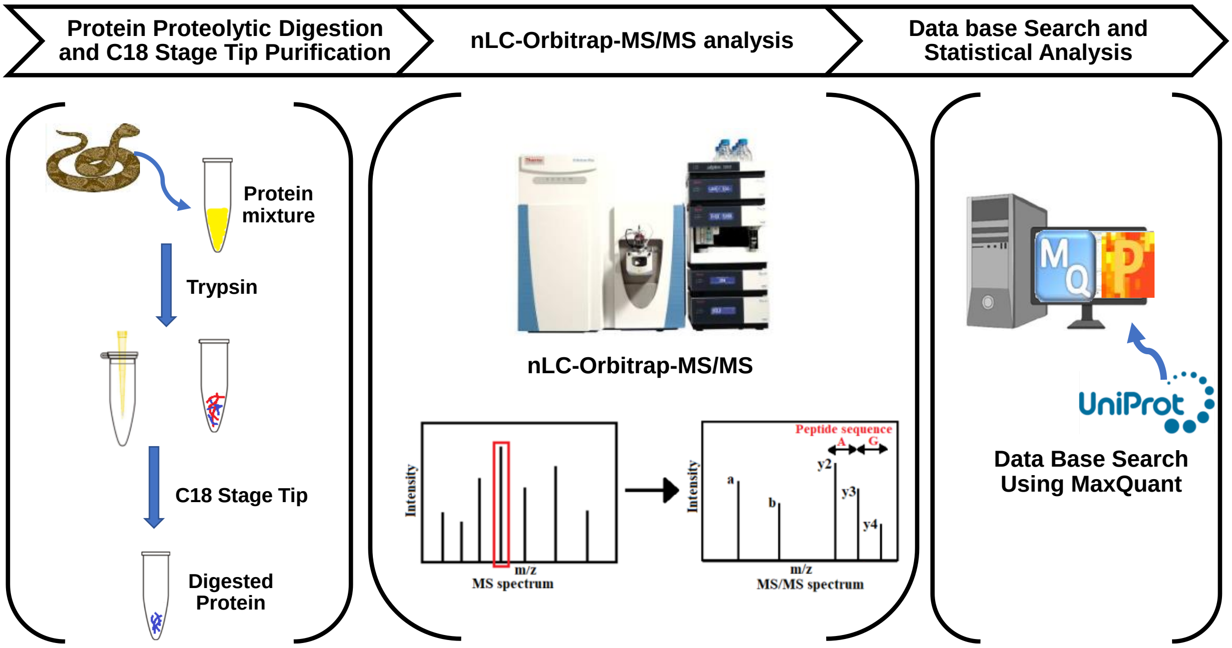


Figure 12: Protein Identification and Characterization using nLC-Orbitrap-MS/MS based Bottom-up Proteomics

1.10. MALD-TOF-MS Based Proteomics

MALDI-TOF-MS is a soft ionization that involves striking the matrix/sample mixture with a high intensity pulse of laser beam, allowing the ablation and desorption of analyte and matrix in a very short time, the ionized matrix molecule assist by absorbing laser energy and the peptide ionized by protonation or deprotonation in the hot plume of the ablation gas, allowing the peptide ion to transfer into the gas phase with minimal fragmentation and without producing thermal decomposition. MALDI-TOF-MS is appropriate for the analysis of small and large molecules that are thermally unstable and hardly volatile.

TOF can be used in linear mode or reflective mode according to the targeted molecule. MALDI-TOF-MS in reflectron positive mode is convenient for proteomic profiling of masses less than five kDa, while in linear mode, it can detect proteins larger than five kDa, using a compatible matrix (Liang, Macher et al. 2014, Woods, Sokolowska et al. 2019). MALDI-TOF-MS can preferably fragment at the amide bond without the need for complex instrumental setup such as collision cell, to produce a singly charged peptide [M + 1] or [MH]+ peaks, where their m/z values correspond to the actual molecular mass (Thiede, Höhenwarter et al. 2005, Voshol, Hoving et al. 2007, Liang, Macher et al. 2014, Woods, Sokolowska et al. 2019). MALDI-TOF-MS basic fragmentation technique were improved by the addition of additional TOF and LIFT device Figure 13, to allow tandem MS/MS to select and fragment a precursor ion obtained from the MS spectrum (Liang, Macher et al. 2014, Woods, Sokolowska et al. 2019). LIFT is a device for raising the potential energy of the ions providing further velocity focusing stage (Kaya 2020).

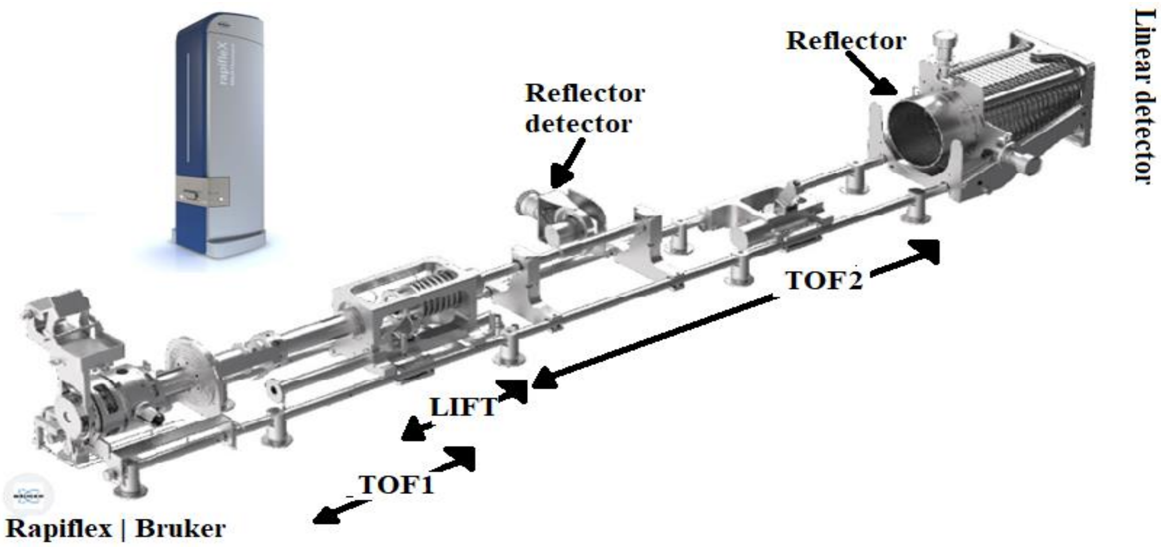


Figure 13: 3D scheme of Rapiflex MALDI-LIFT-MS/MS – Bruker Daltonics. Ions of specific m/z are selected in the first TOF, fragmented in the collision cell, and the masses of the fragments are separated in the second TOF. LIFT device is used for raising the potential energy of the ion, to enhance the fragment separation.

In MALDI-TOF-MS, the protein mixture is co-crystallized under acidic condition (positive mode, protonated proteins) with a large excess of UV absorbing matrix, such as α-cyano-4-hydroxycinnamic acid (HCCA), sinapinic acid (SA), 2,5-dihydroxybenzoic acid (DHB), and 2,5-dihydroxyacetophenone (DHAP),2,5-

dihydroxybenzoic acid (DHB) and spotted on a plate. A co-crystallized homogeneous venom/matrix mixture can absorb a high laser energy threshold, inducing ionization efficiency of low abundant proteins, causing a lower degree of fragmentation and a higher lifetime, thus requiring fewer laser energy shots. Furthermore, the matrix homogeneity is dependent on venom proteome composition. However, salts and larger proteins in venoms can inhibit ionization efficiency and reduce sensitivity for larger proteins (Pan, Xu et al. 2007, Souza, Catharino et al. 2008, Penno, Ernst et al. 2009, Petras, Heiss et al. 2015, Walker, Robinson et al. 2020).

MALDI-MS is fast, robust, easy to perform, sensitive (low fmol range), accurate (low ppm range), quite tolerant to contaminants (Thiede, Höhenwarter et al. 2005). However, it lacks absolute quantitative data and is not able to connect with LC system unlike ESI-MS/MS. In MALDI-MS the abundance of protein is estimated using the intensity data of proteins at a molecular mass, where peak intensity and peak amplitude are not reproducible and have a run-to-run variation originates in part from instrument instability and sample preparation, this measurement used for relative abundance rather than absolute quantitative measurement. Despite the limitation, MALDI-MS have many strength, including the types of biological fluids that may be evaluated, ease of sample preparation, and high-throughput capabilities (White, Chan et al. 2004). Another advantage rises from its high sensitivity to detect the low abundant but not for a high molecular protein.

MALDI-TOF-MS allows the soft ionization of proteins, and it fragments into their native form, directly from the crud sample. This approach has advantages including reducing the consumed time, avoiding loss of minor components, though fractionation process, and preserve the whole protein sequence by avoiding enzymatic digestion. This approach provides complete sequence information about the whole protein structure with the minimal loss of any part of the parent protein, or PTM site, and allows to minimize the experimental variation, regardless of the sample’s complexity. However prior protein purification using C18-stage tip or centrifugal filter can increase the protein intensity, thus improving Protein coverage, particularly before MALDI-MS analysis which lack the ability to ionize large native proteins (White, Chan et al. 2004, Souza, Catharino et al. 2008, Lomonte, Fernández et al. 2014, Petras, Heiss et al. 2015, Tasoulis 2017, Damm, Hempel et al. 2021)

MALDI-TOF-MS is a practical technique for protein and peptide identification, As well as for biomarker identification based on mass finger printing approach (PMF) (Saraswathy and Ramalingam 2011). In PMF peptides are digested frequently using trypsin. The generated MS/MS data are then processed using MS-based proteomic tool (Thiede, Höhenwarter et al. 2005, Saraswathy and Ramalingam 2011), allowing identification based on mass values pattern 'fingerprint' without any sequence information, PMF is an effective approach for identifying relatively pure proteins, but often fails to identify protein mixtures (Voshol, Hoving et al. 2007).

In a venomics study applied on the venom M.l. obtusa and V. raddei from Armenia, the tryptic peptides MALDI-TOF mass fingerprinting, and MS/MS analysis performed for the assignment of the reversed phase fractions. Doubly or triply charged ions of selected peptides from MALDI-TOF mass fingerprint spectra were analyzed in QTrap-MS/MS. The proteome profile was composed of 9 and 11 families, respectively, sharing major and secondary proteins in addition to unique and rare protein families. Venomics analysis demonstrate that each of these venoms have a distinct proteome profile (Sanz, Ayvazyan et al. 2008). In another venomics study applied on the venom of two subspecies of viper Macrovipera lebetina from Southeastern Anatolia and Cyprus by two-dimensional gel electrophoresis (2D-PAGE), followed by MALDI-TOF peptide mass finger printing. The 2D-PAGE revealed the significant difference between these two species proteom profiles. The study suggested that the difference can be considered as specific species biomarker candidate. The proteome profile of M. l. obtusa was composed of 11 protein families including phospholipase A_2, metalloproteinase, serin proteinase, disintegrin, cysteine-rich secretory protein, C-type lectin, vascular endothelial growth factor, nerve growth factor, hyaluronidase, L-amino acid oxidase, and trypsin inhibitor (Igci and Demiralp 2012).

In this study the PMF profile of the venom proteins were obtained using Rapiflex MALDI-MS to create a composition based-biomarker identification tool for the snake species classification. For further information regarding the identity of venom proteom content, MALDI-LIFT-MS/MS were performed, as shown in Figure 14. The high intense precursore ions elected from the MS-PMF spectrum and delivered into the LIFT-MS/MS. The acquired raw data is then processed to identify the peptides sequence using MS-based proteomic software.

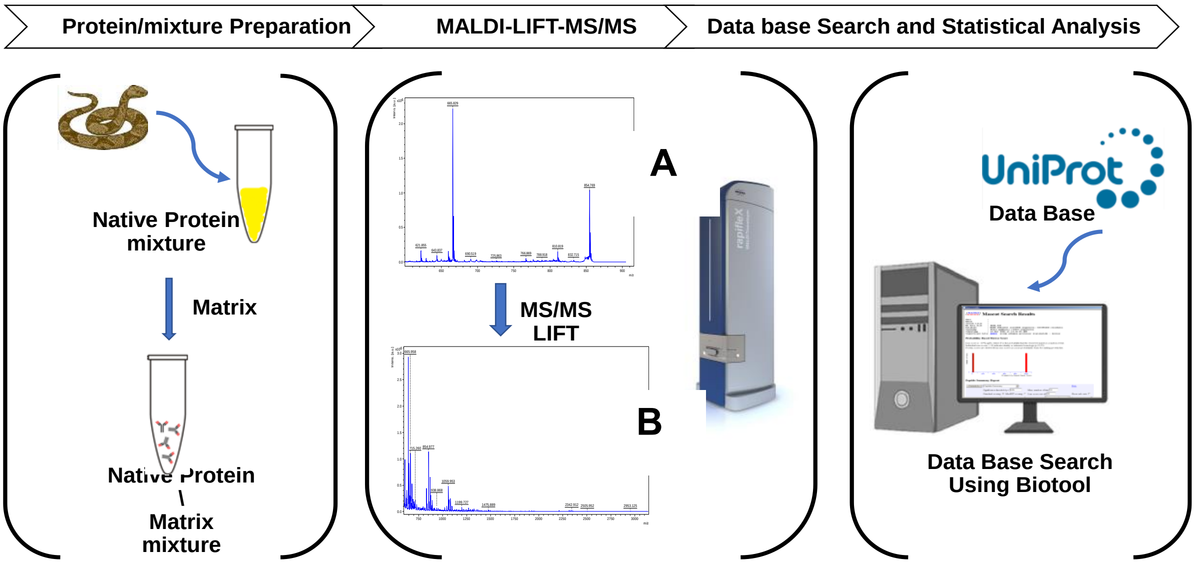


Figure 14: Biomarker Identification using MALDI-TOF-MS based on Peptide Mass Finger Printing approach (A). Native Protein Identification and Characterization using MALDI-TOF-MS/MS based Top-down Proteomics (B)

1.11. MS-based Glycoproteomics

Venom Glycoproteome and glycome were investigated using venomic approach, by applying electrophoresis and lectin affinity biding techniques to separate glycoprotein with specific glycan moiety, followed by examining the experimental MS/MS mass analysis manually or using glycoproteomics software (Aebersold and Mann 2003). Glycomics and glycoproteomics are important to identify the glycosylation modification status of the venoms protein, and the variation in the expression level and the structure at glycan and glycoprotein levels. Therefore, provide insight into the role of Glycosylation modification in proteolytic processing and the changes in enzymatic activity which affects venom pharmacological activities as well as venom composition (Chen, Su et al. 2014, Andrade-Silva, Zelanis et al. 2016, Kayili 2020, Andrade-Silva, Zelanis et al. 2021, Kayili and Salih 2021, Kayili, Ragoubi et al. 2022).

Glycopeptide identification can be achieved by generating diagnostic fragment ions in the MS-mode (without precursor selection) and/or MS/MS-mode (with precursor selection). For precise Glycopeptide detection, N-Glycopeptide fragmentation spectra were monitored by screening the presence of glycan oxonium ions (peptide b- fragment ions in MS/MS spectrum) of (m/z 204.0867 [N-acetyl hexosamine $(HexNAc)_1 + H]^+$, m/z 366.1394 [$HexNAc_1$hexose $(Hex)_1 + H]^+$, m/z 292.1027 [sialic acid $(Sia)_1 + H]^+$, m/z 657.2348 [$HexNAc_1Hex_1Sia_1 + H]^+$) (Wuhrer, Catalina et al. 2007).

ESI-(CID)-MS/MS analysis of glycopeptides is commonly used for glycoproteomics, enabling analyzing protein glycosylation on various levels, including intact glycoproteins, proteolytically generated glycopeptides, providing more information in the form of protein and site specificity. Proteolytic digestion of glycoproteins followed by enrichment of glycopeptides and analysis by MS is a routine method. A common approach is IonTrap (IT)–MS/MS, which allows repetitive ion isolation/fragmentation cycles. Quadrupole–TOF MS/MS has been more widely used for the CID fragment-ion analysis of N-glycopeptides than IT–MS/MS. Quadrupole-ESI-(CID)-MS/MS generates N-glycopeptides precursor dominated by B-type and Y-type fragments of the peptide backbone cleavage providing information of peptide sequence as well as information on the glycosite. Besides exhibit glycosidic linkages revealing information on the glycan moiety structure. Low energy CID results predominantly in cleavage of glycosidic bonds, whilst the peptide backbone remains largely intact. At higher collision energy, the peptides fragments of the observed b-ion series were found to be completely deglycosylated under the applied conditions (Wuhrer, Catalina et al. 2007). In this study glycoproteomics was performed using nLC-Orbitrap-(HCD)-MS/MS which provided rich MS data allowed to identify peptide backbone sequence, and glycan attachment glycosite.

1.12. MS-based Glycomics

Recently soft-ionization techniques, including matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) enabled structural identification of released glycans. Despite the advancements in mass spectrometry for glycoproteomic and Glycomic analysis, it remains challenging, because of the complexity of glycan structures. Heterogeneity in the glycosylation site, where different glycan isomers may attach to the same glycosylation site and the variable glycosylation sites can cause difficulty in analyzing glycoproteins and Glycan profiles. Additionally, negatively charged, and hydrophilic nature of glycan makes their ionization difficult, and their low abundance makes obtaining MS/MS spectra challenging (Chen, Su et al. 2014, Kayili and Salih 2021). This can be overcome using MS/MS system, whereby isomeric ions can be trapped and each species identified on the basis of fragmentation patterns. The isomeric ions can also be separated in the gas phase using ion-mobility spectrometry, a modern and powerful tool to assign glycan structural isomers. (de Haan, Yang et al. 2020)

HILIC-FLD (hydrophilic interaction liquid chromatography with fluorescence detection) combined with MS-system is a standard method for N-glycan detection by their fluorescence interaction and separation based on hydrophilic interaction. Before the MS/MS analysis N-glyacn needs to be labelled by a fluorescence tag from their reducing ends to detect and quantify them by FLD. FLD is a fluorescence detector that offers time-programmable excitation and emission wavelength switching. It hav a high sensitivity and selectivity for the analysis of fluorescence tags trace components. Fluorescence tags such as 2-aminobenzamide, 2-aminobenzoicacid, 2-aminopyridine have been used for the labeling of N-glycans. However, the N-glycan MS/MS spectra can be misinterpreted, in addition it is difficult to obtain the MS/MS spectra of N-glycan due to the low ionization efficiencies of the fluorescence labeled N-glycans. which poses an obstacle for annotations especially for low-abundance N-glycans. Recently, Procainamide was introduced as a fluorescence tag that increase the MS intensities of N-glycans in addition to improve their fluorescence sensitivity, thereby providing more efficient analyses of N-glycans in comparison to other tag reagents (Banazadeh, Veillon et al. 2017, Kayili 2020).

1.12.1.N-glycan Sample Preparation, Purification and Enrichment

To overcome these challenges, a reliable and basic approach is requiring that combine an N-glycan enzymatic release, sensitive florescent tag, and a selective enrichment and isolation process prior to MS/MS analysis (Chen, Su et al. 2014, Banazadeh, Veillon et al. 2017, Kayili 2020) . Glycoprotein is digested by glycosidase enzymes most commonly using PNGase F to release glycan molecules, typically leads to an asparagine to aspartate conversion (hydrolyzes the amide bond of β-aspartylglycosylamine to produce a glycosylamine), with a net of 1 mass unit. The release achieved through overnight incubation at 37 °C (Chen, Su et al. 2014, Banazadeh, Veillon et al. 2017, Woods, Sokolowska et al. 2019). Glycan derivatized by derivatization with hydrophobic reagents, which is routinely achieved via permethylation, reductive amination, and hydrazide chemistry (Banazadeh, Veillon et al. 2017).

A following step to the sample preparation of Glycopeptides and N-glycan including proteolytic digestion, deglycosylation with PNGase-F, labelling of N-glycans, the resulting mixture needs to be purified and/or enriched prior to introduction into the MS. Purification and enrichment of glycoproteins and derivatized released N-glycans is usually applied before MS analysis for efficient ionization and detection of glycopeptides and glycans. This helps to unmask the suppression of N-glycosylated peptides resulting from ordinary peptide signals in nLC-MS analysis (Kayili, Ragoubi et al. 2022). The most used enrichment methods were developed based on several interaction modes, such as hydrophilic interaction liquid chromatography (HILIC), graphitized carbon solid-phase extraction (SPE), hydrazide coupling, lectin-based affinity, ion-pairing, and size exclusion methods. HILIC solid phase extraction SPE has been found to be particularly useful for the enrichment of tryptic N-glycopeptides (Selman, Hemayatkar et al. 2011, Kayili, Barlas et al. 2018, Kayili and Salih 2021).

Selman et. al introduced the use of cotton wool in microtips as versatile HILIC micro-SPE tool to demonstrate the reproducible application of these tips for purification and enrichment of tryptic N-glycopeptides and released N-glycans followed by subclass-specific glycosylation profiling at glycan and glycoprotein levels. Cotton wool HILIC SPE microtips allowed to remove the interfered chemicals through the sample preparation steps such as salts, most nonglycosylated peptieds, and detergent such as SDS. The used cotton wool microtips are Sepharose and microcrystalline cellulose based stationary phase.

Cellulose are nonionic, and HILIC adsorption is, therefore, dominated by hydrogen bonding of the glycan moieties with the stationary phase, while nonglycosylated peptides, lipids, salts, and detergents tend to show low retention. Retention of glycoconjugates is usually achieved with acetonitrile concentrations in the range of 80% while elution is performed with high water content (Selman, Hemayatkar et al. 2011). In this study cotton wool microtips was used for specific isolation and enrichment of digested glycoproteins and derivatized released N-glycans from the venom samples.

In this study MS-based glycoproteomics was applied to profile the glycoproteome composition of snake venom, the workflow involves, digestion using trypsin, enrichment using Cotton-Packed Micropipette Tips, followed by nLC-Orbitrap-MS/MS analysis as shown in Figure 15. Enrichment increased the abundance of the glycoproteins in the sample which enhanced the detection efficiency in the MS/MS experiment, thus identification of glycan moiety. Whereas MS-based glycomics was applied to identify N-glycan moiety structures. Sample preparation involved enzymatic release of the glycan by the PNGase F enzyme, then derivatized using a fluorescent agent (procainamide labeling), followed by purification using Cotton-Packed Micropipette Tips. The released labeled glycans injected into HPLC-ESI-FLD-MS/MS which includes 1260 FLD detector as shown in Figure 16. The data processed and searched for peptide spectrum match PSM using both protein and glyco database (Wuhrer, Catalina et al. 2007, Selman, Hemayatkar et al. 2011, Kayili and Salih 2022)

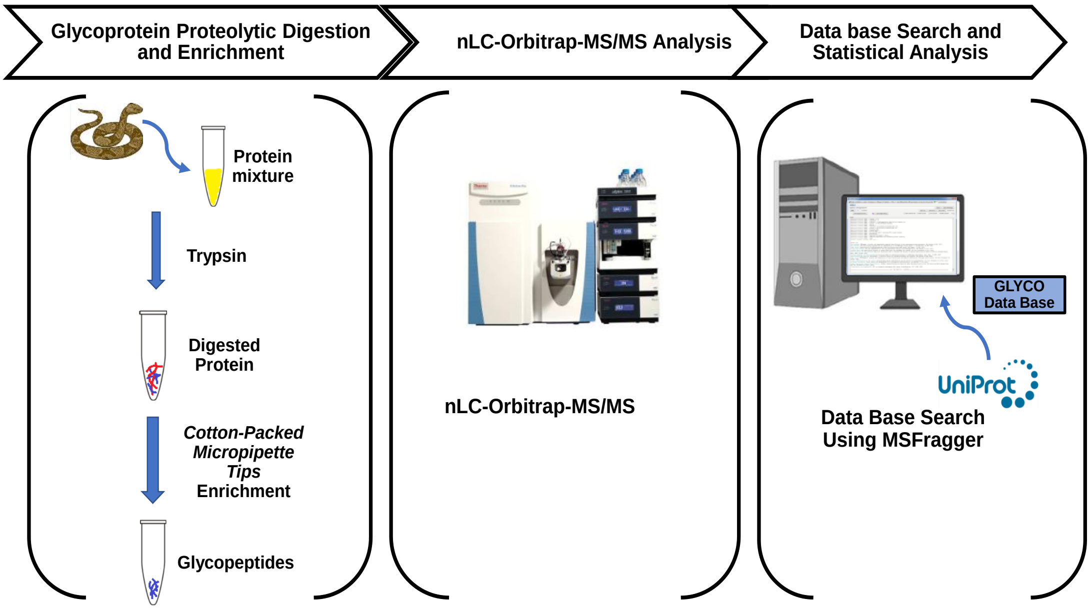


Figure 15. Glycoprotein Identification and Characterization using nLC-Orbitrap-MS/MS based Glycoproteomics.

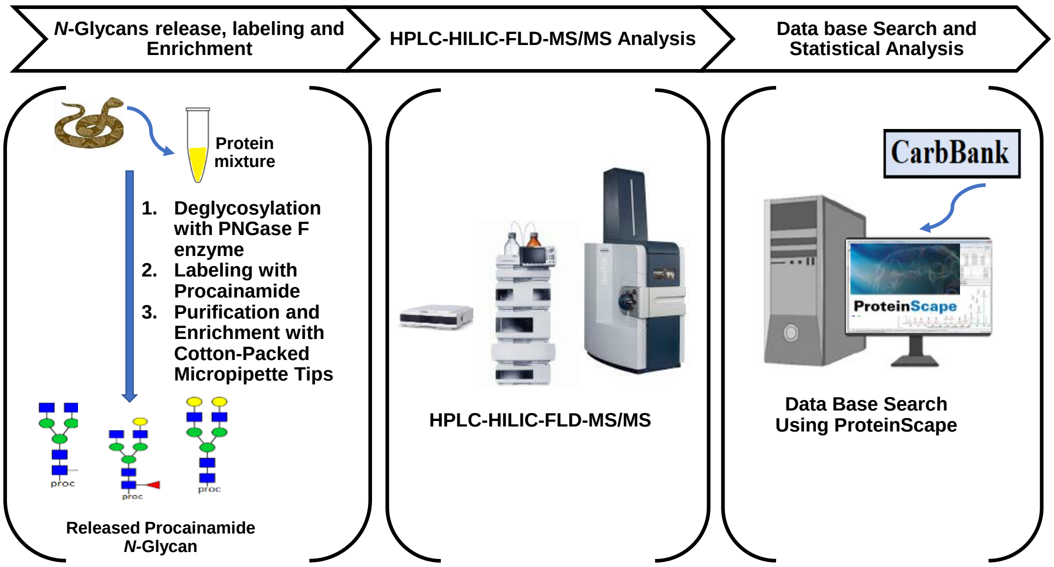


Figure 16. N-Glycans Identification and Characterization using HPLC-HILIC-FLD-MS/MS based Glycomics.

1.13. Data Analysis and Interpretation

MS-based Proteomic software is used for the interpretation of MS and MS/MS spectra of the digested proteins. The obtained information allows identification and quantification of venom proteom, glycoproteome and glycome components. Several commercially and free software are available such as Biotool (Bruker

Daltonics), ProteinScape (Bruker Daltonics), Proteom Discover (Thermo Fisher Scientific), and MaxQuant, and such as MSFragger tools for the latter. These tools allows data analysis of large MS dataset in batch mode speeding up the data analysis process (Banazadeh, Veillon et al. 2017, Woods, Sokolowska et al. 2019, Li, Xu et al. 2020, Kayili and Salih 2021).

1.13.1. Database Search – Qualitative Analysis

MS-based Proteomics software are used to identify the amino acid sequences of the fragmented peptides, or glycans, using search engines algorithm such as Mascot (Perkins, Pappin et al. 1999), Andromeda (Cox, Neuhauser et al. 2011), Byonic (Bern, Kil et al. 2012), and GlycoQuest. Database search commonly uses cross-correlation method, where a theoretical mass spectrum of digested peptide masses stored in public database is compared with measured mass spectrum obtained from MS/MS raw mass data to determine the best spectrum match PSM (protein hit). This provides sequence information for matched peptides and their hit protein, observed modified peptides and their modifiecation sites, determine the unique and common peptides…etc. The comparison validated with a score which reflects the statistical significance of the match between the experimental and theoretical spectrum (Aebersold and Mann 2003).

There are many available public databases such as non-redundant protein UniProt KB (Universal protein knowledge base), NCBI (National Center for Biotechnology Information), glycan Byonic, glycan Carbank and Glycan GlycTouCan. These databases can be uploaded into the MS-based proteomics software for database matching process. The available peptides in public database are primary amino acid sequence that were identified as part of the parent protein using experimental (previously investigated protein sample) or theoretical (in silico predicted proteins using the known specificity of the enzyme, and the masses of the intact peptides calculated, peptide molecular ions fragment preferentially at certain points along the backbone), digested proteomic approaches. Therefore, the database matching of MS/MS spectra mainly identifies digested peptide, not the parent protein. This appears with various errors difficulty in deducing the correct parent protein sequence particularly when interpreting MS-based proteomics data of complex mixtures, and the high molecular weight proteins. This may result with a no assigned match hit or low sequence coverage (loss of information about the parent protein sequence and the post translational modification sites) (Calvete, Juarez et al. 2007, Voshol, Hoving et al. 2007, Cottrell 2011, Petras, Heiss et al. 2015, Tasoulis, Pukala et al. 2021). Regardless these drawbacks these weak matches for the native peptides were accepted considering the molecular mass matching (Cottrell 2011).

Moreover, the available peptides in the database, in most cases, are part of abundant protein. The LC-MS/MS techniques have a significant detection efficiency for high abundant peptides but have poor ionization and detection of low abundant proteins, which is often considered as noise and eliminated from the mass data. Additionally, it might not be identified by search engine because public databases are massively poor with information regarding the low abundant proteins. This mainly influences the quantification analysis which some amount regarding the unidentified low abundant proteins in addition to the new proteins will be missed (Calvete, Juarez et al. 2007, Cottrell 2011).

Public databases also lack a comprehensive specific species proteins entry, limiting the search space to lower number of primary peptide hits. This presented in mass discrepancy (lack of similarity) in proteom since protein with a great difference in sequence homology may not matched and similarly protein with high sequence similarity may not be distinguished due to the missed peptide match in database (Petras, Heiss et al. 2015) (Tasoulis, Pukala et al. 2021) (Calvete, Juarez et al. 2007). This limits the studies ability to identify the diversity of proteomic composition and unique biomarker.

Glycoprotein and N-Glycan are very challenging to identify both the glycan and the peptide attached to it, since glycosylation affect glycan structure without changing peptide backbone sequence, thus glycoproteins may have the same peptide sequence but with different glycans attached on the glycosylation site (Banazadeh, Veillon et al. 2017).

To date proteomic studies on snake venoms characterized large number of proteins but with an incomplete coverage sequence. This mainly occurs because of the shortage in sequence information in snake venom databases. LC-MS/MS and MALDI-MS proteomic profiling approaches is practical to report the proteome and glycoproteome in snake venoms, and to enrich the SV databases with additional peptides sequences, and information about the whole protein.

1.13.2. Quantitative Analysis

MS-based software also predicts and compare the relative intensities of fragment ions. Quantitative analysis demonstrate the relative abundances of venom proteome and glycoproteome components, variation in composition between venom species (Woods, Sokolowska et al. 2019, Tasoulis, Pukala et al. 2021). There are two main methods used to acquire quantity, Label-based and label-free quantification (LFQ), they are simple and low cost (Xie, Liu et al. 2011). LFQ workflow is used for proteomic and glycomics quantification analysis, however, Proteins and glycoproteins are unlike N-glycan, do not require special handling, such as tag or isotope labeling (Woods, Sokolowska et al. 2019).

LFQ can be achieved by spectral counting or measurement of MS precursor ion intensity (Ono, Shitashige et al. 2006, Negishi, Ono et al. 2009). In spectral counting, one measures the number of spectra that correspond with peptides that are part of one protein (Liu, Sadygov et al. 2004, Qian, Jacobs et al. 2005). In MS precursor ion intensity approach achieved by calculating the standard curve or chromatographic peak area corresponding to particular peptides (Strittmatter, Ferguson et al. 2003, Petyuk, Jaitly et al. 2008) and are commonly used in the proteomic LC-ESI-MS and MADLI-MS based analysis (Woods, Sokolowska et al. 2019). In LC-MS/MS the sum of the total intensity of the peptide spectra matched to a given protein are normalized by the total intensity of all the peptide ions assigned to a given peptide and then transform into percentages of the total peptide ion intensities (Calvete, Juarez et al. 2007). While in MALDI-MS the normalized intensity of the precursor ion corresponds to the mass peaks of the digested peptide or whole proteins is converted into protein ratio abundance (Calvete, Juarez et al. 2007, Woods, Sokolowska et al. 2019, Walker, Robinson et al. 2020, Damm, Hempel et al. 2021).

To investigate the diversity of venom proteom glycoproteom and glycom components, and to study the variation in venom composition between different snake species. LFQ intensities are used to claculate the relative abundances of the proteom and glycoproteom components in snake venoms using the total LFQ intensity normalization or total area normalization. The result viewed as a column or Pie diagram to demonstrate the high abundant and low abundant components in each venom. Morover Venn diagram is obtained by using the InteractiveVenn online software (Heberle, Meirelles et al. 2015) to investigate the differences and similarities between venom using.

Whereas, to inspect correlation betewen venom proteom and glycoproteom components proteom, as well to obtain molecular and biological function information of each snake venom , MS-based proteomic and glycoproteomic software such as MaxQuant, Perseus (Tyanova, Temu et al. 2016), MATLAB, FragPipe-Analyst (Polasky, Yu et al. 2020), and FunRich(Pathan, Keerthikumar et al. 2015) are used to process various statitical analysis such as principle componentes analysis PCA, hierarchical clustering analysis.

Composition profiles is essential to define a unique biomarker for species classification that is independent of geographical, phylogenetic, and morphological factors (Calvete, Juárez et al. 2007, Andrade-Silva, Zelanis et al. 2016). In this study the composition-based Clustering and PCA analysis enables classification of venom species into different groups presenting their taxonomical classification.

Determining the abundance of the identified SV proteins is challenging due to several variations.

1. Venom proteom related variation, such as protein composition variation, solubility, net charge, etc., which can impact the peptides ionization and detection efficiency (Calvete, Juarez et al. 2007, Woods, Sokolowska et al. 2019, Walker, Robinson et al. 2020, Damm, Hempel et al. 2021).
2. Experimental and Instrumental related variation, where LFQ analysis mainly depend on the intensity of the abundant proteins while the low abundant proteins may not include in quantification analysis. In addition to

the potential loss of proteins during sample purification (Calvete, Juarez et al. 2007, Woods, Sokolowska et al. 2019).

3. Database related variation (mentioned in section 1.6.1.) it is a particular challenge for venoms in which the same peptide is often present in multiple different protein, leading to ambiguity when determining the identity of protein, and in turn complicating the determination of total number and relative abundance of proteins present in venom (Calvete, Juarez et al. 2007, Melani, Nogueira et al. 2017, Ghezellou, Garikapati et al. 2019, Damm, Hempel et al. 2021, Tasoulis, Pukala et al. 2021).

To reduce this effect and produce reproducible quantitative data with great statistical significance, and to minimize the outlier variation resulted from the differences in the sample proteomes, a minimum of three technical replicate are required, the amount of (n) replicates depend on the area of scientific research.

2. Aim of this Study

The Aims are explained for each part, referred to in the text by their Roman numerals

Part I:

‘’Composition Characterization of Various Viperidae Snake Venoms Using MS-based Proteomics, *N*-glycoproteomics and N-glycomics’’

To study the variation in the whole proteome composition between Viperidae snake species. A comprehensive proteome and glycoproteome characterization was performed for five Viperidae snakes. A further N-glycomics analysis was achieved to investigate the glycosylation level in each venom. The obtained composition profiles were then tested on MS-based proteomics, a machine learning software to differentiate between the venom of the five Viperidae snake species. In addition, enrich the public database with species sequences, particularly the glycoprotein and glycan as well as the low abundant proteins.

Part II

‘’Composition-Based Biomarker Identification for Viperidae Snake Venom Using MALDI-MS/MS Top-Down Proteomics’’

The study aimed to profile the proteins composition diversity of the snake venoms from the Viperidae family, using MALDI-MS/MS based bottom-up proteomic workflow. The venoms MS profiles variability between species from Viperidae and Elapidae families allowed to create a biomarker identification model according to the mass differences of unmatched mass peaks or their relative abundance. The MS/MS proteomic profile demonstrate the difference in sequence of the matched mass peaks, which highlighting the credibility of the MS-based created biomarker model. In addition, shows the composition diversity of the common protein families composed the analyzed venoms.

3. Methods

3.1. Materials and Viperidae Snake Venoms

The reagents and solvents used in this study were purchased from Sigma-Aldrich (St Louis, MO, USA) and were used without any additional purification. MALDI-MS matrixes including *α*-cyano-4-hydroxycinnamic acid (HCCA), sinapinic acid (SA), 2,5-dihydroxybenzoic acid (DHB), and 2,5-dihydroxyacetophenone (DHAP),2,5-dihydroxybenzoic acid (DHB) were purchased from Bruker Daltonics (Bremen, Germany). Microcon centrifugal filter was purchased from Merck (Ireland). Empore Disk C18 (product number 2215) was purchased from 3M, MN. EPPENDORF nonsterile micropipette tips with a capacity of 0.1–10 µL were purchased from Sigma-Aldrich (St. Louis, MO USA). Raw silk from *Bombyx mori* cocoons was obtained from local store of Turkey. The PNGase F enzyme was obtained from New England Biolabs (Ipswich, Massachusetts, USA). Procainamide hydrochloride (Proc) was obtained from Abcam (Cambridge, UK). The purified water was acquired using an Expe-Ultrapure Water System (Mirae St Co., Korea). Trypsin/Lys-C mixture, Mass Spec Grade was obtained from Promega Corporation (Madison, USA).

Lyophilized snake venoms from Macrovipra lebetina obtusa (L1126Ml), Montivipera xanthina (L1134Mx), Vipera ammodytes ammodytes (L117Vaa), Vipera ammodytes montandoni (L1118Vam), and Vipera berus berus (L1121Vbb) were collected in Azerbaijan, Turkey, the Balkans, Bulgaria, and Eastern Russia, respectively were acquired through procurement from Latoxan Laboratory (https://latoxan.com). Within the laboratory, these venoms were taken from animals maintained on-site or captured in the wild. The snake venom pool was collected from individual snakes of the same species. The venom samples were subjected to air drying at ambient temperatures, lyophilized, and then securely stored in sealed glass vials within a controlled environment until their utilization. As stated by Latoxan laboratory, venom protein was purified from its venom with purity ranging from 95% to 98%.

3.2. MALDI Sample/ Matrix Preparation

Four different matrices solutions were prepared and were evaluated against various concentrations of *M. l. obtusa* Ml, *M. xanthina* Mx, *V. a. ammodytes* Vaa, *V. a. montandoni* Vam, *V. b. berus* Vbb and *W. aegyptia* Wa, crud venoms. In this study, these four MALDI matrices were used for protein evaluation. HCCA was used for the peptide evaluation. The dried droplet method was applied for all the matrices as described in **Table 1.**

Table 1. Names, preparation protocol, and spotting instruction for the four matrices used in this study.

Matrix	Preparation	Spotting instruction
HCCA	5 mg/mL prepared in 85% ACN: 15% Water, v/v containing 0.1% TFA	MALDI samples were prepared by mixing the venom samples with the freshly prepared matrix solutions in a 1:1 for protein analysis, and in a 1:10 for peptide analysis v/v). 1 µL of the venom/matrix solution was spotted on the ground steel target plate and allowed to dry at room temperature
SA	10 mg/mL prepared in 30% ACN:70 Water, v/v containing 0.1% TFA	
DHB	20 mg/mL prepared in 70% ACN: 30% Water, v/v containing 0.1% TFA	
DHAP	7.6 mg/mL prepared in 37.5% ETOH: 12.5% of 18mg/ml Diammonium hydrogen citrate solution	

3.3. ***Proteolytic Digestion of Crude Snake Venoms***

A crude snake venom sample with a concentration of 2 µg/µL (dissolved in 8M urea) was subjected to proteolytic digestion using reagents prepared in a 25 mM ammonium bicarbonate (ABC) solution. The reduction of disulfide bonds was accomplished by treating 50 µg of the sample with three µL of 5 mM 1,4-Dithiothreitol (DTT) at 37 °C for one hour, followed by alkylation with 4.65 µL of 15 mM iodoacetamide (IAA) at room temperature in the absence of light for 20 min. The enzyme Lys-C was added to the samples at a ratio of (1:100) enzyme to protein by weight, and the mixture was incubated at 37 °C for 4h. Subsequently, trypsin was added to the samples at a ratio of (1:30, w/w), and the samples were incubated overnight at 37 °C. The digestion was stopped by adding one µL of 100% acetic acid, followed by centrifugation at 14000 rpm for 2 min. Trichloroacetic acid TCA Precipitation of snake venom were performed by addition of equal amount of 100% TCA to SV samples and vortexed well. The mixture was centrifuged at 5000 rpm for 10 min at 20 °C. The supernatant was collected using a 30 kD microcon centrifugal filter (Merck, Ireland). Then the samples were desalted using C18-containing stage tips, following a previously established protocol (Rappsilber, Mann et al. 2007). Samples were stored at -20 °C until analysis with nLC-Orbitrap-MS/MS.

For *N*-glycopeptide analysis, the enzymatic digestion of N-glycopeptides started with the reduction of disulfide bonds, involving the treatment of a 200 µg venom sample with 5 mM 1,4-Dithiothreitol (DTT) at 60 °C for 20 minutes. This was followed by alkylation using 15 mM iodoacetamide (IAA) at room temperature in the absence of light for 20 minutes. Subsequently, the sample was incubated overnight at 37 °C with Trypsin/Lys-C at a weight ratio of 1:100. The enzymatic digestion was halted by the addition of 1 µL of 5.0% trifluoroacetic acid (TFA), followed by centrifugation at 14,000 rpm for 2 minutes. After that, glycopeptides were subjected to purification using a cotton-HILIC stage-tips methodology, as described previously (Selman, Hemayatkar et al. 2011). Samples were stored at -20 °C until subsequent analysis via nLC-Orbitrap-MS/MS.

3.4.N-glycan release and Procainamide Labeling

A previous method was followed with minor modifications (Kayili and Salih 2021). The crude venom samples (100 µg) were subjected to denaturation via incubation at 70 °C for 10 min with 10 µL of 1% sodium dodecyl sulphate (SDS). Subsequently, five µL of 4% nonionic polyoxyethylene surfactant (NP-40) and five µL of 5× phosphate-buffered saline (PBS) were added to the samples, which were then subjected to overnight incubation with 50 U of PNGase F enzyme at 37 °C in thermomixer.

Procainamide solution (110 mg mL^{-1}, dimethyl sulfoxide DMSO/ Acetic Acid AA, 10/3, v/v) and sodium cyanoborohydride solution ($NaCNBH_3$, 60 mg mL^{-1}, DMSO/AA, 10/3, v/v) were prepared for labeling. These solutions were mixed in a (1:1) volume ratio, and then 40 µL of the labeling mixture was added to the glycan release samples. The resulting samples were kept at 65 °C for two hours. Subsequently, procainamide-labeled *N*-glycans were purified using a stage-tips approach (Selman, Hemayatkar et al. 2011). The methodology outlined previously validated using a standard reference glycoprotein source, specifically IgG.

3.5. Purification of Digested Peptides Using C18 Stage-Tip

Reagents. Buffers used were buffer A (Methanol), buffer B (0.5% acetic acid), buffer C (0.5% acetic acid, 60% acetonitrile), and buffer B (0.5% acetic acid, 80% acetonitrile), in water (v/v).

This protocol was performed for both the reduced and nonreduced samples. Purifying reduced and nonreduced peptides were achieved using a previously described procedure, with minor modifications (Rappsilber, Mann et al. 2007, Kayili, Ragoubi et al. 2022). All experiments were carried out using standard 300 µL yellow pipette tips containing a C18 disk. A small piece of the C18 disk was corked out using a small hollow tool, such as the standard 10 ml blue pipet tip, and inserted at the bottom of the pipette tips using a blunt needle. All solutions were loaded from the top of the tip using a pipet. The micro pipet was conditioned respectively with 200 µL of buffer A, buffer D, and buffer B twice, by centrifuging at 4000 rpm for two min. SV samples were loaded twice into the stage tip. In each cycle, the same loading solution was placed into the stage tips and centrifuged at 4000 rpm for two min. Thereafter, the stage tip was washed using 200 µL of buffer B two times by centrifuging at 4000 rpm for two min. Finally, the samples bound to the C18 stage tip were eluted respectively with 200 µL of buffer C and 200 µL of buffer D twice by centrifuging the stage tips at 4000 rpm for 2 min. The eluted samples were directly analyzed by MALDI-TOF-MS, and 1 µL of the eluted sample was mixed with 1 µL of HCCA. 1 µL of the venom/matrix solution was spotted on the ground steel target plate and allowed to dry at room temperature. Samples were dried in a speed vac and stored at −20 °C for further analysis.

3.6. Purification of Procainamide Labeled N-Glycans and N-Glycopeptides Enrichment by Cotton-Packed Micropipette Tips

Purification of glycopeptides and procainamide-labeled *N*-glycans was achieved using a procedure described previously (Selman, Hemayatkar et al. 2011) with minor modifications. The micropipette tip was packed with approximately 0.1 mg of silk fibroin by pushing into the end side of the micropipette tip using a needle tightly. Before loading the sample, the micropipette tip containing the cotton was washed by aspirating and dispensing 20 µL of water, followed by 20 µL of 85% acetonitrile (ACN), in five cycles. The solution containing labeled N-glycans and the resulting peptide mixture was adjusted to an 85% acetonitrile (ACN) content before loading. Each sample was loaded into a silk-packed micropipette tip by pipetting up and down approximately 20 times. then was washed five times with 20 µL of 85/14/1, ACN/Water/ trifluoroacetic acid (TFA), (v/v/v) and 85/15, ACN/Water, (v/v). Finally, the loaded *N*-glycans were eluted from the cotton wool by pipetting up and down ten times with 25 µL of water, repeated twice. The same protocol was employed for the glycopeptides enrichment. Samples were stored at −20 °C for further analyses.

3.7. MALDI-TOF-MS/MS Analysis for Top-Down Proteomics

Rapiflex MALDI-TOF-MS (Bruker Daltonik GmbH, Bremen, Germany) was employed to *conduct the mass spectrometry analysis.* Venom protein was mixed with MALDI-matrix in a 1:1, v/v, then the mixture was spotted on the Ground steel target plate and allowed to dry by air.

MS and MS/MS data were collected automatically using AutoXecute (version 4), A 25 kV acceleration voltage was applied with a 160 ns extraction delay. MS data acquired with an average of 4000 laser shots at 80%- 90 Hz% Hz laser power. MS/MS data acquired with an average of 4000 laser shots at a 100 % Hz laser power. The instrument was calibrated with a Protein1CalibStandard (mono ovalbumin peptide calibrant) for the MS experiment and using angiotensin II for the LIFT-MS/MS experiment

Protein MS profiles were acquired using 2,5-dihydroxyacetophenone, DHAP matrix in positive linear ion mode in the mass range of 5 kDa to 60 kDa. Peptides MS profiles was acquired using *α*-cyano-4-hydroxycinnamic acid HCCA matrix in positive reflectron ion mode in the mass range of 500 Da to 5 kDa.

MS/MS experiments were conducted to select the high intense precursor ion obtained from the MS spectra. MS/MS peptides spectrum was acquired using HCCA matrix in LIFT-MS/MS mode. The mass calibration of the instrument was achieved using a tune mixture solution (Agilant Technologies, Santa Clara, USA) before each sequence. To evaluate the instrument's performance, technical duplicates were prepared and analyzed in parallel.

3.8. nLC-Orbitrap-MS/MS Analysis for Bottom-up Proteomics and Glycoproteomics

An integrated Thermo Q-Exactive Plus instrument with an easy-nano source and Ultimate 3000 RSLC nano liquid chromatography system (Dionex/Thermo Scientific) was employed to conduct mass spectrometric analyses. Peptide mixtures (0.5 µg) were injected into the device. Chromatographic separation was achieved using an Acclaim PepMap 100 trap column (100 µm × 5 cm, particle size 5 µm, Dionex/Thermo Scientific) and an Acclaim PepMap RSLC C18 nano separation column (75 µm × 75 cm, particle size three µm, Dionex/Thermo Scientific), with elution solvents A (2% Acetonitrile ACN containing 0.1% formic acid) and B (80% ACN containing 0.1% formic acid). The gradient program was modified to achieve a linear increase in solvent B from 5% to 40% over 120 min, followed by 95% mobile phase B over 15 min, and re-equilibration in 5% mobile phase B. For the parameter of MS analysis, a full mass spectrum was acquired in the range of 375-2000 (m/z) using HCD mode under optimum mass spectrum parameters, where the highest intensity was achieved with NCE=29. The AGC target values for MS1 and MS2 in proteomic analyses were set as $3X10^6$ and $2X10^5$, respectively. MS/MS data were collected automatically using Xcalibur Qual Browser (Thermo Xcalibur version 4.1, Thermo Fisher Scientific, Waltham, MA, USA) and deconvolution of isotopically resolved spectra was conducted by using the XTRACT algorithm of Xcalibur Qual Browser. The mass calibration of the instrument was achieved using a tune mixture solution (Agilent Technologies, Santa Clara, USA) before each sequence. Technical duplicates were prepared and analyzed in parallel to evaluate the instrument's performance.

A volume of five microliters from the prepared samples was injected for glycoproteomics experiments. The setup included an Acclaim PepMap 100 trap column (100 µm × 2 cm, particle size five µm, Dionex/Thermo Scientific) and an Acclaim PepMap RSLC C18 nano separation column (75 µm × 50 cm, particle size three µm, Dionex/Thermo Scientific). Mobile phase A consisted of a 0.1% formic acid solution, while mobile phase B comprised 80% acetonitrile (ACN) with a 0.1% formic acid solution. The gradient program was designed to transition from 3% mobile phase B to 40% mobile phase B over 90 minutes. In the analysis, the Top5 method was employed. Full mass spectra were acquired within the range of 500 to 2,000 m/z. A Stepped HCD mode was applied for site-specific glycosylation analysis, utilizing collision energies of 20, 30, and 40.

3.9. HPLC-HILIC-FLD-MS/MS analysis for Glycomics

A previously described method for analyzing procainamide-labeled *N*-glycans of crude snake venoms was used (Kayili and Salih 2022). A QTOF (TIMSTOF) mass spectrometer (Bruker Daltonik, GmbH) in combination with an Agilent 1200 series HPLC system, including a 1260 FLD detector, was used for this purpose. A Waters glycan BEH amide 2.5 µm (2.1 mm ID x 15 cm L) column was used to separate the labeled *N*-glycans. The excitation and emission wavelengths of the fluorescence detector were set to 310 nm and 370 nm, respectively. The mobile phases comprised 100% ACN and 50 mM ammonium format (pH:4.4) for phases (A) and (B), respectively. Mobile phase (A) gradually decreased from 75% to 53% over 60 min with a flow rate of 0.35 mL/min during the analytical separations. Before sample injection, 25 µL of the purified procainamide labeled *N*-glycans were mixed with 75 µL of ACN for proper loading conditions and the injection volume was 30 µL. Hystar 4.1 (Bruker Daltonik, GmHB) was used to control the LC and MS operations. For the parameter of MS analysis, the capillary voltage was maintained at 4.5 kV, and the source temperature was set to 250°C. The nebulizer gas was adjusted to 1.7 bar, and the drying gas was set to 6 L/min. The MS spectra were acquired within a range of 50 to 2800 Da at a frequency of 1 Hz. MS/MS experiments were conducted to select the two most abundant precursor ions at spectra rates ranging from 0.5 Hz to 2 Hz. The mass calibration of the instrument was achieved using a tune mixture solution (Agilent Technologies, Santa Clara, USA) before each sequence.

*3.*10. MS Data Processing

3.10.1. MALDI-TOF-MS/MS Top-Down Proteomics

MS data were exported to excel sheet data including mass, intensity, signal/noise ratio and area information of all peaks using Bruker Flex Analysis software. The baseline subtraction (Flex Analysis, Bruker Daltonics) was applied for each spectrum and only mass peaks with a signal/noise ratio≥3 were considered in the data processing.

MS/MS data were searched against a homologous protein of Viperidae or Elapidae snake venom proteins database (downloaded from UniProtKB) using proteomic search software (Biotool, BrukerDaltonics). Top-scoring peptide matches were obtained based on non-enzyme, Peptide mass tolerance was set to ± 0.3 Da, Fragment mass tolerance was set to ± 0.5 Da, and the carbamidomethyl (C) was set as variable modifications and then exported to excel sheet. Results from the Biotool analysis yielded information on each detected protein, including matched MS/MS arrangement, accession number, protein family/ subtype, protein coverage, and scores, and other related data. The identified proteins were sorted by their protein families and illustrated using pie chart according to their relative abundances.

The relative abundances of the most abundant mass peaks (precursor ion) obtained from MALDI-MS were calculated using the total area normalization approach. The normalized peak area value was divided into the sum of the total normalized area values*.* The relative abundances of the protein families were estimated as the ratio of the sum of the normalized areas of the proteins belonging to the same protein family to the total normalized area values. Venom protein composition were investigated by comparing the experimental relative abundances with the literature.

MALDI-TOF-MS accuracy and reproducibility were investigated by performing a triplicate technical replicate in parallel, using 500µg of the crude venom samples (Supporting Material, Table S2, and Table S3).

3.10.2. nLC-Orbitrap-MS/MS Buttom-up Proteomics

The proteomics MS spectra obtained from nLC-Orbitrap-MS/MS were loaded into MaxQuant (v1.6.17) software for Identification and quantification analysis. To identify digested proteins fragmented by Orbitrap-HCD, MS spectra were searched against the non-redundant protein UniProt database of Viperidae snake family protein. For quantification analysis, a label-free quantification (LFQ) workflow was applied. Default parameters were used for "Orbitrap" devices with few modifications. The shared peptides including ''razor+unique'' between different protein groups used for the quantification analysis. Protein groups identified by a minimum of two peptides, including at least one unique and razor peptide were included in the analysis. The minimum LFQ peptide was set at 1, and the enzymes Lys-C/trypsin were selected for digestion specificity. A false discovery rate (FDR) <0.01% was set at the PSM (peptide spectrum match) and protein levels. The output table provided information on each detected protein, including, Majority protein ID, peptide sequences, Fasta headers, molecular weight [kDa], score, sequence coverage [%], LFQ intensity, and other related data.

3.10.3. nLC-Orbitrap-MS/MS Glycoproteomics

The MS raw data obtained from nLC-orbitrap glycoproteomics analysis were processed using MSFragger software, employing default Glyco approach described previously (Polasky, Yu et al. 2020). To identify the glycopeptide sequences, MS spectra were searched against the non-redundant protein UniProt database of Viperidae snake family. Quantification analysis was employed using label-free (MS1) quantitation of enriched glycopeptide fragmented by CID/HCD (Glyco-N-LFQ) workflow. For precise qualitative detection, N-Glycopeptide fragmentation spectra were monitored by screening for the presence of glycan oxonium ions (204.0867 [N-acetyl hexosamine $(HexNAc)_1 + H]^+$, 366.1394 $[HexNAc_1hexose (Hex)_1 + H]^+$, 292.1027 [sialic acid $(Sia)_1 + H]^+$, 657.2348 $[HexNAc_1Hex_1Sia_1 + H]^+$). The confirmation of peptide sequences was achieved by assessing the presence of peptide b- and y-fragment ions in MS/MS spectrum. Default parameters were used with minor modifications, Trypsin and Lys-C digestion with up to 2 missed cleavages, with a mass tolerance for precursors and fragments set at 20 and 20 ppm, respectively. Cysteine carbamidomethylating +57.02146 was determined as a variable modification. A false discovery rate (FDR) <0.01% analysis was performed using a decoy database composed of 50% of protein entries of the non-redundant UniProt database. The entire analysis was performed in triplicate. Yielding comprehensive information for each peptide including the peptide sequence, assigned modification, charge, protein accession number, description, and LFQ (label-free quantitation) intensities.

3.10.4. HPLC-HILIC-FLD-MS/MS Glycomics

The N-glycomics MS spectra of proc-labeled-N-glycans obtained from HPLC-HILIC-FLD-MS/MS were processed for each mass spectrum using Data Analysis software (Bruker Daltonik, GmbH), and then loaded into the Protein Scape software version 4 (Bruker Daltonik, GmHB). To identify N-glycan structure, MS spectra were searched against the glycan Carbbank database using GlycoQuest Search Engine. Parameters for MS and MS/MS tolerances were set to 20 ppm and 0.05 Da, respectively, and threshold score was set to 30. The recognition of procainamide-labeled *N*-glycans was achieved by scanning specific fragment ions in the obtained MS spectra, which were specific to the procainamide-labeled *N*-glycans. Identified N-glycans were manually verified in FLD (Fluorescence Detector) and BPC (Base Peak Chromatogram) chromatograms using the Data Analysis software (Bruker Daltonik, GmbH). Results from ProteinScape analysis provide comprehensive information for each detected N-glycan, including structure, N-glycan type, score, Area, and other related data.

3.11. Statistical Analysis

Proteomic data were subjected to statistical analysis using the Perseus software (Tyanova, Temu et al. 2016). First, contaminated and reverse-matched proteins were filtered from the identified proteins obtained from MaxQuant. Then, LFQ intensities were log2 transformed, and the data was meticulously filtered based on the detection of proteins. Sixty percent of the total proteins found in the total group were included in the analysis. The hierarchical cluster analysis was achieved using the Pearson correlation approach for distances between columns and rows. Clustering analysis for the glycoproteomics data was performed using the online software FragPipe (FragPipe-Analyst (nesvilab.org)), and a similar data prefiltering was processed before applying Pearson correlation. In addition, Principal component analysis (PCA) was performed using Perseus software. The analysis considered the five venoms as variables and the protein as the data observation. The singular value decomposition algorithm was used to generate the principal components. Five-fold cross-validation was used for machine-learning analysis to evaluate the models' performance with the PCA-enabled mode. The same module was applied for proteomics and glycomics data to assess the cluster results.

Relative quantification of protein families exists in each venom, involving utilizing LFQ intensity values for all identified proteins in the analysis. Protein groups identified to contain at least two LFQ values were included in the analysis. Considering the relatively high redundancy of the snake venom proteins, MaxQuant identifies proteins unambiguously by reporting the identified peptides as protein groups. In this study, the first entry of each group was used as a representative. In addition, only protein groups identified to contain at least two peptides, being at least one ‘’razor+unique’’ were included in the analysis. The relative abundance of each protein group was achieved using total LFQ intensity normalization. Then the identified protein groups were categorized into snake protein families by their types. This same methodology was applied to quantify glycosylated protein families in each venom, using LFQ intensity values obtained from MSFragger-*glyco* analysis.

For the quantification of proc-labeled *N*-glycan peaks, FLD chromatogram was automatically integrated using a Data Analysis software algorithm, and manual integration was applied to detect the areas of some peaks. Then, the total area normalization approach was used for relative abundance calculation. The relative abundances of the detected *N*-glycan were illustrated as a column chart created using GraphPad Prism.

The web-based InteractiVenn tool (http://www.interactivenn.net/) was used to investigate the differences and similarities between the analyzed snake venoms (Heberle, Meirelles et al. 2015). FunRich analysis software was used to acquire information about the molecular and biological function of each snake venom based on the composition of the identified proteins (Pathan, Keerthikumar et al. 2015).

4. Results and Discussion

Part I:

‘’Composition Characterization of Various Viperidae Snake Venoms Using MS-based Proteomics, *N*-glycoproteomics and N-glycomics’’

4.1. Proteomic Analysis of Viperidae Snake Venoms

To explore the diversity and variation of the venom proteome among the Viperidae species, the crude venom of *Macrovipra lebetina obtusa (Ml), Montivipera xanthina (Mx), Vipera ammodytes ammodytes (Vaa), Vipera ammodytes montandoni (Vam), Vipera berus berus (Vbb)* were digested with trypsin, followed by nLC-Orbitrap-MS/MS analysis. The MS spectra were matched against the UniProt protein database specific to the Viperidae snake family for protein identification. Proteins were then categorized under non-redundant groups encompassing diverse Viperidae venoms. This analytical approach, utilizing nLC-Orbitrap for the analysis of crude venom, enabled the comprehensive identification and quantification of numerous proteins across the five-venom species. As a result, a total of 144 protein groups were identified across the five Viperidae species as shown in (Table S1, Supporting Information). The identified proteins were classified for each venom species based on their LFQ intensity values, resulting in a variable number of protein groups of 56, 85, 67, 47, and 40 were identified unambiguously from the venom of *Ml, Mx, Vaa, Vam*, and *Vbb* respectively. To investigate the protein families distribution for each venom (Figure 1), The identified protein groups were classified by their types into protein families and the relative abundances of each protein group were calculated using total intensity normalization method. The identified protein groups were assigned to 8, 15, 5, 8, and 11 protein families as given in (Tables S2-S6, Supporting Information).

The venoms of the five Viperidae snake species showed a comparable protein families distribution pattern. Identified protein families were sorted by their abundance into major, secondary, minor, and rare families. Major families were shared by the venom of the five species including, snake venom serien protaese (SVSPs), snake venom metalloproteinase (SVMPs), phospholipase A2 (PLA2s), and C-type lectin and C-type lectin-like (CTL/SNACLEC). Whereas secondary families presented in high abundance in two to three of the five venom species including, L-Amino Acid Oxidases (LAAOs), fibrinogenase (FPG), disintegrin (DIS), Cysteine-rich secretory protein (CRISP), Kunitz-Type Serine Protease Inhibitors (KSPI), and Vascular endothelial growth factors (VEGFs). It is notable that a high proportion of the venom proteome composition was made up of major and secondary protein families, in addition to their significant differences among all venoms as given in (Figure 1). Minor and rare families found in a very low amount and among three or one of the venoms including, Venom phosphodiesterase (PDE), Nerve growth factors (NGFs), Nucleotidases (Nts), Snake Venom Metalloproteinase Inhibitors (SVMPI), Natriuretic Peptides (NP), Glutaminyl cyclases (QCs), and Renin. Result was in accordance with the findings described in a recent review by Damm. M, *et al*. (Damm, Hempel et al. 2021). They had overviewed identified protein families exist in 89 Viperinae venom proteomes and sorted them by their general abundance into major, secondary, minor, and rare families.

Additionally reported the large variation in the major protein families ratio between genera while the secondary families were highly abundant in one genus or another (Damm, Hempel et al. 2021). The obtained protein families distribution profiles of the five venoms provide evidence that MS-based proteomics approach produced a diverse composition profile containing most of the typical representative protein families of the Viperidae species. In addition to identification of minor and rare protein families that are usually detected in ‘’venom gland transcriptomic studies’’ but not proteomic analysis.

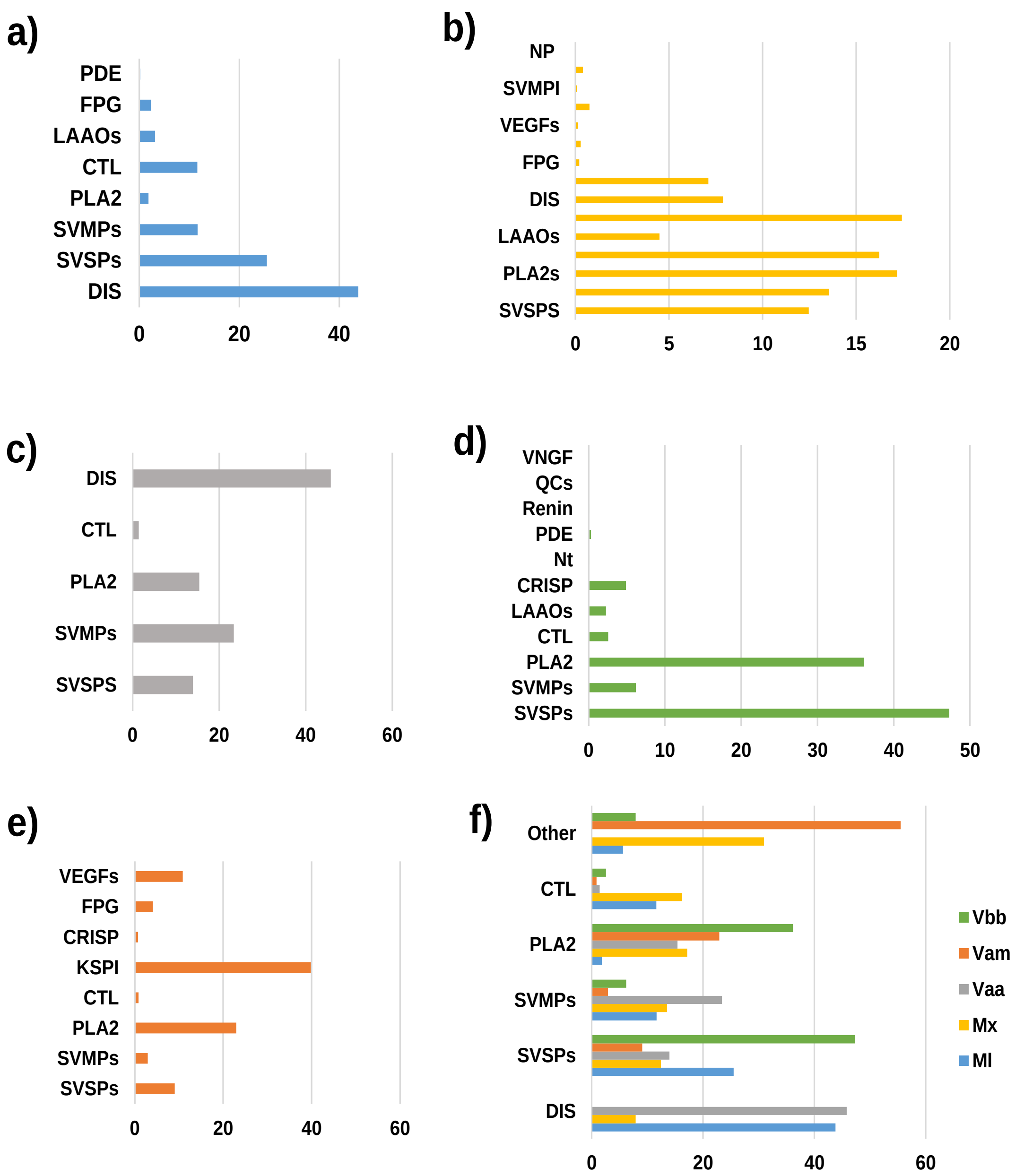


Figure 1. Distribution of protein families identified in the proteom of (A) *M. lebetina* (*Ml*), *(b) M. xanthina* (*Mx*), (c) *V. a. ammodytes* (*Vaa*), (d) *V. b. berus* (*Vbb*), (e)*V. a. montandoni (Vam*), and (f) Distribution of shared families among the venoms. L-amino acid oxidases (LAAOs), C-type lectin and C-type lectin-like (CTL/SNACLEC), disintegrin (DIS), fibrinogenase (FPG), snake venom serien protaese (SVSPs), snake venom metalloproteinase (SVMPs), phospholipase A2 (PLA2s), Vascular endothelial growth factors (VEGFs), Venom phosphodiesterase (PDE), Kunitz-Type Serine Protease Inhibitors (KSPI), Snake Venom

The interactiVenn analysis revealed the Distribution of the unique proteins identified in the venom proteome, grouped by venom. The identified protein groups were sorted by their shared and unique proteins. Unique proteins that appeared only in one of the venoms were 11, 20, 4, and 7 for each venom of *Ml, Mx, Vaa,* and *Vbb*, respectively except for the venom of *Vam* which showed no unique protein, as shown in (Figure 2A). Whereas seven shared proteins were detected between all venoms. Moreover, there were proteins shared at least between 2 venoms. For instance, 37 proteins were shared between venoms of *Ml* and *Mx*, 28 proteins between venoms of *Ml* and *Vbb*, 44 between venoms of *Mx* and *Vbb,* and 38 between venoms of *Vbb* and *Vam*. It is notable that most of the unique proteins identified for each venom belong to major protein families, specifically SVSP, and SVMP. The venom of *Mx* was observed to have the highest number of unique protein groups.

A comparative assessment of protein family distribution profiles revealed variations in the number of unique and shared proteins among identified protein groups. This was in accordance with the fact venom proteome peptides can be shared between homologous proteins, leading to presence of shared and distinct proteins within the same protein family. This resulted from the different evolutionary pathways and presence of diverse isoforms. Both protein families distribution profile and interactiVenn analysis showed that each venom exhibited a distinct proteome composition. Specifically, the venom of *Mx*, and *Vbb* where can be distinguished from other venom based on the presence of rare protein families as in the venom of *Mx* and *Vbb.* Snake Venom Metalloproteinase Inhibitors (SVMPI), and Natriuretic Peptides (NP) were only identified in the venom of *Mx*. While Glutaminyl cyclases (QCs), and Renin were only identified in the venom of *Vbb*. Moreover, the venom of *Ml* and *Vam* can be distinguished by the remarkably higher amount of CTL/SNACLAC and KSPI, in each respectively.

A similar proteome composition pattern was reported for a closely related Viperidae species. All the proteomics studies had successfully shown the presence of major and secondary protein families. However, the variation in abundances of protein families was a notable difference, as well, the presence or absence of some secondary or rare protein families.

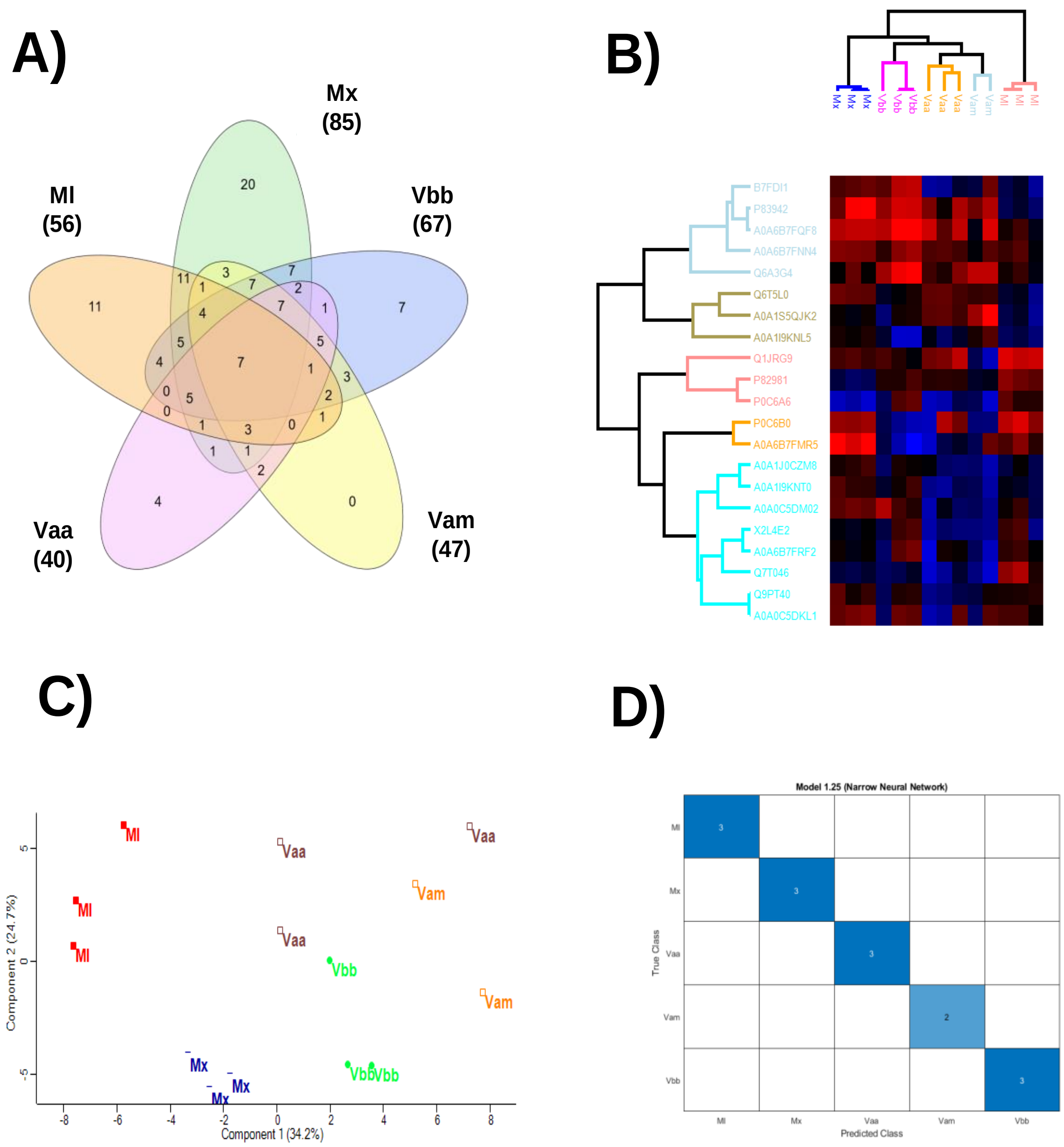


Figure 2. Venn diagram of the distribution of unique and shared proteins among the venoms from Viperidae species (A). Viperidae venom clustering based on proteome composition. Hierarchical clustering of venom proteome characterization. For each venom, a given protein is either present (red) or absent (blue) (B). Two components PCA clustering of venoms proteome based on proteome composition (C). A 5-fold cross-validation approach using the Wide Neural Network model, the detected proteins have a positive linear

In a previous venomics study of *Ml* venom from Turkey, they had reported similar protein families, in addition to the presence of NGF, CRISP, VEGF, and Hyaluronidase while this study identified PDE, and FPG proteins instead. (Igci and Demiralp 2012). A similar composition pattern was observed in the venom proteome of *Ml* from Tunisia and Armenia (Bazaa, Marrakchi et al. 2005, Sanz, Ayvazyan et al. 2008). Regarding the venom of *Mx*, in a venomics study on the venom of *Radde's mountain viper* and *Mount Bulgar viper* from Turkey, a closely related species at the *Montivipera* genus level, the identified proteins were divided into PLA2, SVSPs, SVMP, VEGF, CRISP, CTL, DIS and LAAO families (Nalbantsoy, Hempel et al. 2017). In another proteomics study on the venom of *V. raddei* from Armenia, Nt, KSPI, and VEGF proteins were identified in a low amount (Sanz, Ayvazyan et al. 2008). However, we identified the presence of a low amount of PDE, NGF, SVMPI, and NP in the venom of *Mx snake*.

In comparison with the protein composition profile of the *Vaa* venom from Croatia, and northwest Bulgaria, the NGF, QC, NP, SVMPI, Bradykinin potentiating peptides (BPPs), and Phospholipase B (PLB) proteins were absent in this study proteomics analysis (Georgieva, Risch et al. 2008, Leonardi, Sajevic et al. 2019). In a proteomics study of the venom of *Vbb* from Russia, the result showed not contain Renin and FBG proteins which were observed in this study. (Al-Shekhadat, Lopushanskaya et al. 2019). However, in another venomics study on a *Vbb* from Russia, the Fibrinogenolytic activity assay revealed that *Vbb* venom was able to cleave fibrinogen due to the presence of α-fibrinogenase (FBG) proteins. The same study revealed the presence of two Renin-like Aspartic protease (AspP), which mainly regulates systemic hypertension (Latinović, Leonardi et al. 2016). Regarding the venom of *Vam,* in a venomics study applied to the venom of *Vam* from Turkey, PLA2 was the abundant protein component, followed by VEGF, SVSP, LAAO, CRISP, SVMP, CTL, and Aps respectively (Hempel, Damm et al. 2018). A similar protein families distribution was found in this study, in addition to the detection of high amounts of KSPI and FBG proteins.

4.2. Proteom composition-based venom classification

The composition of the whole proteome was used to classify the venoms by a hierarchical cluster based on similarities in their protein composition profiles in a binary matrix assignment using Pearson correlation. Before the statistical analysis, data cleansing, including normalization, filtering, and missing values handling was performed to ensure that different samples were comparable and enable reliable estimation of the percentage of proteins. This strategy overcomes the challenges arising from the pairwise sequence similarity search, and the missing values (Tyanova, Temu et al. 2016, Tyanova, Temu et al. 2016). Accordingly, the data were filtered using Perseus software, and then clustering analysis was applied using 60% of the LFQ values found in the group. Where, 21 shared proteins (12.5% of the initially covered) populated by SVSPs, SVMP, and CTL/SNACLEC were used for the clustering analysis as listed in (Table S7, Supporting Information). The cluster analysis enabled the classification of the venom based on the absence or presence of a given protein group. Figure 2B showed that venom proteomes of *Ml* and *Mx* formed a distinct cluster from other venom. While the venom of *Vbb* showed a quite different cluster to the venoms of *Vaa and Vam*.

MATLAB software's classification learner algorithms were applied to investigate the correlation between the venoms and their composition and to evaluate the hierarchical clustering result. Principal component analysis (PCA) was applied to the same data obtained from the Perseus software data set (Table S7, Supporting Information). The PCA plot of two principle components as given in (Figure 2C), showed that venom of *Ml* clustered in the upper left quadrant, *Mx* clustered in the left lower quadrant, *Vbb* in the right lower quadrant while venoms of *Vaa,* and *Vam* in the right upper quadrant. The separation of venom proteomics data into four distinct groups by the PCA plot using classification machine learning algorithms confirmed the clustering results obtained from the hierarchical dendrogram. The venom of *Ml*, *Mx*, and *Vbb* were separated into three different groups. While the fourth group included both *Vaa* and *Vam* venoms. In a previous proteomic study applied to the venom from Bothrops snakes, the clustering and the PCA analysis were used to study the relation between the phylogenetic classification and the composition of seven Bothrops venoms. The seven venoms were classified into three groups based on the composition of the unique proteins (Andrade-Silva, Zelanis et al. 2016). The classification pattern obtained from clustering analysis and PCA analysis is likely related to the different genera that the five species are coming from. Specifically, *Vaa, Vam,* and *Vbb* belong to the *Vipera* genera, while *Ml* and *Mx* belong to the *Macrovipra* and *Montivipera* genera respectively. The quality and robustness of the proteomics result was validated using a 5-fold cross-validation approach, and the “Narrow Neural Network” model was found to consistently outperform the other models, with an average accuracy of 100% and an AUC value of 1.00 (Figure 2D). As a result, the applied machine learning algorithm module can effectively classify venoms proteomics data based on their composition profiles.

4.3. Functional Enrichment Analysis of Viperidae Snake Venoms

The functional enrichment analysis including molecular function and biological process analysis was achieved with Funrich software. The non-redundant protein UniProt database of the Viperidae family was used for gene enrichment analysis. The functional enrichment analysis confirmed the significant contribution of the abundant protein families (Figure 3C) to the overall biological function of the Viperidae venoms envenomation. it was observed that Trypsin-like serine protease (Tryp_SPc) protein domain was the most abundant, followed by Phospholipase A2 (PA2c), Disintegrins (DISIN), and ADAM Cysteine-Rich (ACR) among the venom of *Ml, Vaa, Vam*, and *Vbb* except for the venom *Mx*, in which it proteome was dominant by PLA2 protein domain.

The variation of protein families abundance contribution to the total venom functions was revealed by the molecular function and biological process profiles. The molecular function analysis results as given in (Figure 3A), revealed that proteins among the five snake venoms exhibited high toxin activity ($p<0.001$), followed by low to moderate serine-type endopeptidase and peptidase activities. Despite the low amount of PLA2 presented in their proteome, all venom possesses high toxin activity. Knowing that high toxic activity of venom is typically caused by the PLA2 (Mohamed Abd El-Aziz, Soares et al. 2019). Furthermore, SVSPs and SVMPs are not considered toxic, but they contribute to the toxic effect when combined with other proteins. These proteolytic enzymes are well known to affect the hemostatic system through different catalytic mechanisms such as the fibrinogen cleavage to fibrin, platelet aggregation inhibition, and prothrombin activating (Mohamed Abd El-Aziz, Soares et al. 2019).

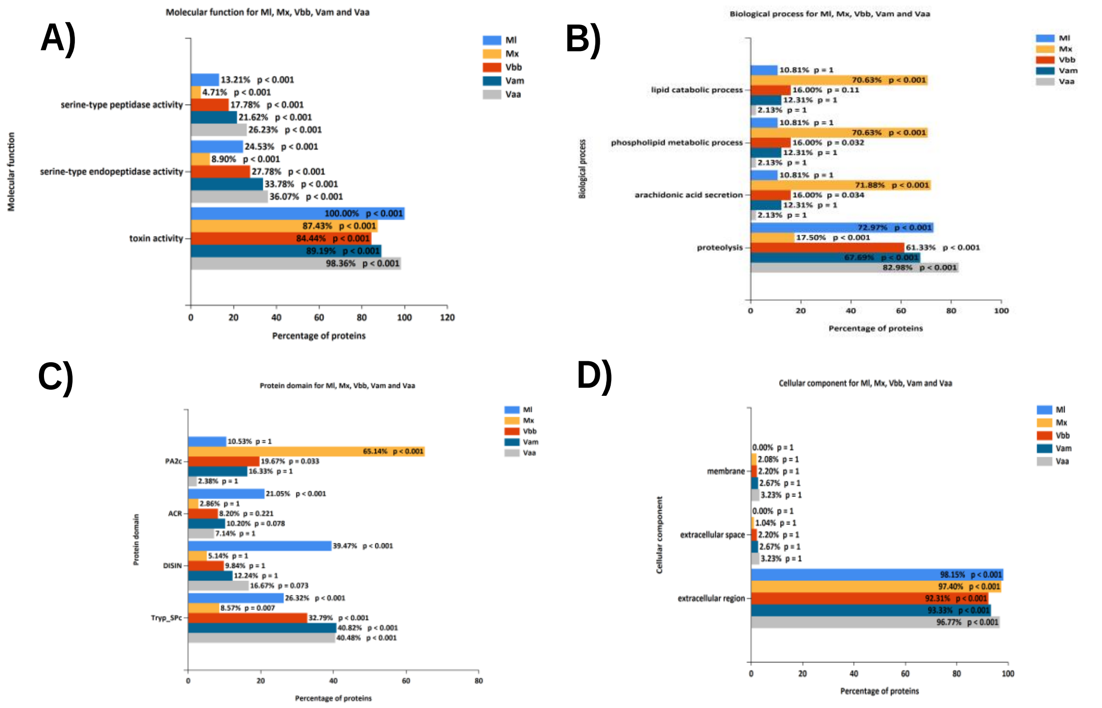


Figure 3: Schematic representation of Viperidae venom functional enrichment analysis based on their proteom composition. Enzymatic and toxic molecular function (A). Proteolysis as a dominant biological process (B). Abundant protein families which have a major contribution on the venom function (C). Viperidae venoms Cellular components (D).

The biological process result also was found consistent with the molecular function profile. Figure 3B showed that Proteolysis was the dominant biological process associated mainly with the presence of high amounts of SVMPs, and SVSPs. Followed by low phospholipid metabolic process and arachidonic acid secretion which mainly was associated with the lower amount of PLA2 in the venom of *Ml, Vaa, Vam,* and *Vbb* species. Interestingly the venom of *Mx.* showed lower proteolysis activity and higher arachidonic acid secretion, Phospholipid metabolic process, and Lipid catabolic process from other venoms. This distinction could be attributed to the higher amount of PLA2 and lower amount of proteolytic enzymes specifically, SVSP and DIS as observed in (Figure 3C). Finally, most of proteins detected in snake venom were in the extracellular region (Figure 3D). The functional enrichment analysis underscores the unique proteome composition, molecular function, and biological process profile of the venom of *Mx* species. While the venom of *Ml* can be distinguished regarding the higher amount of DIS, and ACR to other venoms.

The proteomic analysis revealed that several protein groups belonging to SVSP, and SVMP were detected in high amounts in the venom of *Ml, Mx, Vaa, Vam,* and *Vbb* species. The identified SVSP groups included thrombin-like enzymes, serein proteinase SP-(2,3,6, VLSP), Factor V activator, and coagulation factor subclasses. The identified SVMPs mostly belong to the type P-III, and peptides from zinc metalloproteinase and disintigrin-like were found among the venoms. Most of the identified SVMP peptides among all venom exhibited significant similarity to those from *Vaa*, Echis, Crotalus, and Bothrops. Similarly, SVSPs peptides showed resemblance to venom from *Ml,* and *Vaa* in addition to *Bothrops jararaca* and *Agkistrodon contortrix contortrix* venom.

Protein groups belonging to PLA2 were also detected in a high amount in all venom except for the venom of *Ml* snake species. Different PLA2 subclasses were identified including Phospholipase, basic phospholipase A2, Acidic phospholipase A2, and Ammodytin. The venom of *Mx was* observed to possess high arachidonic acid secretion, Phospholipid metabolic process, and Lipid catabolic process (Figure 3B). It is worth highlighting that the venom of *Mx* was found to contain Ammodytoxin (Atx), a secreted PLA2 enzyme, extracted from the venom of the *Vaa.* This enzyme is well-recognized for its presynaptic toxicity, and possess affinity for cellular membrane allows to increase the toxin activity (Ivanušec, Šribar et al. 2022). Furthermore, Cytotoxicity effect of *Mx* venom on cancer cells was well studied due to its medical importance. A previous study was applied to the venom of *Mx* from Turkey to investigate the cytotoxic and antimicrobial properties against various cancer and microbial cells. They reported notable cytotoxicity to MCF-7 and LNCaP cells and suggested that the cytotoxicity effects on cancer cells were concentration, time, and cancer cell line dependent (Yalcın, Ozen et al. 2014). The identified PLA2 peptides among the venom were found to have a similar sequence mainly to those from *Vam*, and *Vaa*, in addition to several PLA2 described from the Viperidae snake species, such as Vipera aspis aspis, Daboias, Bothrops, and Eristicophis macmahoni. In overall major protein families were reported to present in high amounts in most of Viperidae species, which reflects their essential role in snake envenomation. In addition, their contribution to the evolutionary process by undergoing different modifications to produce several protein isomers.

4.4. Glycoproteomic Analysis of Viperidae Snake Venoms

To get further information about the diversity and variation of the Glycosylated proteins exist in the glycoproteome of Viperidae species, an MS-based glycoproteomic approach was applied. The venom crud was digested using two proteolytic enzymes (Trypsin and Lys-C) and analyzed using nLC-orbitrap-MS/MS. MSFragger-*glyco* software identified *N*-glycopeptides sequence and their peak intensity, and only those with at least two peptides were considered reliable for identification and quantification analysis. This approach allowed the identification of many enriched glycosylated peptides.

Detected *N*-glycopeptides were grouped into protein families and their abundances were quantified using total area normalization approach. A list of the identified and quantified glycopeptides from each snake species was given in (Table S8-12, Supporting Information). The result showed a variable number of glycosylated proteins were identified as 11, 25, 19, 18, and 10 and assigned to 6, 9, 8, 7, and 7 protein families from the venom of *Ml, Mx, Vaa, Vam*, and *Vbb*, respectively. The identified glycosylated peptides can be sorted by abundance as follows, Major families including SVSP, SVMPs, SNACLAC, and LAAO. Secondary including PDE, Nt, and Hyaluronidase. Aminopeptidase (APs) and Calmin-like were found only in the venom of *Mx,* while Calreticulin was found only in the venom of *Vaa* as given in (Figure 4A). Those major and secondary families were shared among all venoms except the absence of LAAO in the venom of the *Ml* snake.

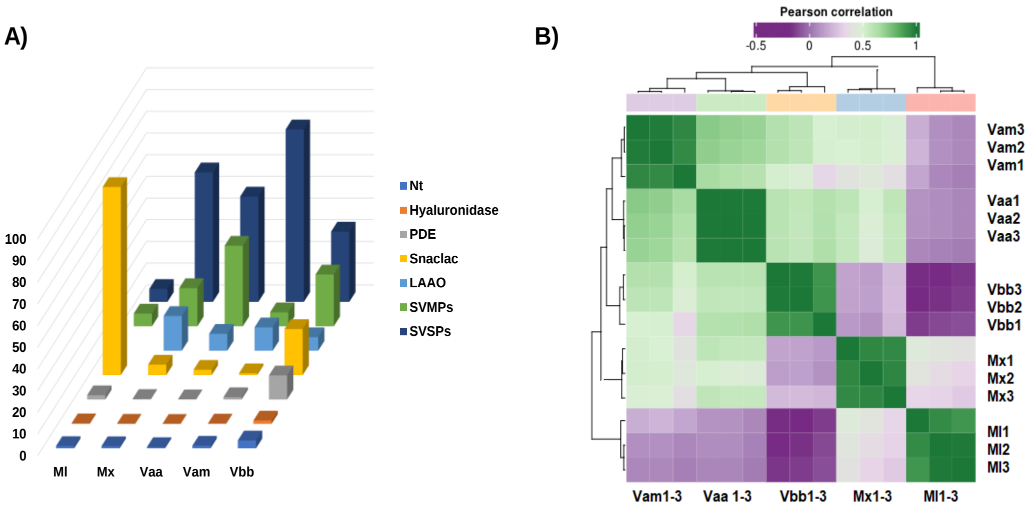


Figure 4: Viperidae venom protein families identified by MS-based glycoproteomics (A). A graphical visualization of heat map of venoms proteom characterization. The Viperidae venom replicates have a positive linear correlation. For each venom, the shared protein is either present (green) or absent (violet) (B). Abbreviation: L-amino acid oxidases (LAAO), C-type lectin (SNACLEC), snake venom serien protaese (SVSPs), snake venom metalloproteinase (SVMPs), Venom phosphodiesterase (PDE), *M. lebetina* (*Ml*), *M. xanthina* (*Mx*), *V. a. ammodytes* (*Vaa*), mV. *a. montandoni*

Glycosylation represents a pivotal attribute within the repertoire of snake venom components, affording a substantial advantage in the preservation of glandular lumen homeostasis. This advantage is principally derived from its capacity to enhance protein solubility, thereby enabling the snake to generate an exceedingly concentrated venomous secretion (Andrade-Silva et al., 2018). In addition, linked glycosylation may guarantee the accurate folding of critical functional domains (Soares and Oliveira, 2009). Moreover, glycans that incorporate sialic acid are involved in modulating the functionality of proteases found in snake venom. It has been observed that the proteolytic activity of nine different Bothrops snake venoms on various substrates decreased when sialic acid was enzymatically removed (Brás-Costa et al., 2023). Therefore, the characterization of the glycosylation pattern of snake venoms are crucial to understand the function of glycans in the proteolytic activity.

The glycoproteomic profiles were observed to share the typical glycosylated protein families present in Viperidae species such as SVSPs, and SVMPs which are known to be glycosylated. The venoms of *Mx, Vaa, Vam*, and *Vbb* were abundant by SVSPs, and SVMPs, while the venom of *Ml* was abundant with SNACLAC proteins. This pattern was very similar to that observed for Viperidae species and reported so far in, *Macrovipera lebetina obtusa Ml* (Samel, Subbi et al. 2002, Igci and Demiralp 2012, Gopcevic, Karadzic et al. 2021)*, Vipera ammodytes ammodytes Vaa (Georgieva, Risch et al. 2008, Leonardi, Sajevic et al. 2019), Vipera ammodytes montandoni Vam* (Hempel, Damm et al. 2018)*,* and *Vipera berus berus Vbb* (Bocian, Urbanik et al. 2016, Al-Shekhadat, Lopushanskaya et al. 2019) snake species venoms. CTL is recognized to contain a carbohydrate domain (Morita 2005). LAAOs were frequently glycosylated, but the glycan moiety is not well understood (Guo, Liu et al. 2012, Andrade-Silva, Zelanis et al. 2016). Notably, glycoproteomics analysis enabled the identification of the rare Hyaluronidase protein among all venom. Hyaluronidase were reported in a previous proteomics study in the venom of Viperidae species (Igci and Demiralp 2012), Snake venom hyaluronidases are glycoproteins isolated from the venom of Cerastes cersastes viper, and have catalytic function. It facilitates the venom diffusion of other proteins by degrading the extracellular matrix in the victim's tissue and therefore, enhancing the effects of the venom proteins (Boldrini-Franca, Cologna et al. 2017)

4.5. N-glycoproteome composition-based venom classification

To study the correlation between the venom proteomes, Pearson correlation analysis was applied using FragPipe LFQ-analyst. The identified proteins have a positive linear correlation for all venom replicates, as illustrated by a heat map in (Figure 4B), indicating the overlapping of the shared peptides among the venom The clustering analysis classified the venoms into four groups, the first, second, and third group composed only of *Ml, Vbb* and *Mx* in each respectively. The fourth group was composed of the venoms from *Vaa* and *Vam* species. Notably, the glycoproteomics clustering result was similar to the clustering obtained from the Proteomics analysis. Because both were obtained based on the composition of peptide backbone sequences.

Glycoproteomics provided further compositional information about the differences and similarities of proteins exist in venom proteomes. It was excepted to detect a distinct low abundant protein for each venom. Rather, hyaluronidase was detected but was shared between all venom. This confirms that abundant proteins have a major contribution to the biological function of the venoms. While rare or low abundant proteins are secondary metabolites resulting from evolutionary and/or modification processes. Moreover, glycoprotein profiles showed mass differences of shared glycosylated peptides among venoms despite having a similar sequence. Indicating the presence of different *N*-glycan moieties attached to the glycopeptides.

Glycoproteomics revealed the homology in protein backbone sequences. The high number of variable *N*-glycans confirmed the population of venom proteome with diverse glycoprotein isomers. The glycosylation level variation was confirmed by the detection of several variable N-glycan with complex structures.

4.6. N-glycomics Analysis of Viperidae Snake Venoms

To investigate the diversity and variation of the N-glycan moiety in the venom of the five Viperidae species, as well as their glycosylation modification pattern the *N-glycan* profiles were obtained using by *N*-glycomic approach. *N*-glycans were released from venom proteins, subjected to fluorescent labeling, and then analyzed by HILIC-FLD-MS/MS. The MS spectra were searched against the glycan Carbbank database using Protein Scape software. The applied approach allowed identification and quantification of the *N*-glycans attached to the proteins at the position of glycan sequon (N-X-S/T) in each protein from the five snake species venom. A list of the identified *N*-glycans for each snake species was given in (Table S13-17, Supporting Information). A representative FLD chromatogram of the proc-labeled-*N*-glycan was given in (Figure S2A-6A, Supporting Information).

Results revealed identification of a total of 78 distinct *N*-glycan structures across all venom samples. A variable *N*-glycan moiety of 22, 27, 20, 22, and 46, were detected in the venoms of *Ml, Mx, Vaa, Vam*, and *Vbb*, respectively. The distribution of the unique identified *N*-glycan moiety in venom proteome, among the five venoms was illustrated by Venn diagram in (Figure 5A). Notably, one *N*-glycan moiety (Hex5HexNAc2) was shared in all venom, while some were unique to a specific snake species. Specifically, venoms of *Ml, Mx, Vaa, Vam*, and *Vbb* had 6, 2, 2, 3, and 28 unique *N*-glycan moieties, respectively. The venom of *Vbb* displayed the highest number of identified *N*-glycan and unique *N*-glycan structures. This suggests that the venom of *Vbb* has the highest level of glycosylation among the five venoms. The interactiVenn analysis demonstrated the distinct N-glycan pattern of each venom.

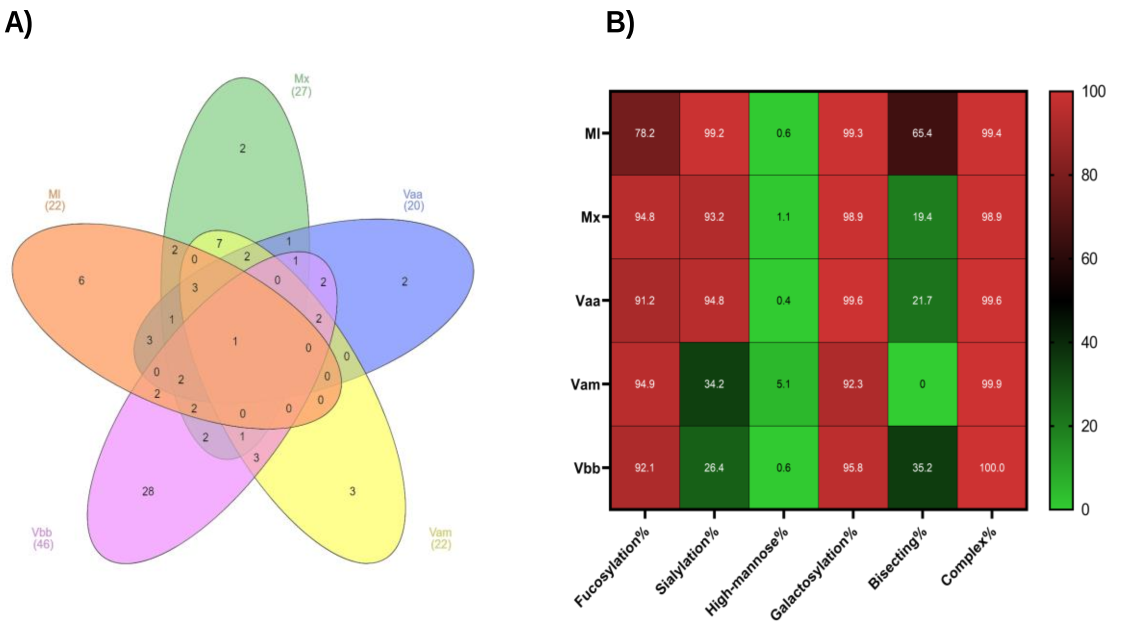


Figure 5: Venn diagram of the distribution of the unique and shared *N*-glycan among the venom from Viperidae species (A). A graphical visualization of two hierarchical clustering of venoms *N*-glycan trait characterization. For each venom, a given *N*-glycan trait is either present

Quantitative analysis was achieved using the total area normalization approach of each *N*-glycan peak belonging to venom species, as shown in (Figure S2B-6B, Supporting Information). Quantitative analysis of *N*-glycans from each venom revealed that the venom of *Ml* and *Mx* had the same *N*-glycan with Hex5HexNAc5NeuAc2dHex1-proc structure as the most abundant N-glycan at 39.7% and 27.9% of the total N-glycan composition. The Hex5HexNAc4NeuAc2dHex1-pro and Hex5HexNAc4dHex1-proc were the most abundant at 48.07% and 35.9% in the venom of *Vaa*, and *Vam* respectively. While in the venom of *Vbb* both Hex5HexNAc5NeuAc2-proc and

Hex5HexNAc5dHex3-proc were found in a high abundance at 15.41% and 8.91% of the total N-glycan composition. Indicating that the venom of *Ml, Mx,* and *Vaa* were rich in N-glycans containing sialic acid moiety.

The *N*-glycans composition profiles of the five venoms were found to be made up of similar *N*-glycan types, with some exceptions. The identified *N*-glycans were classified into high-mannose, bisecting, and complex types based on their structural features for each venom. It was found that most of the *N*-glycans possess complex structures, followed by Bisecting structures. While a low number of high-mannose N-glycans were found across the venom. The Bisecting was absent in the venom of *Vam* as given in (Table S13-17, Supporting Information). Furthermore, the *N*-glycans were categorized according to the presence of fucose on their core and sialic acid contents. To confirm the presence of core fucosylation and bisecting type *N*-glycan structures, diagnostic fragments were monitored in each *N*-glycan MS/MS spectra for structural validation. Specifically, the identification of core fucosylation was confirmed by the presence of a fragment with a mass-to-charge ratio (m/z) of 587.3, corresponding to proc+NF. Likewise, bisecting type *N*-glycan structures was confirmed by detecting two distinct fragments: *m/z* 1009.5 for proc+HN3 and *m/z* 1155.5 for proc+HN3F.

In a glycomics investigation involving various species of Bothrops Vipers, analogous compositional characteristics have been reported. Predominantly, the prevalent structural type consisted of hybrid/complex N-glycans, with many compositions featuring the presence of sialic acid and fucose residues (Andrade-Silva et al., 2018). A prior investigation on Russell's viper venom revealed a significant abundance of N-glycan chains containing sialic acid residues linked to beta-galactosyl residues, along with the presence of bisecting N-acetylglucosamine residues (Gowda et al., 1994; Soares and Oliveira, 2009). In our research, we detected the presence of sialic acids (NeuAc) within the N-glycan component of Viperidae venoms. These keto sugars, originating from neuraminic acids, are typically situated at the outermost extremity of glycan chains (Andrade-Silva et al., 2018; Brás Costa et al., 2023).

The cluster analysis evaluated the similarities in N-glycan traits among the snake venoms and differentiated the venom by N-glycan traits structure features. The complex *N*-glycan were galactosylated and fucosylated abundantly, while sialylated *N*-glycans, and bisecting *N*-glycans ratios were varying among venom species, and high-mannose *N*-glycans were found in a low ratio across the venoms as given in (Figure 5B). Most of the complex *N*-glycans were found to carry a fucose residue at the 1-6 position of the *N*-glycan core, the venom *N*-glycans fucosylation ratio was high except for *Ml*, which had a relatively low ratio (78.2%). The sialylation ratio was dramatically high in the venoms of *Ml, Mx*, and *Vaa* when compared to the venoms of *Vam* and *Vbb*. In contrast, high-mannose content was found in a low amount for all species. The bisecting ratio was found to possess a low ratio except for the venom of *Ml* (65%). Interestingly, the bisecting type *N*-glycans were not detected in the venom of the *Vam* snake species.

4.7. N-glycome composition-based venom classification

The composition of the *N*-glycan traits was used to classify venoms by hierarchical clustering. The Hierarchical diagram showed that *Ml* formed a distinct cluster, while the venoms of *Mx* and *Vaa,* and the venom of *Vam* and *Vbb* exhibited a similar cluster. Accordingly, venoms were classified into three different groups based on similarities in their *N*-glycan composition profiles as presented in (Figure 6A). Group one contained only the venom of *Ml,* the second contained the venom of *Mx* and *Vaa,* third contained the venom of *Vam* and *Vbb* snakes. The principal component analysis (PCA) was also performed to further elucidate the relationships between venom samples based on their *N*-glycan trait compositions using MATLAB software. PCA analysis provided a better result than clustering analysis as given in (Figure 6B). The placement of the venoms in the PCA plot showed their distinct *N*-glycan profiles and classified venom into four distinct groups. In which three of the five venoms clustered in different quadrants except for venoms of *Vaa* and *Mx* species. Accordingly, the five venoms can be classified into four groups based on their *N*-glycome composition, Group1 is composed of venoms of *Mx* and *Vaa,* whereas venom of *Ml, Vam,* and *Vbb* were placed in three different groups. This is consistent with what has been found in a previous glycomics study on the venom of Bothrops species. This suggested that the diversity in the *N*-glycan composition reflects the divergent pathway of each species during the evolutionary process. On the other hand, some venom's *N*-glycan content seems to have evolved as traits with a conserved composition through the evolutionary process such as in the case of the venom of *Vaa* and *Mx* species (Andrade-Silva, Zelanis et al. 2016, Andrade-Silva, Ashline et al. 2018). The substantial quantity of glycans detected in the venoms validates the findings obtained through glycoproteomic analysis.

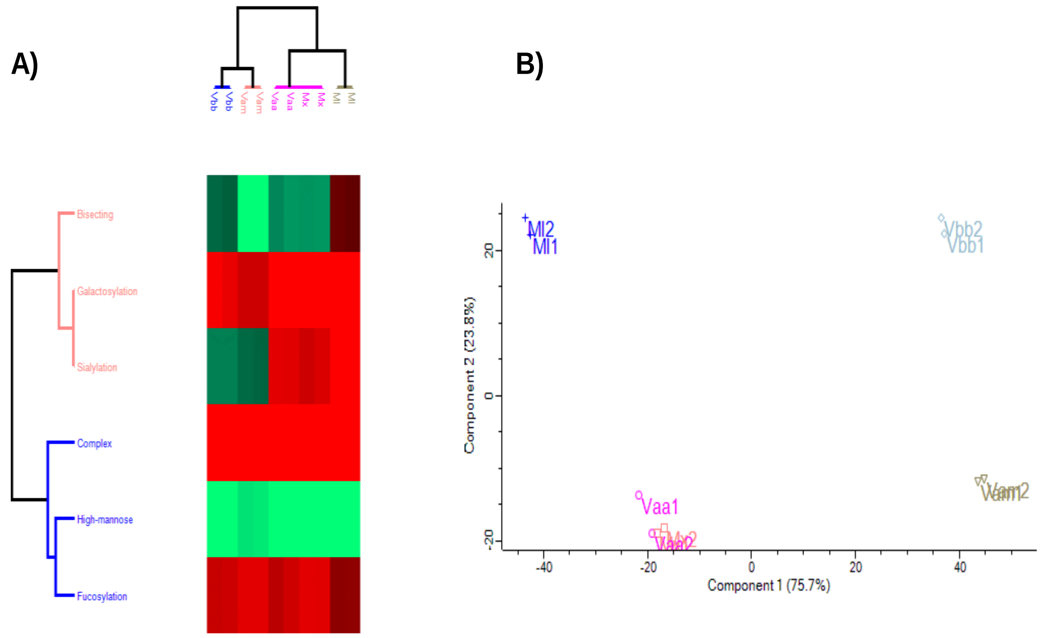


Figure 6: Viperidae venom clustering based on the *N*-glyacn traits composition. A graphical visualization of two hierarchical clustering of venom *N*-glyacn traits characterization. For each venom, a given *N*-glyacn trait is either present (red) or absent (green) (A). A graphical visualization of two components PCA clustering of venoms *N*-glyacn traits (B).

Part II

‘’Composition-Based Biomarker Identification for Viperidae Snake Venom Using MALDI-MS/MS Proteomics’’

4.8. MS-based composition profiles of Viperidae venoms using MALDI-TOF-MS

Protein profiles of five crude venoms obtained using HCCA matrix at reflectron mode in the mass range of 500 Da to 5 kDa and DHAP matrix at linear mode along the mass range of 5 Da to 60 kDa. Figure S1-2, Supporting information compared MALDI-TOF-MS spectra of the venoms obtained in the mass range of 5 kDa to 40 kDa, and the mass range of 500 Da to 5 kDa respectively. MS spectra reported only in the mass range of 5 to 40 kDa because we could not observe protein peaks in this region at all concentrations. The protein profiles of venoms along full mass range of 500 Da to 60 kD were found different and showed several unmatched and matched mass peaks. MALDI-MS data were validated using a triplicate technical replicate in parallel, using 500µg of the venoms (Table S18a-b, supporting information). Raw data were manually statistically processed to calculate the relative abundances in the mass range of 5 kDa to 40 kDa, and the mass range of 500 Da to 5 kDa respectively, for each venom sample by total intensities normalization as given in Table S19a-e, and Table S20a-e and compared as shown in Figure 7a-e, and Figure 8a-e.

The mass spectrum of the venoms demonstrated a like peaks distribution pattern, this can be explained by the presence of the typical snake venom protein families in the venom proteomes. Table S21, supporting information shows the overall proteins mass peaks distribution at the mass range of 500 Da to 40 kDa for Ml, Mx, Vaa, Vbb, Vam, and Wa crude venoms. The MS of protein less than five kDa display resolved peaks while in the mass range of 5 kDa to 30 kDa show an overlapping broadened peak, which was challenging to interpret, this indicate the presence of high molecular weight proteins which is difficult to resolve using MALDI-MS. The MS spectral intensity and ionization features vary among the five venoms; this occurs because of the composition variation within snake species.

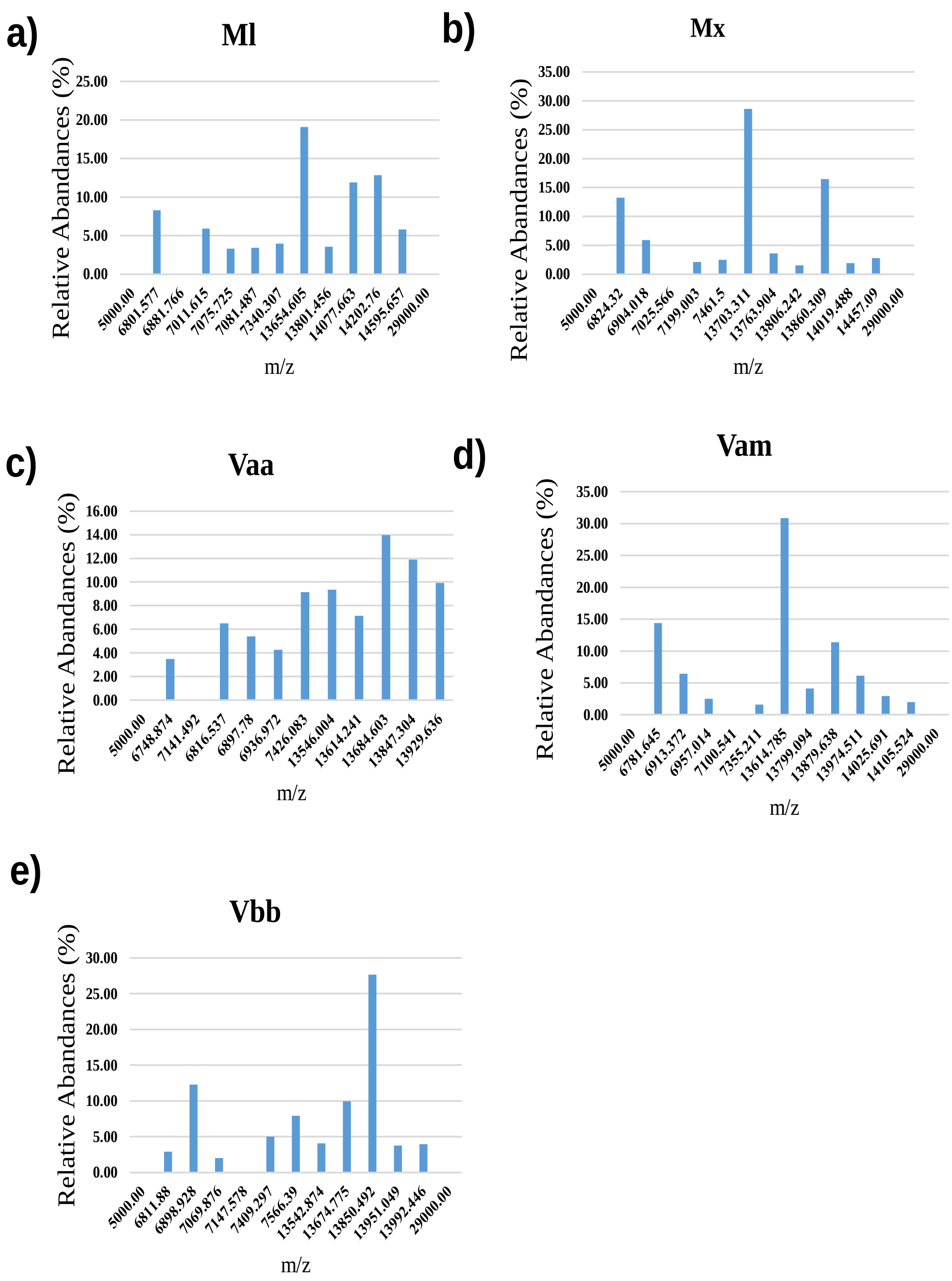


Figure 7. Mass finger printing protein profiles in the mass range of 5 kDa to 60 kDa. The column charts describe the relative abundance values of the most abundant 10 protein peaks of in the proteom (A) *M. lebetina* (*Ml*), *(b) M. xanthina* (*Mx*), (c) *V. a. ammodytes* (*Vaa*), (d) *V. b. berus* (*Vbb*), (e)*V. a. montandoni (Vam*).

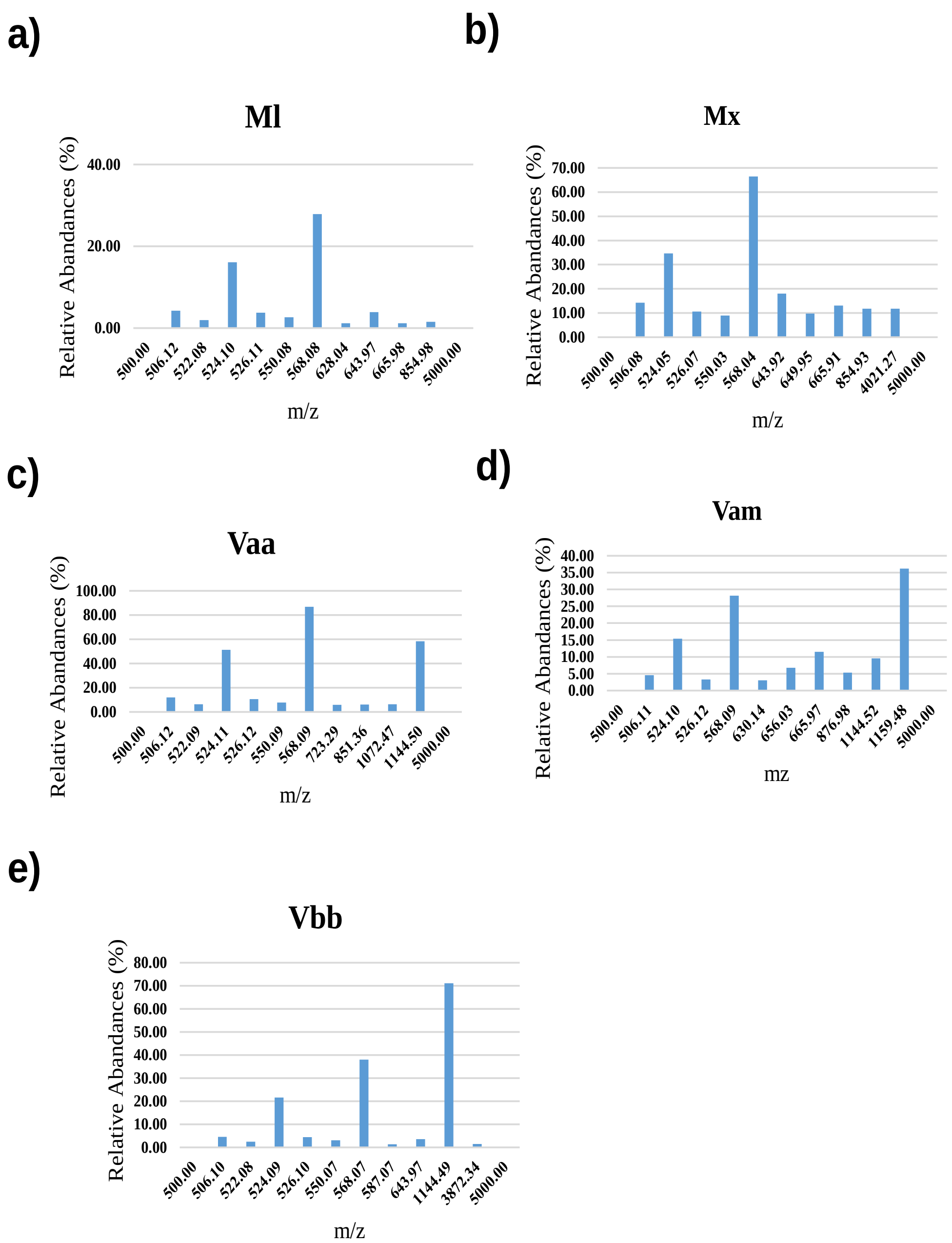


Figure 8. Mass finger printing protein profiles in the mass range of 500 Da to 5 kDa. The column charts describe the relative abundance values of the most abundant 10 protein peaks of in the proteom (A) *M. lebetina* (*Ml*), *(b) M. xanthina* (*Mx*), (c) *V. a. ammodytes* (*Vaa*), (d) *V. b. berus* (*Vbb*), (e)*V. a. montandoni (Vam*).

By comparing the high abundant 10 proteins along the whole mass range at 500 Da to 40 kDa by their mass differences and relative abundances of the five venoms a flow chart was created for biomarker assignment as shown in Figure 9. The mass peaks were divides into three groups (matched peaks, Unmatched mass peaks with a mass difference more than 10 Da, Unmatched mass peaks with a mass difference less than 10 Da). The main criterion is the mass difference with 1 Da for the protein in the mass range of 500 to 5kDa and with 10 Da for protein in the mass range of 5kDa to 40kDa. The second criterion is the relative abundance difference, when the mass difference is less than 1, 10 Da for proteins in the mass range of 500 to 5kDa, 5kDa to 40kDa, respectively. In the case of matched peaks, MS/MS analysis is required. To validate the classification, model the SDEV of the matched and unmatched peaks were calculated As shown in Table S22a-c, supporting information. FigureS3, supporting information demonstrate the similar MS spectrum of the matched mass peak values with SDEV values less than 0.5 at m/z of 568, 644, and 1144 kDa of different analyzed venoms.

The result showed a different MS-based protein profiles and several unmatched and matched mass peaks of the venom of *Ml, Mx, Vaa, Vbb,* and *Vam*, along the full mass range of 500 Da to 40 kD showed. The first group composed Unmatched mass peaks, which have a significance mass difference with a mass difference less than 10 Da and can be used as Biomarker candidate, these peaks are unique and can be observed by naked eye regarding their highest relative abundance. Second group is Unmatched mass peaks with a mass difference more than 10 Da but have a significant difference in their relative abundances. Third group includes matched mass peaks which were found in all venom species.

Accordingly, the designed flow chart is a valid model to assign MS-based species biomarker candidate according to the mass difference or relative abundance of matched and unmatched peaks. Additionally, this model established using the abundant protein mass peaks. Abundant protein is typically present in venoms of different species in high amount, consequently they are easily to detect, and have high intensities in all venom replicates.

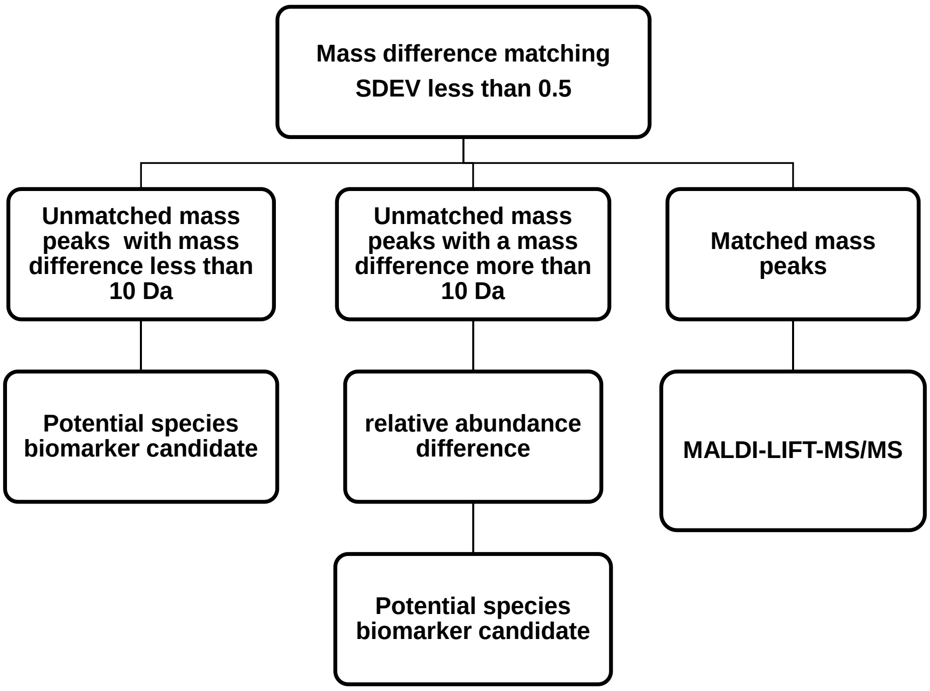


Figure 9. A flow chart describes biomarker identification according to the mass differences of unmatched peaks or their relative abundance. 10 Da mass difference is applicable for peptides with a molecular mass of s more than 5 kDa. for the peptides less than 5 kDa the mass difference of 1 Da is applicable. 1 Da for the protein in the mass range of 500 to 5kDa and with 10 Da for the protein in the mass range of 5kDa to 60kDa

4.9. Top-Down Proteomics Analysis of Viperidae venoms using MALDI-TOF-MS/MS

To confirm the similarity of the MS-based matched peaked, and to investigates the diversity and composition variation of the venoms belonging to Viperidae family using MALDI-LIFT-MS/MS. MS/MS approach allowed identification of the venom peptide sequences assigned to the five venoms using Bio tool (Bruker), and then protein family profiles were semi-quantified. An average of twenty-five precursor ions of highest intensity were selected from MS spectra and analyzed by MALDI-LIFT-MS/MS. Biotool (Bruker) search software was used to process the MS/MS data against a non-redundant of protein UniProtKB specific for Viperidae snake database. The identified protein peaks m/z, matched peptide MS/MS arrangement, Accession Number, Protein Family/ Subtypes, Protein coverage, and Scores were reported in Table S23a-e, supporting information.

A total of 19, 17, 18, 15, and 26 precursor ions were assigned to 13, 13, 13, 3, 12 protein hits. The identified proteins were classified into 6, 7, 5,5 and 6 protein families, respectively to the venom of *Ml, Mx, Vaa, Vam*, and *Vbb* snake species. The score/ Protein sequence coverage is ranging from 1/ 1% to 14/ 95% for all matched proteins. Number of the precursor ion were identified by their MS/MS arrangements but were not assigned to protein hits.

The interactiVenn analysis revealed the Distribution of the unique proteins identified in the venom proteome, grouped by venom. The identified protein groups were sorted by their shared and unique proteins. Unique proteins that appeared only in one of the venoms were 3, 3, 3, 1 and 2 for each venom of *Ml, Mx, Vaa, Vam* and *Vbb*, respectively as shown in (Figure10**)**. Whereas 3 shared proteins were detected between all venoms. Moreover, only one protein was shared between venoms of *Ml* and *Mx*, between venoms of *Ml* and *Vbb*, between venoms of *Mx* and *Vbb,* and between venoms of *Vbb* and *Vam*.

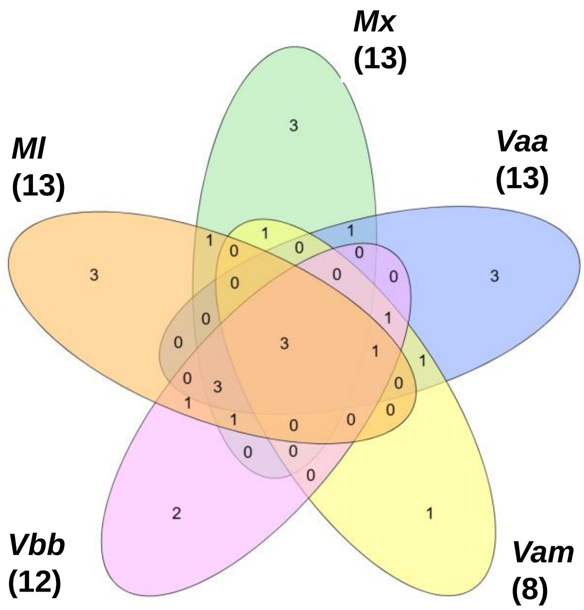


Figure 10. Distribution of the unique and shared proteins among the venoms from Viperidae species

To investigate the composition diversity within venoms, identified proteins were classified into protein families and then relative abundance of protein families were calculated for each venom as shown Figure 11a-e. The result revealed that distinct protein families profiles for each snake species. The protein profiles showed a resemblance to the Viperidae composition reported in the literature. The venom of the five species was found to share major and secondary protein families including, Disintegrin (DIS), snake venom serien protaese (SVSPs), snake venom metalloproteinase (SVMPs), phospholipase A2 (PLA2s), Bradykinin-potentiating peptides (BPPs) as shown in Figure 11e. SVSP and SVMP was abundant in the five venoms. Minor proteins also were detected by this TD proteomics approach, where Snake Venom Metalloproteinase Inhibitors(SVMPI) was detected in the venom of Mx at low amount. Whereas Nerve growth factors (NGFs) was detected in the venom of Mx and Vam. Interestingly 3FToxin was also detected in the venom of Ml, and Vbb, 3Ftoxin are not typically found in the venom of Elapidae family not Viperidae venoms. These unique protein families can be considered as a biomarker candidate for each species.

Moreover, by comparing the protein subtypes by mass, the study revealed that the number of unique proteins found were 10, 7, 7, 6, 17 and 6 for Ml, Mx, Vbb, Vam, Vaa, and Wa, respectively. On the other hand, six proteins were common by mass between the six crude snake species. As well, as other ten proteins were found to be common in between at least two of the venom species, as shown in Table S24 which compared the common protein subtypes per protein family for the crude snake species. Fig. S5-11, supporting information displayed MS/MS spectra of the common protein subtypes generated from the crude snake venom species.

The study revealed that three of the matched proteins by mass have the same MS/MS arrangement in *Ml, Mx, Vaa, Vam*, and *Vbb*. While other 13 showed a diverse arrangement, for example at 1066.253 m/z, *Ml* and *Vbb, Mx* and *Vaa* had the same MS/MS arrangement but different from *Vam* arrangement as given in Table S24. The differences in amino acid arrangement reflects diversity of snake venom proteome. Considering the difference in MS/MS arrangement, these proteins can be assigned as a biomarker candidate. The result demonstrated the interspecies similarity between species at the genera and family level.

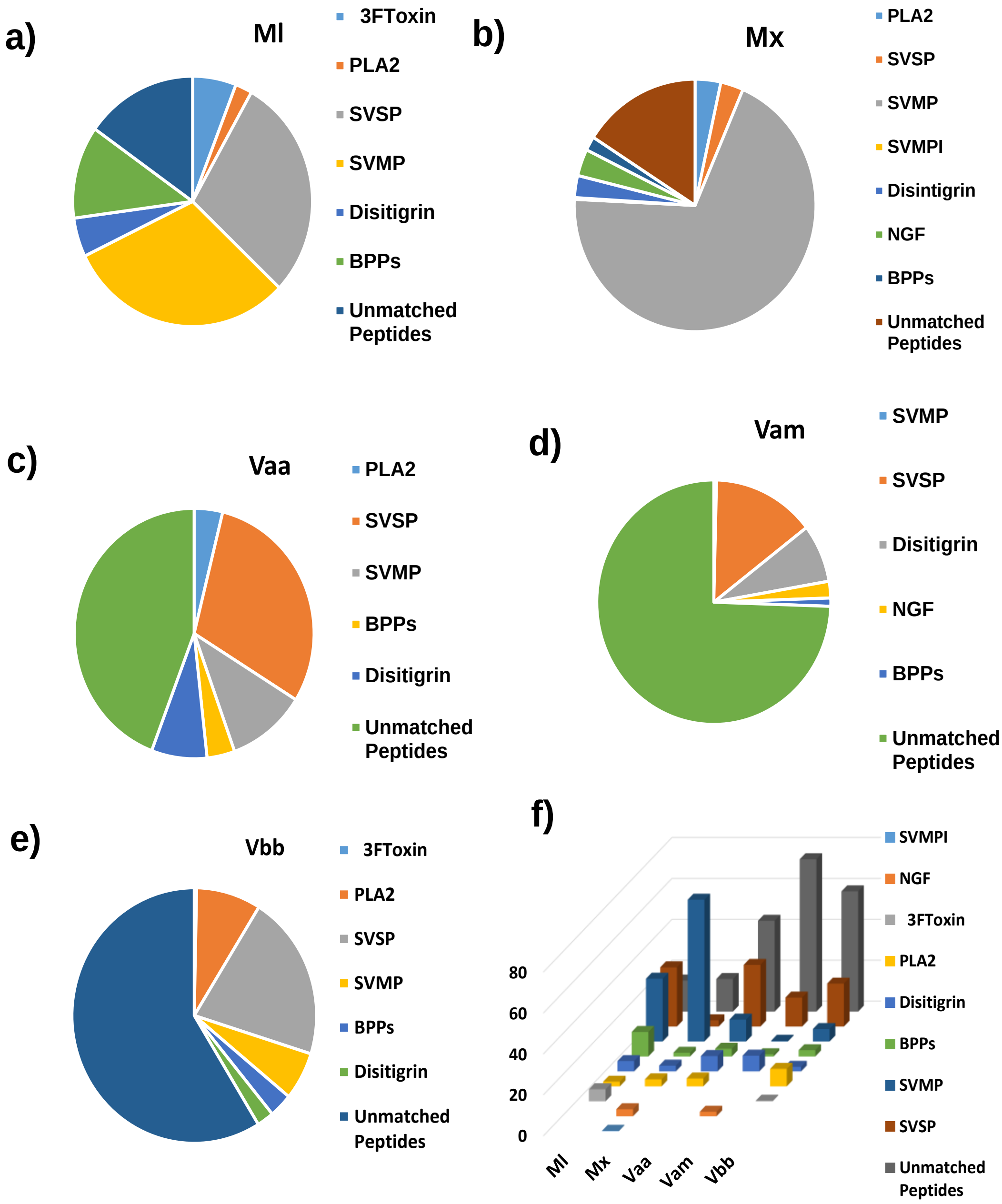


Figure 11. Distribution of protein families identified in the proteom of (A) *M. lebetina* (*Ml*), *(b) M. xanthina* (*Mx*), (c) *V. a. ammodytes* (*Vaa*), (d) *V. b. berus* (*Vbb*), (e)*V. a. montandoni (Vam*), and (f) Distribution of shared families among the venoms. Disintegrin (DIS), snake venom serien protaese (SVSPs), snake venom metalloproteinase (SVMPs), phospholipase A2 (PLA2s), Snake Venom Metalloproteinase Inhibitors(SVMPI), Nerve growth factors (NGFs), Bradykinin-potentiating peptides (BPPs), 3FToxin.

The protein profiles were found to exhibit similarity to that observed for Viperidae species reported in the literature so far. In a venomics study on the venom of *Ml* from Turkey (Igci and Demiralp 2012), and European *Vaa* venom (Gopcevic, Karadzic et al. 2021)(Georgieva, Risch et al. 2008)(Leonardi, Sajevic et al. 2019).

In a bottom-up approach applied on the venom of *Vam* from Turkey (Hempel, Damm et al. 2018) and on the venom of Russian *Vbb* (Al-Shekhadat, Lopushanskaya et al. 2019). The electrophoretic protein distribution of the venom of *Montivipera xanthina Mx* from Turkey was reported by (Arikan, Gocmen et al. 2008). Whereas Nalbantsoy, A. *et. al*. reported the mass profile of two Montivipera species *M. bulgardaghica* and *M. raddi* from Turkey (Nalbantsoy, Hempel et al. 2017). Our finding mainly found to share the major and secondary abundant protein families with the previous studies findings. However, the composition abundance is different mainly because of the different analysis approach including sample preparation and MS system.

A high proportion of the proteins were not assigned to protein hits, this can be explained by the drawbacks in the workflow approach and the Database (UniProtKB). On one hand MALDI-MS can not ionize low abundant and large proteins, consequently quantitative analysis performed only on abundant proteins. In addition, shortage of the specific species proteins available in database for the *Ml, Mx, Vaa, Vam,* and *Vbb* venoms, regardless the frequent database enriching with proteins from different snake species. Moreover, the available protein in database are a tryptic peptide which is a part of the parent protein, this influence the search result of the MS/MS obtained for native proteins, including low score and coverage percentage of the identified protein hits and result in only partial sequence, in the worse scenario it may result with not or false assignment to proteins hits. The obtained information is sufficient for a relative characterization of the protein composition. Additionally, the absence of some typical venom components reported in Viperidae venoms makes it difficult to compare the venom composition at the species level.

5. Conclusion

In this study, a comparative analysis was conducted between the glycoproteomic results and the glycomic outputs. The objective was to ascertain the degree of concordance between the glycan types identified in glycoproteomic experiments and those obtained from glycomic experiments. Our investigation revealed a noteworthy alignment between the two datasets, with the most prevalent glycan types observed in the glycoproteomic experiments closely corresponding to the most abundant glycan types elucidated through glycomic experiments. Detailed findings of this alignment can be found in Table S8-17.

This extensive MS-based clustering workflow showed that the *N*-glycomics data made it possible to classify the venoms into five individuals. The Glycomics profile delivered significantly better results due to the greater variation of *N*-glycan moieties among the venom. Contrary to the findings of the proteomics and glycoproteomics results, which verified to produce the same clustering results. The overall result of the classification was in parallel with the phylogeny cladograms of the Eurasian vipers reported by Freitas et al. (Freitas, Ursenbacher et al. 2020). Which places the five Viperidae species into 3 different clades. *Ml* was in *Macrovipera*, *Mx* was in *Montivipera*, while *Vbb, Vaa,* and *Vam* were in the *Vipera* clade, it showed that *Vbb* was classified in a different subspecies of the *Vaa*, and *Vam* species.

In our research, we employed a multi-omic methodology to comprehensively elucidate the proteomic, glycoproteomic, and glycomic profiles of venom samples derived from Viperidae snake species. This holistic approach has facilitated a profound exploration of the significance of protein compositions present within snake venoms. Furthermore, our meticulous characterization of the glycosylation patterns within these venoms has provided novel insights into the functional roles of post-translational modifications that are prevalent in venomous secretions. This wealth of information holds great promise for advancing the development of strategies to mitigate the deleterious effects of snakebite envenomation. By affording a more nuanced understanding of the composition and biological roles of the identified proteins, our study serves as a valuable resource for researchers seeking to devise innovative approaches for treating snakebite incidents.

Additionally, the comprehensive data generated by this investigation, encompassing protein, glycoprotein, and glycan abundances across the five distinct venom species, can be harnessed to advance the classification of snake venoms. In a broader academic context, our multi-omic approach not only contributes to the fundamental knowledge regarding venom composition but also opens avenues for potential applications within the biomedical industry. The intricate details gleaned from our study may serve as a foundation for the development of novel therapeutic modalities and biomedical innovations, further underscoring the far-reaching implications of our research findings.

The comparison studies are conducted based on abundant proteins. Although widely accepted, it suffers from some limitations due to the different applied approaches starting from sample preparation, LC-MS technique, search engine, specific species database, and venom collection. Another limitation involves the number of identified proteins and the absence of low abundant proteins in the obtained profiles, which is often excluded from the identification through the proteomic workflow. Overall, this study's basic findings of the whole proteome composition in the venoms of Viperidae species are consistent with previous studies' findings. Moreover, the applied workflow verified the presence of low abundant proteins, which lead to good proteomics-based clustering analysis, even if the classification could not distinguish all venoms as individuals.

This comprehensive MS-based -omics pipeline successfully uncovered the distinct proteome and glycoproteome composition for each Viperidae species. The protein and glycoprotein distribution profiles among venoms were consistent with typical Viperidae components, offering a comprehensive understanding of the specific proteins in venoms' proteome and glycoproteome. The glycosylation level variation was confirmed by the detection of several variable N-glycan with complex structures. Comparison of different composition profiles by clustering analysis revealed different evolutionary pathways of venom proteomes and glycoproteomes. The applied bioinformatics enabled fast classification of venoms based on pure proteome and glycoproteome MS-based data, without any complex fractionation process, particularly *N*-glycan MS data. The clustering result was in line with reported phylogeny cladograms. Applying clustering analysis for classification, using some well-established global database rich with specific species protein information for Viperidae species can serve as a useful tool for snake species identification and consequently, early diagnosis of snakebite clinical symptoms and the preclinic assessment of envenomation. The obtained mass spectrometry data were deposited in the public data repository, alongside the active demand to enrich the public database with additional specific species protein information.

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7. Appendix I: Supporting Information Figures

Part I

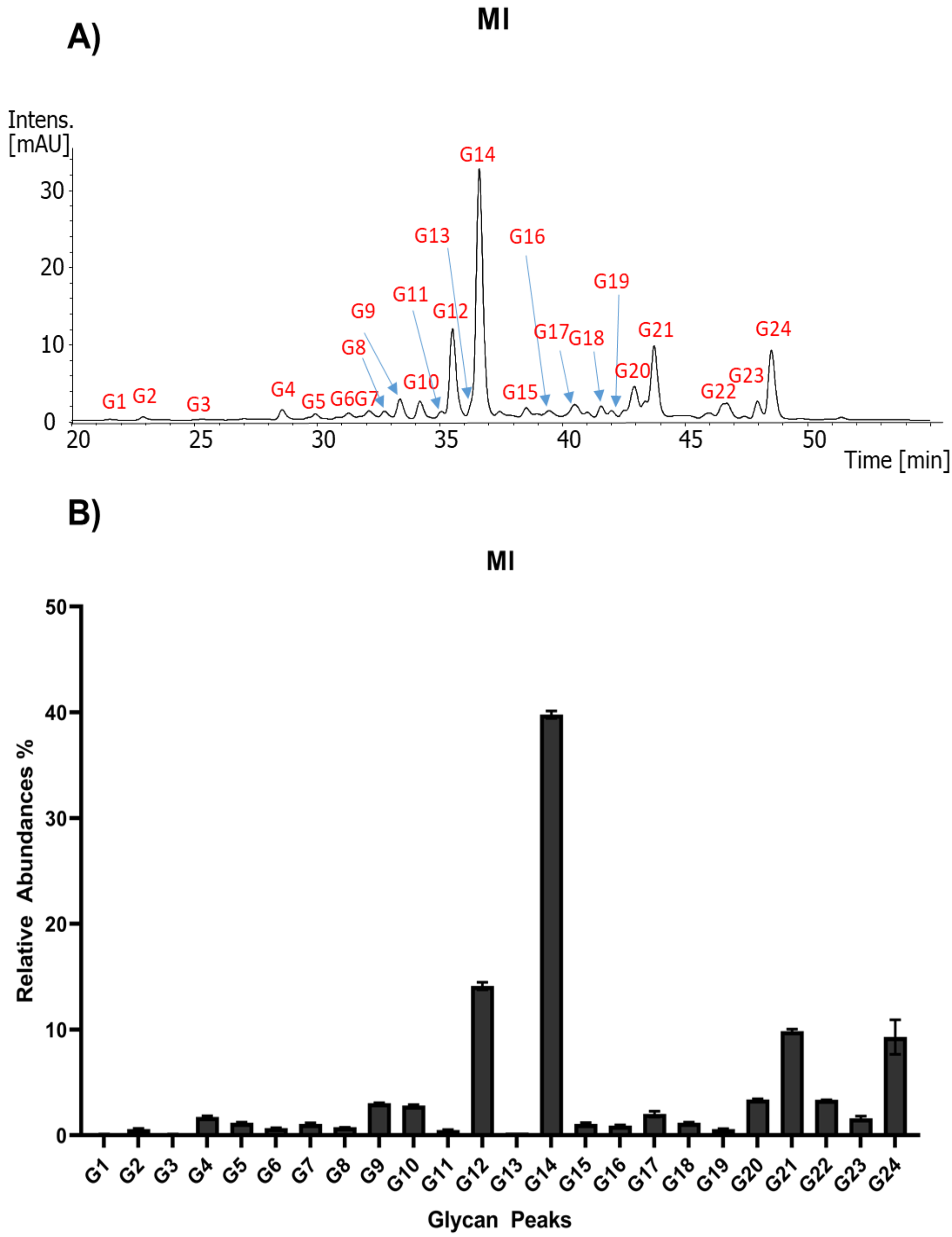


Figure S1. The FLD chromatogram of the *N*-glycan peaks of the snake venom *Ml* (A) and, the relative abundances of the *N*-glycan peaks (B).

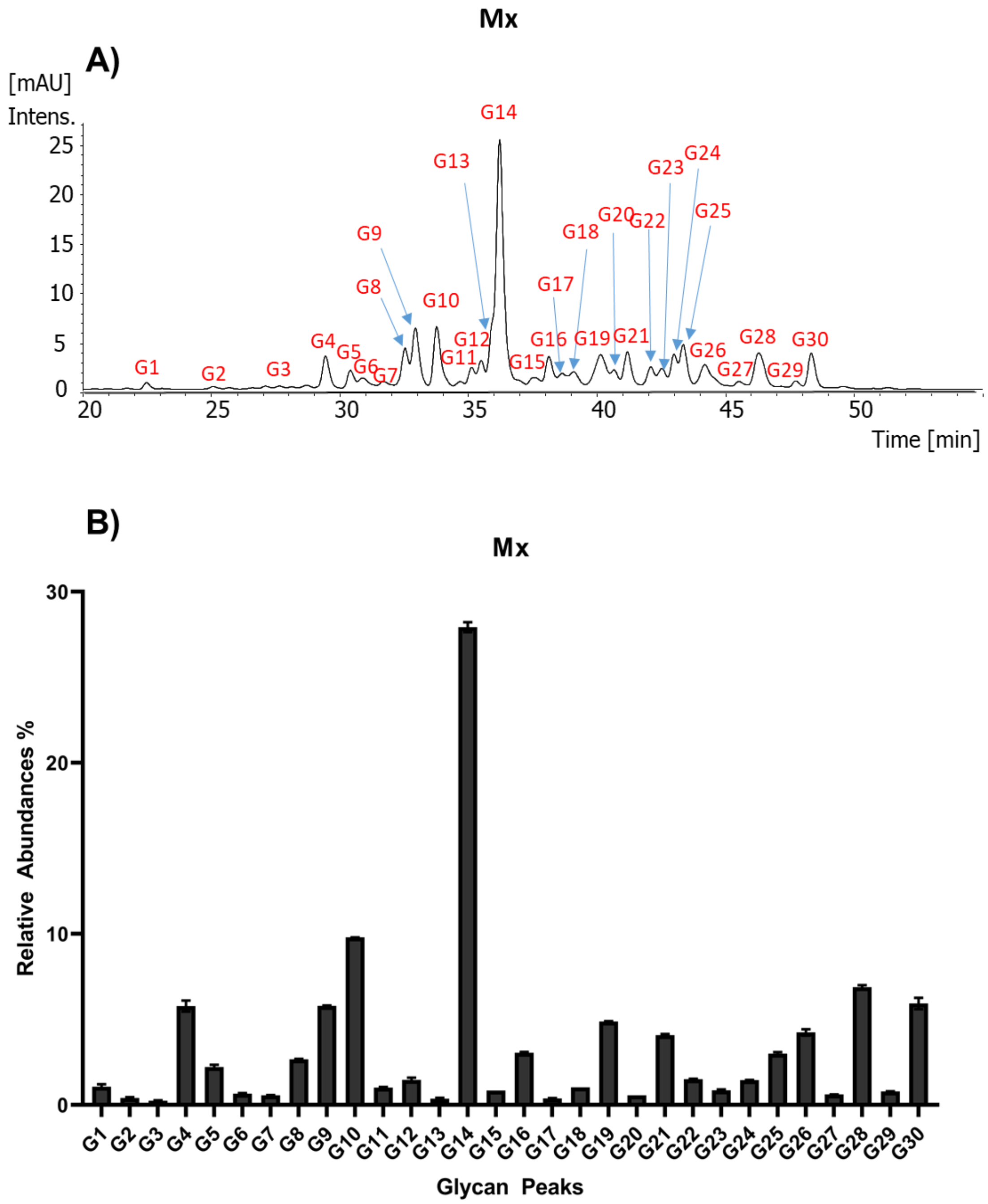
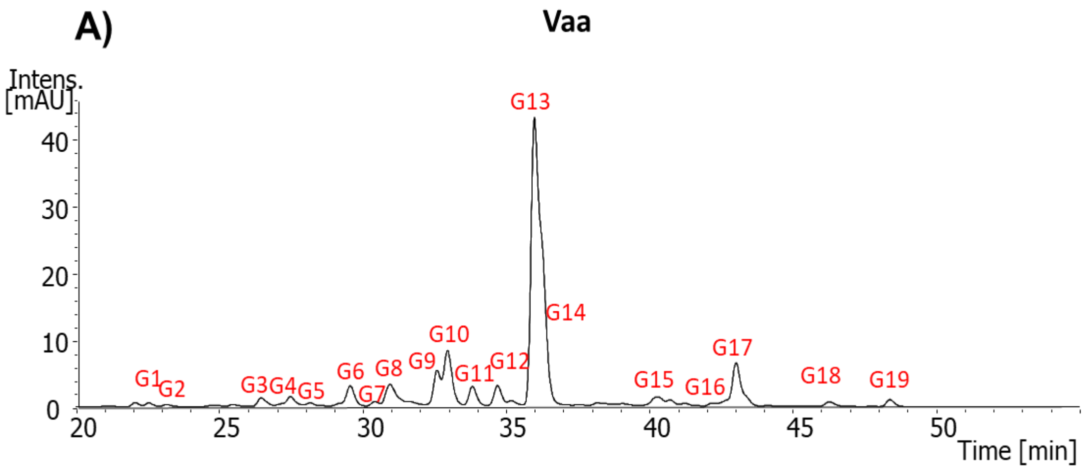


Figure S2. The FLD chromatogram of the *N*-glycan peaks of the snake venom *Mx* (A) and, the relative abundances of the *N*-glycan peaks (B).



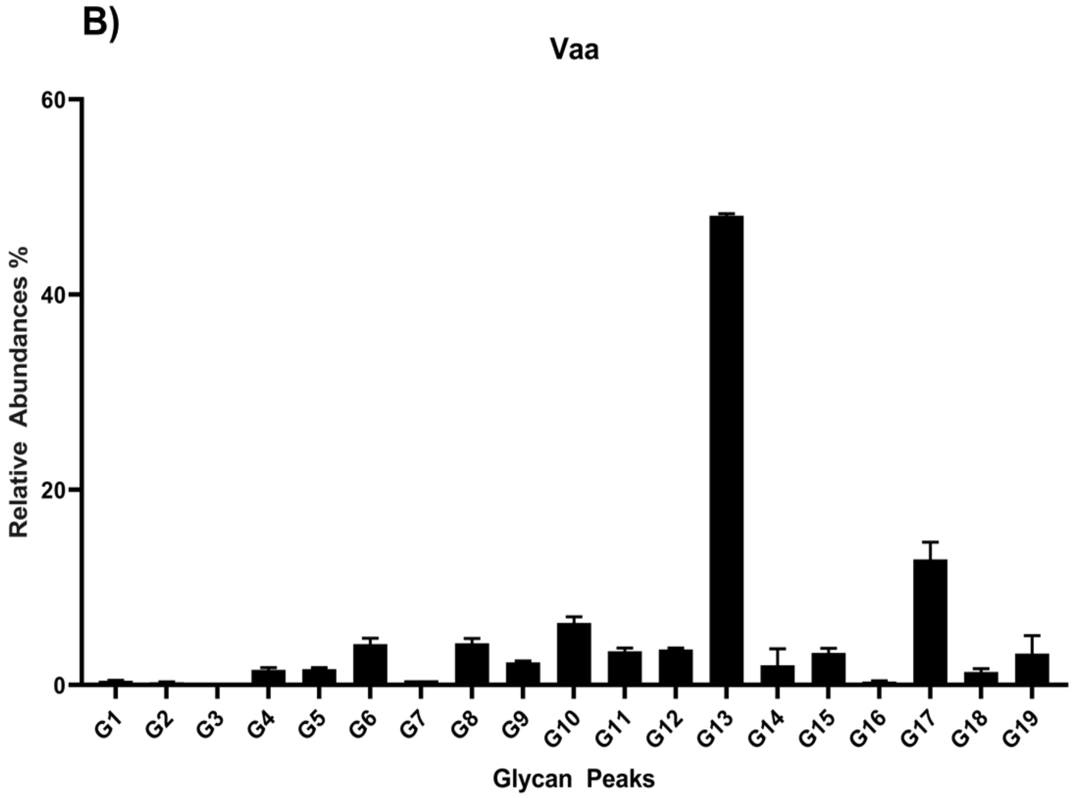
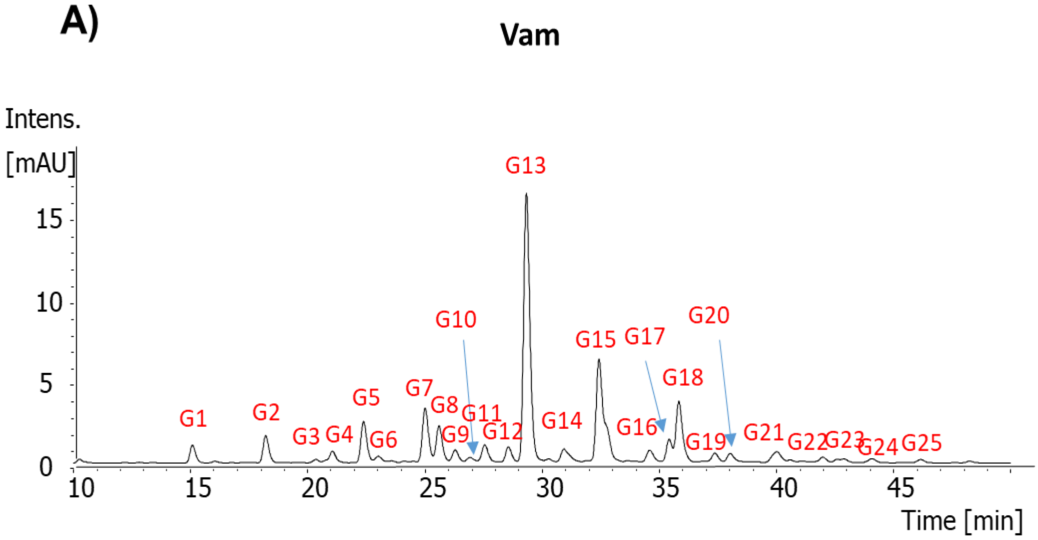


Figure S3. The FLD chromatogram of the *N*-glycan peaks of the snake venom *Vaa* (A) and, the relative abundances of the *N*-glycan peaks (B).

A)



B)

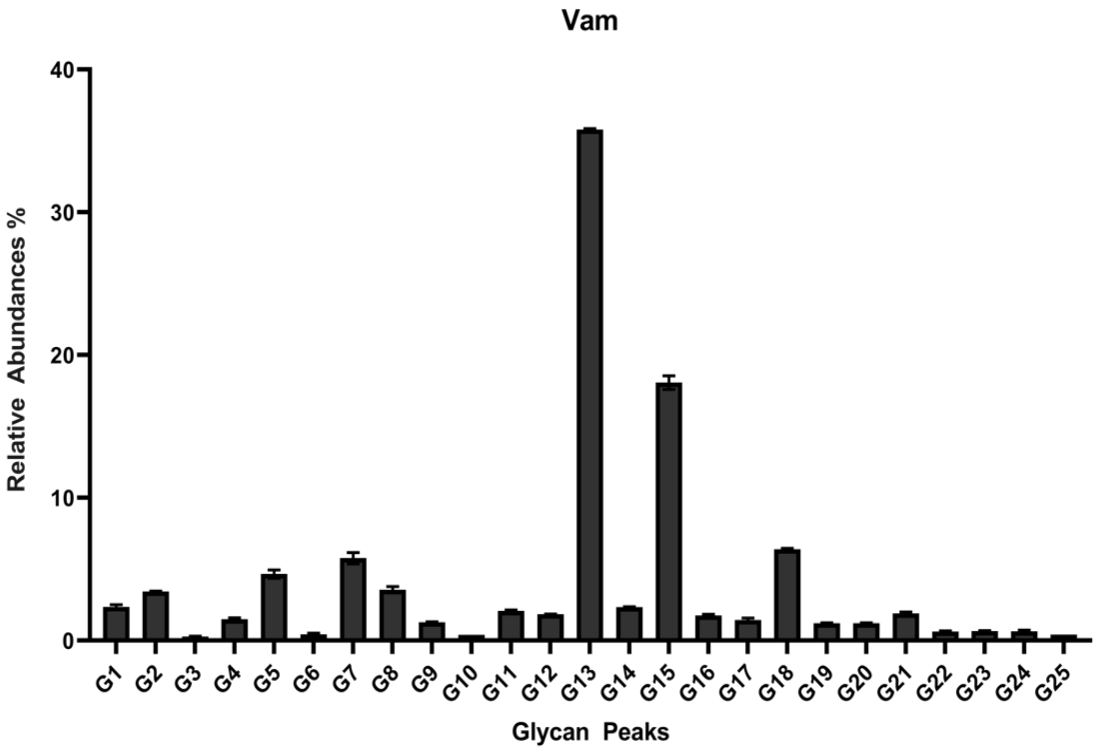


Figure S4. The FLD chromatogram of the *N*-glycan peaks of the snake venom *Vam* (A) and, the relative abundances of the *N*-glycan peaks (B).

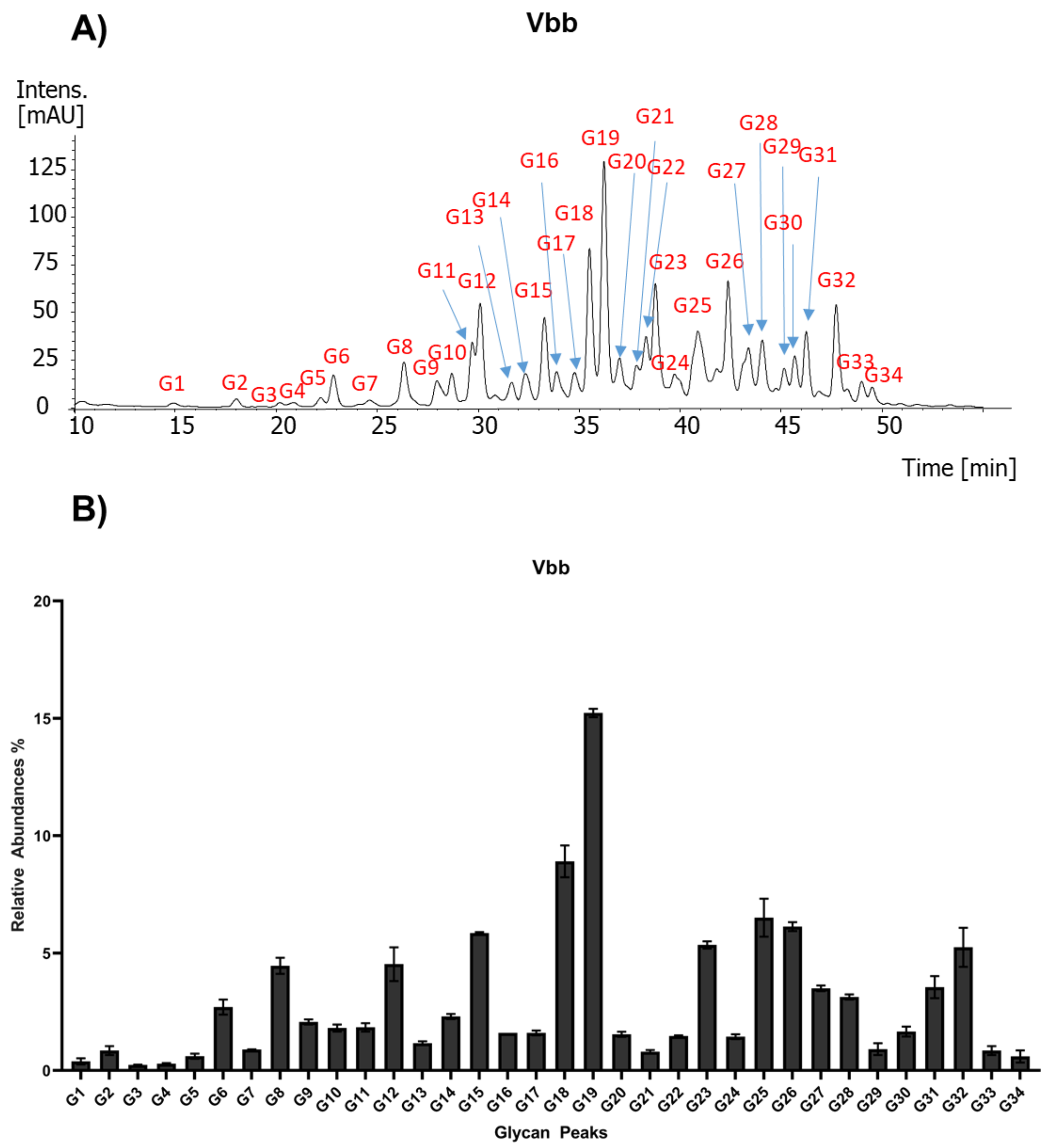


Figure S5. The FLD chromatogram of the *N*-glycan peaks of the snake venom *Vbb* (A) and, the relative abundances of the *N*-glycan peaks (B).

7. Appendix I: Supporting Information Figures

Part II

Fig S6. MALDI-TOF-MS spectra of whole free p of Ml, Mx, Vaa, Vam, Vbb, and Wa using DHAP in the mass range of 5 – 60 kDa. A comparison of ML, MX, Vaa, Vam, Vbb, and Wa crude venoms proteins composition in the mass range of 5 kDa to 60 kDa.

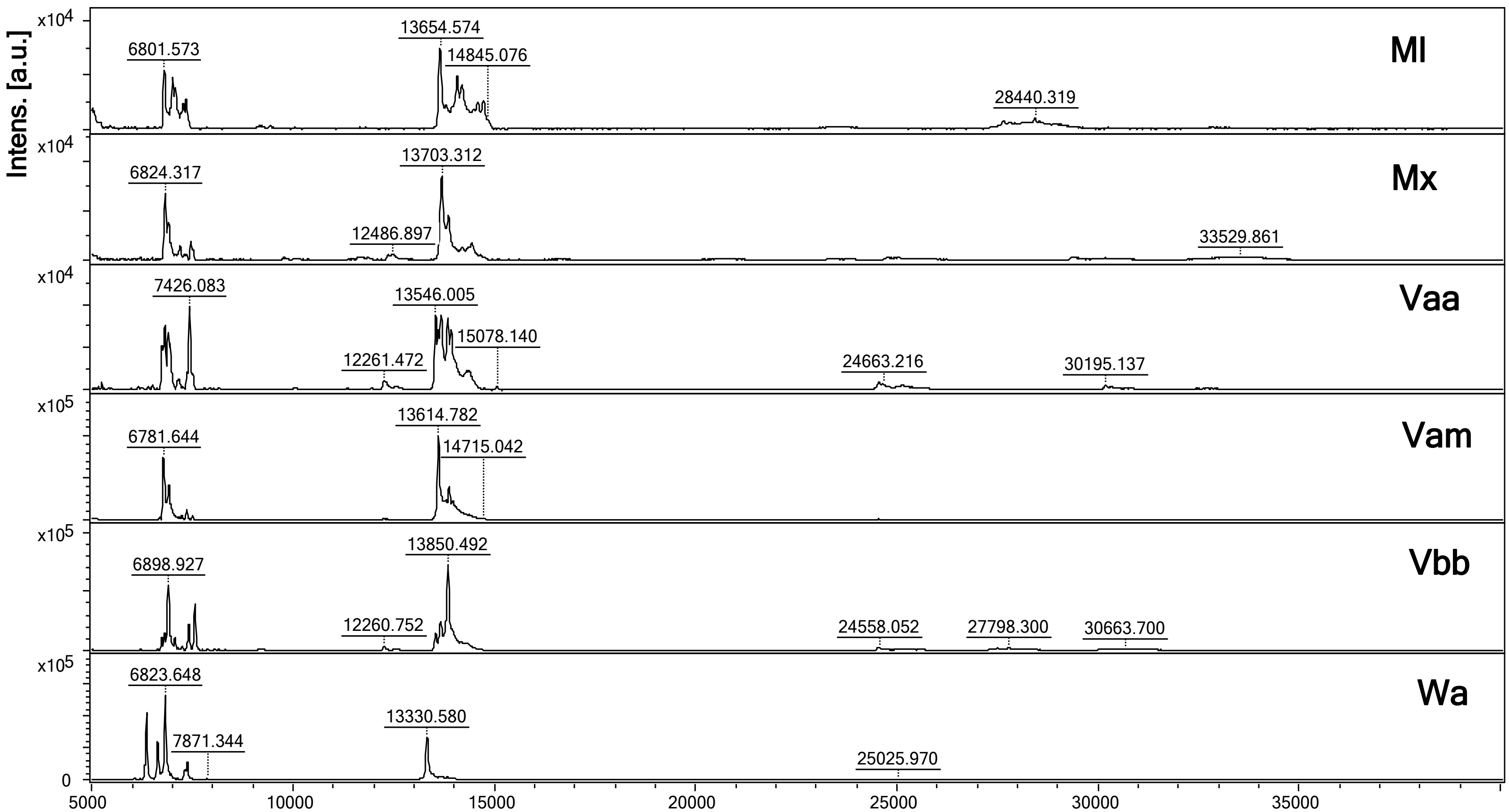


Fig S7. MALDI-TOF-MS spectra of whole free peptides of Ml, Mx, Vaa, Vam, Vbb, and Wa, using HCCA in the mass range of 0.5 - 5 kDa. A comparison of ML, MX, Vaa, Vam, Vbb, and Wa crude venoms peptides composition in the mass range of 500 Da to 5 kDa.

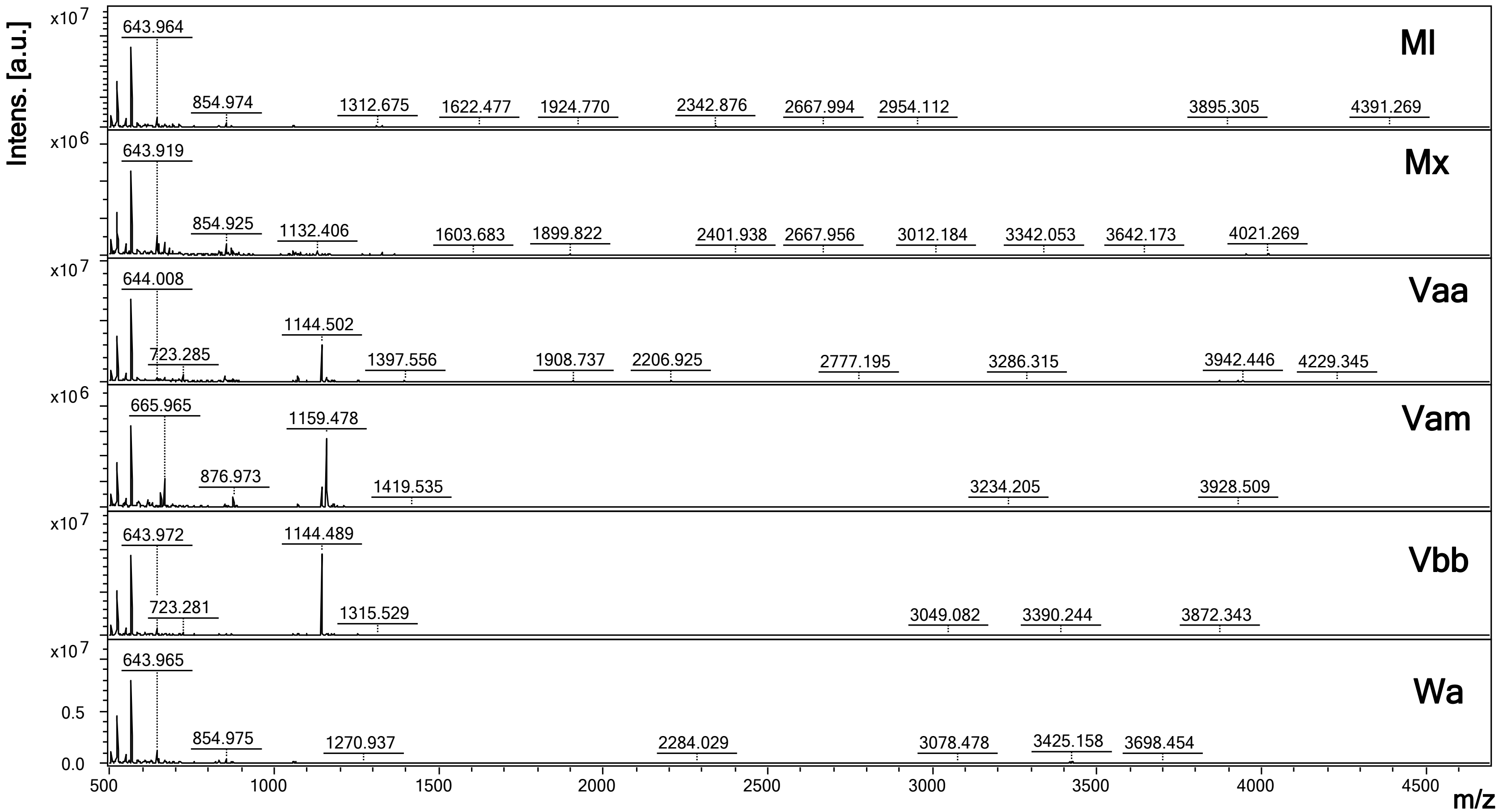


Figure S8. A comparison of the peptides matched mass peaks values of ML, MX, Vaa, Vam, and Vbb crude venoms.

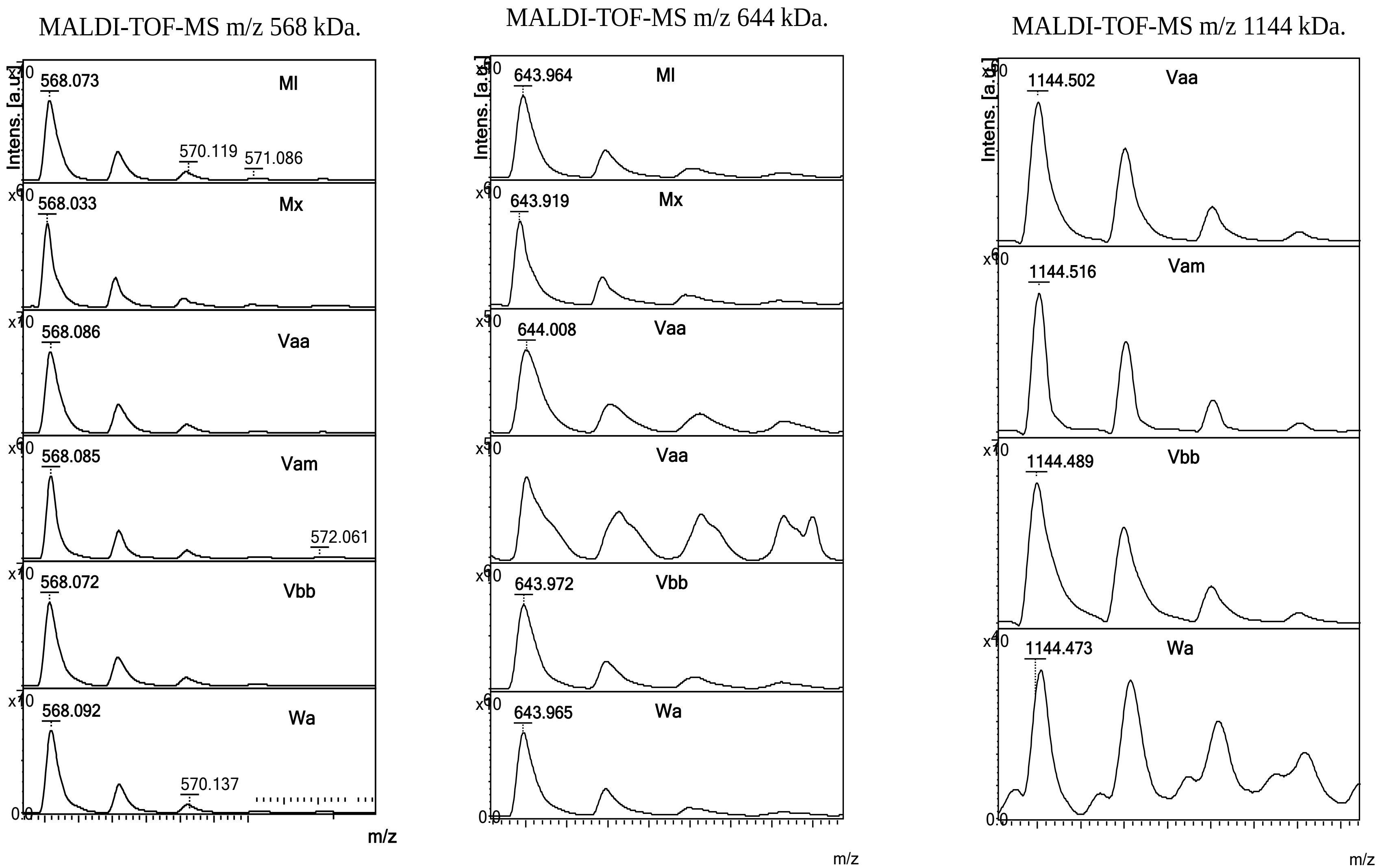


Fig S9. MS/MS m/z 568 Da. Comparison of shared peptidomes subtypes per protein family identified in *M. lebetina Ml, M. xanthina Mx, V. a. ammodytes Vaa, V. a. montandoni Vam, V. b. berus Vbb and W. aegyptia Wa*, crude venoms.

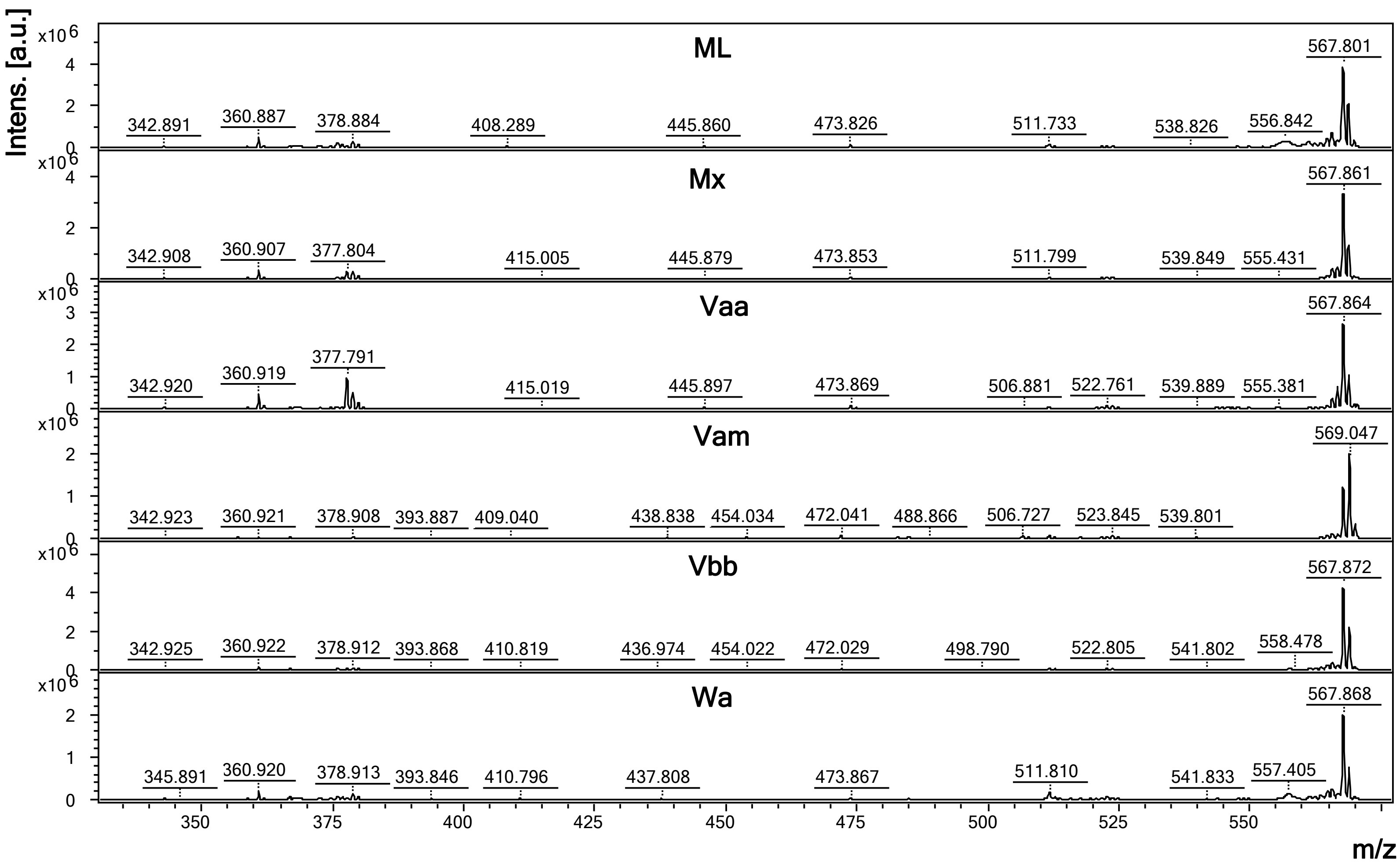


Fig S10: MS/MS m/z 586 Da. A Comparison of shared peptidomes subtypes per protein family identified in M. lebetina Ml, M. xanthina Mx, V. a. ammodytes Vaa, V. a. montandoni Vam, V. b. berus Vbb and W. aegyptia Wa, crude venoms.

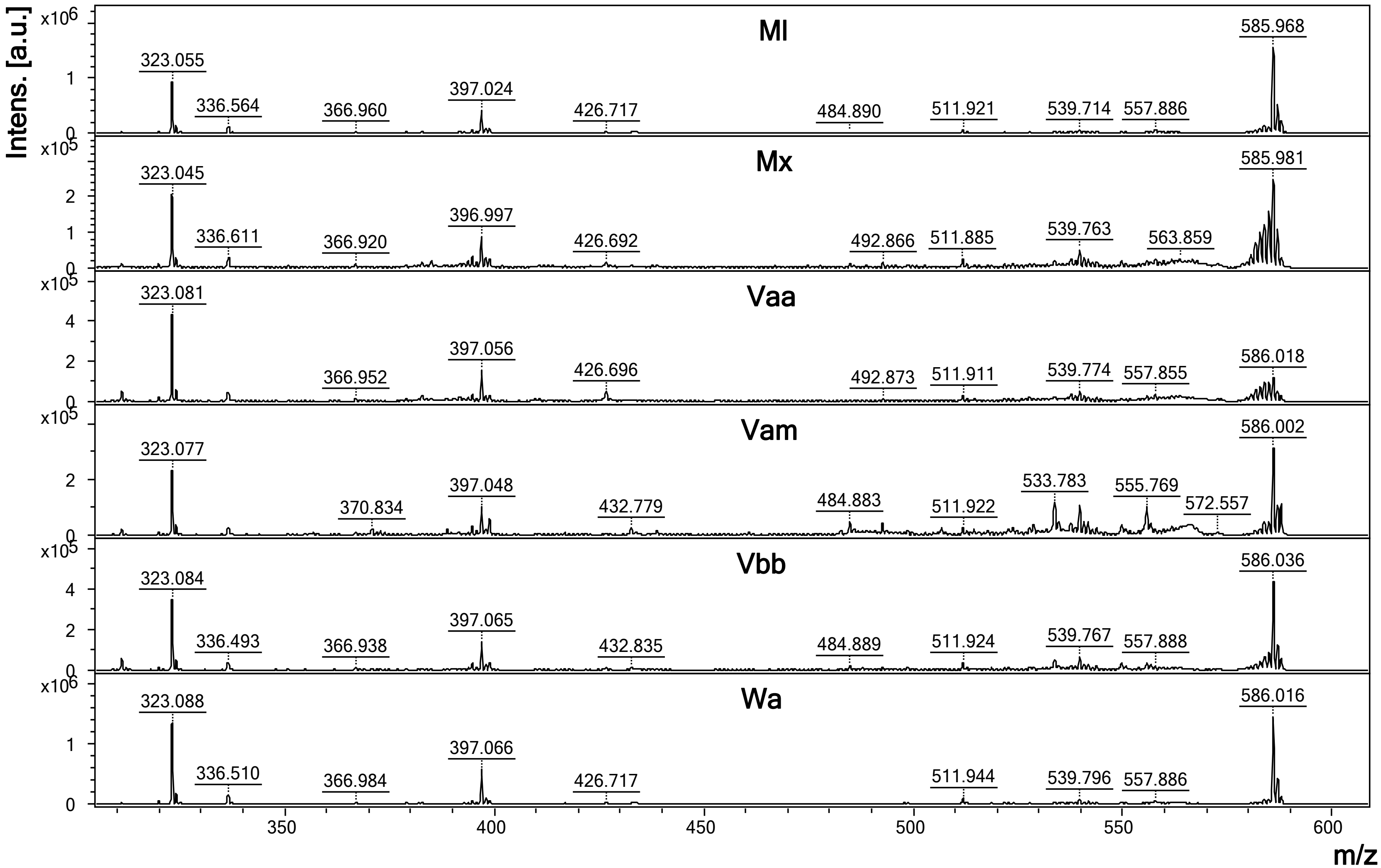


Fig S11: MS/MS m/z 644 Da. A Comparison of shared peptidomes subtypes per protein family identified in M. lebetina Ml, M. xanthina Mx, V. a. ammodytes Vaa, V. a. montandoni Vam, V. b. berus Vbb and W. aegyptia Wa, crude venoms.

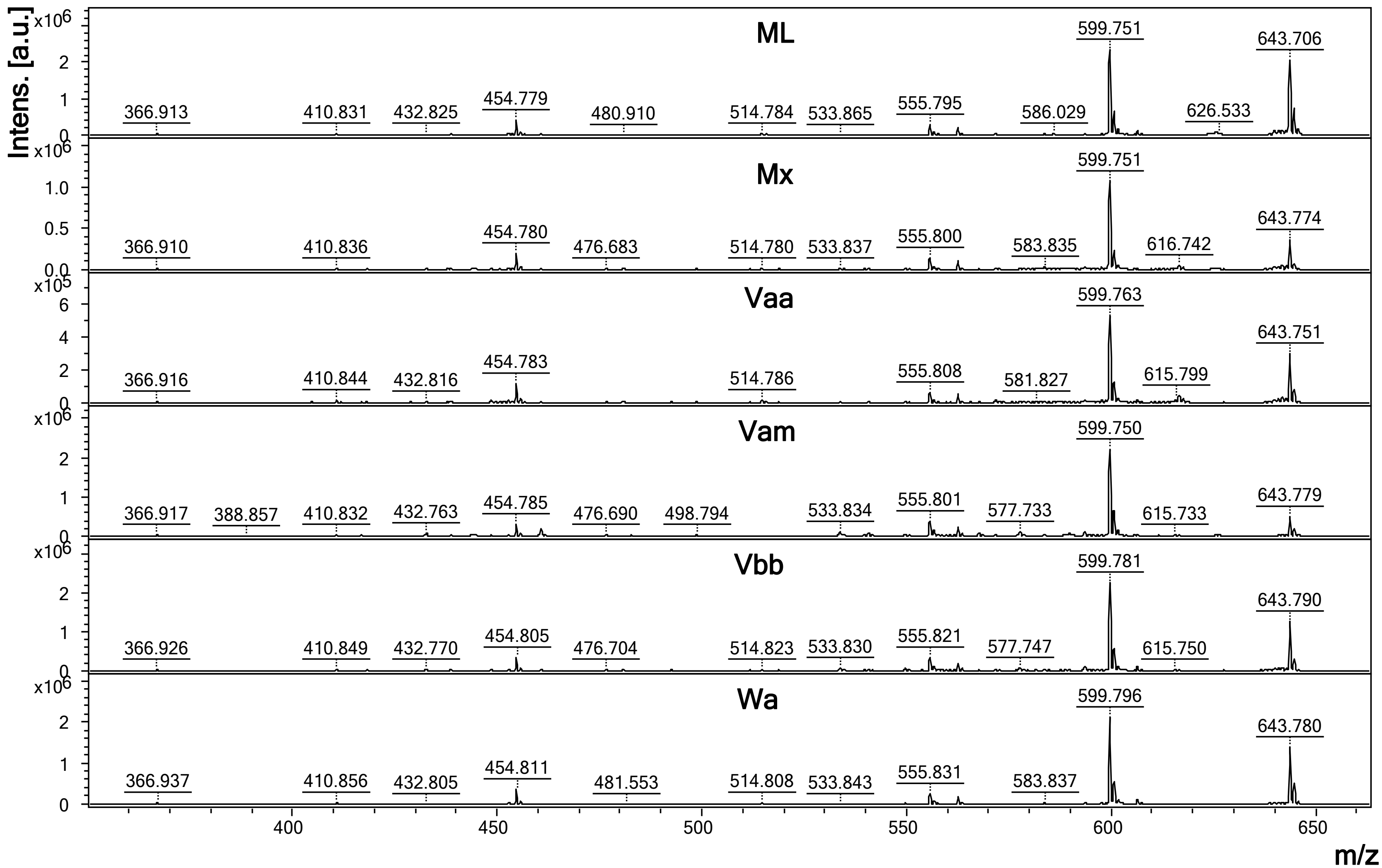


Fig S12: MS/MS m/z 855 Da. A Comparison of shared peptidomes subtypes per protein family identified in M. lebetina Ml, M. xanthina Mx, V. a. ammodytes Vaa, V. a. montandoni Vam, V. b. berus Vbb and W. aegyptia Wa, crude venoms.

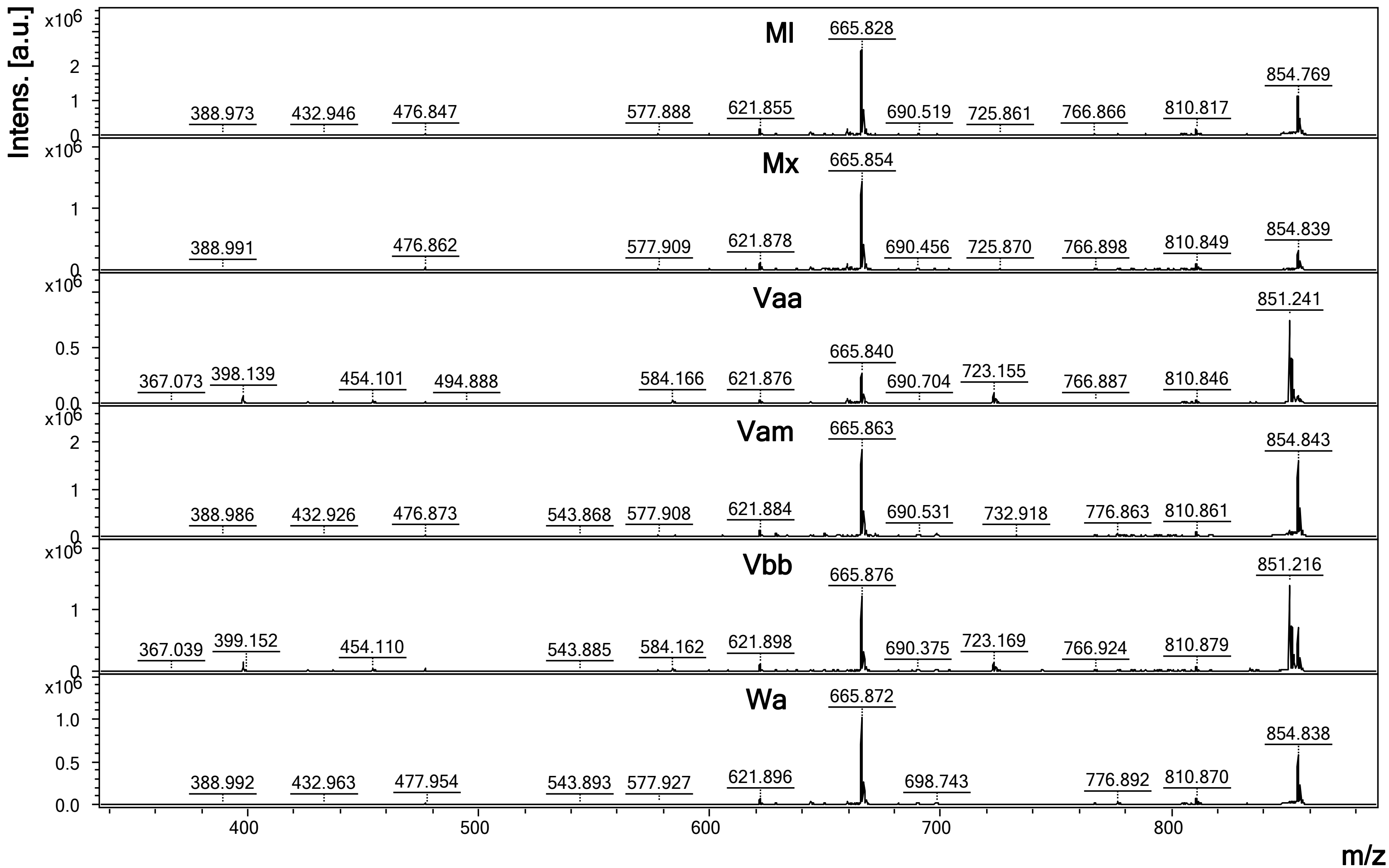


Fig S13: MS/MS m/z 1060 Da. A Comparison of shared peptidomes subtypes per protein family identified in M. lebetina Ml, M. xanthina Mx, V. a. ammodytes Vaa, V. a. montandoni Vam, V. b. berus Vbb and W. aegyptia Wa, crude venoms.

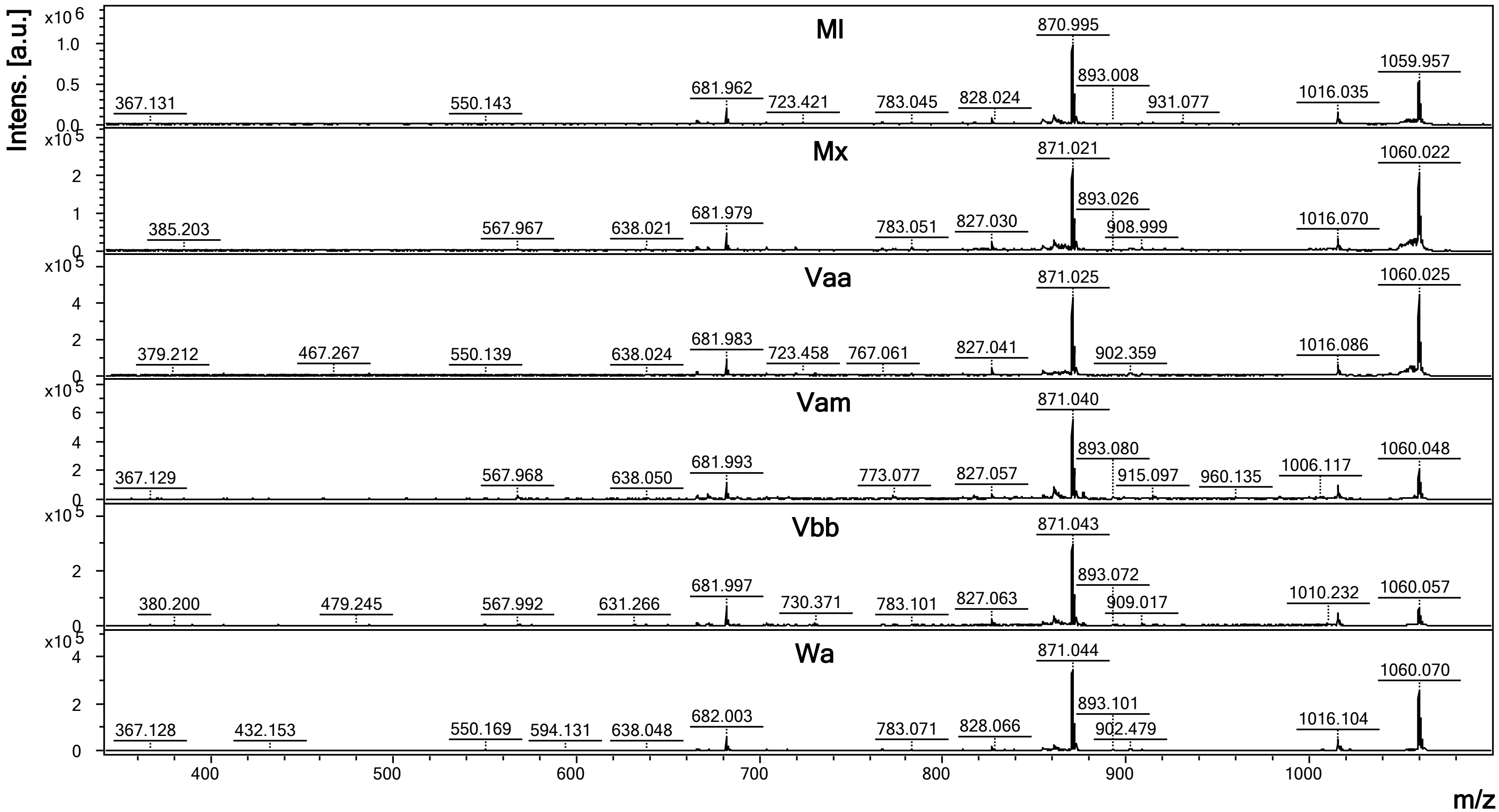


Fig S14: MS/MS m/z 1066 Da. A Comparison of shared peptidomes subtypes per protein family identified in M. lebetina Ml, M. xanthina Mx, V. a. ammodytes Vaa, V. a. montandoni Vam, V. b. berus Vbb and W. aegyptia Wa, crude venoms.

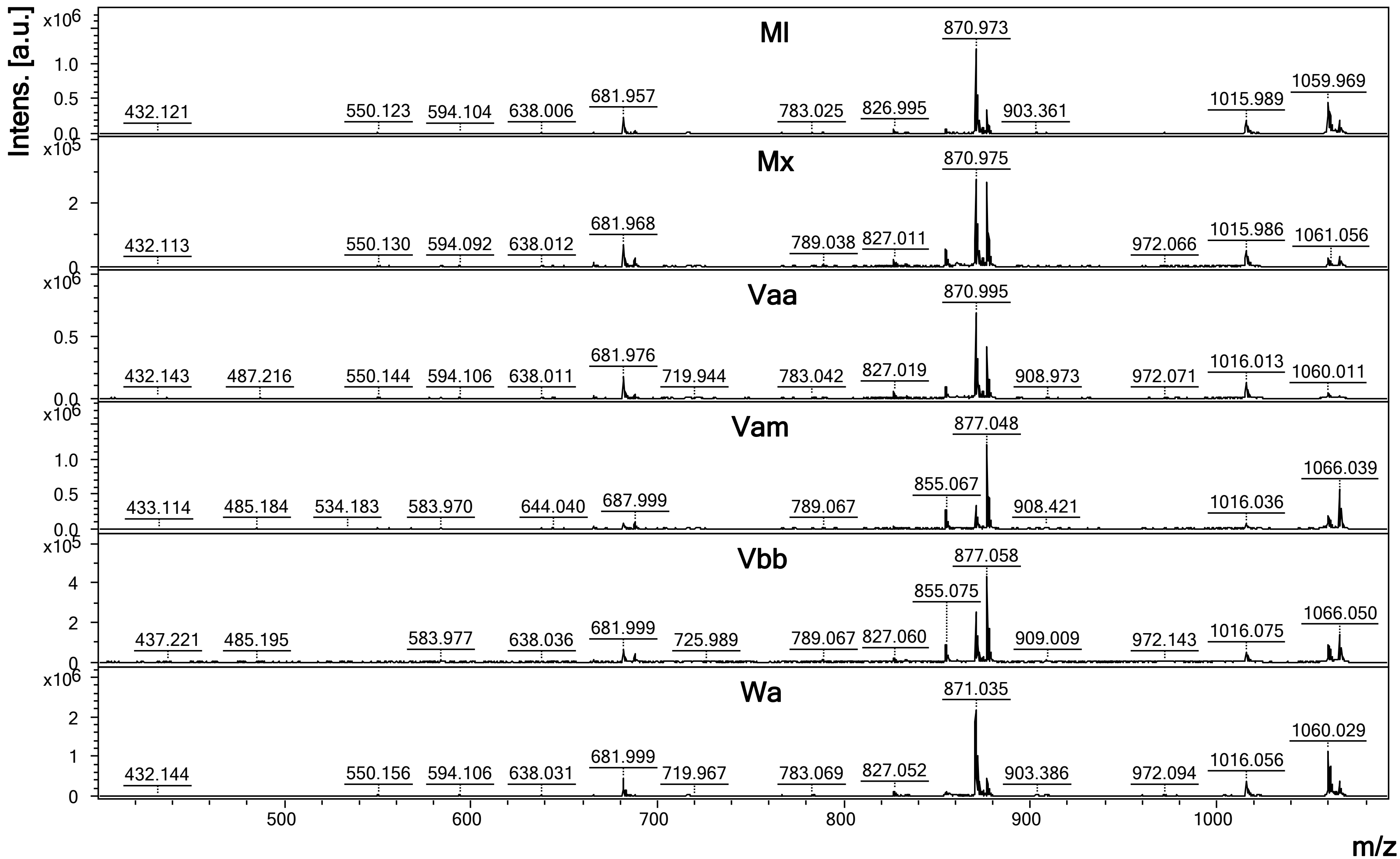


Fig S15: MS/MS m/z 1144 Da. A Comparison of shared peptidomes subtypes per protein family identified in M. lebetina Ml, M. xanthina Mx, V. a. ammodytes Vaa, V. a. montandoni Vam, V. b. berus Vbb and W. aegyptia Wa, crude venoms.

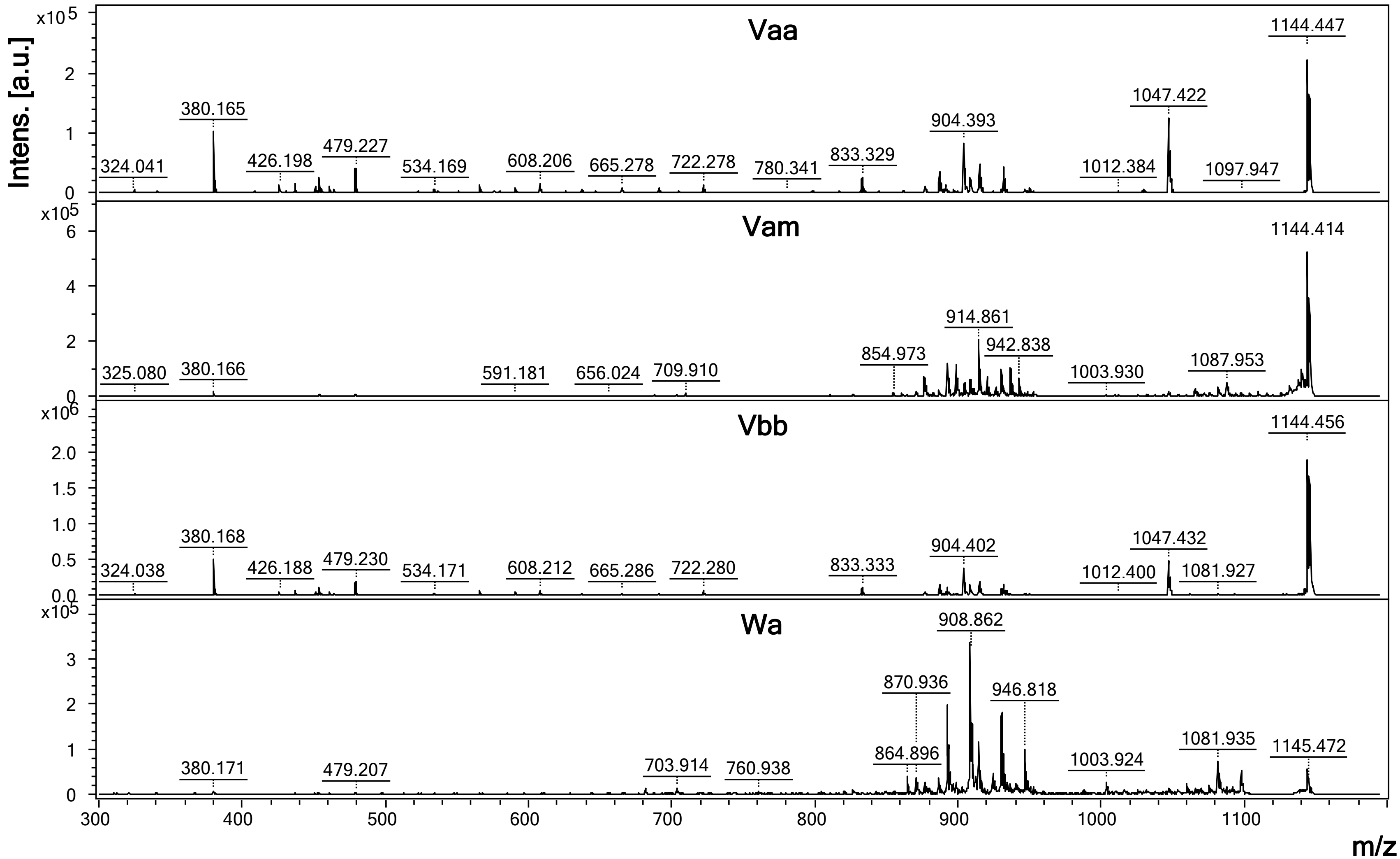
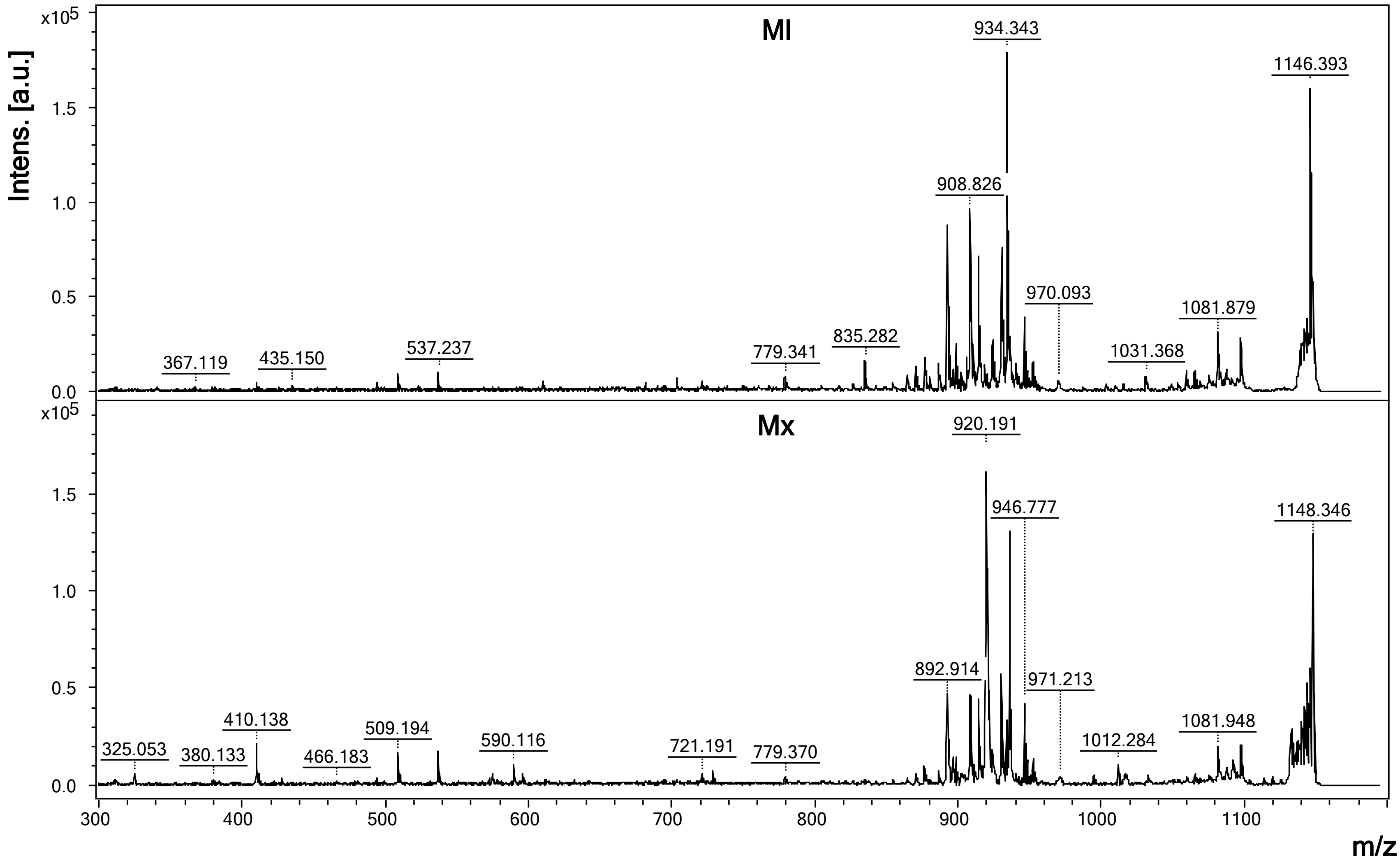


Fig S16: MS/MS m/z 1145 Da. A Comparison of shared peptidomes subtypes per protein family identified in M. lebetina Ml, M. xanthina Mx, V. a. ammodytes Vaa, V. a. montandoni Vam, V. b. berus Vbb and W. aegyptia Wa, crude venoms.



8. Appendix II: Supporting Information Tables

Part I

Table S1. A list of the identified protein groups for crude snake venoms collected from, ML= Macrovipra lebetina obtusa, Mx = Montivipera xanthina, Vaa = Vipera ammodytes ammodytes, Vam = Vipera ammodytes montandoni, Vbb = Vipera berus berus.

											LFQ intensity														
Majority protein IDs	Number of	Peptides	Razor + unique	Sequence	Unique + razor	Unique sequence	Mol. weight [kDa]	Sequence length	Score	Intensity	Ml1	ML2	Ml3	Mx1	Mx2	Mx3	Vbb1	Vbb2	Vbb3	Vam1	Vam2	Vaa1	Vaa2	Vaa3	Peptide sequences
A0A077L6L4	3	10	2	13.7	1.6	1.6	57.38	505	11.607	9E+08	7E+07	1E+08	1E+08	0	0	0	1E+08	1E+08	8E+07	0	0	0	0	0	EGWYANLGPMR;EGWYANL
A0A0A1WD34	1	2	1	13.3	6.2	6.2	13.6	113	6.2344	4E+08	0	0	0	7E+07	1E+08	5E+07	1E+07	2E+07	0	0	0	0	0	0	FCTEQVK;TTDNQWLR
A0A0A1WDS1;A0A0A1WCD4	2	2	2	20.3	20.3	20.3	18.48	158	16.031	4E+08	0	0	0	0	0	0	6E+07	5E+07	0	3E+07	0	0	0	0	SEWSDGSSVSYDNLHK;TWF

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FVTAGTV CRPAR;L KPGAEC	GSHLASI HSSEEEA FVSK;SVS	GYLEWVT LSCEDK; GYLEWVT	AQYCISK; FCTEQAN GGHLVSI	AWNEGT NCFVFK; DGVIWM	AWSDEP NCYVAK; DCHWGW
0	0	0	0	9E+07	0
0	0	0	0	0	0
0	0	0	4E+08	0	0
0	0	0	0	0	0
0	0	0	3E+08	2E+07	0
0	0	0	0	1E+09	0
6E+07	3E+07	0	4E+08	3E+08	0
6E+07	3E+07	0	4E+08	2E+08	0
0	3E+07	7E+07	4E+08	3E+08	2E+08
0	0	5E+07	5E+08	5E+08	4E+08
0	3E+07	1E+08	6E+08	4E+08	2E+08
0	4E+07	5E+08	3E+08	1E+08	7E+08
0	3E+07	1E+09	3E+08	2E+08	2E+09
0	0	3E+08	1E+08	5E+07	3E+08
3E+08	4E+08	3E+09	9E+09	7E+09	9E+09
23.318	19.64	80.196	98.838	50.929	110.72
614	150	156	158	148	150
68.74	17.12	17.67	18.08	17.04	17.46
8.5	16.7	27.6	30.4	8.8	30
8.5	16.7	32.7	55.1	33.1	37.3
9.9	16.7	32.7	55.1	33.1	37.3
3	2	4	7	4	6
4	2	4	7	4	6
1	9	1	9	1	5
A0A0B4U 9L8	A0A0C5D GP5;K9JD K6;K9JDF 6;K9JBV3; A0A1L8D6 F1;Q7T2Q 0;Q8JIV8; A0A194AT	A0A0C5D KK6	A0A0C5D KL1;W5X CJ6;B4XS Z0;B4XSY 9;K9JDF2; K9JBV0	A0A0C5D M02	A0A0C5D QX8

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A0A0C5E 3E9	1	3	2	31	26.6	26.6	18.03	158	16.885	6E+08	0	5E+07	3E+07	0	1E+08	2E+08	0	0	0	0	0	0	0	0	FCTEQAN GGHLVSI ENVGEAN
A0A194A RW5;A0A 194AQ13; F8S109;A 0A6H0QW 67;A0A6H 0QT00;J3 S831;A0A	9	3	1	8.2	3.8	3.8	66.86	599	6.4597	6E+07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	LHSWVE CESGEC CQQCR;Q
A0A1I9KN L5	1	5	5	20.3	20.3	20.3	28.88	261	31.476	3E+09	0	0	0	2E+08	1E+08	2E+08	9E+06	9E+06	0	3E+08	2E+08	7E+07	1E+08	0	ASYALPP R;DNDIML IK;GDSG
A0A1I9KN M0	1	11	2	64.4	20.5	12.6	26.52	239	34.321	2E+08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	DFVYGQ GASPANA VVGHYTQ
A0A1I9KN N1;K9JBU 0	2	3	1	18.2	8.4	8.4	17.4	154	14.747	2E+09	0	0	0	5E+08	4E+08	4E+08	0	0	0	0	0	0	0	0	GYLEWVT LPCGDK; TWEDAE
A0A1I9KN N4	6	7	7	26.5	26.5	4.7	28.17	257	54.274	1E+10	0	0	0	0	0	0	8E+08	4E+08	5E+08	8E+08	5E+07	2E+09	1E+09	1E+09	AAYPWLL ER;CGQP GNPGVYT

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A0A1I9KNP1	1	6	6	35.1	35.1	13.5	17.51	148	97.328	1E+09	0	0	0	1E+08	2E+08	1E+08	0	0	0	0	0	0	0	0	AWSDEPNCYGAK;FCTEEVK;
A0A1I9KNP6	1	4	3	27.9	21.4	21.4	17.63	154	21.44	1E+09	0	0	0	5E+07	4E+07	5E+07	0	0	0	0	0	0	0	0	ANFVAELVTLTK;ANFVAELVT
A0A1I9KNR4;A0A1I9KNR7;Q3BK13	3	6	4	35.2	28.1	18.8	13.98	128	91.394	4E+09	7E+08	3E+08	9E+08	2E+08	2E+08	6E+07	0	0	0	0	0	0	7E+07	1E+08	AMLDGLNDYCTGISSDCPR;F
A0A1I9KNR6	1	8	8	18.1	18.1	13.5	68.39	607	114	6E+09	0	0	0	7E+08	5E+08	7E+08	7E+06	4E+07	0	0	0	0	0	0	HDNAQLLTAIDFDR;HDNAQLL
A0A1I9KNS2	1	3	2	26.4	19.6	11.5	17.22	148	7.217	4E+08	3E+08	0	0	0	1E+08	5E+07	0	0	0	0	0	0	0	0	AWSDKPNCYVAK;EEMNWE
A0A1I9KNS3	27	6	5	11.3	8.2	8.2	53.07	478	33.881	1E+09	0	0	0	0	0	0	0	0	0	0	0	1E+09	6E+07	2E+08	LGEHCVSGPCCR;NPQCILNK;

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A0A1I9KN T0;A0A1I9 KNS1	3	6	6	13.2	13.2	8.2	53.48	478	53.275	4E+09	4E+07	8E+07	1E+08	2E+08	2E+08	3E+08	3E+08	1E+08	7E+07	2E+07	0	5E+07	0	0	GDWNDD YCTGISS DCPR;GD
A0A1J0CZ M6	1	3	2	33.1	16.9	10.1	17.14	148	12.36	6E+07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	AWNEGIN CFVFEIAK ;DGVIWM
A0A1J0CZ M7;B4XT0 5	3	10	10	49.3	49.3	22.3	17.29	148	82.467	4E+09	0	0	0	3E+08	3E+08	3E+08	0	0	0	0	0	0	0	0	ADLVWIG LR;ADLV WIGLRDF
A0A1J0CZ M8;A0A1J 0CZM4	4	4	4	23.4	23.4	14.6	18.05	158	29.335	2E+09	4E+07	1E+08	5E+07	3E+08	2E+08	2E+08	1E+08	2E+08	0	0	0	0	1E+08	0	EGESQM CQGLAK;I IYVNWK;S
A0A1S5Q JK2;A0A1 S5QK73;A 0A1S5QK N1;A0A1S 5QJJ1	10	6	6	54.8	54.8	9.7	10.38	93	49.748	1E+10	0	0	0	1E+08	9E+07	1E+08	2E+08	1E+08	0	3E+09	8E+08	3E+08	4E+08	4E+08	EFIYGGC R;EFIYGG CRGNAN
A0A1Y0DI B4	5	3	1	11.9	5	5	28.57	260	7.4711	9E+07	0	0	0	2E+07	1E+07	2E+07	9E+06	0	0	0	0	0	0	0	FFCLSSK; IMGWGTI SSTK;SIIA

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A0A6B2F4 F1	1	2	1	15.8	10.4	10.4	26.55	240	6.316	9E+07	0	0	0	2E+07	3E+07	1E+07	0	0	0	0	0	0	0	0	DFTFGVG ANSPNAV TGHFTQI
A0A6B7F MP6	3	6	1	31	6.3	6.3	18.08	158	8.4285	1E+09	0	0	0	0	0	0	2E+08	1E+08	0	2E+08	6E+06	2E+08	0	3E+08	AQYCISK; CGDDYPF VCK;NCF
A0A6B7F MQ9	12	8	8	17.5	17.5	17.5	64.31	553	56.958	3E+08	0	1E+06	0	2E+06	0	2E+06	2E+07	2E+07	0	0	0	0	0	0	FTAYAIN GPPVEK; HQGLPES
A0A6B7F MR4	4	14	14	28.2	28.2	25.9	68.84	609	112.34	7E+09	6E+07	1E+08	8E+07	5E+08	5E+08	6E+08	0	0	0	9E+07	0	6E+07	0	0	ASECDLP EYCTGQS ADCPTDH
A0A6B7F MR5	2	9	8	27.2	27.2	24.4	36.84	324	61.287	3E+10	2E+08	6E+08	3E+08	3E+09	2E+09	3E+09	4E+06	0	0	4E+08	0	7E+07	0	0	ENDVPIP CAPEDIK; HCVDVTT
A0A6B7F NN4;R4N NL0	23	19	19	36.4	36.4	17	68.35	616	178.87	2E+10	1E+08	3E+08	2E+08	5E+08	5E+08	6E+08	8E+08	6E+08	2E+08	3E+08	1E+08	3E+08	3E+08	3E+08	AGTVCRP ANGECD VSDVCTG

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HPCAQP HLPAFYT K;IYDYSV	IDRIGSVS GLGCNK;I GSVSGLG	CAGTLIN QEWVLTA AHCNGK;	EANLSPG AVDMIGD LLNEDSG	ATVAEDS CFEENLK; AVCGNEI	CIYDHSP R;DFVYG QGASPA
0	0	3E+08	1E+08	0	0
2E+08	0	2E+09	0	0	0
0	0	1E+09	1E+08	0	0
0	0	9E+07	0	0	0
2E+08	5E+06	2E+09	0	2E+07	0
0	0	1E+09	0	4E+07	0
2E+08	0	3E+09	4E+08	9E+07	0
2E+08	0	3E+09	5E+08	9E+07	0
0	8E+07	1E+09	1E+08	0	2E+07
0	1E+08	1E+09	9E+07	0	2E+07
0	9E+07	1E+09	1E+08	0	2E+07
0	0	1E+08	2E+08	5E+06	0
0	0	3E+08	2E+08	7E+06	0
0	0	9E+07	1E+08	0	0
3E+09	7E+08	5E+10	9E+09	6E+08	9E+07
30.569	14.203	120.09	144.46	58.618	6.352
260	137	257	504	613	224
28.8	15.04	28.32	57.1	69.22	25.04
11.9	10.2	0	15.7	13.5	3.6
14.6	10.2	32.3	34.9	15.8	3.6
14.6	10.2	32.3	34.9	24	37.9
4	2	9	14	6	1
4	2	9	14	15	6
1	5	25	1	5	1
A0A6B7F PJ0	A0A6B7F PT0;A0A1 J0CZN1;A 0A1I9KNP 4;A0A6B7 FNM6;A0A 1I9KNP8	A0A6B7F QF8	A0A6B7F RF2	A0A6B7F RK6	A0A6G5Z UA3

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ASGNPILLNK;QVPVVQAYAFG	FLNAGTICQYAR;GDDMNDY	FFCLSSK;IILGVHSK;IMGWGTI	FTFGGCAGNANNFK;PVTDLC	CQRVHPELPAK;EYTMWDK;F	FFCLSSK;VIGGDECDINEHR
0	2E+09	0	0	4E+09	0
0	2E+09	0	0	0	0
0	9E+08	0	0	4E+09	0
0	0	0	0	0	0
1E+07	0	0	0	4E+09	0
0	0	0	0	7E+09	0
1E+07	0	0	0	4E+09	0
7E+06	0	0	0	4E+09	0
8E+06	0	0	0	0	0
0	0	0	0	0	0
1E+07	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
8E+07	4E+09	3E+08	3E+07	3E+10	9E+06
8.1794	7.6247	6.287	22.322	19.85	6.5192
298	82	260	192	257	235
32.56	9.029	28.93	21.75	28.1	25.11
4	14.6	8.5	24	9.3	5.5
4	14.6	8.5	24	12.1	5.5
14.4	52.4	25	24	29.2	8.5
1	1	1	3	3	1
4	5	6	3	9	2
1	6	4	1	99	9
A0A6G5ZVN1	A0A6G5ZVR7;A0A1J0CZN0	A0A6G5ZVX7;A0A6G5ZUR6;A0A1I9KNP0;A0A6G5ZUW7	A0A6G5ZW53	A0A6G5ZW78	A1E2S3;A1E236;Q9PRY9;A1E238;Q6IWF1;Q9YGS1;Q90Z47;A1E2S2;A1E237

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A8CG87;A8CG78	2	5	2	24.6	10.1	0	15.59	138	30.601	1E+10	0	0	0	2E+09	3E+09	9E+08	0	0	0	0	0	0	0	0	AVCECDRVAAICFR;CCFVHDC
B4XSY6;B4XSY5	2	4	3	25	20.5	11.5	17.74	156	30.121	6E+09	0	0	0	1E+09	1E+09	9E+08	0	0	0	0	0	0	0	0	EANFVAELVSQNIK;FCTEQAN
B4XSY8;W5XDM0;B4XSY7;B4XSZ1	4	4	3	31.3	26	0	15.31	131	22.152	5E+09	0	0	0	7E+08	5E+08	6E+08	0	0	0	0	0	0	2E+07	2E+07	CFGLDQK;DQDCLPGWSFYE
B4XT06	1	6	1	38.4	9.6	9.6	15.04	125	8.8273	3E+08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	AWSDEPNCFVAK;DCHMGW
B4XT08;B4XT03;B4XT02;B4XT01;B4XT00	5	2	1	14.2	9.5	9.5	16.6	148	5.9708	1E+08	0	0	0	3E+07	2E+07	9E+06	7E+06	0	0	0	0	0	0	0	SWADAEK;VWNQCDWGWSN
B6EWW6;A0A6B2F4S9;A0A6B2F1E9;T1DNX8;A0A0K8S2L4;B6EWW5;A0A1W7R	7	2	2	2.4	2.4	2.4	106.1	928	15.377	3E+07	0	0	0	5E+06	0	5E+06	0	0	0	0	0	0	0	0	AQIIDDAFNLAR;DLIVPNDVYR

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B7FDI1;B7FDI0	39	13	13	65.3	65.3	21.3	26.51	239	154.12	1E+10	0	0	0	4E+08	4E+08	3E+08	1E+09	1E+09	3E+08	5E+08	0	6E+07	2E+07	0	CGENIYMSTSPMK;CILSHSP
C0LZJ5	1	3	2	20.5	12.2	12.2	22.63	205	13.133	7E+08	2E+08	2E+08	2E+08	0	0	0	0	0	0	0	0	0	0	0	FVTAGTECR;LHSWVECESGE
C3W4R6;B6CQR5;Q98996;B5U6Z2	5	9	3	60.1	36.2	36.2	15.55	138	20.418	5E+08	3E+08	1E+07	1E+08	0	0	0	0	0	0	0	0	0	0	0	AVCECDRVAAICFGENMNTY
D8MIA2;D8MIA3	2	3	2	6.6	3.9	3.9	28.2	257	12.237	6E+07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	FFCLSNK;SRTLCAGIPR;TLCA
E0Y418	1	4	4	21.9	21.9	21.9	28.7	260	31.183	1E+09	6E+07	8E+07	5E+07	2E+08	2E+08	8E+07	0	0	0	3E+07	0	0	0	0	AYGGLPEK;HPCAQPQLPAFY
E0Y419;A0A1I9KNR8	2	3	2	12.8	10.1	10.1	28.3	257	95.171	4E+09	2E+08	3E+08	3E+08	3E+08	2E+08	3E+08	0	0	0	3E+07	0	9E+07	0	0	AAHPWLPAQSR;FFCLSSK;TL

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CAGTLIN QEWVLTA AHCNGK;	FCYLPAE PGECNAY MPSFYYD	FFCLSSK; IMGWGSI TSPK;SYT	CVDVNTA Y;LTPGS QCADGE	FLTNFKP DCTLIR;H CVDVTTA	LHSWVE CESGEC CDQCR;T
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
2E+08	0	0	0	0	0
3E+08	3E+08	0	0	0	0
4E+08	3E+08	0	0	0	0
0	0	0	8E+07	1E+08	0
0	0	0	8E+07	9E+07	0
0	0	0	1E+08	0	0
0	0	4E+07	5E+07	2E+07	0
0	0	8E+07	2E+07	3E+07	0
0	0	0	0	0	0
2E+09	1E+09	2E+08	8E+08	4E+08	9E+07
9.4364	7.2687	7.6318	12.04	12.706	24.299
257	95	258	568	610	607
28.22	10.43	28.5	64	69.13	68.43
4.3	27.4	4.3	3	0	2.6
4.3	27.4	4.3	3	3.6	2.6
25.7	35.8	9.7	6.3	10.2	5.4
1	1	1	1	2	1
7	3	3	3	6	2
1	1	1	9	18	1
E5AJX2	E5AJX3	E5L0E3	E9JG27;Q2UXR0;E9JG35;A0A0A1WDV1;E9KJX7	E9JG34;E9JG58;Q2UXQ1;E9KJZ2;Q2UXQ9	E9JG41

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E9JG51;E9KNB4;A0A6H0QV24	3	2	2	6.6	6.6	3.1	31.9	290	6.4181	1E+08	0	0	0	2E+07	3E+07	2E+07	0	0	0	0	0	0	0	0	IPCAPEDEK;LGNSYAYCRK
E9JG53;E9JG44	2	2	1	6.5	6.5	6.5	27.7	247	6.3253	4E+08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	FLTNFKPDCTLIR;FLTNFKPD
E9JG63;E9JGB0;A0A0A1WCJ8	4	6	2	9.4	3.5	0	56.95	511	13.565	7E+08	0	5E+07	3E+07	1E+08	5E+07	6E+07	0	0	0	0	0	0	0	0	CVDVNTAY;INVLPEAK;LYCFD
E9JG93;E9JG87	6	3	2	7.2	5	1.8	61.36	541	17.567	8E+07	0	2E+07	2E+07	0	0	0	0	0	0	0	0	0	0	0	KGTDYFYCRK;LHSWVECES
E9JGA1;E9JG79;E9JG90	6	3	3	6.7	6.7	4.5	51.7	462	21.94	4E+08	0	2E+07	2E+07	4E+06	0	5E+06	8E+07	7E+07	0	0	0	0	0	0	ESVGIVQDHSK;NIPCAPQDVK
E9JGC3;E9JGC2	2	3	3	2.4	2.4	2.4	46.5	424	20.813	2E+08	0	0	0	0	0	0	4E+07	2E+07	0	0	0	0	0	0	GTDDFYCR;GTDDFYCRK;KG

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CPLTLYQCR;LHSWVECESGE	GEECDCGSPANCR;LHSWV	MEWYPEAAANAER;RSVNPT	AVCECDRVAAICFGENLNTYD	IPCAPQDVK;KIPCAPQDVK;R	EGWYANLGPMR;HDDIFAYE
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	7E+06	0	0	0
0	0	0	0	0	0
0	3E+07	0	0	1E+08	0
0	7E+07	0	0	1E+08	0
0	2E+07	4E+07	1E+07	0	1E+07
0	2E+07	0	0	0	0
0	2E+07	3E+07	1E+07	0	2E+07
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	2E+08
2E+07	3E+08	1E+08	4E+07	6E+08	6E+08
6.6758	72.059	7.2719	14.979	6.3529	16.37
515	553	220	137	388	504
58.27	62.61	24.74	15.32	43.05	56.89
1.7	2.4	4.5	14.6	5.2	2.6
1.7	7.1	4.5	14.6	5.2	7.9
5	9.9	15.9	48.2	7.7	18.8
1	3	1	1	1	2
2	4	4	7	3	9
10	16	13	3	4	1
E9KJY6;Q2UXQ5;Q2UXQ4;E9KJY7;E9KJY5;E9KJY4;E9KJY9;E9KJY8;E9KJY3;E9	E9KJZ6;Q2UXQ2;E9KJZ5;E9JG29	F2Q6G0;F2Q6G1;F2Q6F3;F2Q6F1;F2Q6E9;F2Q6E8;F2Q6E7;F2Q6E6;F2Q6E5	F8QN53;Q6A3C2;Q6A3A4	F8S111;J3RY90;A0A1W7RBB1;A0A0F7Z792	G8XQX1

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AVDNVLLR;WFIVENR	HDNAQLLTAIDFDGR;QCVDV	IIYVNWK;SWADAEK;WDYVN	GVPILHLIPSPFPR;LIFFDGEE	FFCLSSK;IMGWGTISTSK;TLC	FCYLPADPGR;FCYLPADPGR
0	0	0	0	0	0
0	0	0	0	1E+09	0
0	0	0	0	5E+08	0
0	0	0	0	0	3E+08
0	0	0	1E+07	1E+09	3E+09
0	0	0	0	0	0
4E+06	0	0	6E+06	2E+09	9E+07
4E+06	0	0	8E+06	2E+09	9E+07
0	6E+07	0	0	1E+09	2E+07
0	7E+07	0	0	6E+08	2E+07
0	1E+08	0	0	1E+09	2E+07
0	4E+07	0	0	0	0
0	5E+07	0	0	0	0
0	0	0	0	0	0
2E+07	1E+09	4E+07	1E+08	2E+10	7E+09
12.006	46.605	7.1308	41.939	14.37	33.502
438	601	158	368	257	90
50.38	67.33	18.18	42.12	27.89	9.831
3.4	2.7	7	20.9	4.3	28.9
3.4	4.2	7	20.9	4.3	38.9
3.4	4.2	15.8	20.9	11.3	38.9
2	3	1	6	1	3
2	3	3	6	3	4
6	6	3	22	8	4
J3RY60;T1DH21;A0A1W7RJX6;A0A194ATT6;A0A194ARU1;A0A194AQM0	J3SDW5;J3S3W1;A0A6H0QVI1;A0A6H0QSU1;A0A194ARX9;A0A194ARK5	K9JDK1;K9JCR7;K9JBU9	M9NCG3;U3TDL2;A0A1W7RH88;T2HQ78;T1DH29;Q90YA8;M9ND11;Q9YIB5;M9N	O13069;Q71QJ0;Q71QI7;O13062	P00991;A0A1S5QLZ2

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FCYLPADPGR;FIYGGCR;HTC	AAAICLGENVNTYDK;AVCEC	GEHCVSGPCCR;NSANPCCD	FLNAGTICK;GDDMNDYCTGI	FLNAGTICK;GEHCVSGPCC	FLRAGTVCK;NSGNPCCDPVT
0	0	1E+08	1E+07	0	0
1E+08	0	1E+08	1E+07	0	0
0	0	2E+08	0	0	0
0	3E+09	0	0	0	0
0	1E+10	0	0	0	0
2E+08	2E+08	0	0	0	0
4E+08	4E+07	0	3E+08	0	0
2E+08	2E+07	0	4E+08	0	0
6E+07	0	0	1E+07	0	0
9E+07	6E+06	0	2E+07	0	0
1E+08	0	0	9E+06	0	0
0	0	0	4E+08	9E+07	2E+07
0	0	0	2E+08	3E+07	2E+07
0	0	0	2E+08	0	0
1E+09	7E+10	5E+08	3E+09	2E+08	7E+07
22.264	154.67	15.899	95.715	27.868	12.189
93	122	64	64	65	64
10.33	13.64	7.023	7.009	7.108	7.005
0	28.7	23.4	31.2	24.6	23.4
8.6	86.1	23.4	68.8	24.6	23.4
37.6	86.1	42.2	87.5	56.9	68.8
2	7	2	5	2	1
5	7	5	8	8	3
2	7	1	8	1	1
P00992;A0A1S5QJJ2	P04084;A4VBF0;Q8JFG1;Q715Z8;Q6A3P1;Q10754;Q6A3N6	P0C6A5	P0C6A6	P0C6A8	P0C6A9

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FLNPGTICK;GEHCVSGPCC	HLASIEGLGK;SWVDAEK	DQDCLPGWSYFEK;FCTEEV	KPEIQNEIIDLHNSLRR;MEWY	CCFVHDCCYGK;ENLDTYNK	CCFVHDCCYGR;DATDRCCFV
1E+09	0	0	0	0	0
0	0	0	0	0	0
5E+08	0	0	0	0	0
0	0	0	0	0	0
3E+07	0	0	2E+08	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
8E+08	7E+07	8E+07	1E+08	2E+08	0
7E+08	8E+07	6E+07	1E+08	3E+08	0
9E+08	8E+07	0	1E+08	3E+08	0
1E+09	0	0	0	0	0
2E+09	0	0	0	0	0
6E+08	0	0	0	0	0
1E+10	5E+08	2E+08	2E+09	1E+09	2E+10
14.088	13.084	10.914	31.791	6.1167	83
69	107	125	220	123	122
7.67	12.13	15.16	24.7	14.16	13.83
34.8	15.9	10.4	19.1	6.5	52.5
47.8	15.9	10.4	19.1	6.5	65.6
88.4	15.9	36	25.5	15.4	65.6
2	2	1	3	1	7
9	2	5	5	2	8
1	1	1	2	216	22
P0C6B0	P0DJL4	P0DJL5	P0DMT4;A0A0A1WCN2	P0DUN1;B0LSF5;C0HJP9;T1DP65;P45881;P24027;P20476;P20474;P06859;P0062	P14420;Q6A346;Q8JFG0;Q6YCP1;Q6YCP0;Q6A352;Q7T1C6;Q6A336;Q10755;Q

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P18964;P18965	2	5	2	11.4	5.9	5.9	26.18	236	16.355	7E+06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	FPNGLDK;NEDEQIRVPR;NIRN
P24293;P24294	2	4	2	37.2	20.7	14.9	13.53	121	17.865	4E+09	0	0	0	1E+09	1E+09	0	0	1E+07	0	0	0	0	0	0	CCFVHDCCYGR;GKPLDATDR
P31854	1	8	6	61.6	53.6	40.6	15.72	138	62.876	6E+09	0	0	0	1E+08	4E+08	1E+08	5E+08	3E+09	4E+08	0	0	0	0	0	CCFVHDCCYGR;HAVWSYLSY
P81375	2	8	1	60.7	22.4	22.4	12.44	107	9.0862	5E+08	1E+08	6E+07	4E+07	2E+07	2E+07	9E+06	4E+07	1E+08	0	0	0	0	0	0	ADDKNPLEECFREDDYEEFLEI
P82475;P0DW97	6	5	1	53.6	8.2	8.2	12.57	110	9.3165	2E+09	0	0	0	4E+08	2E+08	3E+08	0	0	0	0	0	0	0	0	CSGCCTDESLK;ETLVSILQEY
P82972;Q7LZ09	2	2	2	37.8	37.8	37.8	3.944	37	16.306	1E+08	0	0	0	1E+07	3E+07	2E+07	0	0	0	0	0	0	0	0	IDRIGSHSGLGCNK;IGSHSGL

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P82981;P0DMH5;A0A2I7YS66;A0A1W7RJU2;Q8QHK2;A0A194AS18;A0A194AP79	34	2	1	15	5.6	5.6	25.41	234	41.921	5E+09	4E+08	4E+08	7E+08	0	0	0	3E+08	2E+08	2E+08	9E+07	7E+07	2E+08	2E+08	2E+08	VFDYNDWIQSIIAGNTAATCP
P83253	1	5	1	36.9	9	9	12.1	111	18.216	5E+09	7E+08	2E+09	1E+09	2E+07	2E+07	2E+07	0	0	0	0	0	0	0	0	FLNAGTICNR;GDDMNDYCT
P83469	3	3	3	73.2	73.2	73.2	4.401	41	26.503	2E+09	2E+08	2E+08	2E+08	0	0	0	0	0	0	0	0	0	0	0	LKPAGTTCWK;SCDCPLYPG;
P83942;P67863;A0A1J0CZM5	3	6	6	60.9	60.9	15.5	12.56	110	110.66	2E+10	0	0	0	3E+09	3E+09	4E+08	2E+09	2E+09	7E+08	1E+09	5E+08	1E+09	5E+08	1E+08	CSGCCTDESLK;ETLVSILQEY
P86389;A0A2D1UXN7;U5HRG7;A0A0H3U209;Q7ZTA8;Q7ZTA6;B0LSG7;B0LSG	20	2	1	14.5	8.9	0	14.19	124	37.113	5E+09	0	0	0	1E+09	1E+09	8E+07	0	0	0	0	0	0	0	0	CCFVHDCCYGK;VAAICFR
Q0NZX9	1	3	1	18.3	6.4	6.4	24.29	218	8.2957	8E+08	0	7E+07	4E+07	1E+08	1E+08	1E+08	0	0	0	0	0	0	0	0	IVSPPVCGNELLEK;LHSWVE

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Q1JRG9	1	9	9	87.7	87.7	0	7.133	65	189.48	1E+10	2E+09	1E+09	2E+09	2E+08	3E+08	3E+08	1E+08	2E+08	3E+08	0	0	1E+09	5E+08	5E+08	FLRAGTV CK;GEHC VSGPCC
Q38L02;K 9JCR1	2	3	1	12.3	7.8	7.8	17.51	154	6.088	3E+08	0	1E+08	6E+07	0	0	0	0	0	0	0	0	0	0	0	TWEDAE K;YHAWI GLR;YHA
Q3ZD74	1	5	3	29.5	18.4	18.4	24.49	217	24.462	2E+09	1E+08	4E+08	4E+08	0	0	0	0	0	0	0	0	0	0	0	SYQFSDC SK;TWAH QLVNNIIV
Q4VM07	2	8	5	16.8	9.8	8.3	68.84	614	69.903	4E+08	0	3E+07	4E+07	0	0	0	2E+07	2E+07	0	0	0	0	0	0	AGTVCRP ANGECD VSDLCTG
Q4VM08	33	13	2	26.9	7.5	0	68.71	616	41.052	5E+08	6E+07	0	0	0	0	0	0	0	0	5E+07	0	0	0	0	AGTVCRP ANGECD VSDLCTG
Q696W1	2	3	3	26.6	26.6	26.6	18.09	158	24.652	5E+09	3E+08	9E+08	1E+09	0	0	0	2E+07	3E+07	0	0	0	0	0	0	FITHFWIG LR;SEWS DGSSVSY

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Q6A363;Q6A362;Q6A361;Q6A360;P14424;P11407;P00626;Q6A364;Q6A359	10	5	5	35.8	35.8	35.8	15.35	137	46.83	2E+09	0	0	0	3E+08	4E+08	3E+08	0	0	0	0	0	0	0	0	AAAICFRK;CCFVHDCCYGNL
Q6A370;Q6A371;Q6A368;Q6A367;Q6A369	7	8	1	46.4	5.1	5.1	15.62	138	6.24	7E+09	0	0	0	0	0	0	2E+09	2E+09	1E+09	0	0	1E+09	0	0	AAAICFRENLK;CCFVHSCCY
Q6A394;Q6A392;Q6A390;Q6A389;Q6A388;Q6A391;F8QN50;Q6A385	8	9	9	60.1	60.1	18.8	15.57	138	147.1	7E+09	0	0	0	1E+07	0	0	1E+09	1E+09	4E+08	0	0	3E+07	0	0	AAAICFRENLK;CCFVHSCCY
Q6A3B4	1	6	1	59.1	19.7	19.7	15.28	137	13.204	2E+08	0	0	0	0	7E+07	7E+07	0	0	0	0	0	0	0	0	CCFVHDCCYGR;CCFVHDCCY
Q6A3F4;Q6A3E3;P34180;Q6A3A9;Q6A3B1;Q6A3C7;Q6A3C9;Q6A3D9;Q6YCN9;	58	10	8	75.9	70.8	0	15.28	137	72.884	6E+09	0	0	0	3E+08	3E+08	2E+08	4E+08	4E+08	7E+08	4E+08	0	0	0	0	AVCECDRVAAICFGENLNTYD
Q6A3G1;Q6A3I0;Q7T1D4;Q7T1D3;Q7T1D2;Q6A3J4;Q6A3I9;Q6A3M5;Q6A3H7;Q	30	10	1	68.8	16.7	16.7	15.4	138	6.8827	6E+07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	AVCECDRVAAICFGENMNTY

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Q6A3G4; Q6A3G5; Q6A3K0	3	13	13	80.4	80.4	16.7	15.44	138	192.93	5E+10	1E+08	2E+08	1E+08	1E+08	4E+08	1E+08	3E+09	2E+09	8E+08	1E+09	1E+09	6E+08	3E+08	2E+08	AVCECDRVAAICFGENMNTY
Q6A3L1	1	10	1	61.6	9.4	9.4	15.43	138	7.7684	2E+08	0	0	0	2E+07	2E+07	3E+07	2E+07	9E+06	0	2E+07	0	0	0	0	AVCECDRVAAICFGENMNTY
Q6A3N2;Q6A3N1	2	5	1	57.8	11.7	11.7	14.3	128	10.556	6E+07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	AAAICLGENVNTYDK;AQDCC
Q6A3N9	1	5	1	42.8	6.5	6.5	15.46	138	6.6419	3E+07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	AAAICLGENVNTYDK;AVCEC
Q6T5L0;O93421;Q9PRW2;P85109;Q7SZE2;P26324;Q9PT51;P09872;P33589;P	18	2	1	8.4	5.5	5.5	26.42	238	24.87	6E+09	0	0	0	4E+08	4E+08	4E+08	2E+08	1E+08	7E+07	2E+08	2E+08	3E+08	4E+08	4E+08	FFCLSSK;VIGGDECNINEHR
Q7LZQ5;Q6A3D8	3	3	2	75	40.4	40.4	5.875	52	21.292	2E+10	0	0	0	0	0	0	2E+09	3E+09	1E+09	2E+09	0	2E+09	3E+09	4E+09	CCFVHDCCYG;NLYQFGNMIF

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Q7LZQ7	4	5	3	82	40	40	5.605	50	47.818	7E+09	0	0	0	0	0	0	0	0	0	1E+09	0	4E+08	1E+09	1E+09	CCFVHDC C;HLSQF GDMINK;
Q7T045	1	6	6	29.5	29.5	29.5	16.58	146	66.777	3E+09	3E+08	3E+08	4E+08	0	0	0	0	0	0	0	0	0	0	0	ALAEESY CLLINTHK ;ALAEESY
Q7T046	2	21	21	32.8	32.8	21.4	68.77	612	199.12	1E+10	2E+08	1E+09	7E+08	7E+07	5E+07	6E+07	8E+07	4E+07	0	8E+06	0	0	0	0	ATVAEDS CFQENQ K;CILNPP
Q7T3T5	4	4	2	32.3	21.5	21.5	14.83	130	23.958	1E+08	0	0	0	1E+07	1E+07	2E+07	1E+07	8E+06	0	6E+06	0	0	0	0	AVCECDR VAAICFR;I VCGGDD
Q7ZZN9	5	5	1	22.5	5.4	0	26.87	240	15.314	1E+08	0	0	0	2E+07	0	2E+07	0	0	0	0	0	0	0	0	CGENIYM SPYPMK; KPEIQNEI
Q8JH85	1	9	9	34.5	34.5	20.5	28.61	258	73.419	3E+09	0	3E+08	1E+08	0	0	0	4E+08	0	0	0	0	0	0	0	AIYGSLP AK;ETLPY VPHCANI

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Q8JI40	1	4	1	15.8	5.4	5.4	26.91	240	18.472	6E+08	0	0	0	1E+07	0	9E+06	0	0	0	6E+07	4E+07	0	9E+07	1E+08	CGENIYMSPIPMK;MEWYPEA
Q910A1;Q6YC90;Q6A3N0;Q6A3M8;Q6A3K8;Q6A3K7;Q6A3G8;Q7T1D1;Q6A3M9;	14	13	1	80.4	16.7	0	15.44	138	23.034	9E+08	0	0	0	0	0	0	1E+08	9E+07	1E+08	1E+08	0	0	0	0	AVCECDRVAAICFGENMNTY
Q98995	22	6	3	20.3	12.3	12.3	53.48	478	23.103	7E+08	2E+08	3E+08	1E+08	0	0	0	0	0	0	0	0	0	0	0	FLRAGTVCK;MHDHAQLLTGI
Q9PT40	5	9	8	30	27.3	13.5	28.89	260	75.122	6E+09	2E+08	2E+08	2E+08	2E+08	1E+08	4E+08	2E+08	2E+08	0	1E+08	0	0	0	0	FFCLSSK;IILGVHSK;IMGWGTI
Q9PT41	1	9	9	43.2	43.2	34	28.6	259	96.379	2E+10	7E+08	2E+09	2E+09	0	0	0	8E+06	3E+06	0	2E+07	0	0	0	0	DTCEGDSGGPLICNGQIQGIV
Q9YGJ2;A0A8T1N5 Q9	2	2	1	8.1	5.4	0	28.68	260	7.0823	7E+06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	FFCLSSK;LVIGGDECNINEHR

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EGWYANLGPMR;EGWYANL	AGFIDDAFALAR;ITEMPQLR;	EGWYANLGPMR;EGWYANL	ASGNPILLNK;HANFPILSANIR	IQTAGTECRPAWDECDVPEY	ALTMEGNQASWR;HWNSYCT
0	0	0	0	0	0
0	0	0	0	2E+08	8E+06
0	0	6E+07	0	0	0
0	0	0	0	0	0
0	0	0	1E+07	2E+07	3E+07
0	0	0	0	0	0
0	9E+06	2E+07	2E+07	0	2E+07
0	1E+07	2E+07	2E+07	0	2E+07
0	0	5E+06	1E+07	0	4E+07
0	0	0	2E+07	0	3E+07
0	0	6E+06	2E+07	0	4E+07
0	0	5E+07	0	0	0
0	0	0	0	0	0
0	0	5E+07	0	0	0
4E+08	1E+08	2E+08	2E+08	4E+08	8E+08
6.6285	56.739	6.4481	14.714	33.382	73.572
516	953	505	504	614	243
58.09	109.5	57.13	55.52	68.66	27.28
0	6.9	0	0	9.8	22.6
2.5	6.9	2	7.5	9.8	22.6
15.3	6.9	16	19.6	11.2	22.6
1	7	1	2	3	6
10	7	11	8	4	6
1	13	2	12	1	39
T2HQ57	T2HQN1;A5HUI5;T2HQ95;T1E7B8;A0A6B2FB96;A0A6B2F080;A0A2H4N3C7;A0A	T2HRS5;T2HQ90	U3T7C6;T2HRS9;B6EWW8;F8S0Z7;A0A1W7RF85;A0A194APL9;A0A0F7Z1K6;A0	V5TBK6	V9I168;A0A6G5ZVU9;P25428;A0A1L8D608;B1Q3K2;A0A8T1N336;A0A6B2FCN7;

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W8E7D1;A0A077LA64;A0A6B2F597	11	16	16	26.2	26.2	16.1	96.18	851	178.04	1E+09	0	4E+07	2E+07	8E+06	2E+06	1E+07	3E+07	3E+07	0	2E+07	0	0	0	0	AATYFWPGSEVK;AGYLETW
W8EFS0	1	14	14	37.5	37.5	17.2	45.03	408	127.75	2E+09	0	5E+06	4E+06	4E+07	3E+07	4E+07	4E+07	4E+07	0	3E+07	0	0	0	0	ASGNPILLNK;CRVPTYVPLEM
X2L4E2	1	12	8	16.5	11.4	0	56.35	498	85.348	6E+09	2E+07	3E+08	2E+08	1E+08	8E+07	1E+08	3E+08	2E+08	5E+07	0	0	0	0	0	EGWYANLGPMR;EGWYANL

Table S2 : Protein groups identified in proteom of the *M. l. obtusa* (Ml) snake venom with their protein families and the calculated relative abundances.

Majority protein IDs	Protein Family	Fasta headers	Peptide sequences	Sequence coverage	Mol. weight [kDa]	Score	Ml1	Ml2	Ml3	Ml1	Ml2	Ml3	Ml1	Ml2	Ml3	Peptide is razor	Intensity	Normalized Intensity	Ml1	Ml2	Ml3	Ml1	Ml2	Ml3	Protein Family	Avg	STD

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	0.04	0.06	0.24	0.03	0.06
	0.87	0.33	1.95	0.24	2.00
	L-Amino Acid Oxidases,	L-Amino Acid Oxidases,	L-Amino Acid Oxidases,	CTL/SNA CLEC, C-type lectin	CTL/SNA CLEC, C-type lectin
Relative Abundance %	0.82	0.26	1.71	0.27	2.06
	0.91	0.39	2.18	0.21	1.94
	1.87	2.57			3.30
LFQ intensity	1E+08	4E+07	2E+08	4E+07	3E+08
	1E+08	6E+07	3E+08	3E+07	3E+08
	7E+07	1E+08			1E+08
	0.32	0.19	2.05	0.14	3.38
	8.55E+08	5.03E+08	5.56E+09	3.71E+08	9.17E+09
	False;Fals	True;False	True;True;	True;True	True;True;
Unique peptides	1	3	2	1	0
	2	3	2	1	0
	1	2	0	0	0
Razor + unique peptides	1	4	3	1	1
	2	4	4	1	1
	1	2	1	0	1
Peptides	6	4	3	2	3
	7	4	4	2	3
	4	2	1	1	2
	11.607	9.0862	85.348	19.64	98.838
	57.375	12.435	56.348	17.117	18.079
	13.7	60.7	16.5	16.7	55.1
	EGWYANLGPMR;EGWYANLGPMRLPE	ADDKNPLEECFREDDYEEFLEIAK;FDEIV	EGWYANLGPMR;EGWYANLGPMRLPE	GSHLASIHSSEEEAFVSK;SVSFVCK	AQYCISK;FCTEQANGGHLVSIESVEEAE
	tr|A0A077L6L4|A0A077L6L4	sp|P81375|OXLA_MACLB L-	sp|X2L4E2|OXLA_BOTPC L-	tr|A0A0C5DGP5|A0A0C5DGP5	tr|A0A0C5DKL1|A0A0C5DKL1
	L-Amino Acid Oxidases,	L-Amino Acid Oxidases,	L-Amino Acid Oxidases,	CTL/SNA CLEC, C-type lectin	CTL/SNA CLEC, C-type lectin
	A0A077L6L4	P81375	X2L4E2	A0A0C5DGP5;K9JD	A0A0C5DKL1;W5X

Table S2 : Protein groups identified in proteom of the *M. l. obtusa* (Ml) snake venom with their protein families and the calculated relative abundances.

0.24	0.14	1.62	2.50	0.12	2.35
0.62	0.61	8.13	####	1.41	####
CTL/SNA CLEC, C-type lectin	CTL/SNA CLEC, C-type lectin	CTL/SNA CLEC, C-type lectin	Disintegrin , DIS	Disintegrin , DIS	Disintegrin , DIS
0.38	0.47	9.75	8.74	1.53	13.97
0.86	0.75	6.51	13.73	1.29	9.27
1.15		8.69	18.08		
5E+07	6E+07	1E+09	1E+09	2E+08	2E+09
1E+08	1E+08	9E+08	2E+09	2E+08	1E+09
4E+07		3E+08	7E+08		
0.87	0.11	1.73	1.80	0.69	4.93
2.35E+09	2.95E+08	4.7E+09	4.89E+09	1.87E+09	1.34E+10
True;True;	False;Fals	True;True;	True;False	True;True;	True;True;
0	1	0	0	1	2
2	2	0	0	1	2
1	2	0	0	0	1
2	1	7	5	1	2
4	3	9	6	1	2
2	2	3	3	0	1
2	2	7	5	6	2
4	4	9	6	9	2
2	2	3	3	3	1
29.335	6.088	24.652	18.216	26.503	189.48
18.052	17.507	18.093	12.099	4.4011	7.1331
23.4	12.3	26.6	36.9	73.2	87.7
EGESQMCQGLAK;IIYVNWK;SWADAEK;	TWEDAEK;YHAWIGLR;YHAWIGLRDQ	FITHFWIGLR;SEWSDGSSVSYDNLLK;S	FLNAGTICNR;GDDMNDYCTGISSDCP	LKPAGTTCWK;SCDCPLYPG;TSLTSHY	FLRAGTVCK;GEHCVSGPCCR;MNSAN
tr|A0A1J0CZM8|A0A1J0CZM8	sp|Q38L02|SLA_DABSI Snaclec	sp|Q696W1|SLLC2_MACLB	sp|P83253|DID1A_MACLB	sp|P83469|DIS_MACLO	tr|Q1JRG9|Q1JRG9_MACLN
CTL/SNA CLEC, C-type lectin	CTL/SNA CLEC, C-type lectin	CTL/SNA CLEC, C-type lectin	Disintegrin , DIS	Disintegrin , DIS	Disintegrin , DIS
A0A1J0CZM8;A0A1J	Q38L02;K9JCR1	Q696W1	P83253	P83469	Q1JRG9

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2.51	0.10	0.76	0.21	0.02	2.13
4.46	1.53	2.04	0.43	0.13	####
Disintegrin , DIS	Disintegrin , DIS	Disintegrin , DIS	Disintegrin , DIS	Disintegrin , DIS	Disintegrin , DIS
6.97	1.63	2.79	0.64	0.15	8.82
1.94	1.44	1.28	0.23	0.11	13.08
17.86		4.79			
9E+08	2E+08	4E+08	9E+07	2E+07	1E+09
3E+08	2E+08	2E+08	3E+07	2E+07	2E+09
7E+08		2E+08			
1.62	0.25	1.14	0.08	0.03	3.71
4.4E+09	6.89E+08	3.09E+09	2.26E+08	68733000	1.01E+10
True;False	True;False	True;True;	False;Fals	False;True	True;False
2	2	2	1	1	0
2	3	2	1	1	0
3	1	2	0	0	0
2	2	2	1	1	1
2	3	2	1	1	1
3	1	2	0	0	0
6	2	3	2	3	3
6	3	3	3	3	3
5	1	3	1	1	1
91.394	13.133	95.715	27.868	12.189	14.088
13.983	22.634	7.0089	7.1081	7.0049	7.6697
35.2	20.5	87.5	56.9	68.8	88.4
AMLDGLN DYCTGIS SDCPR;F LNPGTIC	FVTAGTE CR;LHSW VECESGE CCDQCR;	FLNAGTI CK;GDDM NDYCTGI SSDCPR;	FLNAGTI CK;GEHC VSGPCC R;MNSGN	FLRAGTV CK;NSGN PCCDPVT CQPR;RA	FLNPGTI CK;GEHC VSGPCC R;MNSAN
tr|A0A1I9K NR4|A0A1 I9KNR4_V	sp|C0LZJ5 |VM3LC_ MACLN	sp|P0C6A 6|DID7A_ VIPBB	sp|P0C6A 8|DID4_M ACLO	sp|P0C6A 9|VM25A_ MACLO	sp|P0C6B 0|DID5B_ MACLO
Disintegrin , DIS	Disintegrin , DIS	Disintegrin , DIS	Disintegrin , DIS	Disintegrin , DIS	Disintegrin , DIS
A0A1I9KN R4;A0A1I9	C0LZJ5	P0C6A6	P0C6A8	P0C6A9	P0C6B0

Table S2 : Protein groups identified in proteom of the *M. l. obtusa* (Ml) snake venom with their protein families and the calculated relative abundances.

E0Y419;A 0A1I9KNR	Fibrinogen ase, FPG	sp|E0Y419 |VSPBF_ MACLB	AAHPWLP AQSR;FF CLSSK;TL CAGILQG	12.8	28.297	95.171	1	4	3	0	1	1	0	0	0	True;False	4.37E+09	1.61		3E+08	3E+08		2.32	2.29	Fibrinogen ase, FPG	2.31	0.02
A0A6B7F RK6	Metallopro teinase, SVMPs		ATVAEDS CFEENLK; AVCGNEI WEEGEE	24	69.217	58.618	0	2	2	0	1	1	0	1	1	True;True;	6.12E+08	0.23		7E+06	5E+06		0.05	0.04	Metallopro teinase, SVMPs	0.04	0.00
E9JG27;Q 2UXR0;E9	Metallopro teinase, SVMPs	tr|E9JG27| E9JG27_E CHCS	CVDVNTA Y;LTPGS QCADGE CCDQCR;	6.3	63.998	12.04	3	5	5	2	2	2	0	0	0	False;True	7.66E+08	0.28		2E+07	5E+07		0.14	0.34	Metallopro teinase, SVMPs	0.24	0.10
E9JG34;E 9JG58;Q2	Metallopro teinase, SVMPs	tr|E9JG34| E9JG34_E CHCS	FLTNFKP DCTLIR;H CVDVTTA Y;IPCAPE	10.2	69.125	12.706	3	7	6	0	2	1	0	2	1	True;False	3.7E+08	0.14		3E+07	2E+07		0.18	0.12	Metallopro teinase, SVMPs	0.15	0.03
E9JG63;E 9JGB0;A0	Metallopro teinase, SVMPs	tr|E9JG63| E9JG63_E CHCO	CVDVNTA Y;INVLPE AK;LYCFD NLPEHK;	9.4	56.945	13.565	1	3	3	0	1	1	0	1	1	True;False	6.89E+08	0.25		5E+07	3E+07		0.35	0.22	Metallopro teinase, SVMPs	0.29	0.06
E9JGA1;E 9JG79;E9	Metallopro teinase, SVMPs	tr|E9JGA1| E9JGA1_ ECHCO	ESVGIVQ DHSK;NIP CAPQDVK ;PSYQFS	6.7	51.7	21.94	3	9	8	1	2	2	0	1	1	True;True;	3.94E+08	0.15		2E+07	2E+07		0.14	0.13	Metallopro teinase, SVMPs	0.13	0.01

Table S2 : Protein groups identified in proteom of the *M. l. obtusa* (Ml) snake venom with their protein families and the calculated relative abundances.

0.01	0.09	0.20	0.03	0.00	0.09
0.16	0.39	2.96	0.23	0.33	0.62
Metallopro teinase, SVMPs	Metallopro teinase, SVMPs	Metallopro teinase, SVMPs	Metallopro teinase, SVMPs	Metallopro teinase, SVMPs	Metallopro teinase, SVMPs
0.15	0.31	2.76	0.26	0.33	0.72
0.16	0.48	3.16	0.20	0.33	0.53
		2.59			1.17
2E+07	4E+07	4E+08	4E+07	4E+07	1E+08
2E+07	7E+07	4E+08	3E+07	5E+07	8E+07
		1E+08			4E+07
0.03	0.31	0.70	0.15	0.38	1.44
75622000	8.36E+08	1.91E+09	3.99E+08	1.03E+09	3.92E+09
True;True;	True;False	False;Fals	False;True	True;True;	True;True;
1	1	1	3	0	2
1	1	1	3	0	3
1	1	1	2	0	1
1	1	1	3	8	2
1	1	1	3	9	3
1	1	1	2	3	1
5	2	4	3	8	3
3	2	4	3	9	4
3	1	2	2	3	2
17.567	8.2957	24.462	69.903	46.605	53.275
61.364	24.286	24.486	68.843	67.329	53.478
7.2	18.3	29.5	16.8	4.2	13.2
KGTDYFY CRK;LHS WVECES GECCQQ	IVSPPVC GNELLEK; LHSWVE CESGEC	SYQFSDC SK;TWAH QLVNNIIV FYR;YDG	AGTVCRP ANGECD VSDLCTG QSAECPT	HDNAQLL TAIDFDG R;QCVDV TTAY;RH	GDWNDD YCTGISS DCPR;GD WNDDYC
tr|E9JG93| E9JG93_E CHCO	sp|Q0NZX 9|VM3B2_ BOTJA	sp|Q3ZD7 4|VM1L4_ MACLB	sp|Q4VM0 7|VM3VB_ MACLB	tr|J3SDW5 |J3SDW5_ CROAD	tr|A0A1I9K NT0|A0A1I 9KNT0_VI
Metallopro teinase, SVMPs	Metallopro teinase, SVMPs	Metallopro teinase, SVMPs	Metallopro teinase, SVMPs	Metallopro teinase, SVMPs	Metallopro teinase, SVMPs
E9JG93;E 9JG87	Q0NZX9	Q3ZD74	Q4VM07	J3SDW5;J 3S3W1;A0	A0A1I9KN T0;A0A1I9

Table S2 : Protein groups identified in proteom of the *M. l. obtusa* (Ml) snake venom with their protein families and the calculated relative abundances.

A0A1L8D6 89;A0A1L	Metallopro teinase, SVMPs	tr|A0A1L8 D689|A0A 1L8D689	LHSWVE CQSGEC CDQCR	2.8	67.598	16.004	0	4	4	0	3	3	0	3	3	TRUE	9.81E+08	0.36		7E+06	2E+07		0.05	0.12	Metallopro teinase, SVMPs	0.08	0.03
A0A6B7F MR4	Metallopro teinase, SVMPs	tr|A0A6B7 FMR4|A0A 6B7FMR4	ASECDLP EYCTGQS ADCPTDH FDR;DSC	28.2	68.843	112.34	2	3	3	2	3	3	2	3	3	True;True;	7.14E+09	2.63	6E+07	1E+08	8E+07	1.54	0.87	0.60	Metallopro teinase, SVMPs	0.74	0.14
A0A6B7F MR5	Metallopro teinase, SVMPs	tr|A0A6B7 FMR5|A0A 6B7FMR5	ENDVPIP CAPEDIK; HCVDVTT AY;KEND	27.2	36.837	61.287	2	5	4	2	5	4	0	0	0	True;False	2.86E+10	10.54	2E+08	6E+08	3E+08	5.07	4.33	2.16	Metallopro teinase, SVMPs	3.24	1.08
A0A6B7F NN4;R4N	Metallopro teinase, SVMPs	tr|A0A6B7 FNN4|A0A 6B7FNN4	AGTVCRP ANGECD VSDVCTG QSAECPT	36.4	68.345	178.87	3	6	6	3	6	6	3	6	6	True;True;	1.58E+10	5.81	1E+08	3E+08	2E+08	2.77	2.36	1.72	Metallopro teinase, SVMPs	2.04	0.32
Q6A3G4; Q6A3G5;	PLA2, Ammodyti n, Atn	tr|Q6A3G4 |Q6A3G4_ VIPAM	AVCECDR VAAICFG ENMNTY DK;AVCE	80.4	15.436	192.93	10	20	14	10	20	14	5	10	7	True;True;	4.67E+10	17.20	1E+08	2E+08	1E+08	2.52	1.63	1.07	PLA2, Ammodyti n, Atn	1.35	0.28
C3W4R6; B6CQR5;	PLA2s, phospholip ase A2	sp|C3W4R 6|PA2A1_ MACLB	AVCECDR VAAICFG ENMNTY DK;AVCE	60.1	15.55	20.418	0	8	4	0	8	4	0	5	3	False;Fals	5.17E+08	0.19		1E+07	1E+08		0.09	0.86	PLA2s, phospholip ase A2	0.47	0.39

Table S2 : Protein groups identified in proteom of the *M. l. obtusa* (Ml) snake venom with their protein families and the calculated relative abundances.

0.38	0.10	0.00	1.17	0.04	0.89
1.49	0.49	1.14	6.24	####	4.04
SVSP, Serine proteinase	SVSP, Serine proteinase	SVSP, Serine proteinase	SVSPs, factor X activatorC	SVSPs, Factor V activator	SVSPs, snake venom
1.10	0.39	1.15	5.07	12.07	4.93
1.87	0.59	1.14	7.40	12.15	3.15
2.32	1.57	4.84		17.28	
1E+08	5E+07	2E+08	7E+08	2E+09	7E+08
3E+08	8E+07	2E+08	1E+09	2E+09	4E+08
9E+07	6E+07	2E+08		7E+08	
17.99	0.52	2.16	4.53	7.25	1.72
4.88E+10	1.41E+09	5.87E+09	1.23E+10	1.97E+10	4.67E+09
True;True;	True;True;	False;True	True;True;	True;True;	False;True
1	2	4	3	4	0
3	3	4	5	4	0
1	1	5	0	2	0
1	6	8	6	5	2
3	6	8	12	5	3
1	4	7	0	3	0
4	7	8	6	5	2
6	7	8	12	5	3
3	5	7	0	3	0
120.09	31.183	75.122	199.12	96.379	41.921
28.317	28.702	28.893	68.774	28.595	25.413
32.3	21.9	30	32.8	43.2	15
CAGTLIN QEWVLTA AHCNGK; CQRVHP	AYGGLPE K;HPCAQ PQLPAFY TK;LNSPV	FFCLSSK; IILGVHSK; IMGWGTI TTTK;NVP	ATVAEDS CFQENQ K;CILNPP LR;CILNP	DTCEGDS GGPLICN GQIQGIV SGGSDP	VFDYND WIQSIIAG NTAATCP P;VVGGD
tr|A0A6B7 FQF8|A0A 6B7FQF8	sp|E0Y418 |VSP1_MA CLB	sp|Q9PT4 0|VSPH2_ MACLB	sp|Q7T04 6|VM3CX_ MACLB	sp|Q9PT4 1|VSPF5_ MACLB	sp|P82981 |VSP2_AG KCO
SVSP, Serine proteinase	SVSP, Serine proteinase	SVSP, Serine proteinase	SVSPs, factor X activatorC	SVSPs, Factor V activator	SVSPs, snake venom
A0A6B7F QF8	E0Y418	Q9PT40	Q7T046	Q9PT41	P82981;P 0DMH5;A0

Table S2 : Protein groups identified in proteom of the *M. l. obtusa* (Ml) snake venom with their protein families and the calculated relative abundances.

W8E7D1; A0A077LA	Venom phosphodi esterase,	sp|W8E7D 1|PDE_M ACLB	AATYFWP GSEVK;A GYLETW DSLMPNI	26.2	96.18	178.04	3	8	6	1	5	3	0	0	0	True;True;	1.1E+09	0.41		4E+07	2E+07		0.25	0.12	Venom phosphodi esterase,	0.18	0.06

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

Majority protein IDs	Protein Family	Fasta headers	Peptide sequences		Sequence coverage [%]	Mol. weight [kDa]	Score		Mx2	Mx3	Mx1	Mx2	Mx3	Mx1	Mx2	Mx3	Peptide is razor	Intensity	Normalized Intensity	Mx1	Mx2	Mx3	Mx1	Mx2	Mx3	Protein Family	Avg	STD
								Peptides			Razor +			Unique peptides						LFQ intensity			Relative Abundance %					
A0A6B7FRF2	L-Amino	tr|A0A6B7FRF2|A0A6B7FRF	EANLSP GAVDMI GDLLNE		13.3	13.596	6.2344	2	2	2	1	1	1	1	1	1	True;Fals	3.91E+08	0.116793	6.56E+07	1.32E+08	4.59E+07	0.28	0.62	0.27	L-Amino Acid	0.28	0.16

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

0.12	0.23	2.40	0.10	0.13	0.26
2.39	1.82	3.27	0.44	0.82	2.42
L-Amino Acid	L-Amino Acid	CTL/SNA CLEC, C-	CTL/SNA CLEC, C-	CTL/SNA CLEC, C-	CTL/SNA CLEC, C-
2.26	2.03	0.44	0.34	0.91	2.62
2.33	2.13	4.64	0.31	0.60	2.01
2.53	1.61	6.10	0.53	0.73	2.22
3.83E+08	3.45E+08	7.51E+07	5.75E+07	1.54E+08	4.46E+08
4.94E+08	4.52E+08	9.83E+08	6.68E+07	1.27E+08	4.25E+08
5.86E+08	3.73E+08	1.41E+09	1.24E+08	1.68E+08	5.13E+08
2.734778	2.180126	1.526965	0.308502	0.846656	0.684336
9.17E+09	7.31E+09	5.12E+09	1.03E+09	2.84E+09	2.29E+09
True;True	True;True	True;Fals	True;True	True;True	True;Fals
1	2	0	2	2	1
1	1	0	1	2	1
1	2	0	2	2	1
5	4	1	2	2	1
3	3	1	2	2	1
4	4	1	2	2	1
5	4	2	2	2	3
3	3	2	2	2	3
4	4	2	2	2	2
98.838	50.929	37.113	46.605	31.476	14.747
18.079	17.042	14.194	67.329	28.884	17.398
55.1	33.1	14.5	4.2	20.3	18.2
EGWYAN LGPMR;E GWYANL	EGWYAN LGPMR;E GWYANL	FCTEQV K;TTDNQ WLR	AQYCISK ;FCTEQA NGGHLV	AWNEGT NCFVFK; DGVIWM	AWSDEP NCYVAK; DCHWG
tr|T2HRS 5|T2HRS 5_PROFL	sp|X2L4E 2|OXLA_ BOTPC L-	tr|A0A0A1 WD34|A0 A0A1WD	tr|A0A0C5 DKL1|A0 A0C5DKL	tr|A0A0C5 DM02|A0 A0C5DM0	tr|A0A0C5 DQX8|A0 A0C5DQ
L-Amino	L-Amino	CTL/SNA	CTL/SNA	CTL/SNA	CTL/SNA
T2HRS5; T2HQ90	X2L4E2	A0A0A1W D34	A0A0C5D KL1;W5X	A0A0C5D M02	A0A0C5D QX8

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

A0A0C5E 3E9	CTL/SNA	tr|A0A0C5 E3E9|A0A 0C5E3E9	FCTEQA NGGHLV SIENVGE		35.1	17.505	97.328	6	5	4	6	5	4	2	2	2	True;True	1.33E+09	0.395405	1.33E+08	1.52E+08	1.14E+08	0.57	0.72	0.67	CTL/SNA CLEC, C-	0.62	0.06
A0A1I9K NN1;K9J	CTL/SNA	tr|A0A1I9 KNN1|A0 A1I9KNN	GYLEWV TLPCGD K;TWEDA		10.2	15.039	14.203	2	2	2	2	2	2	2	2	2	True;True	6.80E+08	0.202828	9.06E+07	1.49E+08	8.47E+07	0.39	0.70	0.50	CTL/SNA CLEC, C-	0.44	0.13
A0A1I9K NP1	CTL/SNA	tr|A0A1I9 KNP1|A0 A1I9KNP	AWSDEP NCYGAK; FCTEEVK		27.9	17.626	21.44	3	2	2	2	1	1	2	1	1	True;True	1.00E+09	0.298716	4.92E+07	4.22E+07	5.44E+07	0.21	0.20	0.32	CTL/SNA CLEC, C-	0.27	0.05
A0A1I9K NP6	CTL/SNA	tr|A0A1I9 KNP6|A0 A1I9KNP	ANFVAEL VTLTK;A NFVAELV		35.2	13.983	91.394	5	4	5	3	2	3	2	1	1	True;Fals	4.40E+09	1.312556	1.59E+08	2.20E+08	5.61E+07	0.69	1.04	0.33	CTL/SNA CLEC, C-	0.51	0.29
A0A1I9K NS2	CTL/SNA	tr|A0A1I9 KNS2|A0 A1I9KNS	AWSDKP NCYVAK; EEMNWE		18.1	68.387	114	5	3	5	5	3	5	3	1	3	True;True	6.02E+09	1.79567	6.81E+08	4.58E+08	6.91E+08	2.94	2.16	4.07	CTL/SNA CLEC, C-	3.51	0.78
A0A1J0C ZM7;B4X	CTL/SNA	tr|A0A1J0 CZM7|A0 A1J0CZM	ADLVWIG LR;ADLV WIGLRDF		13.2	53.478	53.275	2	3	3	2	3	3	2	3	2	True;True	3.92E+09	1.169567	1.99E+08	2.05E+08	3.25E+08	0.86	0.97	1.91	CTL/SNA CLEC, C-	1.39	0.47

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

0.15	0.11	0.01	0.03	0.73	3.50
1.05	1.50	0.08	0.08	3.03	14.24
CTL/SNA CLEC, C-	CTL/SNA CLEC, C-	Cysteine-rich	Cysteine-rich	Cysteine-rich	Cysteine-rich
0.89	1.59	0.10	0.08	3.79	16.56
0.89	1.33	0.06	0.14	2.19	7.99
1.20	1.42	0.07	0.08	2.28	11.93
1.52E+08	2.70E+08	1.62E+07	1.41E+07	6.44E+08	2.81E+09
1.88E+08	2.81E+08	1.37E+07	3.00E+07	4.65E+08	1.69E+09
2.79E+08	3.28E+08	1.65E+07	1.92E+07	5.28E+08	2.76E+09
0.702116	1.063094	0.02816	0.028055	2.130842	8.53339
2.35E+09	3.56E+09	9.44E+07	9.40E+07	7.14E+09	2.86E+10
True;True	True;True	False;Fals	True;Fals	True;True	True;Fals
1	2	1	1	11	5
1	2	1	1	7	4
1	2	1	1	8	3
2	6	1	1	12	6
3	7	1	1	9	4
2	5	1	1	9	4
2	6	3	2	12	7
3	7	3	2	9	5
2	5	3	2	9	5
29.335	82.467	7.4711	6.316	112.34	61.287
18.052	17.285	28.574	26.546	68.843	36.837
23.4	49.3	11.9	15.8	28.2	27.2
EANFVA ELVSQNI K;FCTEQ	CFGLDQ K;DQDCL PGWSFY	CGENIY MSTSPM K;CILSHS	KPEIQNE IIDLHNSL RR;MEW	CGENIY MSPIPMK ;MEWYP	CIYDHSP R;DFVYG QGASPA
sp|B4XSY 6|SLAB_ MACLB	sp|B4XSY 8|SLAD_ MACLB	sp|B7FDI 1|CRVP_ VIPBE	sp|P0DM T4|CRVP ECHCO	sp|Q8JI40 |CRVP_G LOBL	tr|A0A6G 5ZUA3|A0 A6G5ZUA
CTL/SNA	CTL/SNA	Cysteine-	Cysteine-	Cysteine-	Cysteine-
B4XSY6; B4XSY5	B4XSY8; W5XDM0;	B7FDI1;B 7FDI0	P0DMT4; A0A0A1W	Q8JI40	A0A6G5Z UA3

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

<table>
<tr><td>P0C6A6</td><td>Disintegri</td><td>sp|P0C6A6|DID7A_VIPBB</td><td>FLNAGTICK;GDDMNDYCT</td><td></td><td>36.4</td><td>68.345</td><td>178.87</td><td>10</td><td>8</td><td>11</td><td>10</td><td>8</td><td>11</td><td>2</td><td>1</td><td>2</td><td>True;True</td><td>1.58E+10</td><td>4.704045</td><td>5.07E+08</td><td>5.39E+08</td><td>5.96E+08</td><td>2.19</td><td>2.54</td><td>3.51</td><td>Disintegrin, DIS</td><td>2.85</td><td>0.56</td></tr>
<tr><td>P0C6B0</td><td>Disintegri</td><td>sp|P0C6B0|DID5B_MACLO</td><td>FLNPGTICK;GEHCVSGPCC</td><td></td><td>32.3</td><td>28.317</td><td>120.09</td><td>5</td><td>5</td><td>4</td><td>5</td><td>5</td><td>4</td><td>0</td><td>0</td><td>0</td><td>True;True</td><td>4.88E+10</td><td>14.56977</td><td>1.31E+09</td><td>1.31E+09</td><td>1.07E+09</td><td>5.66</td><td>6.16</td><td>6.31</td><td>Disintegrin, DIS</td><td>5.98</td><td>0.28</td></tr>
<tr><td>P83253</td><td>Disintegri</td><td>sp|P83253|DID1A_MACLB</td><td>FLNAGTICNR;GDDMNDYC</td><td></td><td>34.9</td><td>57.102</td><td>144.46</td><td>7</td><td>6</td><td>8</td><td>7</td><td>6</td><td>8</td><td>0</td><td>0</td><td>0</td><td>True;True</td><td>9.40E+09</td><td>2.805631</td><td>1.23E+08</td><td>9.15E+07</td><td>1.37E+08</td><td>0.53</td><td>0.43</td><td>0.80</td><td>Disintegrin, DIS</td><td>0.67</td><td>0.16</td></tr>
<tr><td>Q1JRG9</td><td>Disintegri</td><td>tr|Q1JRG9|Q1JRG9_MACLN</td><td>FLRAGTVCK;GEHCVSGPCC</td><td></td><td>37.9</td><td>25.042</td><td>6.352</td><td>2</td><td>2</td><td>2</td><td>1</td><td>1</td><td>1</td><td>1</td><td>1</td><td>1</td><td>True;Fals</td><td>8.86E+07</td><td>0.026417</td><td>1.80E+07</td><td>2.03E+07</td><td>1.70E+07</td><td>0.08</td><td>0.10</td><td>0.10</td><td>Disintegrin, DIS</td><td>0.09</td><td>0.01</td></tr>
<tr><td>A0A1I9KNR4;A0A</td><td>Disintegri</td><td>tr|A0A1I9KNR4|A0A1I9KNR</td><td>AMLDGLNDYCTGISSDCPR;</td><td></td><td>14.4</td><td>32.557</td><td>8.1794</td><td>2</td><td>2</td><td>3</td><td>1</td><td>0</td><td>1</td><td>1</td><td>0</td><td>1</td><td>False;Fals</td><td>8.09E+07</td><td>0.024139</td><td>9.62E+06</td><td></td><td>8.06E+06</td><td>0.04</td><td></td><td>0.05</td><td>Disintegrin, DIS</td><td>0.04</td><td>0.00</td></tr>
<tr><td>E0Y419;A0A1I9KN</td><td>Fibrinoge</td><td>sp|E0Y419|VSPBF_MACLB</td><td>AAHPWLPAQSR;FFCLSSK;</td><td></td><td>22.6</td><td>27.284</td><td>73.572</td><td>5</td><td>4</td><td>6</td><td>5</td><td>4</td><td>6</td><td>5</td><td>4</td><td>6</td><td>True;True</td><td>7.61E+08</td><td>0.227001</td><td>3.97E+07</td><td>2.89E+07</td><td>3.82E+07</td><td>0.17</td><td>0.14</td><td>0.23</td><td>Fibrinogenase,</td><td>0.20</td><td>0.04</td></tr>
</table>

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

0.23	0.45	0.33	0.09	0.14	0.36
1.13	4.80	3.14	1.78	0.58	1.68
Metalloproteinase	Metalloproteinase	Metalloproteinase	Metalloproteinase	Metalloproteinase	Metalloproteinase
1.07	5.36	3.25	1.69	0.47	1.98
1.61	4.76	2.46	1.66	0.80	1.14
1.18	4.25	3.02	1.86	0.69	1.37
1.82E+08	9.10E+08	5.53E+08	2.88E+08	8.05E+07	3.37E+08
3.41E+08	1.01E+09	5.22E+08	3.51E+08	1.69E+08	2.42E+08
2.74E+08	9.85E+08	7.00E+08	4.32E+08	1.61E+08	3.17E+08
0.361067	1.832603	1.591135	4.401838	0.4213	1.304084
1.21E+09	6.14E+09	5.33E+09	1.48E+10	1.41E+09	4.37E+09
False;Tru	True;True	True;True	True;True	True;True	True;Fals
1	2	0	1	2	1
1	2	0	1	3	1
1	2	0	1	2	1
1	3	3	4	2	1
1	3	3	4	3	1
1	3	3	4	2	1
2	3	4	4	2	2
2	4	4	4	3	2
2	3	3	4	2	2
6.1167	30.121	22.152	154.12	31.183	95.171
14.159	17.738	15.308	26.509	28.702	28.297
15.4	25	31.3	65.3	21.9	12.8
HDNAQLLTAIDFDGR;QCV	GDWNDDYCTGISSDCPR;GD	LHSWVECQSGECCDQCR	ENDVPIPCAPEDIK;HCVDVT	FLTNFKPDCTLIR;HCVDVTT	IPCAPEDEK;LGNSYAYCRK
tr|J3SDW5|J3SDW5_CROA	tr|A0A1I9KNT0|A0A1I9KNT0	tr|A0A1L8D689|A0A1L8D689	tr|A0A6B7FMR5|A0A6B7FMR	tr|E9JG34|E9JG34_ECHCS	tr|E9JG51|E9JG51_ECHCS
Metallopr	Metallopr	Metallopr	Metallopr	Metallopr	Metallopr
J3SDW5;J3S3W1;	A0A1I9KNT0;A0A1	A0A1L8D689;A0A1	A0A6B7FMR5	E9JG34;E9JG58;Q2	E9JG51;E9KNB4;A

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

0.04	0.02	0.07	0.00	1.11	0.45
0.44	0.10	0.18	0.08	4.94	2.06
Metalloproteinase	Nucleotidases Nt	Nucleotidases Nt	PLA2, Ammodyti	PLA2, Ammodyti	PLA2, Ammodyti
0.46	0.12	0.25	0.08	5.69	2.59
0.35	0.11			2.98	1.76
0.41	0.07	0.11	0.08	4.19	1.53
7.82E+07	2.11E+07	4.23E+07	1.28E+07	9.66E+08	4.39E+08
7.50E+07	2.30E+07			6.31E+08	3.74E+08
9.60E+07	1.73E+07	2.64E+07	1.77E+07	9.70E+08	3.56E+08
0.228573	0.102891	0.043821	0.180158	4.903925	1.825025
7.66E+08	3.45E+08	1.47E+08	6.04E+08	1.64E+10	6.12E+09
False;Tru	True;True	False;Fals	False;Fals	False;Tru	False;Tru
1	1	1	0	1	1
1	0	0	0	1	1
1	1	1	0	1	1
1	3	1	1	1	1
1	1	0	0	1	1
1	3	1	1	1	1
3	4	4	8	2	2
3	2	3	6	2	2
2	4	4	7	2	2
12.04	72.059	7.2719	16.37	14.37	24.87
63.998	62.611	24.743	56.887	27.894	26.416
6.3	9.9	15.9	18.8	11.3	8.4
ESVGIVQDHSK;NIPCAPQD	ASGNPILLNK;QVPVVQAYA	ASGNPILLNK;HANFPILSANI	AVCECDRVAAICFGENLNT	AVCECDRVAAICFGENMNT	AVCECDRVAAICFGENMNT
tr|E9JGA1|E9JGA1_ECHCO	tr|A0A6G5ZVN1|A0A6G5ZVN	tr|U3T7C6|U3T7C6_OVOOK	tr|Q6A3F4|Q6A3F4_VIPAP	tr|Q6A3G4|Q6A3G4_VIPAM	tr|Q6A3L1|Q6A3L1_VIPAP
Metallopr	Nucleotid	Nucleotid	PLA2,	PLA2,	PLA2,
E9JGA1; E9JG79;E	A0A6G5ZVN1	U3T7C6;T2HRS9;B	Q6A3F4; Q6A3E3;	Q6A3G4; Q6A3G5;	Q6A3L1

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

0.31	0.02	0.49	0.03	0.06	0.43
1.48	0.05	4.05	0.40	0.62	1.63
PLA2, Ammodyti	PLA2s, phospholi	PLA2s, phospholi	PLA2s, phospholi	PLA2s, phospholi	PLA2s, phospholi
1.83	0.07	4.43	0.44	0.68	1.65
1.74	0.07	3.24	0.38	0.69	0.71
1.13	0.04	3.67	0.36	0.56	1.61
3.11E+08	1.11E+07	7.53E+08	7.47E+07	1.16E+08	2.79E+08
3.69E+08	1.55E+07	6.87E+08	8.14E+07	1.47E+08	1.51E+08
2.61E+08	8.72E+06	8.50E+08	8.42E+07	1.31E+08	3.73E+08
0.636454	0.922372	3.001185	0.136351	0.548	0.564109
2.13E+09	3.09E+09	1.01E+10	4.57E+08	1.84E+09	1.89E+09
True;True	True;True	True;Fals	True;True	False;Fals	False;Fals
2	0	0	2	1	1
5	0	0	2	1	1
3	0	0	2	1	1
2	1	1	2	1	1
5	1	1	2	1	1
3	1	1	2	1	1
2	2	5	2	2	4
5	2	5	2	2	4
3	2	5	2	2	4
46.83	95.715	14.088	13.084	31.791	9.3165
15.353	7.0089	7.6697	12.125	24.699	12.574
35.8	87.5	88.4	15.9	25.5	53.6
AAAICFR K;CCFVH DCCYGN	CCFVHD CCYGK;V AAICFR	FTAYAIN GPPVEK; HQGLPE	AVCECD RVAAICF R;CCFVH	CCFVHD C	AVCECD RVAAICF GENLNT
tr|Q6A363 |Q6A363_ VIPAP	sp|P8638 9|PA2A2_ BOTAS	tr|A0A6B7 FMQ9|A0 A6B7FM	sp|A8CG8 7|PA2A2_ DABRR	sp|B3A0N 3|PA2B3_ BOTMA	sp|F8QN5 3|PA2A2_ VIPRE
PLA2,	PLA2s,	PLA2s,	PLA2s,	PLA2s,	PLA2s,
Q6A363; Q6A362;	P86389;A 0A2D1UX	A0A6B7F MQ9	A8CG87; A8CG78	B3A0N3	F8QN53; Q6A3C2;

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

0.48	0.11	0.01	0.02	4.72	0.11
0.71	1.15	0.06	0.10	7.39	0.70
PLA2s, phospholi	PLA2s, phospholi	SVSPS, Serine	SVSPS, Serine	SVSPS, Serine	SVSPS, Serine
0.84	1.02	0.05	0.11	2.36	0.81
1.71	1.20	0.08	0.08	12.33	0.57
0.58	1.28	0.07	0.08	12.42	0.59
1.42E+08	1.74E+08	9.19E+06	1.95E+07	4.00E+08	1.38E+08
3.63E+08	2.54E+08	1.71E+07	1.61E+07	2.61E+09	1.20E+08
1.35E+08	2.98E+08	1.73E+07	1.85E+07	2.88E+09	1.37E+08
1.843939	1.785497	0.150149	1.458767	7.092165	0.249358
6.18E+09	5.98E+09	5.03E+08	4.89E+09	2.38E+10	8.36E+08
False;Tru	True;Fals	True;Fals	True;Fals	True;True	True;Fals
0	0	1	1	0	1
0	0	1	1	0	1
0	0	1	1	0	1
1	1	1	1	3	1
1	2	1	1	3	1
1	2	1	1	3	1
2	2	7	2	3	2
3	4	5	2	3	2
2	3	6	2	3	2
62.876	72.884	9.0862	18.216	110.66	8.2957
15.716	15.279	12.435	12.099	12.561	24.286
61.6	75.9	60.7	36.9	60.9	18.3
CCFVHD CCYGR;H AVWSYL	AVCECD RVAAICF R;IVCGG	VFDYTD WIQSIIA GNTAAT	FCYLPAD PGR;FIY GGCR;HT	FCYLPAD PGR;FCY LPADPG	CAGTLIN QEWVLT AAHCNG
sp|P3185 4|PA2B_V IPBB	sp|Q7T3T 5|PA2AB_ DABSI	tr|A0A1W 7RJU0|A0 A1W7RJ	sp|P0099 2|VKT3_V IPAA	sp|P0099 1|VKT1_V IPAA	tr|A0A6B7 FQF8|A0 A6B7FQF
PLA2s,	PLA2s,	SVSPS,	SVSPS,	SVSPS,	SVSPS,
P31854	Q7T3T5	A0A1W7 RJU0;A0	P00992;A 0A1S5QJ	P00991;A 0A1S5QL	A0A6B7F QF8

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

0.26	0.04	0.13	0.01	0.00	0.66
1.28	0.13	1.07	0.09	0.05	1.58
SVSPS, Serine	SVSPS, Serine	SVSPs, snake	SVSPs, factor X	SVSPs, RVV-V,	SVSPs, Venom
1.57	0.17	1.21	0.10	0.05	2.20
1.51	0.09				0.68
0.99	0.10	0.94	0.07	0.06	0.97
2.66E+08	2.90E+07	2.05E+08	1.63E+07	9.00E+06	3.73E+08
3.19E+08	1.87E+07				1.44E+08
2.29E+08	2.21E+07	2.18E+08	1.73E+07	1.28E+07	2.25E+08
3.99641	0.056572	2.677529	0.038529	0.176133	1.750413
1.34E+10	1.90E+08	8.98E+09	1.29E+08	5.90E+08	5.87E+09
True;True	False;Fals	True;True	True;Fals	True;Fals	False;Tru
0	1	1	0	1	1
0	1	1	0	0	2
0	1	1	0	1	2
4	1	2	1	1	3
4	1	1	0	0	6
4	1	2	1	1	6
4	2	2	4	4	4
4	3	1	3	3	7
4	2	2	4	4	7
189.48	7.7684	110.72	15.314	18.472	75.122
7.1331	15.428	17.458	26.865	26.914	28.893
87.7	61.6	37.3	22.5	15.8	30
VFDYTE WIR	FFCLSSK ;IILGVHS K;IMGWG	VFDYTD WIR	ATVAED SCFQEN QK;CILN	VIGGDEC NINEHPF LA	SIIAGNT AVTCPP
tr|A0A8T1 N163|A0A 8T1N163	sp|Q9PT4 0|VSPH2 MACLB	sp|Q7SYF 1|VSPP_ CERCE	sp|Q7T04 6|VM3CX MACLB	sp|P8653 0|VSP1_ DABRR	sp|Q9YGJ 8|VSPPA GLOBR
SVSPS,	SVSPS,	SVSPs,	SVSPs,	SVSPs,	SVSPs,
A0A8T1N 163	Q9PT40	Q7SYF1; D5KRX9	Q7T046	P86530	Q9YGJ8; K0J2I0;A0

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

0.00	0.02	0.04	0.10	4.48	0.02
0.08	0.05	0.20	0.55	7.10	0.07
Vascular endothelia	Vascular endothelia	Venom phosphodi	Venom phosphodi	KSPI, Kunitz-	SVMPI, Snake
0.08	0.07	0.24	0.62	5.13	0.09
0.09		0.16	0.38	15.97	0.05
0.09	0.03	0.16	0.49	9.06	0.05
1.34E+07	1.11E+07	4.00E+07	1.04E+08	8.71E+08	1.55E+07
1.81E+07		3.37E+07	8.04E+07	3.39E+09	1.10E+07
1.98E+07	7.77E+06	3.79E+07	1.13E+08	2.10E+09	1.06E+07
0.067252	0.329235	0.492958	1.659095	4.336206	0.03258
2.25E+08	1.10E+09	1.65E+09	5.56E+09	1.45E+10	1.09E+08
False;Tru	True;True	True;True	True;True	True;Fals	False;Tru
0	4	4	0	0	1
0	1	2	0	0	1
0	2	3	0	0	1
2	7	11	6	2	1
1	1	7	5	2	1
2	3	7	6	2	1
6	7	11	10	3	3
5	1	7	9	4	3
5	3	7	10	3	3
14.714	178.04	127.75	85.348	30.601	23.958
55.516	96.18	45.03	56.348	15.586	14.828
19.6	26.2	37.5	16.5	24.6	32.3
CSGCCT DESLK;E TLVSILQ	CSGCCT DESLK;E TLVSILQ	AATYFW PGSEVK; AGYLET	ASGNPIL LNK;CRV PTYVPLE	EFIYGGC R;EFIYG GCRGNA	IDRIGSV SGLGCN K;IGSVS
sp|P8247 5|TXVE2_ MACLB	sp|P8394 2|TXVE_ VIPAP	sp|W8E7 D1|PDE_ MACLB	sp|W8EF S0|V5NT D_MACL	tr|A0A1S5 QJK2|A0 A1S5QJK	tr|A0A6B7 FPT0|A0A 6B7FPT0
Vascular	Vascular	Venom	Venom	KSPI,	SVMPI,
P82475;P 0DW97	P83942;P 67863;A0	W8E7D1; A0A077L	W8EFS0	A0A1S5Q JK2;A0A1	A0A6B7F PT0;A0A1

Table S3 : Protein groups identified in proteom of the *M. xanthina* (*Mx*) snake venom.

V9I168;A0A6G5ZV	Nerve	tr|V9I168|V9I168_VIPUR	ALTMEGNQASWR:HWNSY		9.4	56.945	13.565	4	4	5	2	2	2	0	0	0	True;Fals	6.89E+08	0.205566	9.59E+07	5.36E+07	6.45E+07	0.41	0.25	0.38	Nerve growth	0.40	0.07
P82972;Q7LZ09	NP,	sp|P82972|VNP_PSEPC	IDRIGSHSGLGCNK:IGSHS		6.7	51.7	21.94	2	0	2	2	0	2	1	0	1	True;True	3.94E+08	0.117533	4.08E+06		4.59E+06	0.02		0.03	NP, Natriuretic	0.02	0.00
T1DKS7;A0A6B2F	T-	tr|T1DKS7|T1DKS7_CROHD	EGIVALR		16	57.128	6.4481	9	8	9	1	0	1	0	0	0	False;Fals	1.73E+08	0.051718	6.30E+06		4.65E+06	0.03		0.03	T-complex	0.03	0.00
J3S993	Peptidyl-	tr|J3S993|J3S993_CROAD	YGDILAAR		48.2	15.316	14.979	2	2	2	1	0	1	1	0	1	False;Fals	4.00E+07	0.011946	1.04E+07		1.01E+07	0.04		0.06	Peptidyl-glycine	0.05	0.01

Table S4 : Protein groups identified in proteom of the *V. a. ammodytes* (*Vaa*) snake venom.

Majority protein IDs	Protein Family	Fasta headers	Peptide sequences	Sequence coverage [%]	Mol. weight [kDa]	Score	Peptides Vaa1	Peptides Vaa2	Peptides Vaa3	Razor + unique peptides Vaa1	Razor + unique peptides Vaa2	Razor + unique peptides Vaa3	Unique peptides Vaa1	Unique peptides Vaa2	Unique peptides Vaa3	Peptide is razor	Intensity	LFQ intensity Vaa1	LFQ intensity Vaa2	LFQ intensity Vaa3	Relative Abundance % Vaa1	Relative Abundance % Vaa2	Relative Abundance % Vaa3	Avg	STD
P82981;P0DMH5;A0	SVSPs,	sp|P82981	VFDYND WIQSIIAG	15	25.413	41.921	2	3	2	2	3	2	0	0	0	False;True	46684000	1.84E+08	1.84E+08	1.95E+08		14.02	13.89	13.96	0.09
B4XSY8; W5XDM0;	CTL/SNA	sp|B4XSY	CFGLDQK ;DQDCLP	31.3	15.308	22.152	4	4	2	4	4	2	0	0	0	True;True;	53335000		1.78E+07	2.08E+07		1.35	1.48	1.42	0.09
Q1JRG9	Disintegrin	tr|Q1JRG9	FLRAGTV CK;GEHC	87.7	7.1331	189.48	3	3	4	2	1	2	2	1	2	True;True;	13396000	1.26E+09	4.62E+08	5.48E+08		35.14	39.07	37.10	2.78

Table S4 : Protein groups identified in proteom of the *V. a. ammodytes* (*Vaa*) snake venom.

P0C6A5	Disintegrin DIS	sp|P0C6A	GEHCVS GPCCR;N	42.2	7.0229	15.899	1	3	3	0	1	1	0	0	0	False;True	52138000		1.01E+08	1.37E+08		7.66	9.76	8.71	1.48
A0A6B7F NN4;R4N	Metallopro	tr|A0A6B7	AGTVCRP ANGECD	36.4	68.345	178.87	1	4	3	1	4	3	0	0	0	True;True;	15768000		2.99E+08	3.37E+08		22.77	24.02	23.39	0.88
Q6A3G4; Q6A3G5;	PLA2,	tr|Q6A3G4	AVCECDR VAAICFG	80.4	15.436	192.93	3	3	4	3	3	4	0	0	0	True;True;	46696000	5.89E+08	2.51E+08	1.65E+08		19.06	11.78	15.42	5.14

Table S5: Protein groups identified in proteom of *V. a. montandoni* (*Vam*) snake venom.

Majority protein IDs	Protein Family	Fasta headers	Peptide sequences	Sequence coverage [%]	Mol. weight [kDa]	Peptide is razor	Intensity	Normalized Intensity	Vam1	Vam2	Vam1	Vam2	Protein Family	Avg	STD
									LFQ intensity		Relative Abundance %				

Table S5: Protein groups identified in proteom of *V. a. montandoni* (*Vam*) snake venom.

A0A1I9KNL5	SVSPs,Serine proteinase	tr|A0A1I9	ASYALPPR;DNDIMLIK;GDSGGPLICNGEIQGIVSYGK;LCAGIPR;NGAPSIYTK	20.3	28.884	True;True;True;Tru	2.84E+09	1.55	3.37E+08	1.56E+08	2.77	4.44	SVSPs,Serine	3.60	1.18
A0A1I9KNN4	SVSPs,Serine proteinase	tr|A0A1I9	AAYPWLLER;CGQPGNPGVYTK;KVLNEDEETREPTEK;SRTLCAGILR;TLCAGILR;TSTHIAPLSLPSSPPS	26.5	28.168	True;True;True;Tru	1.29E+10	7.08	8.35E+08	4.62E+07	6.87	1.31	SVSPs,Serine	4.09	3.93
A0A6B7FQF8	SVSPs, Serine proteinase	tr|A0A6B7	CAGTLINQEWVLTAAHCNGK;CQRVHPELPAK;FFCLSSK;GRVVCAGIWQGGK;IELGVHDK;IMGWGTISSTK;	32.3	28.317	True;True;True;Tru	4.88E+10	26.74	1.82E+09	9.03E+07	14.92	2.57	SVSPs, Serine	8.75	8.74
P82981;P0DMH5;A0A2I7YS66;A0A1W7RJU2;Q8QHK2;A0A194AS18;A0A194APZ9;A0	SVSPs, snake venom thrombin-like enzymes	sp|P8298	VFDYNDWIQSIIAGNTAATCPP;VVGGDECNINEHR	15	25.413	False;True	4.67E+09	2.56	8.72E+07	7.12E+07	0.72	2.02	SVSPs, snake	1.37	0.92
A0A1S5QJK2;A0A1S5QK73;A0A1S5QKN1;A0A1S5QJJ1	KSPI, Kunitz-Type Serine Protease Inhibitors	tr|A0A1S5	EFIYGGCR;EFIYGGCRGNANNFK;FCYLPADPGR;FYYNPASNK;HTCVASGIQPR;TWDECRHTCVASGIQ	54.8	10.375	True;True;True;Tru	1.18E+10	6.45	3.42E+09	8.05E+08	28.13	22.87	KSPI, Kunitz-	25.50	3.72
P00991;A0A1S5QLZ2	KSPI, Kunitz-Type Serine Protease Inhibitors	sp|P0099	FCYLPADPGR;FCYLPADPGRCK;FIYGGCPGNANNFK;FYYDSASNK	38.9	9.8312	False;True;True;Tr	7.25E+09	3.97	2.52E+09	2.76E+08	20.75	7.86	KSPI, Kunitz-	14.30	9.11

Table S5: Protein groups identified in proteom of *V. a. montandoni* (*Vam*) snake venom.

####	0.96	0.40	3.00	0.21	2.85
22.92	0.84	0.75	4.10	2.93	10.84
PLA2, Ammodyti	CTL/SNA CLEC, C-	Cysteine-rich	Fibrinoge nase,	Metallopr oteinase	Vascular endothelia
35.58	0.16	1.04	6.23	3.08	12.85
10.27	1.53	0.47	1.98	2.78	8.82
1.25E+09	5.71E+06	3.65E+07	2.19E+08	1.08E+08	4.52E+08
1.25E+09	1.86E+08	5.69E+07	2.41E+08	3.38E+08	1.07E+09
25.56	0.77	0.32	3.35	8.63	13.01
4.67E+10	1.41E+09	5.9E+08	6.12E+09	1.58E+10	2.38E+10
True;True ;True;Tru	False;Tru e;False;F	True;Fals e;False;F	False;Tru e	True;True ;True;Tru	True;True ;True;Tru
15.436	18.081	26.914	26.416	68.345	12.561
80.4	31	15.8	8.4	36.4	60.9
AVCECD RVAAICF GENMNT YDK;AVC ECDRVA AICFGEN MNTYDK K;CCFVH DCCYGR; CCFVHD CCYGRV NGCDPK;	AQYCISK ;CGDDYP FVCK;NC FGLEK;T SADYVWI GLWNQR ;TSADYV WIGLWN QRK;WT DGSSVIY K	CGENIY MSPIPMK ;MEWYP EAAANA ER;RSVN PTASNM LK;SVNP TASNML K	FFCLSSK ;VIGGDE CNINEHR	AGTVCR PANGEC DVSDVC TGQSAE CPTDQF QR;DCQ NPCCNA ATCK;ET DLLNR;H DNAQLLT GINFNGP TAGLGYI	CSGCCT DESLK;E TLVSILQ EYPDEIS DIFRPSC VAVLR;F TEHTAC ECRPR;H TVDLQIM R;PFLEV HER;PSC VAVLR
tr|Q6A3G 4|Q6A3G	tr|A0A6B7 FMP6|A0	sp|Q8JI40 |CRVP_C	sp|Q6T5L 0|VSPSH	tr|A0A6B7 FNN4|A0	sp|P8394 2|TXVE
PLA2, Ammodyti n, Atn	CTL/SNA CLEC, C-type lectin and C-type lectin-like	Cysteine-rich secretory protein, CRISP	Fibrinoge nase, FPG	Metallopr oteinase	Vascular endothelia l growth factors (VEGFs)
Q6A3G4; Q6A3G5; Q6A3K0	A0A6B7F MP6	Q8JI40	Q6T5L0; O93421;Q 9PRW2;P 85109;Q7 SZE2;P26 324;Q9PT 51;P0987 2;P33589; P0DJG5;	A0A6B7F NN4;R4N NL0	P83942;P 67863;A0 A1J0CZM 5

Table S6: Protein groups identified in proteom of the *V. b. berus* (*Vbb*) snake venom.

Majority protein IDs	Protein Family	Fasta headers	Peptide sequences	Sequence coverage [%]	Mol. weight [kDa]	Score	Peptides Vbb1	Peptides Vbb2	Peptides Vbb3	Razor + unique peptides Vbb1	Razor + unique peptides Vbb2	Razor + unique peptides Vbb3	Unique peptides Vbb1	Unique peptides Vbb2	Unique peptides Vbb3	Peptide is razor	Intensity	Normalized Intesity	LFQ intensity Vbb1	LFQ intensity Vbb2	Ralative Abundance % Vbb1	Ralative Abundance % Vbb2	Protein Family	Avg	STD
A0A077L 6L4	L-Amino	tr|A0A077 L6L4|A0A	EGWYAN LGPMR;E GWYANL GPMRLP EK;FWED	13.7	57.375	11.607	6	6	1	1	2	1	1	2	1	False;Fals	85542000	0.26	1.36E+08	1.30E+08	0.57	0.52	L-Amino Acid	0.54	0.04
A0A6B7F RF2	L-Amino	tr|A0A6B7 FRF2|A0	EANLSP GAVDMI GDLLNE DSGYYV SFIESLK;	34.9	57.102	144.46	7	3	0	7	3	0	7	3	0	True;True	94045000	2.83	5.03E+08	3.51E+08	2.10	1.39	L-Amino Acid	1.74	0.50
Q7LZK6;T 2HPQ8;P	CTL/SNA	sp|Q7LZK 6|SLA_P	TWEDAE R	17.5	4.7544	5.9949	2	2	0	2	2	0	0	0	0	TRUE	74082000	0.02	2.22E+07	1.76E+07	0.09	0.07	CTL/SNA CLEC, C-	0.08	0.02

Table S6: Protein groups identified in proteom of the *V. b. berus* (*Vbb*) snake venom.

0.05	0.01	0.13	0.06	0.01	0.00
0.22	0.06	1.64	0.58	0.24	0.45
CTL/SNA CLEC, C-	CTL/SNA CLEC, C-	CTL/SNA CLEC, C-	CTL/SNA CLEC, C-	Metallopr oteinase	Metallopr oteinase
0.18	0.06	1.73	0.62	0.25	0.45
0.26	0.05	1.55	0.53	0.23	0.45
4.66E+07	1.54E+07	4.37E+08	1.56E+08	6.31E+07	1.13E+08
6.27E+07	1.21E+07	3.71E+08	1.28E+08	5.63E+07	1.07E+08
0.11	0.12	2.76	0.71	0.09	0.18
35699000	39149000	91670000	23535000	30487000	60143000
True;True	True;Fals	True;True	True;True	True;True	False;Fals
0	0	0	1	0	0
1	1	0	3	1	6
1	1	0	2	1	7
1	0	2	1	0	0
2	1	3	3	1	6
2	1	2	3	1	7
1	0	2	1	0	0
2	3	3	3	3	6
2	2	2	3	3	7
16.031	6.2344	98.838	29.335	23.318	6.3529
18.476	13.596	18.079	18.052	68.744	43.047
20.3	13.3	55.1	23.4	9.9	7.7
SEWSDG SSVSYD NLHK;TW FHMYCA DSYPFV	FCTEQV K;TTDNQ WLR	AQYCISK ;FCTEQA NGGHLV SIESVEE AEFVAQL	EGESQM CQGLAK; IIYVNWK; SWADAE K;WDYVS	FVTAGTV CRPAR;L KPGAEC GDGVCC YQCR;Q	IPCAPQD VK;KIPCA PQDVK;R HDNAQL LTAIDFD
tr|A0A0A1 WDS1|A0	tr|A0A0A1 WD34|A0	tr|A0A0C5 DKL1|A0	tr|A0A1J0 CZM8|A0	tr|A0A0B4 U9L8|A0A	tr|F8S111| F8S111
CTL/SNA	CTL/SNA	CTL/SNA	CTL/SNA	Metallopr	Metallopr
A0A0A1W DS1;A0A	A0A0A1W D34	A0A0C5D KL1;W5X	A0A1J0C ZM8;A0A	A0A0B4U 9L8	F8S111;J 3RY90;A0

Table S6: Protein groups identified in proteom of the *V. b. berus* (*Vbb*) snake venom.

0.10	0.37	0.36	0.74	0.02	0.02
0.10	0.80	0.56	2.99	0.36	0.31
Metalloproteinase	Metalloproteinase	Metalloproteinase	Metalloproteinase	Metalloproteinase	Metalloproteinase
0.17	0.55	0.30	2.47	0.34	0.29
0.03	1.06	0.82	3.51	0.37	0.32
4.31E+07	1.37E+08	7.61E+07	6.21E+08	8.69E+07	7.41E+07
7.12E+06	2.55E+08	1.96E+08	8.43E+08	8.96E+07	7.58E+07
1.81	1.18	0.29	4.74	0.18	0.12
60191000	39204000	98078000	15768000	61200000	39397000
True;True	True;True	TRUE	True;True	True;True	True;True
0	0	0	0	1	0
6	3	0	4	3	1
7	3	0	4	4	1
1	0	6	0	1	0
14	3	6	9	4	1
15	3	6	11	5	1
1	0	6	0	1	0
14	3	6	9	7	4
15	3	6	11	9	4
114	53.275	16.004	178.87	58.618	21.94
68.387	53.478	67.598	68.345	69.217	51.7
18.1	13.2	2.8	36.4	24	6.7
HDNAQLLTAIDFDR;HDNAQLLTAIDFDRETIGL	GDWNDDYCTGISSDCPR;GDWNDDYCTGISSDC	LHSWVECQSGECCDQCR	AGTVCRPANGECDVSDVCTGQSAECPTDQF	ATVAEDSCFEENLK;AVCGNEIWEEGEECDCG	ESVGIVQDHSK;NIPCAPQDVK;PSYQFSDCSK
tr|A0A1I9KNR6|A0	tr|A0A1I9KNT0|A0	tr|A0A1L8D689|A0A	tr|A0A6B7FNN4|A0	tr|A0A6B7FRK6|A0	tr|E9JGA1|E9JGA1
Metallopr	Metallopr	Metallopr	Metallopr	Metallopr	Metallopr
A0A1I9KNR6	A0A1I9KNT0;A0A1	A0A1L8D689;A0A1	A0A6B7FNN4;R4N	A0A6B7FRK6	E9JGA1;E9JG79;E

Table S6: Protein groups identified in proteom of the *V. b. berus* (*Vbb*) snake venom.

E9JGC3; E9JGC2	Metallopr	tr|E9JGC 3|E9JGC3	GTDDFY CR;GTDD FYCRK;K GTDDFY CRK	2.4	46.499	20.813	4	4	0	4	4	0	4	4	0	True;True	17882000	0.05	3.58E+07	2.02E+07	0.15	0.08	Metallopr oteinase	0.11	0.05
E9KJZ6;Q 2UXQ2;E	Metallopr	tr|E9KJZ6 |E9KJZ6	GEECDC GSPANC R;LHSWV ECESGE CCDQCR	9.9	62.611	72.059	5	5	4	2	2	1	1	1	1	True;True	34489000	0.10	6.95E+07	2.74E+07	0.29	0.11	Metallopr oteinase	0.20	0.13
Q4VM07	Metallopr	sp|Q4VM 07|VM3V	AGTVCR PANGEC DVSDLC TGQSAE CPTDQF	16.8	68.843	69.903	12	12	7	12	12	7	4	4	2	False;Tru	39901000	0.12	2.11E+07	1.58E+07	0.09	0.06	Metallopr oteinase	0.08	0.02
Q6A3F4; Q6A3E3;	PLA2,	tr|Q6A3F4 |Q6A3F4	AVCECD RVAAICF GENLNT YDK;CCF VHDCCY	75.9	15.279	72.884	3	2	2	1	1	0	1	1	0	True;Fals	59850000	1.80	3.77E+08	4.22E+08	1.57	1.67	PLA2, Ammodyti	1.62	0.07
Q6A370; Q6A371;	PLA2,	tr|Q6A370 |Q6A370	AAAICFR ENLK;CC FVHSCC YAK;DAT DRCCFV	46.4	15.616	6.24	2	2	0	2	2	0	1	1	0	False;Fals	67379000	2.03	1.82E+09	2.04E+09	7.57	8.10	PLA2, Ammodyti	7.83	0.38
Q6A394; Q6A392;	PLA2,	tr|Q6A394 |Q6A394	AAAICFR ENLK;CC FVHSCC YAK;DAT DRCCFV	60.1	15.569	147.1	3	2	0	3	2	0	3	2	0	True;True	69335000	2.08	1.23E+09	1.20E+09	5.12	4.76	PLA2, Ammodyti	4.94	0.25

Table S6: Protein groups identified in proteom of the *V. b. berus* (*Vbb*) snake venom.

A0A0A1W C86;A0A6	PLA2s,	tr|A0A0A1 WC86|A0	AAALCFR	5.8	13.353	6.4173	4	2	0	4	2	0	4	2	0	TRUE	62533000	1.88	1.11E+09	1.30E+09	4.63	5.17	PLA2s, phospholi	4.90	0.38
A0A6B7F MQ9	PLA2s,	tr|A0A6B7 FMO9|A0	FTAYAIN GPPVEK; HQGLPE SYNFDF VTMK;NG	17.5	64.31	56.958	2	2	1	1	1	0	1	1	0	True;True	25325000	0.08	1.83E+07	2.01E+07	0.08	0.08	PLA2s, phospholi	0.08	0.00
P04084;A 4VBF0;Q	PLA2s,	sp|P0408 4|PA2HA	AAAICLG ENVNTY DK;AVCE CDRAAAI CLGENV	86.1	13.639	154.67	2	2	2	1	1	1	1	1	1	True;True	70626000	21.24	1.59E+07	4.34E+07	0.07	0.17	PLA2s, phospholi	0.12	0.07
P31854	PLA2s,	sp|P3185 4|PA2B_V	CCFVHD CCYGR;H AVWSYL SYGCYC GWGGQ	61.6	15.716	62.876	3	3	2	3	3	2	1	2	1	False;Tru	61809000	1.86	5.18E+08	2.55E+09	2.16	10.12	PLA2s, phospholi	6.14	5.63
Q910A1; Q6YC90;	PLA2s,	sp|Q910A 1|PA2A1	AVCECD RVAAICF GENMNT YDK;AVC ECDRVA	80.4	15.435	23.034	6	6	1	5	5	0	1	1	0	False;Fals	88633000	0.27	1.17E+08	9.48E+07	0.49	0.38	PLA2s, phospholi	0.43	0.08
Q7LZQ5; Q6A3D8	PLA2s,	tr|Q7LZQ 5|Q7LZQ	CCFVHD CCYG;NL YQFGNM IFK;SALL SYSNYG	75	5.8747	21.292	6	6	2	5	5	1	5	4	1	True;True	20913000	6.29	2.13E+09	2.82E+09	8.87	11.20	PLA2s, phospholi	10.03	1.65

Table S6: Protein groups identified in proteom of the *V. b. berus* (*Vbb*) snake venom.

Q7T3T5	PLA2s, phospholi	sp|Q7T3T5|PA2AB	AVCECDRVAAICFR;IVCGGDDPCLR;VAAICFR;	32.3	14.828	23.958	6	6	4	5	5	3	0	0	0	False;Tru	10921000	0.03	1.16E+07	7.69E+06	0.05	0.03	PLA2s, phospholi	0.04	0.01
A0A1I9KNN4	SVSPs, Serine	tr|A0A1I9KNN4|A0	AAYPWLLER;CGQPGNPGVYTK;KVLNEDEET	26.5	28.168	54.274	5	5	2	5	5	2	2	2	1	True;True	12927000	3.89	8.30E+08	3.52E+08	3.46	1.40	SVSPs, Serine	2.43	1.46
A0A2I7YS70	SVSPs, Serine	tr|A0A2I7YS70|A0A	IMGWGTISDTK	4.2	28.389	6.4108	6	6	0	5	5	0	4	4	0	TRUE	41266000	0.01	7.40E+06	4.83E+06	0.03	0.02	SVSPs, Serine	0.03	0.01
A0A6B7FQF8	SVSPs, Serine	tr|A0A6B7FQF8|A0	CAGTLINQEWVLTAAHCNGK;CQRVHPELPAK;	32.3	28.317	120.09	8	6	3	1	1	1	1	1	1	True;True	48838000	14.69	3.33E+09	2.81E+09	13.87	11.15	SVSPs, Serine	12.51	1.92
A0A6G5ZW78	SVSPs, Serine	tr|A0A6G5ZW78|A	CQRVHPELPAK;EYTMWDK;FFCLSSK;GRVVC	29.2	28.102	19.85	9	7	4	9	7	4	2	2	2	False;Tru	29875000	8.98	4.33E+09	4.44E+09	18.06	17.61	SVSPs, Serine	17.84	0.32
E5AJX2	SVSPs, Serine	sp|E5AJX2|VSP_VI	CAGTLINQEWVLTAAHCNGK;CQGVHPELPAK;	25.7	28.215	9.4364	10	7	5	10	7	5	1	1	0	False;Tru	15585000	0.47	3.79E+08	2.53E+08	1.58	1.00	SVSPs, Serine	1.29	0.41

Table S6: Protein groups identified in proteom of the *V. b. berus* (*Vbb*) snake venom.

0.31	0.19	0.08	0.38	0.59	0.01
1.45	0.58	0.72	0.95	9.49	0.07
SVSPs, Serine	SVSPs, Serine	SVSPs, Serine	SVSPs ,snake	SVSps, snake	Nucleotid ases Nt
1.24	0.72	0.66	0.68	9.08	0.08
1.67	0.45	0.78	1.21	9.91	0.06
3.11E+08	1.81E+08	1.67E+08	1.71E+08	2.29E+09	2.08E+07
4.01E+08	1.08E+08	1.88E+08	2.91E+08	2.38E+09	1.53E+07
0.93	4.03	1.76	1.40	4.94	0.07
30918000	13396000	58674000	46684000	16438000	22543000
True;True	True;True	False;Tru	False;Tru	False;Tru	False;Tru
0	0	1	0	0	0
0	1	1	1	0	0
0	1	2	2	0	0
1	0	1	0	0	0
1	1	1	1	3	4
1	1	2	2	3	5
6	3	1	0	1	0
7	5	2	2	4	4
10	8	3	3	4	5
95.715	189.48	75.122	41.921	14.37	14.714
7.0089	7.1331	28.893	25.413	27.894	55.516
87.5	87.7	30	15	11.3	19.6
FLNAGTICK;GDDMNDYCTGISSDCPR;GEHCV	FLRAGTVCK;GEHCVSGPCCR;MNSANPCCDPIT	FFCLSSK;IILGVHSK;IMGWGTITTTK;NVPNEDQ	VFDYNDWIQSIIAGNTAATCPP;VVGGDECNIN	FFCLSSK;IMGWGTISTSK;TLCAGILQGGK	ASGNPILLNK;HANFPILSANIRPK;IIALGHSGFF
sp|P0C6A6|DID7A	tr|Q1JRG9|O1JRG	sp|Q9PT40|VSPH2	sp|P82981|VSP2_A	sp|O13069|VSP2_B	tr|U3T7C6|U3T7C6
SVSPs,	SVSPs,	SVSPs,	SVSPs	SVSps,	Nucleotid
P0C6A6	Q1JRG9	Q9PT40	P82981;P0DMH5;A	O13069;Q71QJ0;Q7	U3T7C6;T2HRS9;B

Table S6: Protein groups identified in proteom of the *V. b. berus* (*Vbb*) snake venom.

0.01	0.00	0.00	0.01	0.01	0.56
0.04	0.01	0.10	0.17	0.12	4.90
Nucleotid ases Nt	Venom phosphodi	Venom phosphodi	Venom phosphodi	Renin	Cysteine-rich
0.04	0.01	0.10	0.16	0.11	4.50
0.03	0.02	0.11	0.17	0.13	5.29
1.08E+07	3.51E+06	2.56E+07	4.10E+07	2.77E+07	1.13E+09
7.26E+06	3.68E+06	2.58E+07	4.18E+07	3.03E+07	1.27E+09
0.02	0.01	0.33	0.50	0.03	4.44
80913000	18473000	11036000	16524000	98383000	14755000
False;Fals	True;True	True;True	True;True	TRUE	True;True
1	0	0	0	0	0
1	0	5	3	1	0
1	0	4	4	1	0
1	0	0	0	0	2
1	1	9	10	1	1
1	1	10	11	1	2
6	0	0	0	0	2
7	7	9	10	4	2
7	7	10	11	6	3
8.1794	12.006	178.04	127.75	6.551	154.12
32.557	50.376	96.18	45.03	13.186	26.509
14.4	3.4	26.2	37.5	8.6	65.3
ASGNPIL LNK;QVP VVQAYA FGK;VGII GYTTK;Y	AVDNVLL R;WFIVE NR	AATYFW PGSEVK; AGYLET WDSLMP NINK;CS	ASGNPIL LNK;CRV PTYVPLE MEK;DIP EDQVVK;	TAPTILT NFR	CGENIY MSTSPM K;CILSHS PR;DFVY GQGASP
tr|A0A6G 5ZVN1|A0	tr|J3RY60 |J3RY60	sp|W8E7 D1|PDE	sp|W8EF S0|V5NT	tr|A0A0A1 WCW0|A	sp|B7FDI 1|CRVP
Nucleotid	Venom	Venom	Venom	Renin	Cysteine-
A0A6G5Z VN1	J3RY60;T 1DH21;A0	W8E7D1; A0A077L	W8EFS0	A0A0A1W CW0	B7FDI1;B 7FDI0

Table S6: Protein groups identified in proteom of the *V. b. berus* (*Vbb*) snake venom.

M9NCG3; U3TDL2;	Glutaminy	tr|M9NCG 3|M9NCG	GVPILHLI PSPFPPR; LIFFDGE EAFVR;M WQNDLH	20.9	42.116	41.939	2	2	0	1	1	0	0	0	0	True;True	99597000	0.03	7.65E+06	5.69E+06	0.03	0.02	Glutaminy l cyclases	0.03	0.01
V9I168;A 0A6G5ZV	Venom	tr|V9I168| V9I168_V	ALTMEG NQASWR ;HWNSY CTTTDTF VR;IDTA	22.6	27.284	73.572	7	6	1	1	1	0	0	0	0	True;True	76091000	0.23	1.83E+07	1.95E+07	0.08	0.08	Venom nerve	0.08	0.00
T1E782;J 3SCG9;A	V-type	tr|T1E782| T1E782	IMGSHD NLSTK	9.3	13.757	7.3155	6	5	1	3	3	1	0	0	0	TRUE	65229000	0.02	1.66E+07	2.27E+07	0.07	0.09	V-type proton	0.08	0.01

Table S7. The dataset used for the PCA and machine learning analysis.

LFQ intensity Ml_exp1	LFQ intensity Ml_exp2	LFQ intensity Ml3_exp3	LFQ intensity Mx_exp1	LFQ intensity Mx_exp2	LFQ intensity Mx_exp3	LFQ intensity ubb_exp1	LFQ intensity ubb_exp2	LFQ intensity ubb_exp3	LFQ intensity Vam_exp1	LFQ intensity Vam_exp2	LFQ intensity Waa_exp1	LFQ intensity Waa_exp2	LFQ intensity Waa_exp3	C: Reverse	C: Potential contaminant	C: Taxonomy IDs	N: Peptides	N: Razor + unique	N: Unique peptides	N: Sequence coverage	N: Unique + razor	N: Unique sequence	N: Mol. weight [kDa]	N: Q-value	N: Score	N: Intensity	N: MS/MS count	T: Protein IDs	T: Majority protein IDs		T: id

Table S7. The dataset used for the PCA and machine learning analysis.

12	13	26	38	39	45	57
CTL/SN	CTL/SN	SVMPs	SVMPs	SVMPs	KSPI	SVMPs
A0A0C5DKL1:W	A0A0C5DM02	A0A1I9KNL5	A0A1I9KNT0:A	A0A1J0CZM8:A	A0A1S5OJK2:A	A0A6B7FMR5
A0A0C5DKL1:W	A0A0C5DM02	A0A1I9KNL5	A0A1I9KNT0:A	A0A1J0CZM8:A	A0A1S5OJK2:A	A0A6B7FMR5:T
46	44	23	30	17	24	44
916700	730780	283800	392040	235350	117840	286040
98.838	50.929	31.476	53.275	29.335	49.748	61.287
0	0	0	0	0	0	0
18.079	17.042	28.884	53.478	18.052	10.375	36.837
30.4	8.8	20.3	8.2	14.6	9.7	24.4
55.1	33.1	20.3	13.2	23.4	54.8	27.2
55.1	33.1	20.3	13.2	23.4	54.8	27.2
2	2	5	4	2	1	6
7	4	5	6	4	6	8
7	4	5	6	4	6	9
-1	-1	-1	-1	-1	-1	-1
24.17	26.45	24.98	24.01	25.02	28.50	25.49
23.65	23.62	26.53	24.35	26.69	28.42	23.16
28.53	25.03	26.07	25.46	24.79	27.93	26.16
24.97	25.20	27.22	25.29	24.94	29.58	25.31
28.33	24.31	28.33	23.88	23.63	31.67	28.63
25.03	29.95	25.96	26.00	24.99	24.70	25.56
28.70	28.00	23.14	27.03	27.22	27.10	23.69
28.47	27.39	23.16	27.93	26.93	27.24	21.91
28.51	28.36	27.20	28.28	27.18	26.93	31.39
28.88	28.75	26.92	27.61	27.49	26.49	30.66
29.13	28.48	27.33	27.57	28.05	27.00	31.36
28.03	26.97	25.15	26.51	25.61	25.49	28.10
28.03	27.80	25.65	26.17	26.87	26.12	29.19
26.90	25.48	24.66	25.40	25.39	24.08	27.52

Table S7. The dataset used for the PCA and machine learning analysis.

58	60	61	78	126	130	133
SVMPs	SVSPS	SVSPS	CTL/SN	SVSPS	LAAOs	DIS
A0A6B7FNN4:R	A0A6B7FOF8	A0A6B7FRF2	B7FDI1;B7FDI0	Q6T5L0:O9342	P0C6A6	P0C6B0
A0A6B7FNN4:R	A0A6B7FOF8:A	A0A6B7FRF2	B7FDI1;B7FDI0:	Q6T5L0:O9342	P0C6A6:P83254	P0C6B0
121	89	99	80	19	25	15
157680	488380	940450	147550	611750	309180	100600
178.87	120.09	144.46	154.12	24.87	95.715	14.088
0	0	0	0	0	0	0
68.345	28.317	57.102	26.509	26.416	7.0089	7.6697
17	0	15.7	21.3	5.5	31.2	34.8
36.4	32.3	34.9	65.3	5.5	68.8	47.8
36.4	32.3	34.9	65.3	8.4	87.5	88.4
8	0	5	4	1	1	1
19	9	14	13	1	5	2
19	9	14	13	2	8	9
-1	-1	-1	-1	-1	-1	-1
28.33	28.38	27.13	24.18	28.57	23.70	29.99
28.16	30.49	22.78	23.95	28.51	23.36	23.38
27.94	30.26	26.91	25.92	28.01	25.46	28.81
26.69	26.43	25.20	25.65	27.71	25.26	24.66
28.33	30.76	23.38	28.76	27.84	23.08	24.62
27.54	30.06	24.92	28.34	26.14	25.21	25.44
29.21	31.39	28.39	30.08	26.70	28.21	24.26
29.65	31.63	28.91	30.24	27.22	28.58	23.52
29.15	30.00	27.03	28.10	28.71	23.40	29.49
29.00	30.28	26.45	28.39	28.48	23.89	29.36
28.92	30.29	26.87	28.69	28.41	23.06	29.66
27.77	27.13	27.57	25.45	25.93	28.47	30.13
28.32	27.98	27.89	26.11	26.33	27.43	30.79
26.65	26.40	26.75	24.03	24.29	27.44	29.23

Table S7. The dataset used for the PCA and machine learning analysis.

145	148	151	163	172	178	189
CTL/SN	PLA2s	PLA2s	CRISP	SVMPs	SVSPS	LAAOs
P82981; P0DMH	P83942; P67863:	Q1JRG 9	Q6A3G 4:O6A3	Q7T046	Q9PT40	X2L4E2
P82981; P0DMH	P83942; P67863:	Q1JRG 9	Q6A3G 4:O6A3	Q7T046 :A0A6G	Q9PT40 :P0DPS	X2L4E2
20	50	68	149	78	52	49
466840	237730	133960	466960	123070	586740	556130
41.921	110.66	189.48	192.93	199.12	75.122	85.348
0	0	0	0	0	0	0
25.413	12.561	7.1331	15.436	68.774	28.893	56.348
5.6	15.5	0	16.7	21.4	13.5	0
5.6	60.9	87.7	80.4	32.8	27.3	11.4
15	60.9	87.7	80.4	32.8	30	16.5
1	2	0	1	10	4	0
1	6	9	13	21	8	8
2	6	9	13	21	9	12
-1	-1	-1	-1	-1	-1	-1
27.54	26.97	29.03	27.30	24.11	24.76	25.00
27.46	29.00	28.78	27.90	23.08	24.32	23.73
27.45	29.87	30.23	29.13	25.95	25.49	24.61
26.09	28.75	25.35	30.22	24.53	25.66	24.60
26.38	30.00	23.40	30.22	23.01	27.09	24.31
27.36	29.40	28.12	29.63	25.11	25.24	25.59
27.35	30.60	27.43	31.12	25.42	27.32	27.84
28.12	30.51	26.68	31.47	26.17	27.49	28.21
25.68	28.58	27.99	26.84	25.74	28.47	26.64
24.96	31.28	28.25	28.56	25.45	27.10	26.26
25.26	31.42	27.77	26.87	25.98	27.74	26.75
29.29	25.31	30.79	27.09	29.33	27.18	27.76
28.74	26.02	30.29	27.78	29.97	27.27	28.21
28.39	24.88	30.49	26.51	27.29	27.45	24.34

Table S8: The identified N-glycopeptides in the glycoproteome from *M. l. obtusa* (*Ml*) snake venom .

										MaxLFQ Intensity			Ralative Abundance %			
Protein ID	**Protein Description**	**Peptide Sequence**	**Modified Sequence**	**Glycan Composition**	**Prev AA**	**Next AA**	**Start**	**End**	**Peptide Length**	**Charges**	**M. l. obtusa (Ml)-1 MaxLFQ Intensity**	**M. l. obtusa (Ml)-2 MaxLFQ Intensity**	**M. l. obtusa (Ml-)3 MaxLFQ Intensity**	**Ml1**	**Ml2**	**Ml3**	**Avg**
Q7T046	Coagulation factor X-activating enzyme heavy chain	NCICNDSSCIMSAVLSSQPSK	NC[57.0215]IC[57.0215]N[2407.8518]DSSC[57.0215]IMSAVLS	HexNAc(5)Hex(5)NeuAc(2) % 2407.8519	K	L	349.00	369.00	21.00	4.00	1.18E+07	0.00E+00	3.52E+06	0.18		0.03	0.07
	Coagulation factor X-activating enzyme heavy chain	NCICNDSSCIMSAVLSSQPSK	NC[57.0215]IC[57.0215]N[2553.9099]DSSC[57.0215]IMSAVLS	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	L	349.00	369.00	21.00	3,4	1.34E+08	0.00E+00	1.82E+08	2.04		1.36	1.13
Q9PT40	Snake venom serine protease homolog 2	KPVNDSTHIVPLSLPSSPPSVG	KPVN[2553.9099]DSTHIVPLSLPSSPPSVGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	L	120.00	145.00	26.00	4.00	6.17E+07	0.00E+00	8.48E+07	0.94		0.63	0.52

Table S8: The identified N-glycopeptides in the glycoproteome from *M. l. obtusa* (*Ml*) snake venom .

	Snake venom serine protease homolog 2	KPVNDSTHIVPLSLPSSPPSVG	KPVN[3007.0581]DSTHIVPLSLPSSPPSVGSVC[57.0215]R	HexNAc(5)Hex(6)Fuc(1)NeuAc(3) % 3007.0580	K	L	120.00	145.00	26.00	4.00	3.93E+07	2.69E+07		0.60	0.20		0.27
	Snake venom serine protease homolog 2	LKKPVNDSTHIVPLSLPSSPPS	LKKPVN[3866.3650]DSTHIVPLSLPSSPPSVGSVC[57.0215]R	HexNAc(7)Hex(7)Fuc(1)NeuAc(4) % 3866.3650	R	L	118.00	145.00	28.00	4,5	1.46E+08	3.16E+08		2.21	2.37		1.53
A0A194A	Snake venom serine protease	LDSPVNNSAHIAPLSLPSSPPS	LDSPVN[3448.2427]NSAHIAPLSLPSSPPSVGSVC[57.0215]R	HexNAc(9)Hex(10) % 3448.2427	R	L	118.00	145.00	28.00	4.00	7.92E+07	0.00E+00	7.03E+07	1.20		0.52	0.58
A0A6B7FMQ3	Serine proteinase SP-7	LNSSVTYNTHIAPLSLPSSPPR	LN[2553.9099]SSVTYNTHIAPLSLPSSPPR	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	R	V	115.00	136.00	22.00	3,4,5	7.02E+07	6.88E+07	9.55E+07	1.07	0.52	0.71	0.76
	Serine proteinase SP-7	LNSSVTYNTHIAPLSLPSSPPR	LN[3064.0796]SSVTYNTHIAPLSLPSSPPR	HexNAc(6)Hex(6)NeuAc(3) % 3064.0795	R	V	115.00	136.00	22.00	4.00	5.77E+07	4.37E+07	6.86E+07	0.88	0.33	0.51	0.57
W8E7D1	Venom phosphodiesterase	INGSYPTIYK	IN[1378.4757]GSYPTIYK	HexNAc(2)Hex(6) % 1378.4757	K	V	258.00	267.00	10.00	2,3	0.00E+00	1.40E+08	6.14E+07		1.05	0.46	0.50

Table S8: The identified N-glycopeptides in the glycoproteome from *M. l. obtusa* (*Ml*) snake venom .

	Venom phosphodiesterase	LKPAPNNGTHGSLNHLLK	LKPAPNN[1702.5814]GTHGSLNHLLK	HexNAc(2)Hex(8) % 1702.5814	K	N	506.00	523.00	18.00	3,4,5	0.00E+00	4.62E+07	5.04E+07		0.35	0.37	0.24
	Venom phosphodiesterase	LKPAPNNGTHGSLNHLLK	LKPAPNN[1864.6342]GTHGSLNHLLK	HexNAc(2)Hex(9) % 1864.6342	K	N	506.00	523.00	18.00	3,4,5	5.42E+07	1.77E+08	1.87E+08	0.82	1.33	1.39	1.18
	Venom phosphodiesterase	LKPAPNNGTHGSLNHLLK	LKPAPNN[2026.6871]GTHGSLNHLLK	HexNAc(2)Hex(10) % 2026.6871	K	N	506.00	523.00	18.00	3,4,5	0.00E+00	5.23E+07	7.81E+07		0.39	0.58	0.32
A0A0C5DGP5	C-type lectin-like protein 4B	VIEPLKNWTDAEK	VIEPLKN[1954.7036]WTDAEK	HexNAc(5)Hex(4)NeuAc(1) % 1954.7036	K	F	41.00	53.00	13.00	3.00	0.00E+00	8.91E+07	5.50E+07		0.67	0.41	0.36
	C-type lectin-like protein 4B	VIEPLKNWTDAEK	VIEPLKN[2407.8518]WTDAEK	HexNAc(5)Hex(5)NeuAc(2) % 2407.8519	K	F	41.00	53.00	13.00	3.00	0.00E+00	1.27E+08	1.00E+08		0.95	0.74	0.56
Q7T045	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[1460.5288]WTDAEK	HexNAc(4)Hex(4) % 1460.5288	K	F	47.00	53.00	7.00	2,3	9.19E+06	3.95E+07	2.86E+07	0.14	0.30	0.21	0.22

Table S8: The identified N-glycopeptides in the glycoproteome from *M. l. obtusa* (*Ml*) snake venom .

	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[1501.5553]WTDAEK	HexNAc(5) Hex(3) % 1501.5553	K	F	47.00	53.00	7.00	2,3	7.86E+07	3.45E+08	1.93E+08	1.19	2.59	1.44	1.74
	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[1622.5817]WTDAEK	HexNAc(4) Hex(5) % 1622.5817	K	F	47.00	53.00	7.00	2,3	3.55E+07	1.43E+08	9.50E+07	0.54	1.07	0.71	0.77
	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[1663.6082]WTDAEK	HexNAc(5) Hex(4) % 1663.6082	K	F	47.00	53.00	7.00	2,3	1.19E+08	5.27E+08	2.80E+08	1.80	3.96	2.08	2.61
	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[1784.6345]WTDAEK	HexNAc(4) Hex(6) % 1784.6345	K	F	47.00	53.00	7.00	3.00	0.00E+00	7.89E+06	1.01E+07		0.06	0.07	0.04
	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[1825.6610]WTDAEK	HexNAc(5) Hex(5) % 1825.6610	K	F	47.00	53.00	7.00	2,3	4.16E+07	1.65E+08	1.19E+08	0.63	1.24	0.89	0.92
	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[1954.7036]WTDAEK	HexNAc(5) Hex(4)NeuAc(1) % 1954.7036	K	F	47.00	53.00	7.00	2,3	1.09E+09	3.43E+09	2.42E+09	16.57	25.76	17.97	20.10

Table S8: The identified N-glycopeptides in the glycoproteome from *M. l. obtusa* (*Ml*) snake venom .

	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[2116.7563]WTDAEK	HexNAc(5)Hex(5)NeuAc(1) % 2116.7564	K	F	47.00	53.00	7.00	2,3	7.13E+08	1.80E+09	1.54E+09	10.83	13.52	11.48	11.94
	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[2204.7725]WTDAEK	HexNAc(4)Hex(5)NeuAc(2) % 2204.7725	K	F	47.00	53.00	7.00	2,3	1.39E+08	1.74E+08	1.60E+08	2.11	1.31	1.19	1.54
	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[2278.8091]WTDAEK	HexNAc(5)Hex(6)NeuAc(1) % 2278.8092	K	F	47.00	53.00	7.00	3.00	0.00E+00	1.57E+08	1.53E+08		1.18	1.14	0.77
	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[2407.8518]WTDAEK	HexNAc(5)Hex(5)NeuAc(2) % 2407.8519	K	F	47.00	53.00	7.00	2,3	3.03E+09	4.05E+09	6.37E+09	45.93	30.38	47.31	41.21
	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[2569.9048]WTDAEK	HexNAc(5)Hex(6)NeuAc(2) % 2569.9047	K	F	47.00	53.00	7.00	3.00	1.52E+08	1.44E+08	2.56E+08	2.31	1.08	1.90	1.77
	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[2772.9839]WTDAEK	HexNAc(6)Hex(6)NeuAc(2) % 2772.9841	K	F	47.00	53.00	7.00	3.00	0.00E+00	1.02E+07	1.96E+07		0.08	0.15	0.07

Table S8: The identified N-glycopeptides in the glycoproteome from *M. l. obtusa* (*Ml*) snake venom .

	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[3064.0796]WTDAEK	HexNAc(6)Hex(6)NeuAc(3) % 3064.0795	K	F	47.00	53.00	7.00	3.00	2.78E+08	4.01E+07	1.91E+08	4.22	0.30	1.42	1.98
A0A1I9KNR6	Metalloproteinase of class P-III MPIII-1	YNNNSTAIR	YNN[1694.6028]NSTAIR	HexNAc(3)Hex(4)Fuc(1)NeuAc(1) % 1694.6028	K	T	219.00	227.00	9.00	3.00	2.85E+07	1.09E+08	6.89E+07	0.43	0.82	0.51	0.59
	Metalloproteinase of class P-III MPIII-1	YNNNSTAIR	YNN[1872.6506]NSTAIR	HexNAc(3)Hex(6)NeuAc(1) % 1872.6505	K	T	219.00	227.00	9.00	2,3	0.00E+00	9.74E+07	8.32E+07		0.73	0.62	0.45
	Metalloproteinase of class P-III MPIII-1	YNNNSTAIR	YNN[2018.7085]NSTAIR	HexNAc(3)Hex(6)Fuc(1)NeuAc(1) % 2018.7085	K	T	219.00	227.00	9.00	2,3	0.00E+00	7.88E+07	5.19E+07		0.59	0.39	0.33
	Metalloproteinase of class P-III MPIII-1	YNNNSTAIR	YNN[2553.9099]NSTAIR	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	T	219.00	227.00	9.00	3.00	6.61E+07	1.95E+08	1.77E+08	1.00	1.46	1.32	1.26
	Metalloproteinase of class P-III MPIII-1	YNNNSTAIR	YNNN[1694.6028]STAIR	HexNAc(3)Hex(4)Fuc(1)NeuAc(1) % 1694.6028	K	T	219.00	227.00	9.00	2.00	0.00E+00	1.23E+08	6.50E+07		0.92	0.48	0.47

Table S8: The identified N-glycopeptides in the glycoproteome from *M. l. obtusa* (*Ml*) snake venom .

V5TBK6	Zinc metalloproteinase-disintegrin-like protein H4 subunit A	YTGNSTAIR	YTGN[1216.4229]STAIR	HexNAc(2)Hex(5) % 1216.4229	K	T	217.00	225.00	9.00	2,3	4.93E+07	2.02E+08	1.10E+08	0.75	1.51	0.82	1.03
	Zinc metalloproteinase-disintegrin-like protein H4 subunit A	YTGNSTAIR	YTGN[1872.6506]STAIR	HexNAc(3)Hex(6)NeuAc(1) % 1872.6505	K	T	217.00	225.00	9.00	2,3	0.00E+00	1.86E+08	1.46E+08		1.39	1.09	0.83
A3QVN3	Hyaluronidase-1	YIVNVTTAAK	YIVN[1216.4229]VTTAAK	HexNAc(2)Hex(5) % 1216.4229	R	L	354.00	363.00	10.00	2.00	0.00E+00	5.29E+07	2.05E+07		0.40	0.15	0.18
A0A6G5ZVN1	5'-nucleotidase (Fragment)	GPIASNISGYILPYK	GPIASN[2553.9099]ISGYILPYK	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	L	35.00	49.00	15.00	3.00	1.06E+08	1.59E+08	4.65E+07	1.61	1.19	0.35	1.05

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

											MaxLFQ Intensity	**Ralative Abundance %**

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

Protein ID	Protein Description	Peptide Sequence	Modified Sequence	Glycan Composition	Prev AA	Next AA	Start	End	Peptide Length	Charges	M. xanthina (Mx)-1 MaxLFQ	M. xanthina (Mx)-2 MaxLFQ	M. xanthina (Mx)-3 MaxLFQ	Mx2	Mx3	Avg
A0A1W7RJX5	Snake venom serine protease	DIMLIRLDSPVNNSAHIVPLSLPSSPSVGSVCR	DIMLIRLDSPVN[2010.6920]NSAHIVPLSLPSSPSVGSVC[57.0215]R	HexNAc(2)Hex(10)Fuc(1)NeuAc(1) % 2463.8404	K	L	112.00	145.00	34.00	4,5	0.00E+00	6.65E+07	8.72E+07	0.27	0.25	0.17
A0A1W7RJV9	Snake venom serine protease	RPVNKSTHIAPLSLPSSPSLGSVCR	RPVN[2463.8403]KSTHIAPLSLPSSPSLGSVC[57.0215]R	HexNAc(2)Hex(10)Fuc(1)NeuAc(1) % 2463.8404	K	V	120.00	145.00	26.00	4.00	0.00E+00	1.62E+08	2.85E+08	0.65	0.83	0.49
A0A6B7FPJ0	Serine proteinase SP-8	DMFFCLSNK	DM[15.9949]FFC[57.0215]LSN[2553.9099]K	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	S	96.00	104.00	9.00	3.00	0.00E+00	1.63E+08	2.53E+08	0.66	0.74	0.47
A0A6B7FQF8	Serine proteinase SP-6	SIIAGNTTATCPP	SIIAGN[2553.9099]TTATC[57.0215]PP	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	-	245.00	257.00	13.00	3.00	2.69E+08	1.43E+08	2.10E+08	0.58	0.61	0.73
A0A1I9KNL5	Serine proteinase SP-2	SLVLLYNASGFR	SLVLLYN[1216.4229]ASGFR	HexNAc(2)Hex(5) % 1216.4229	R	C	38.00	49.00	12.00	2,3	7.66E+07	6.67E+07	6.68E+07	0.27	0.19	0.25

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

	Serine proteinase SP-2	SLVLLYNASGFR	SLVLLYN[1872.6506]ASGFR	HexNAc(3)Hex(6)NeuAc(1) % 1872.6505	R	C	38.00	49.00	12.00	2,3	2.04E+08	1.35E+08	1.85E+08	0.55	0.54	0.61
A0A1I9KNP0	Vaa serine proteinase homolog 1	RPVNDSTHIAPLSLPSSPPSVGSVCR	RPVN[2553.9099]DSTHIAPLSLPSSPPSVGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	L	120.00	145.00	26.00	3,4,5	6.47E+07	7.24E+07	9.71E+07	0.29	0.28	0.27
B0FXM3	Thrombin-like enzyme gyroxin B1.7	LNIPVRNSTHIAPLSLPSSPPSVGSVCR	LNIPVRN[3009.0737]STHIAPLSLPSSPPSVGSVC[57.0215]R	HexNAc(7)Hex(8)NeuAc(1) % 3009.0737	R	V	115.00	142.00	28.00	4.00	1.53E+08	8.64E+07	0.00E+00	0.35		0.31
J7LCB0	Snake venom serine protease Da-36	LNKPVSNSTHIAPLSLPSSPPSIGSVCR	LNKPVSN[2393.8726]STHIAPLSLPSSPPSIGSVC[57.0215]R	HexNAc(7)Hex(6) % 2393.8726	K	V	118.00	145.00	28.00	3,4,5	9.71E+08	1.06E+09	1.03E+09	4.30	3.00	3.64
	Snake venom serine protease Da-36	LNKPVSNSTHIAPLSLPSSPPSIGSVCR	LNKPVSN[2555.9253]STHIAPLSLPSSPPSIGSVC[57.0215]R	HexNAc(7)Hex(7) % 2555.9254	K	V	118.00	145.00	28.00	4,5	5.50E+07	8.86E+07	1.49E+08	0.36	0.43	0.33
Q71QI2	Snake venom serine protease CL2	LNRPVNNSVHIAPLSLPSSPPSVGSVCR	LNRPVN[2352.8459]NSVHIAPLSLPSSPPSVGSVC[57.0215]R	HexNAc(6)Hex(7) % 2352.8461	R	L	116.00	143.00	28.00	4,5	6.25E+07	3.55E+09	4.44E+09	14.36	12.92	9.17

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

	Snake venom serine protease CL2	LNRPVNNSVHIAPLSLPSSPPSVGSVCR	LNRPVN[2555.9253]NSVHIAPLSLPSSPPSVGSVC[57.0215]R	HexNAc(7)Hex(7) % 2555.9254	R	L	116.00	143.00	28.00	3,4,5	4.20E+09	4.02E+09	4.73E+09	16.27	13.75	15.22
E5AJX2	Snake venom serine protease nikobin	LNTPVNNSTHIAPVSLASRPPVVGSVCR	LNTPVN[1856.6556]NSTHIAPVSLASRPPVVGSVC[57.0215]R	HexNAc(3)Hex(5)Fuc(1)NeuAc(1) % 1856.6557	R	L	115.00	142.00	28.00	4.00	7.29E+07	6.04E+07	6.81E+07	0.24	0.20	0.24
	Snake venom serine protease nikobin	LNTPVNNSTHIAPVSLASRPPVVGSVCR	LNTPVN[2059.7351]NSTHIAPVSLASRPPVVGSVC[57.0215]R	HexNAc(4)Hex(5)Fuc(1)NeuAc(1) % 2059.7350	R	L	115.00	142.00	28.00	4.00	1.96E+08	1.22E+08	1.61E+08	0.49	0.47	0.56
	Snake venom serine protease nikobin	LNTPVNNSTHIAPVSLASRPPVVGSVCR	LNTPVN[2100.7615]NSTHIAPVSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) % 2100.7615	R	L	115.00	142.00	28.00	4.00	7.01E+08	1.01E+09	1.46E+09	4.08	4.24	3.64
	Snake venom serine protease nikobin	LNTPVNNSTHIAPVSLASRPPVVGSVCR	LNTPVN[2221.7878]NSTHIAPVSLASRPPVVGSVC[57.0215]R	HexNAc(4)Hex(6)Fuc(1)NeuAc(1) % 2221.7878	R	L	115.00	142.00	28.00	4.00	1.68E+08	0.00E+00	1.71E+08	0.00	0.50	0.37
	Snake venom serine protease nikobin	LNTPVNNSTHIAPVSLASRPPVVGSVCR	LNTPVN[2262.8142]NSTHIAPVSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(1)NeuAc(1) % 2262.8143	R	L	115.00	142.00	28.00	4,5	1.25E+09	1.06E+09	1.53E+09	4.28	4.44	4.46

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

	Snake venom serine protease nikobin	LNTPVNNSTHIAPVSLASRPPVVGSVCR	LNTPVN[2350.8303]NSTHIAPVSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(1)NeuAc(1) % 2350.8304	R	L	115.00	142.00	28.00	3,4,5	1.21E+09	1.36E+09	1.30E+09	5.51	3.78	4.60
	Snake venom serine protease nikobin	LNTPVNNSTHIAPVSLASRPPVVGSVCR	LNTPVN[2553.9099]NSTHIAPVSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	R	L	115.00	142.00	28.00	4,5	2.34E+09	2.54E+09	3.74E+09	10.27	10.88	9.96
	Snake venom serine protease nikobin	LNTPVNNSTHIAPVSLASRPPVVGSVCR	LNTPVN[2555.9253]NSTHIAPVSLASRPPVVGSVC[57.0215]R	HexNAc(7)Hex(7) % 2555.9254	R	L	115.00	142.00	28.00	4.00	2.11E+08	1.08E+08	0.00E+00	0.44		0.41
	Snake venom serine protease nikobin	LNTPVNNSTHIAPVSLASRPPVVGSVCR	LNTPVN[2715.9626]NSTHIAPVSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(6)Fuc(1)NeuAc(2) % 2715.9626	R	L	115.00	142.00	28.00	4.00	7.16E+07	0.00E+00	8.73E+07		0.25	0.17
	Snake venom serine protease nikobin	LNTPVNNSTHIAPVSLASRPPVVGSVCR	LNTPVN[3007.0581]NSTHIAPVSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(6)Fuc(1)NeuAc(3) % 3007.0580	R	L	115.00	142.00	28.00	4,5	1.43E+08	0.00E+00	9.34E+07		0.27	0.27
Q7T045	Snaclec coagulation factor X-activating enzyme	NWTDAEK	N[1622.5817]WTDAEK	HexNAc(4)Hex(5) % 1622.5817	K	F	47.00	53.00	7.00	2,3	2.71E+07	2.24E+07	2.31E+07	0.09	0.07	0.09

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

	Snaclec coagulation factor X-activating enzyme	NWTDAEK	N[1954.7036]WTDAEK	HexNAc(5)Hex(4)NeuAc(1) % 1954.7036	K	F	47.00	53.00	7.00	2,3	3.34E+08	2.34E+08	2.73E+08	0.95	0.79	0.99
	Snaclec coagulation factor X-activating enzyme	NWTDAEK	N[2116.7563]WTDAEK	HexNAc(5)Hex(5)NeuAc(1) % 1954.7036	K	F	47.00	53.00	7.00	2,3	1.71E+08	0.00E+00	2.11E+08		0.61	0.42
	Snaclec coagulation factor X-activating enzyme	NWTDAEK	N[2407.8518]WTDAEK	HexNAc(5)Hex(5)NeuAc(2) % 2407.8519	K	F	47.00	53.00	7.00	2,3	9.51E+08	1.03E+09	9.59E+08	4.18	2.79	3.50
A0A1I9KNS3	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2262.8142]C[57.0215]SK	HexNAc(5)Hex(5)Fuc(1)NeuAc(1) % 2262.8143	R	N	370.00	378.00	9.00	2,3	3.92E+07	0.00E+00	6.66E+07		0.19	0.11
	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2116.7564]C[57.0215]SK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2116.7564	R	N	370.00	378.00	9.00	2,3	5.20E+07	0.00E+00	5.68E+07		0.17	0.12
	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2553.9099]C[57.0215]SK	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	R	N	370.00	378.00	9.00	2,3	9.09E+08	3.70E+08	4.73E+08	1.50	1.38	2.09

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

A0A6B7FRK6	Metalloproteinase of class P-III MPIII-2	YNNDSTAIR	YNN[2350.8303]DSTAIR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	T	217.00	225.00	9.00	2,3	0.00E+00	8.50E+08	1.18E+09	3.44	3.43	2.29
	Metalloproteinase of class P-III MPIII-2	YNNDSTAIR	YNN[2553.9099]DSTAIR	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	T	217.00	225.00	9.00	3.00	9.25E+08	3.65E+08	8.19E+08	1.48	2.38	2.43
E9JG33	Metalloproteinase	YTGNSTAIK	YTGN[1216.4229]STAIK	HexNAc(2)Hex(5) % 1216.4229	K	T	217.00	225.00	9.00	2.00	6.69E+08	4.48E+08	8.98E+08	1.81	2.61	2.31
	Metalloproteinase	YTGNSTAIK	YTGN[1710.5977]STAIK	HexNAc(3)Hex(5)NeuAc(1) % 1710.5977	K	T	217.00	225.00	9.00	2,3	8.87E+07	6.89E+07	1.19E+08	0.28	0.35	0.32
	Metalloproteinase	YTGNSTAIK	YTGN[1856.6556]STAIK	HexNAc(3)Hex(5)Fuc(1)NeuAc(1) % 1856.6557	K	T	217.00	225.00	9.00	2,3	7.12E+07	7.02E+07	9.98E+07	0.28	0.29	0.28
	Metalloproteinase	YTGNSTAIK	YTGN[1872.6506]STAIK	HexNAc(3)Hex(6)NeuAc(1) % 1872.6505	K	T	217.00	225.00	9.00	2,3	3.74E+08	2.07E+08	3.55E+08	0.84	1.03	1.09

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

	Metalloproteinase	YTGNSTAIK	YTGN[2018.7085]STAIK	HexNAc(3)Hex(6)Fuc(1)NeuAc(1) % 2018.7085	K	T	217.00	225.00	9.00	2,3	6.47E+07	2.99E+07	6.14E+07	0.12	0.18	0.18
	Metalloproteinase	YTGNSTAIK	YTGN[2350.8303]STAIK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	T	217.00	225.00	9.00	3.00	1.91E+08	0.00E+00	1.17E+08		0.34	0.35
R4NNL0	Zinc metalloproteinase-disintegrin-like protein	FSNCSQEEHR	FSN[2262.8142]C[57.0215]SQEEHR	HexNAc(5)Hex(5)Fuc(1)NeuAc(1) % 2262.8143	R	K	375.00	384.00	10.00	3.00	3.83E+07	0.00E+00	5.82E+07		0.17	0.10
	Zinc metalloproteinase-disintegrin-like protein	FSNCSQEEHR	FSN[2350.8303]C[57.0215]SQEEHR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	K	375.00	384.00	10.00	2,3,4	1.62E+08	0.00E+00	6.07E+07		0.18	0.26
	Zinc metalloproteinase-disintegrin-like protein	FSNCSQEEHR	FSN[2553.9099]C[57.0215]SQEEHR	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	R	K	375.00	384.00	10.00	3,4	7.97E+08	3.83E+08	1.26E+09	1.55	3.65	2.72
Q2UXQ5	Zinc metalloproteinase-disintegrin-like	LFSNCSEIQYER	LFSN[2553.9099]C[57.0215]SEIQYER	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	F	372.00	383.00	12.00	3.00	1.19E+08	0.00E+00	9.21E+07		0.27	0.24

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

V5TBK6	Zinc metalloproteinase-disintegrin-like protein	YTGNSTAIR	YTGN[1216.4229]STAIR	HexNAc(2)Hex(5) % 1216.4229	K	T	217.00	225.00	9.00	2,3	1.15E+09	5.24E+08	7.95E+08	2.12	2.31	2.90
A0A6B7FRF2	Amine oxidase	TNCSYILNK	TN[2059.7351]C[57.0215]SYILNK	HexNAc(4)Hex(5)Fuc(1)NeuAc(1) % 2059.7350	R	Y	189.00	197.00	9.00	2,3	1.53E+08	1.12E+08	1.91E+08	0.45	0.56	0.53
	Amine oxidase	TNCSYILNK	TN[2100.7615]C[57.0215]SYILNK	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) % 2100.7615	R	Y	189.00	197.00	9.00	2,3	6.44E+07	6.43E+07	9.17E+07	0.26	0.27	0.26
	Amine oxidase	TNCSYILNK	TN[2262.8142]C[57.0215]SYILNK	HexNAc(5)Hex(5)Fuc(1)NeuAc(1) % 2262.8143	R	Y	189.00	197.00	9.00	3.00	3.03E+08	2.96E+08	3.65E+08	1.20	1.06	1.13
	Amine oxidase	TNCSYILNK	TN[2350.8303]C[57.0215]SYILNK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	Y	189.00	197.00	9.00	2,3	2.02E+09	1.37E+09	1.94E+09	5.56	5.64	6.24
	Amine oxidase	TNCSYILNK	TN[2553.9099]C[57.0215]SYILNK	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	R	Y	189.00	197.00	9.00	2,3	2.33E+09	1.65E+09	2.44E+09	6.67	7.11	7.48

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

0.15	0.22		7.48E+07	0.00E+00	6.29E+07	3.00	9.00	197.00	189.00	Y	R	HexNAc(5) Hex(6)Fuc (1)NeuAc(2) % 2715.9626	TN[2715.9 626]C[57.0 215]SYILN K	TNCSYIL NK	Amine oxidase	
1.85	1.35		4.64E+08	0.00E+00	1.13E+09	2,3,4	10.00	197.00	188.00	Y	K	HexNAc(4) Hex(5)Fuc (1)NeuAc(2) % 2350.8304	RTN[2350. 8303]C[57. 0215]SYIL NK	RTNCSYI LNK	Amine oxidase	
0.47	0.47	0.45	1.60E+08	1.10E+08	1.35E+08	2,3	13.00	188.00	176.00	F	K	HexNAc(2) Hex(5) % 1216.4229	TIVYLN[12 16.4229]G TTQAC[57 .0215]R	TIVYLNGT TQACR	Snake venom 5'- nucleotida se (Fragment	**W8EFS0**
0.64	0.66	0.55	2.27E+08	1.36E+08	1.91E+08	3.00	15.00	49.00	35.00	L	K	HexNAc(5) Hex(5)Fuc (1)NeuAc(2) % 2553.9098	GPIASN[2 553.9099]I SGYILPY K	GPIASNIS GYILPYK	5'- nucleotida se (Fragment)	**A0A6G5Z VN1**
0.10	0.06	0.13	2.18E+07	3.30E+07	2.62E+07	2.00	10.00	363.00	354.00	L	R	HexNAc(2) Hex(5) % 1216.4229	YIVN[1216 .4229]VTT AAK	YIVNVTTA AK	Hyaluronid ase-1	**A3QVN3**
0.08	0.16	0.10	5.41E+07	2.40E+07	0.00E+00	3.00	14.00	266.00	253.00	A	K	HexNAc(3) Hex(5)Neu Ac(1) % 1710.5977	AN[1710.5 977]FSITL IHLPGYK	ANFSITLI HLPGYK	Aminopept idase	**A0A1W7R JE8**

Table S9: The identified N-glycopeptides in the glycoproteome from *M. xanthina* (*Mx*) snake venom.

W8E7D1	Venom phosphodiesterase	INGSYPTIYK	IN[1378.4757]GSYPTIYK	HexNAc(2)Hex(6) % 1378.4757	K	V	258.00	267.00	10.00	2,3	5.63E+07	7.11E+07	7.94E+07	0.29	0.23	0.24
A0A0F7ZBV7	Calmin-like	NMSVSVK	N[1419.5023]MSVSVK	HexNAc(3)Hex(5) % 1419.5023	R	D	178.00	184.00	7.00	2.00	1.51E+08	7.28E+07	9.35E+07	0.29	0.27	0.38

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

											MaxLFQ Intensity			**Ralative Abundance %**			
Protein ID	**Protein Description**	**Peptide Sequence**	**Modified Sequence**	**Glycan Composition**	**Prev AA**	**Next AA**	**Start**	**End**	**Peptide Length**	**Charges**	**V. a. ammodytes (Vaa)-1 MaxLFQ**	**V. a. ammodytes (Vaa)-2 MaxLFQ**	**V. a. ammodytes (Vaa)-3 MaxLFQ**	**Vaa1**	**Vaa2**	**Vaa3**	**Avg**

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

A0A1I9KNL5	Serine proteinase SP-2	KPVNNSIHIAPLSLPSSPPSEGSVCR	KPVN[2059.7351]NSIHIAPLSLPSSPPSEGSVC[57.0215]R	HexNAc(4)Hex(5)Fuc(1)NeuAc(1) % 2059.7350	K	L	118.00	143.00	26.00	3,4	6.40E+07	3.87E+07	5.72E+07	0.16	0.12	0.14	0.14
	Serine proteinase SP-2	KPVNNSIHIAPLSLPSSPPSEGSVCR	KPVN[2350.8304]NSIHIAPLSLPSSPPSEGSVC[57.0215]R	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	L	118.00	143.00	26.00	3,4,5	6.31E+08	5.97E+08	7.02E+08	1.59	1.86	1.68	1.71
	Serine proteinase SP-2	KPVNNSIHIAPLSLPSSPPSEGSVCR	KPVN[2553.9099]NSIHIAPLSLPSSPPSEGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	L	118.00	143.00	26.00	3,4,5	5.95E+08	5.52E+08	6.30E+08	1.50	1.71	1.51	1.57
A0A2I7YS71	Serine endopeptidase	LKRPVNNSAHIATLSLPSSPPSVGSVCR	LKRPVN[2350.8304]NSAHIATLSLPSSPPSVGSVC[57.0215]R	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	L	118.00	145.00	28.00	4.00	1.87E+08	0.00E+00	1.78E+08	0.47		0.43	0.30
A0A6B7FMQ3	Serine proteinase SP-7	LNSSVTYNTHIAPLSLPSSPPR	LN[2059.7351]SSVTYNTHIAPLSLPSSPPR	HexNAc(4)Hex(5)Fuc(1)NeuAc(1) % 2059.7350	R	V	115.00	136.00	22.00	3.00	1.05E+08	0.00E+00	6.75E+07	0.26		0.16	0.14
	Serine proteinase SP-7	LNSSVTYNTHIAPLSLPSSPPR	LN[2350.8304]SSVTYNTHIAPLSLPSSPPR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	V	115.00	136.00	22.00	3,4,5	6.21E+08	4.07E+08	3.83E+08	1.56	1.27	0.92	1.25

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Serine proteinase SP-7	LNSSVTYNTHIAPLSLPSSPPR	LN[2553.9099]SSVTYNTHIAPLSLPSSPPR	HexNAc(5)Hex(6)Fuc(1)NeuAc(3) % 3007.0580	R	V	115.00	136.00	22.00	3,4,5	1.62E+08	1.28E+08	9.80E+07	0.41	0.40	0.23	0.35
	Serine proteinase SP-7	LNSSVTYNTHIAPLSLPSSPPR	LN[3007.0581]SSVTYNTHIAPLSLPSSPPR	HexNAc(5)Hex(6)Fuc(1)NeuAc(3) % 3007.0580	R	V	115.00	136.00	22.00	3,4	1.69E+08	7.27E+07	0.00E+00	0.43	0.23		0.22
A0A6B7FQF8	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1606.5867]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(4)Hex(4)Fuc(1) % 1606.5867	R	L	115.00	142.00	28.00	4.00	4.90E+07	2.81E+07	0.00E+00	0.12	0.09		0.07
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1694.6028]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(3)Hex(4)Fuc(1)NeuAc(1) % 1694.6028	R	L	115.00	142.00	28.00	3,4	9.14E+08	9.41E+08	8.56E+08	2.30	2.92	2.05	2.42
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1695.6232]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(3)Hex(4)Fuc(3) % 1695.6232	R	L	115.00	142.00	28.00	3,4,5	1.07E+09	1.05E+09	9.30E+08	2.68	3.26	2.23	2.72
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1809.6660]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(4)Fuc(1) % 1809.6661	R	L	115.00	142.00	28.00	4.00	1.78E+08	9.15E+07	1.30E+08	0.45	0.28	0.31	0.35

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[1 856.6556] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(3) Hex(5)Fuc (1)NeuAc(1) % 1856.6557	R	L	115.00	142.00	28.00	4,5	0.00E+00	1.67E+08	1.73E+08		0.52	0.42	0.31
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[1 872.6506] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(3) Hex(6)Neu Ac(1) % 1872.6505	R	L	115.00	142.00	28.00	4.00	0.00E+00	1.21E+07	0.00E+00		0.04		0.01
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[1 897.6821] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(4) Hex(4)Fuc (1)NeuAc(1) % 1897.6821	R	L	115.00	142.00	28.00	3,4,5	9.83E+08	9.13E+08	9.13E+08	2.47	2.84	2.19	2.50
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 018.7085] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(3) Hex(6)Fuc (1)NeuAc(1) % 2018.7085	R	L	115.00	142.00	28.00	4.00	2.38E+08	2.46E+08	2.50E+08	0.60	0.77	0.60	0.65
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 059.7351] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(4) Hex(5)Fuc (1)NeuAc(1) % 2059.7350	R	L	115.00	142.00	28.00	3,4,5	9.80E+08	7.52E+08	9.23E+08	2.47	2.34	2.21	2.34
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 100.7615] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(5) Hex(4)Fuc (1)NeuAc(1) % 2100.7615	R	L	115.00	142.00	28.00	3,4,5	3.00E+09	2.76E+09	2.86E+09	7.54	8.57	6.86	7.66

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 157.7830] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(6) Hex(4)Neu Ac(1) % 2157.7831	R	L	115.00	142.00	28.00	4.00	4.76E+08	6.94E+07	8.05E+07	1.20	0.22	0.19	0.54
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 262.8142] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(5) Hex(5)Fuc (1)NeuAc(1) % 2262.8143	R	L	115.00	142.00	28.00	4,5	4.68E+08	4.42E+08	4.60E+08	1.18	1.37	1.10	1.22
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 350.8304] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(4) Hex(5)Fuc (1)NeuAc(2) % 2350.8304	R	L	115.00	142.00	28.00	3,4,5	4.09E+09	3.54E+09	3.99E+09	10.29	11.00	9.56	10.28
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 407.8518] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(5) Hex(5)Neu Ac(2) % 2407.8519	R	L	115.00	142.00	28.00	4,5	8.02E+08	1.39E+08	7.16E+07	2.02	0.43	0.17	0.87
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 553.9099] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(5) Hex(5)Fuc (1)NeuAc(2) % 2553.9098	R	L	115.00	142.00	28.00	3,4,5	1.54E+09	1.10E+09	1.52E+09	3.87	3.43	3.65	3.65
	Serine proteinase SP-6	SIIAGNTT ATCPP	SIIAGN[20 59.7351]T TATC[57.0 215]PP	HexNAc(4) Hex(5)Fuc (1)NeuAc(1) % 2059.7350	K	-	245.00	257.00	13.00	3.00	2.04E+08	1.26E+08	2.63E+08	0.51	0.39	0.63	0.51

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Serine proteinase SP-6	SIIAGNTT ATCPP	SIIAGN[2350.8303]TTATC[57.0215]PP	HexNAc(4) Hex(5)Fuc (1)NeuAc(2) % 2350.8304	K	-	245.00	257.00	13.00	2,3	1.60E+09	1.02E+09	1.83E+09	4.02	3.16	4.39	3.86
	Serine proteinase SP-6	SIIAGNTT ATCPP	SIIAGN[2553.9099]TTATC[57.0215]PP	HexNAc(5) Hex(5)Fuc (1)NeuAc(2) % 2553.9098	K	-	245.00	257.00	13.00	3.00	2.35E+08	1.06E+08	2.95E+08	0.59	0.33	0.71	0.54
A0A1I9KNP0	Vaa serine proteinase homolog 1	RPVNDST HIAPLSLP SSPPSVG SVCR	RPVN[2350.8304]DSTHIAPLSLPSSPPSVGSVC[57.0215]R	HexNAc(4) Hex(5)Fuc (1)NeuAc(2) % 2350.8304	K	L	120.00	145.00	26.00	3,4,5	3.42E+07	0.00E+00	6.34E+07	0.09		0.15	0.08
	Vaa serine proteinase homolog 1	RPVNDST HIAPLSLP SSPPSVG SVCR	RPVN[2553.9099]DSTHIAPLSLPSSPPSVGSVC[57.0215]R	HexNAc(5) Hex(5)Fuc (1)NeuAc(2) % 2553.9098	K	L	120.00	145.00	26.00	3,4,5	2.68E+08	3.32E+08	3.94E+08	0.67	1.03	0.94	0.88
	Vaa serine proteinase homolog 1	RPVNDST HIAPLSLP SSPPSVG SVCR	RPVN[3007.0581]DSTHIAPLSLPSSPPSVGSVC[57.0215]R	HexNAc(5) Hex(6)Fuc (1)NeuAc(3) % 3007.0580	K	L	120.00	145.00	26.00	4,5	3.12E+08	2.67E+08	3.66E+08	0.78	0.83	0.88	0.83
A0A1I9KNL5	Serine proteinase SP-2	SLVLLYN ASGFR	SLVLLYN[1216.4229]ASGFR	HexNAc(2) Hex(5) % 1216.4229	R	C	38.00	49.00	12.00	2,3	8.06E+07	5.09E+07	6.15E+07	0.20	0.16	0.15	0.17

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Serine proteinase SP-2	SLVLLYN ASGFR	SLVLLYN[1378.4757]ASGFR	HexNAc(2) Hex(6) % 1378.4757	R	C	38.00	49.00	12.00	2,3	5.92E+07	2.48E+07	4.48E+07	0.15	0.08	0.11	0.11
	Serine proteinase SP-2	SLVLLYN ASGFR	SLVLLYN[1872.6506]ASGFR	HexNAc(3) Hex(6)Neu Ac(1) % 1872.6505	R	C	38.00	49.00	12.00	2,3	2.02E+08	7.98E+07	1.38E+08	0.51	0.25	0.33	0.36
A0A1I9KNS3	Metallopro teinase of class P-II MPII-3	SYQFSNC SK	SYQFSN[1403.5074]C[57.0215]SK	HexNAc(3) Hex(4)Fuc (1) % 1403.5074	R	N	370.00	378.00	9.00	2,3	2.61E+07	2.22E+07	2.75E+07	0.07	0.07	0.07	0.07
	Metallopro teinase of class P-II MPII-3	SYQFSNC SK	SYQFSN[1694.6028]C[57.0215]SK	HexNAc(3) Hex(4)Fuc (1)NeuAc(1) % 1694.6028	R	N	370.00	378.00	9.00	2,3	1.13E+08	1.09E+08	1.56E+08	0.28	0.34	0.37	0.33
	Metallopro teinase of class P-II MPII-3	SYQFSNC SK	SYQFSN[1809.6660]C[57.0215]SK	HexNAc(5) Hex(4)Fuc (1) % 1809.6661	R	N	370.00	378.00	9.00	2,3	1.11E+08	7.20E+07	1.13E+08	0.28	0.22	0.27	0.26
	Metallopro teinase of class P-II MPII-3	SYQFSNC SK	SYQFSN[1971.7189]C[57.0215]SK	HexNAc(5) Hex(5)Fuc (1) % 1971.7189	R	N	370.00	378.00	9.00	3.00	2.69E+07	1.23E+07	2.93E+07	0.07	0.04	0.07	0.06

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2059.7351]C[57.0215]SK	HexNAc(4)Hex(5)Fuc(1)NeuAc(1) % 2059.7350	R	N	370.00	378.00	9.00	2,3	6.86E+08	2.72E+08	7.88E+08	1.73	0.85	1.89	1.49
	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2100.7615]C[57.0215]SK	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) % 2100.7615	R	N	370.00	378.00	9.00	2,3	4.15E+08	4.87E+08	7.63E+08	1.04	1.51	1.83	1.46
	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2262.8142]C[57.0215]SK	HexNAc(5)Hex(5)Fuc(1)NeuAc(1) % 2262.8143	R	N	370.00	378.00	9.00	2,3	5.71E+08	4.45E+08	7.36E+08	1.44	1.38	1.76	1.53
	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2350.8303]C[57.0215]SK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	N	370.00	378.00	9.00	2,3	4.32E+09	3.02E+09	4.69E+09	10.88	9.37	11.24	10.50
	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2553.9099]C[57.0215]SK	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	R	N	370.00	378.00	9.00	2,3	1.46E+09	1.00E+09	1.76E+09	3.68	3.12	4.21	3.67
A0A1I9KNR6	Metalloproteinase of class P-III MPIII-1	TGYCYNGTCPK	TGYC[57.0215]YN[2204.7725]GTC[57.0215]PK	HexNAc(4)Hex(5)NeuAc(2) % 2204.7725	K	M	501.00	511.00	11.00	2,3	2.39E+08	1.48E+08	2.48E+08	0.60	0.46	0.60	0.55

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Metalloproteinase of class P-III MPIII-1	YNNNSTAIR	YNN[2350.8303]NSTAIR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	T	219.00	227.00	9.00	3.00	1.75E+08	2.76E+08	3.44E+08	0.44	0.86	0.82	0.71
	Metalloproteinase of class P-III MPIII-2	YNNDSTAIR	YNN[2059.7351]DSTAIR	HexNAc(4)Hex(5)Fuc(1)NeuAc(1) % 2059.7350	K	T	217.00	225.00	9.00	2,3	3.79E+08	2.83E+08	4.78E+08	0.96	0.88	1.15	0.99
	Metalloproteinase of class P-III MPIII-2	YNNDSTAIR	YNN[2350.8303]DSTAIR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	T	217.00	225.00	9.00	2,3	2.03E+09	1.74E+09	2.72E+09	5.10	5.41	6.52	5.68
	Metalloproteinase of class P-III MPIII-2	YNNDSTAIR	YNN[2553.9099]DSTAIR	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	T	217.00	225.00	9.00	3.00	0.00E+00	2.45E+08	4.47E+08		0.76	1.07	0.61
	Metalloproteinase of class P-III MPIII-2	KYNNDSTAIR	KYNN[2350.8303]DSTAIR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	T	216.00	225.00	10.00	3,4	1.92E+09	9.94E+08	9.33E+08	4.84	3.09	2.23	3.39
	Metalloproteinase of class P-III MPIII-2	KYNNDSTAIR	KYNN[2553.9099]DSTAIR	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	T	216.00	225.00	10.00	3.00	2.66E+08	1.66E+08	0.00E+00	0.67	0.52	0.00	0.39

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

E9JG33	Metalloproteinase	YTGNSTAIK	YTGN[1216.4229]STAIK	HexNAc(2)Hex(5) % 1216.4229	K	T	217.00	225.00	9.00	2.00	0.00E+00	4.99E+07	4.64E+07		0.16	0.11	0.09
	Metalloproteinase	YTGNSTAIK	YTGN[1694.6028]STAIK	HexNAc(3)Hex(4)Fuc(1)NeuAc(1) % 1694.6028	K	T	217.00	225.00	9.00	2,3	8.87E+07	0.00E+00	1.08E+08	0.22		0.26	0.16
	Metalloproteinase	YTGNSTAIK	YTGN[1856.6556]STAIK	HexNAc(3)Hex(5)Fuc(1)NeuAc(1) % 1856.6557	K	T	217.00	225.00	9.00	2,3	8.53E+07	1.12E+08	1.17E+08	0.21	0.35	0.28	0.28
	Metalloproteinase	YTGNSTAIK	YTGN[1872.6506]STAIK	HexNAc(3)Hex(6)NeuAc(1) % 1872.6505	K	T	217.00	225.00	9.00	2,3	5.87E+07	6.25E+07	6.99E+07	0.15	0.19	0.17	0.17
	Metalloproteinase	YTGNSTAIK	YTGN[2018.7085]STAIK	HexNAc(3)Hex(6)Fuc(1)NeuAc(1) % 2018.7085	K	T	217.00	225.00	9.00	2,3	0.00E+00	3.45E+07	4.53E+07		0.11	0.11	0.07
V5TBK6	Zinc metalloproteinase-disintegrin-	YTGNSTAIR	YTGN[1216.4229]STAIR	HexNAc(2)Hex(5) % 1216.4229	K	T	217.00	225.00	9.00	2,3	2.49E+08	0.00E+00	2.39E+08	0.63	0.00	0.57	0.40

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Zinc metallopro teinase-disintegrin-	YTGNSTA IR	YTGN[169 4.6028]ST AIR	HexNAc(2) Hex(5) % 1216.4229	K	T	217.00	225.00	9.00	2,3	2.08E+08	1.73E+08	2.25E+08	0.52	0.54	0.54	0.53
	Zinc metallopro teinase-disintegrin-	YTGNSTA IR	YTGN[187 2.6506]ST AIR	HexNAc(2) Hex(5) % 1216.4229	K	T	217.00	225.00	9.00	2,3	2.60E+08	2.05E+08	2.92E+08	0.65	0.64	0.70	0.66
	Zinc metallopro teinase-disintegrin-	YTGNSTA IR	YTGN[201 8.7085]ST AIR	HexNAc(2) Hex(5) % 1216.4229	K	T	217.00	225.00	9.00	2,3	1.09E+08	1.26E+08	1.35E+08	0.27	0.39	0.32	0.33
	Zinc metallopro teinase-disintegrin-	NPCCNAT TCK	NPC[57.02 15]C[57.02 15]N[2350. 8303]ATT C[57.0215] K	HexNAc(4) Hex(5)Fuc (1)NeuAc(2) % 2350.8304	R	L	429.00	438.00	10.00	3.00	2.24E+08	7.84E+08	3.69E+08	0.56	2.44	0.88	1.30
	Zinc metallopro teinase-disintegrin-	NPCCNAT TCK	NPC[57.02 15]C[57.02 15]N[2366. 8252]ATT C[57.0215] K	HexNAc(4) Hex(6)Neu Ac(2) % 2366.8253	R	L	429.00	438.00	10.00	3.00	0.00E+00	2.35E+07	2.72E+07		0.07	0.07	0.05
	Zinc metallopro teinase-disintegrin-	NPCCNAT TCK	NPC[57.02 15]C[57.02 15]N[2553. 9099]ATT C[57.0215] K	HexNAc(5) Hex(5)Fuc (1)NeuAc(2) % 2553.9098	R	L	429.00	438.00	10.00	3.00	0.00E+00	1.23E+08	6.94E+07		0.38	0.17	0.18

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

A0A6G5ZUZ2	Snake venom metalloprotease 2	LFSNCSNHNYQR	LFSN[2350.8303]C[57.0215]SNHNYQR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	Y	204.00	215.00	12.00	3.00	0.00E+00	2.92E+07	5.49E+07	0.00	0.09	0.13	0.07
R4NNL0	Zinc metalloproteinase-disintegrin-	FSNCSQEEHR	FSN[2100.7615]C[57.0215]SQEEHR	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) % 2100.7615	R	K	375.00	384.00	10.00	3.00	0.00E+00	1.40E+08	2.33E+08		0.43	0.56	0.33
	Zinc metalloproteinase-disintegrin-	FSNCSQEEHR	FSN[2350.8303]C[57.0215]SQEEHR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	K	375.00	384.00	10.00	2,3,4	3.33E+08	8.34E+08	1.10E+09	0.84	2.59	2.63	2.02
	Zinc metalloproteinase-disintegrin-	FSNCSQEEHR	FSN[2553.9099]C[57.0215]SQEEHR	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	R	K	375.00	384.00	10.00	3,4	1.89E+08	2.99E+08	3.15E+08	0.48	0.93	0.75	0.72
	Zinc metalloproteinase-disintegrin-	NGHPCQNNNGYCYNGTCPILGK	NGHPC[57.0215]QNNNGYC[57.0215]YN[1216.4229]GTC[57.0215]PILGK	HexNAc(2)Hex(5) % 1216.4229	R	Q	493.00	514.00	22.00	3.00	2.01E+07	1.51E+07	2.01E+07	0.05	0.05	0.05	0.05
	Zinc metalloproteinase-disintegrin-	NGHPCQNNNGYCYNGTCPILGK	NGHPC[57.0215]QNNNGYC[57.0215]YN[1710.5977]GTC[57.0215]PILGK	HexNAc(3)Hex(5)NeuAc(1) % 1710.5977	R	Q	493.00	514.00	22.00	3.00	2.99E+07	0.00E+00	2.56E+07	0.08		0.06	0.05

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Zinc metallopro teinase- disintegrin-	NGHPCQ NNNGYC YNGTCPI LGK	NGHPC[5 7.0215]QN NNGYC[5 7.0215]YN [1872.650 6]GTC[57. 0215]PILG	HexNAc(3) Hex(6)Neu Ac(1) % 1872.6505	R	Q	493.00	514.00	22.00	3.00	2.66E+07	2.12E+07	2.74E+07	0.07	0.07	0.07	0.07
	Zinc metallopro teinase- disintegrin-	NGHPCQ NNNGYC YNGTCPI LGK	NGHPC[5 7.0215]QN NNGYC[5 7.0215]YN [1913.677 0]GTC[57. 0215]PILG	HexNAc(4) Hex(5)Neu Ac(1) % 1913.6771	R	Q	493.00	514.00	22.00	3,4	6.19E+07	3.18E+07	4.94E+07	0.16	0.10	0.12	0.12
	Zinc metallopro teinase- disintegrin-	NGHPCQ NNNGYC YNGTCPI LGK	NGHPC[5 7.0215]QN NNGYC[5 7.0215]YN [2204.772 5]GTC[57. 0215]PILG	HexNAc(4) Hex(5)Neu Ac(2) % 2204.7725	R	Q	493.00	514.00	22.00	3,4	2.48E+08	1.40E+08	2.42E+08	0.63	0.44	0.58	0.55
	Zinc metallopro teinase- disintegrin-	NGHPCQ NNNGYC YNGTCPI LGK	NGHPC[5 7.0215]QN NNGYC[5 7.0215]YN [2350.830 4]GTC[57. 0215]PILG	HexNAc(4) Hex(5)Fuc (1)NeuAc(2) % 2350.8304	R	Q	493.00	514.00	22.00	3,4	0.00E+00	3.18E+07	5.32E+07		0.10	0.13	0.08
Q7T045	Snaclec coagulatio n factor X- activating	NWTDAE K	N[1095.39 66]WTDA EK	HexNAc(3) Hex(3) % 1095.3966	K	F	47.00	53.00	7.00	2.00	0.00E+00	2.49E+07	2.18E+07		0.08	0.05	0.04
	Snaclec coagulatio n factor X- activating	NWTDAE K	N[1257.44 95]WTDA EK	HexNAc(3) Hex(4) % 1257.4495	K	F	47.00	53.00	7.00	2.00	0.00E+00	5.62E+06	9.82E+06		0.02	0.02	0.01

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

Snaclec coagulation factor X-activating	NWTDAEK	N[1419.5023]WTDAEK	HexNAc(3) Hex(5) % 1419.5023	K	F	47.00	53.00	7.00	2.00	1.03E+07	0.00E+00	6.39E+06	0.03		0.02	0.01
Snaclec coagulation factor X-activating	NWTDAEK	N[1501.5553]WTDAEK	HexNAc(5) Hex(3) % 1501.5553	K	F	47.00	53.00	7.00	2,3	2.07E+08	1.73E+08	1.66E+08	0.52	0.54	0.40	0.49
Snaclec coagulation factor X-activating	NWTDAEK	N[1548.5449]WTDAEK	HexNAc(3) Hex(4)NeuAc(1) % 1548.5449	K	F	47.00	53.00	7.00	2.00	1.94E+07	5.22E+07	0.00E+00	0.05	0.16		0.07
Snaclec coagulation factor X-activating	NWTDAEK	N[1622.5817]WTDAEK	HexNAc(4) Hex(5) % 1622.5817	K	F	47.00	53.00	7.00	2,3	3.94E+07	2.83E+07	3.37E+07	0.10	0.09	0.08	0.09
Snaclec coagulation factor X-activating	NWTDAEK	N[1663.6082]WTDAEK	HexNAc(5) Hex(4) % 1663.6082	K	F	47.00	53.00	7.00	2,3	9.38E+07	7.13E+07	1.01E+08	0.24	0.22	0.24	0.23
Snaclec coagulation factor X-activating	NWTDAEK	N[1954.7036]WTDAEK	HexNAc(5) Hex(4)NeuAc(1) % 1954.7036	K	F	47.00	53.00	7.00	2,3	5.93E+08	4.14E+08	5.25E+08	1.49	1.29	1.26	1.35

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

0.15	0.07	0.07	0.09	0.03	0.17
	0.05	0.12	0.12	0.04	0.19
0.23	0.08		0.08	0.04	0.13
0.21	0.08	0.10	0.08		0.18
0.00E+00	2.23E+07	5.10E+07	5.13E+07	1.85E+07	7.98E+07
7.56E+07	2.61E+07	0.00E+00	2.49E+07	1.16E+07	4.22E+07
8.50E+07	3.36E+07	3.79E+07	3.32E+07	0.00E+00	6.98E+07
2,3	2.00	3.00	3.00	2,3	2,3
7.00	7.00	9.00	9.00	9.00	9.00
53.00	53.00	197.00	197.00	197.00	197.00
47.00	47.00	189.00	189.00	189.00	189.00
F	F	Y	Y	Y	Y
K	K	R	R	R	R
HexNAc(4)Hex(5)NeuAc(2) % 2204.7725	HexNAc(2)Hex(3) % 892.3172	HexNAc(3)Hex(4)Fuc(1)NeuAc(1) % 1694.6028	HexNAc(3)Hex(6)NeuAc(1) % 1872.6505	HexNAc(3)Hex(6)NeuAc(1) % 1872.6505	HexNAc(3)Hex(6)Fuc(1)NeuAc(1) % 2018.7085
N[2204.7725]WTDAEK	N[892.3172]WTDAEK	TN[1694.6028]C[57.0215]SYILNK	TN[1856.6556]C[57.0215]SYILNK	TN[1872.6506]C[57.0215]SYILNK	TN[2018.7085]C[57.0215]SYILNK
NWTDAEK	NWTDAEK	TNCSYILNK	TNCSYILNK	TNCSYILNK	TNCSYILNK
Snaclec coagulation factor X-activating	Snaclec coagulation factor X-activating	Amine oxidase	Amine oxidase	Amine oxidase	Amine oxidase
		A0A6B7FRF2			

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Amine oxidase	TNCSYILNK	TN[2059.7351]C[57.0215]SYILNK	HexNAc(4)Hex(5)Fuc(1)NeuAc(1) % 2059.7350	R	Y	189.00	197.00	9.00	2,3	3.36E+08	1.92E+08	3.54E+08	0.85	0.60	0.85	0.76
	Amine oxidase	TNCSYILNK	TN[2100.7615]C[57.0215]SYILNK	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) % 2100.7615	R	Y	189.00	197.00	9.00	2,3	2.29E+08	1.62E+08	2.70E+08	0.58	0.50	0.65	0.58
	Amine oxidase	TNCSYILNK	TN[2262.8142]C[57.0215]SYILNK	HexNAc(5)Hex(5)Fuc(1)NeuAc(1) % 2262.8143	R	Y	189.00	197.00	9.00	3.00	1.40E+08	7.29E+07	1.60E+08	0.35	0.23	0.38	0.32
	Amine oxidase	TNCSYILNK	TN[2350.8303]C[57.0215]SYILNK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	Y	189.00	197.00	9.00	2,3	1.31E+09	1.24E+09	1.96E+09	3.29	3.87	4.69	3.95
	Amine oxidase	TNCSYILNK	TN[2553.9099]C[57.0215]SYILNK	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	R	Y	189.00	197.00	9.00	2,3	1.63E+08	1.54E+08	2.64E+08	0.41	0.48	0.63	0.51
	Amine oxidase	RTNCSYILNK	RTN[1856.6556]C[57.0215]SYILNK	HexNAc(3)Hex(6)NeuAc(1) % 1872.6505	K	Y	188.00	197.00	10.00	3.00	3.89E+07	2.14E+07	0.00E+00	0.10	0.07		0.05

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

	Amine oxidase	RTNCSYILNK	RTN[2350.8303]C[57.0215]SYILNK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	Y	188.00	197.00	10.00	2,3,4	7.70E+08	4.69E+08	4.60E+08	1.94	1.46	1.10	1.50
A0A6G5ZVN1	5'-nucleotidase (Fragment	GPIASNISGYILPYK	GPIASN[2350.8303]ISGYILPYK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	L	35.00	49.00	15.00	3.00	1.02E+08	6.50E+07	9.31E+07	0.26	0.20	0.22	0.23
W8EFS0	Snake venom 5'-nucleotidase	TIVYLNGTTQACR	TIVYLN[1216.4229]GTTQAC[57.0215]R	HexNAc(2)Hex(5) % 1216.4229	K	F	176.00	188.00	13.00	2,3	1.57E+08	9.24E+07	1.17E+08	0.40	0.29	0.28	0.32
W8E7D1	Venom phosphodiesterase	INGSYPTIYK	IN[1378.4757]GSYPTIYK	HexNAc(2)Hex(6) % 1378.4757	K	V	258.00	267.00	10.00	2,3	2.53E+07	1.87E+07	0.00E+00	0.06	0.06		0.04
	Venom phosphodiesterase	LKPAPNNGTHGSLNHLLK	LKPAPNN[1864.6342]GTHGSLNHLLK	HexNAc(2)Hex(9) % 1864.6342	K	N	506.00	523.00	18.00	3,4,5	0.00E+00	2.14E+07	2.98E+07		0.07	0.07	0.05
A3QVN3	Hyaluronidase-1	YIVNVTTAAK	YIVN[1216.4229]VTTAAK	HexNAc(2)Hex(5) % 1216.4229	R	L	354.00	363.00	10.00	2.00	0.00E+00	5.96E+06	1.01E+07		0.02	0.02	0.01

Table S10: The identified N-glycopeptides in the glycoproteome from *V. a. ammodytes* (*Vaa*) snake venom.

A0A0F7ZCK0	Calreticulin	FAEEIGNETWGATKDAEK	FAEEIGN[1695.6232]ETWGATKDAEK	HexNAc(3)Hex(4)Fuc(3) % 1695.6232	K	K	337.00	354.00	18.00	4.00	0.00E+00	1.23E+08	1.10E+08		0.38	0.26	0.22

Table S11. The identified N-glycopeptides in the glycoproteome from *V. a. montandoni* (*Vam*) snake venom .

											MaxLFQ Intensity			**Ralative Abundance %**			
Protein ID	**Protein Description**	**Peptide Sequence**	**Modified Sequence**	**Glycan Composition**	**Prev AA**	**Next AA**	**Start**	**End**	**Peptide Length**	**Charges**	**V. a. montandoni (Vam) 1 MaxLFQ**	**V. a. montandoni (Vam) 2 MaxLFQ**	**V. a. montandoni (Vam) 3 MaxLFQ**	**Vam1**	**Vam2**	**Vam3**	**Avg**
A0A6B7FMO3	Serine proteinase SP-7	IMGWGAITSPNETFPNVPHCANINILHYSVCR	IMGWGAITSPN[2350.8304]ETFPNVPHC[57.0215]ANINILHYSVC[57.0215]R	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	A	143.00	174.00	32.00	4.00	5.32E+07	0.00E+00	5.09E+07	0.26		0.15	0.14

Table S11. The identified N-glycopeptides in the glycoproteome from *V. a. montandoni* (*Vam*) snake venom .

	Serine proteinase SP-7	LNSSVTYNTHIAPLSLPSSPPR	LN[2350.8304]SSVTYNTHIAPLSLPSSPPR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	V	115.00	136.00	22.00	3,4,5	1.62E+08	3.09E+08	4.30E+08	0.79	2.29	1.27	1.45
A0A6B7FPJ0	Serine proteinase SP-8	IMGWGAITSPNETYPDVPHCADIK	IMGWGAITSPN[2350.8304]ETYPDVPHC[57.0215]ADIK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	L	146.00	169.00	24.00	3,4	1.49E+08	0.00E+00	7.65E+06	0.73		0.02	0.25
A0A1I9KNL5	Serine proteinase SP-2	FFCLNSTSNTR	FFC[57.0215]LN[2350.8303]STSNTR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	K	96.00	106.00	11.00	2,3	1.08E+09	3.41E+08	1.00E+09	5.29	2.52	2.97	3.59
	Serine proteinase SP-2	KPVNNSIHIAPLSLPSSPPSEGSVCR	KPVN[2059.7351]NSIHIAPLSLPSSPPSEGSVC[57.0215]R	HexNAc(4)Hex(5)Fuc(1)NeuAc(1) % 2059.7350	K	L	118.00	143.00	26.00	3,4	4.08E+07	1.91E+08	2.38E+08	0.20	1.41	0.70	0.77
	Serine proteinase SP-2	KPVNNSIHIAPLSLPSSPPSEGSVCR	KPVN[2350.8304]NSIHIAPLSLPSSPPSEGSVC[57.0215]R	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	L	118.00	143.00	26.00	3,4,5	1.61E+09	1.44E+09	3.72E+09	7.89	10.65	11.00	9.85
	Serine proteinase SP-2	KPVNNSIHIAPLSLPSSPPSEGSVCR	KPVN[2407.8518]NSIHIAPLSLPSSPPSEGSVC[57.0215]R	HexNAc(5)Hex(5)NeuAc(2) % 2407.8519	K	L	118.00	143.00	26.00	4.00	2.88E+07	0.00E+00	8.77E+07	0.14		0.26	0.13

Table S11. The identified N-glycopeptides in the glycoproteome from *V. a. montandoni* (*Vam*) snake venom .

	Serine proteinase SP-2	KPVNNSIHIAPLSLPSSPPSEGSVCR	KPVN[2553.9099]NSIHIAPLSLPSSPPSEGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	K	L	118.00	143.00	26.00	3,4,5	7.27E+07	0.00E+00	1.93E+08	0.36		0.57	0.31
A0A6B7FQF8	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1241.4545]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(3)Hex(3)Fuc(1) % 1241.4545	R	L	115.00	142.00	28.00	4.00	3.75E+07	0.00E+00	5.95E+07	0.18		0.18	0.12
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1694.6028]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(3)Hex(4)Fuc(3) % 1695.6232	R	L	115.00	142.00	28.00	3,4	6.40E+08	0.00E+00	6.98E+08	3.14		2.06	1.73
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1695.6232]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(3)Hex(4)Fuc(3) % 1695.6232	R	L	115.00	142.00	28.00	3,4,5	0.00E+00	2.92E+08	7.95E+08		2.16	2.35	1.50
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1856.6556]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(3)Hex(5)Fuc(1)NeuAc(1) % 1856.6557	R	L	115.00	142.00	28.00	4,5	1.57E+08	1.54E+08	3.17E+08	0.77	1.14	0.94	0.95
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1872.6506]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(3)Hex(6)NeuAc(1) % 1872.6505	R	L	115.00	142.00	28.00	4.00	4.13E+07	0.00E+00	4.80E+07	0.20		0.14	0.11

Table S11. The identified N-glycopeptides in the glycoproteome from *V. a. montandoni* (*Vam*) snake venom .

	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[1 897.6821] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(4) Hex(4)Fuc (1)NeuAc(1) % 1897.6821	R	L	115.00	142.00	28.00	3,4,5	5.98E+08	3.09E+08	8.10E+08	2.94	2.29	2.39	2.54
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 018.7085] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(3) Hex(6)Fuc (1)NeuAc(1) % 2018.7085	R	L	115.00	142.00	28.00	4.00	1.91E+08	0.00E+00	1.67E+08	0.94		0.49	0.48
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 059.7351] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(4) Hex(5)Fuc (1)NeuAc(1) % 2059.7350	R	L	115.00	142.00	28.00	3,4,5	1.95E+08	5.84E+08	9.89E+08	0.96	4.33	2.92	2.74
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 100.7615] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(5) Hex(4)Fuc (1)NeuAc(1) % 2100.7615	R	L	115.00	142.00	28.00	3,4,5	1.25E+08	4.99E+07	1.87E+08	0.61	0.37	0.55	0.51
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 350.8304] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(4) Hex(5)Fuc (1)NeuAc(2) % 2350.8304	R	L	115.00	142.00	28.00	3,4,5	4.45E+09	4.38E+09	6.65E+09	21.85	32.42	19.66	24.64
	Serine proteinase SP-6	LNTPVNN STHIAPLS LASRPPV VGSVCR	LNTPVN[2 407.8518] NSTHIAPL SLASRPP VVGSVC[57.0215]R	HexNAc(5) Hex(5)Neu Ac(2) % 2407.8519	R	L	115.00	142.00	28.00	4,5	1.94E+08	1.43E+08	4.10E+08	0.95	1.06	1.21	1.07

Table S11. The identified N-glycopeptides in the glycoproteome from *V. a. montandoni* (*Vam*) snake venom .

	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[2553.9099]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	R	L	115.00	142.00	28.00	3,4,5	1.30E+08	0.00E+00	2.08E+08	0.64		0.61	0.42
	Serine proteinase SP-6	NYTMWDKDIMLIR	N[2350.8303]YTMWDKDIMLIR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	L	102.00	114.00	13.00	3,4	6.48E+08	0.00E+00	1.65E+08	3.18		0.49	1.22
	Serine proteinase SP-6	NYTMWDKDIMLIR	N[2350.8303]YTM[15.9949]WDKDIMLIR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	L	102.00	114.00	13.00	3,4	1.63E+09	0.00E+00	1.02E+09	8.03		3.01	3.68
	Serine proteinase SP-6	NYTMWDKDIMLIR	N[2350.8303]YTM[15.9949]WDKDIM[15.9949]LIR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	L	102.00	114.00	13.00	3.00	2.33E+08	0.00E+00	2.59E+08	1.14		0.77	0.64
	Serine proteinase SP-6	SIIAGNTTATCPP	SIIAGN[2350.8303]TTATC[57.0215]PP	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	-	245.00	257.00	13.00	2,3	1.61E+09	1.39E+09	2.96E+09	7.90	10.32	8.76	8.99
A0A1I9KNP0	Vaa serine proteinase homolog 1	RPVNDSTHIAPLSLPSSPSVGSVCR	RPVN[2350.8304]DSTHIAPLSLPSSPSVGSVC[57.0215]R	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	L	120.00	145.00	26.00	3,4,5	1.86E+08	3.10E+08	2.51E+08	0.91	2.29	0.74	1.32

Table S11. The identified N-glycopeptides in the glycoproteome from *V. a. montandoni* (*Vam*) snake venom .

	Vaa serine proteinase homolog 1	RPVNDSTHIAPLSLPSSPPSVGSVCR	RPVN[3007.0581]DSTHIAPLSLPSSPPSVGSVC[57.0215]R	HexNAc(5)Hex(6)Fuc(1)NeuAc(3) % 3007.0580	K	L	120.00	145.00	26.00	4,5	1.73E+08	2.25E+08	6.36E+08	0.85	1.67	1.88	1.47
E9JG24	Serine protease (Fragment)	LRRPVNNSTHIAPVSLPSSPSSPR	LRRPVN[2350.8304]NSTHIAPVSLPSSPSSPR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	S	118.00	141.00	24.00	5.00	0.00E+00	3.77E+07	2.57E+07		0.28	0.08	0.12
	Serine protease (Fragment)	RPVNNSTHIAPVSLPSSPSSPR	RPVNN[2350.8304]STHIAPVSLPSSPSSPR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	S	120.00	141.00	22.00	3,4,5	7.47E+08	4.81E+08	1.01E+09	3.67	3.56	2.99	3.41
	Serine protease (Fragment)	RPVNNSTHIAPVSLPSSPSSPR	RPVN[2018.7085]NSTHIAPVSLPSSPSSPR	HexNAc(3)Hex(6)Fuc(1)NeuAc(1) % 2018.7085	R	S	120.00	141.00	22.00	4.00	2.33E+07	0.00E+00	2.80E+07	0.11		0.08	0.07
A0A1I9KNL5	Serine proteinase SP-2	SLVLLYNASGFR	SLVLLYN[1216.4229]ASGFR	HexNAc(2)Hex(5) % 1216.4229	R	C	38.00	49.00	12.00	2,3	1.31E+08	0.00E+00	3.54E+08	0.64		1.05	0.56
	Serine proteinase SP-2	SLVLLYNASGFR	SLVLLYN[1378.4757]ASGFR	HexNAc(2)Hex(6) % 1378.4757	R	C	38.00	49.00	12.00	2,3	1.58E+08	0.00E+00	2.23E+08	0.77		0.66	0.48

Table S11. The identified N-glycopeptides in the glycoproteome from *V. a. montandoni* (*Vam*) snake venom .

	Serine proteinase SP-2	SLVLLYN ASGFR	SLVLLYN[1548.5449]ASGFR	HexNAc(3) Hex(4)Neu Ac(1) % 1548.5449	R	C	38.00	49.00	12.00	2,3	1.09E+08	0.00E+00	2.25E+08	0.54		0.66	0.40
	Serine proteinase SP-2	SLVLLYN ASGFR	SLVLLYN[1872.6506]ASGFR	HexNAc(3) Hex(6)Neu Ac(1) % 1872.6505	R	C	38.00	49.00	12.00	2,3	2.72E+08	4.54E+08	8.97E+08	1.34	3.36	2.65	2.45
R4NNL0	Zinc metallopro teinase-disintegrin-like protein H3	NGHPCQ NNNGYC YNGTCPI LGK	NGHPC[5 7.0215]QN NNGYC[5 7.0215]YN [1872.650 6]GTC[57. 0215]PILG K	HexNAc(3) Hex(6)Neu Ac(1) % 1872.6505	R	Q	493.00	514.00	22.00	3.00	2.91E+07	0.00E+00	2.77E+07	0.14		0.08	0.07
	Zinc metallopro teinase-disintegrin-like protein H3	NGHPCQ NNNGYC YNGTCPI LGK	NGHPC[5 7.0215]QN NNGYC[5 7.0215]YN [2204.772 5]GTC[57. 0215]PILG K	HexNAc(4) Hex(5)Neu Ac(2) % 2204.7725	R	Q	493.00	514.00	22.00	3,4	8.30E+07	3.45E+08	2.17E+08	0.41	2.56	0.64	1.20
	Zinc metallopro teinase-disintegrin-like protein H3	FSNCSQE EHR	FSN[2350. 8303]C[57. 0215]SQE EHR	HexNAc(4) Hex(5)Fuc (1)NeuAc(2) % 2350.8304	R	K	375.00	384.00	10.00	2,3,4	1.94E+08	0.00E+00	1.29E+09	0.95		3.81	1.59
Q2UXQ5	Zinc metallopro teinase-disintegrin-like EoVMP2	LFSNCSEI QYER	LFSN[235 0.8303]C[5 7.0215]SE IQYER	HexNAc(4) Hex(5)Fuc (1)NeuAc(2) % 2350.8304	K	F	372.00	383.00	12.00	2,3	2.34E+08	0.00E+00	3.14E+08	1.15		0.93	0.69

Table S11. The identified N-glycopeptides in the glycoproteome from *V. a. montandoni* (*Vam*) snake venom .

A0A1I9KNS3	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2059.7351]C[57.0215]SK	HexNAc(4)Hex(5)Fuc(1)NeuAc(1) % 2059.7350	R	N	370.00	378.00	9.00	2,3	1.72E+07	0.00E+00	5.10E+07	0.08		0.15	0.08
	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2350.8303]C[57.0215]SK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	N	370.00	378.00	9.00	2,3	1.02E+09	4.51E+08	1.03E+09	5.00	3.34	3.06	3.80
	Metalloproteinase of class P-II MPII-3	SYQFSNCSK	SYQFSN[2553.9099]C[57.0215]SK	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) % 2553.9098	R	N	370.00	378.00	9.00	2,3	4.66E+07	0.00E+00	7.67E+07	0.23		0.23	0.15
A0A6B7FRK6	Metalloproteinase of class P-III MPIII-2	YNNDSTAIR	YNN[2350.8303]DSTAIR	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	T	217.00	225.00	9.00	2,3	3.49E+07	0.00E+00	2.26E+08	0.17		0.67	0.28
E9JG33	Metalloproteinase	YTGNSTAIK	YTGN[1216.4229]STAIK	HexNAc(2)Hex(5) % 1216.4229	K	T	217.00	225.00	9.00	2.00	2.64E+07	0.00E+00	2.95E+07	0.13		0.09	0.07
	Metalloproteinase	YTGNSTAIK	YTGN[1694.6028]STAIK	HexNAc(3)Hex(4)Fuc(1)NeuAc(1) % 1694.6028	K	T	217.00	225.00	9.00	2,3	5.00E+07	0.00E+00	5.33E+07	0.25		0.16	0.13

Table S11. The identified N-glycopeptides in the glycoproteome from *V. a. montandoni* (*Vam*) snake venom .

	Metalloproteinase	YTGNSTAIK	YTGN[1872.6506]STAIK	HexNAc(3)Hex(6)NeuAc(1) % 1872.6505	K	T	217.00	225.00	9.00	2,3	5.29E+07	0.00E+00	6.53E+07	0.26		0.19	0.15
A0A6B7FRF2	Amine oxidase	TNCSYILNK	TN[2018.7085]C[57.0215]SYILNK	HexNAc(3)Hex(6)Fuc(1)NeuAc(1) % 2018.7085	R	Y	189.00	197.00	9.00	2,3	2.48E+07	0.00E+00	4.07E+07	0.12		0.12	0.08
	Amine oxidase	TNCSYILNK	TN[2059.7351]C[57.0215]SYILNK	HexNAc(4)Hex(5)Fuc(1)NeuAc(1) % 2059.7350	R	Y	189.00	197.00	9.00	2,3	4.92E+07	0.00E+00	1.79E+08	0.24		0.53	0.26
	Amine oxidase	TNCSYILNK	TN[2350.8303]C[57.0215]SYILNK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	R	Y	189.00	197.00	9.00	2,3	9.02E+08	1.26E+09	2.79E+09	4.43	9.33	8.24	7.33
	Amine oxidase	RTNCSYILNK	RTN[2350.8303]C[57.0215]SYILNK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	Y	188.00	197.00	10.00	2,3,4	1.13E+09	0.00E+00	1.24E+09	5.57		3.66	3.08
W8EFS0	Snake venom 5'-nucleotidase (Fragment)	TIVYLNGTTQACR	TIVYLN[1216.4229]GTTQAC[57.0215]R	HexNAc(2)Hex(5) % 1216.4229	K	F	176.00	188.00	13.00	2,3	1.18E+08	0.00E+00	2.06E+08	0.58		0.61	0.40

Table S11. The identified N-glycopeptides in the glycoproteome from *V. a. montandoni* (*Vam*) snake venom .

A0A6G5ZVN1	5'-nucleotidase (Fragment)	GPIASNISGYILPYK	GPIASN[2350.8303]ISGYILPYK	HexNAc(4)Hex(5)Fuc(1)NeuAc(2) % 2350.8304	K	L	35.00	49.00	15.00	3.00	1.24E+08	2.30E+08	1.96E+08	0.61	1.71	0.58	0.96
W8E7D1	Venom phosphodiesterase	LKPAPNNGTHGSLNHLLK	LKPAPNN[1864.6342]GTHGSLNHLLK	HexNAc(2)Hex(9) % 1864.6342	K	N	506.00	523.00	18.00	3,4,5	1.52E+08	0.00E+00	1.03E+08	0.74		0.30	0.35
	Venom phosphodiesterase	INGSYPTIYK	IN[1378.4757]GSYPTIYK	HexNAc(2)Hex(9) % 1864.6342	K	V	258.00	267.00	10.00	2,3	1.17E+08	0.00E+00	1.85E+08	0.57		0.55	0.37
A3QVN3	Hyaluronidase-1	YIVNVTTAAK	YIVN[1216.4229]VTTAAK	HexNAc(2)Hex(5) % 1216.4229	R	L	354.00	363.00	10.00	2.00	4.27E+07	0.00E+00	4.84E+07	0.21		0.14	0.12
Q7T045	Snaclec coagulation factor X-activating enzyme light chain 1	NWTDAEK	N[2204.7725]WTDAEK	HexNAc(4)Hex(5)NeuAc(2) % 2204.7725	K	F	47.00	53.00	7.00	2,3	0.00E+00	1.25E+08	2.47E+08		0.93	0.73	0.55

Table S12. The identified N-glycopeptides in the glycoproteome from *V. b. berus* (*Vbb*) snake venom .

											MaxLFQ Intensity		NRalative Abundance %		
Protein ID	**Protein Description**	**Peptide Sequence**	**Modified Sequence**	**Glycan Composition**	**Prev AA**	**Next AA**	**Start**	**End**	**Peptide Length**	**Charges**	**V. b. berus (Vbb)-2MaxLFQ Intensity**	**V. b. berus (Vbb)-3 MaxLFQ Intensity**	**Vbb2**	**Vbb3**	**Avg**
W8E7D1	Venom phosphodiesterase	INGSYPTIYK	IN[1216.4229]GSYPTIYK	HexNAc(2)Hex(5) % 1216.4229	K	V	258.00	267.00	10.00	2,3	2.34E+08	2.53E+08	2.83	2.69	2.76
	Venom phosphodiesterase	INGSYPTIYK	IN[1378.4757]GSYPTIYK	HexNAc(2)Hex(6) % 1378.4757	K	V	258.00	267.00	10.00	2,3	2.22E+08	2.64E+08	2.68	2.81	2.74
	Venom phosphodiesterase	INGSYPTIYK	IN[2100.7615]GSYPTIYK	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) %	K	V	258.00	267.00	10.00	3.00	3.00E+07	3.42E+07	0.36	0.36	0.36

Table S12. The identified N-glycopeptides in the glycoproteome from *V. b. berus* (*Vbb*) snake venom .

	Venom phosphodiesterase	LKPAPNNGTHGSLNHLLK	LKPAPNN[1864.6342]GTHGSLNHLLK	HexNAc(2)Hex(9) % 1864.6342	K	N	506.00	523.00	18.00	3,4,5	3.08E+08	2.89E+08	3.72	3.07	3.40
	Venom phosphodiesterase	LKPAPNNGTHGSLNHLLK	LKPAPNN[2026.6871]GTHGSLNHLLK	HexNAc(2)Hex(10) % 2026.6871	K	N	506.00	523.00	18.00	3,4,5	1.62E+08	1.59E+08	1.96	1.70	1.83
A0A6B7FQF8	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1930.6924]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(4)Hex(6)Fuc(1) % 1930.6924	R	L	115.00	142.00	28.00	4.00	5.42E+07	4.56E+07	0.65	0.48	0.57
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[1955.7240]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(4)Fuc(2) % 1955.724	R	L	115.00	142.00	28.00	4.00	1.84E+08	1.71E+08	2.22	1.82	2.02
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[2060.7554]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(4)Hex(5)Fuc(3) % 2060.7554	R	L	115.00	142.00	28.00	4.00	3.42E+07	3.68E+07	0.41	0.39	0.40
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[2100.7615]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) %	R	L	115.00	142.00	28.00	3,4,5	5.56E+08	7.87E+08	6.73	8.37	7.55

Table S12. The identified N-glycopeptides in the glycoproteome from *V. b. berus* (*Vbb*) snake venom .

	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[2117.7769]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(2) % 2117.776	R	L	115.00	142.00	28.00	4.00	2.90E+07	3.15E+07	0.35	0.33	0.34
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[2262.8142]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(3) % 2263.8347	R	L	115.00	142.00	28.00	4,5	5.71E+07	6.55E+07	0.69	0.70	0.69
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[2263.8347]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(3) % 2263.8347	R	L	115.00	142.00	28.00	4.00	7.24E+07	6.43E+07	0.88	0.68	0.78
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[2350.7927]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(2)Hex(12) % 2350.7927	R	L	115.00	142.00	28.00	3,4,5	4.80E+07	3.73E+07	0.58	0.40	0.49
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[2553.9099]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) %	R	L	115.00	142.00	28.00	3,4,5	9.07E+08	1.02E+09	10.97	10.89	10.93
	Serine proteinase SP-6	LNTPVNNSTHIAPLSLASRPPVVGSVCR	LNTPVN[2555.9253]NSTHIAPLSLASRPPVVGSVC[57.0215]R	HexNAc(7)Hex(7) % 2555.9254	R	L	115.00	142.00	28.00	3,4,5	5.87E+08	9.57E+08	7.10	10.17	8.64

Table S12. The identified N-glycopeptides in the glycoproteome from *V. b. berus* (*Vbb*) snake venom .

Q7T045	Snaclec coagulation factor X-	NWTDAEK	N[1501.5553]WTDAEK	HexNAc(5)Hex(3) % 1501.5553	K	F	47.00	53.00	7.00	2,3	1.72E+08	1.99E+08	2.08	2.12	2.10
	Snaclec coagulation factor X-	NWTDAEK	N[1622.5817]WTDAEK	HexNAc(4)Hex(5) % 1622.5817	K	F	47.00	53.00	7.00	2,3	6.00E+07	5.23E+07	0.73	0.56	0.64
	Snaclec coagulation factor X-	NWTDAEK	N[1809.6660]WTDAEK	HexNAc(5)Hex(4)Fuc(1) % 1809.6661	K	F	47.00	53.00	7.00	2,3	2.01E+08	2.61E+08	2.43	2.78	2.61
	Snaclec coagulation factor X-	NWTDAEK	N[1954.7036]WTDAEK	HexNAc(5)Hex(4)NeuAc(1) % 1954.7036	K	F	47.00	53.00	7.00	2,3	2.37E+08	2.87E+08	2.87	3.05	2.96
	Snaclec coagulation factor X-	NWTDAEK	N[2100.7615]WTDAEK	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) %	K	F	47.00	53.00	7.00	2,3	1.12E+09	1.05E+09	13.51	11.19	12.35
	Snaclec coagulation factor X-	NWTDAEK	N[2262.8142]WTDAEK	HexNAc(5)Hex(5)Fuc(1)NeuAc(1) %	K	F	47.00	53.00	7.00	3.00	5.45E+07	4.73E+07	0.66	0.50	0.58

Table S12. The identified N-glycopeptides in the glycoproteome from *V. b. berus* (*Vbb*) snake venom .

A0A6B7FRF2	Amine oxidase	TNCSYILNK	TN[1955.7240]C[57.0215]SYILNK	HexNAc(5)Hex(4)Fuc(2) % 1955.7240	R	Y	189.00	197.00	9.00	3.00	4.98E+07	4.06E+07	0.60	0.43	0.52
	Amine oxidase	TNCSYILNK	TN[2100.7615]C[57.0215]SYILNK	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) %	R	Y	189.00	197.00	9.00	2,3	1.60E+08	2.16E+08	1.94	2.29	2.12
	Amine oxidase	TNCSYILNK	TN[2262.8142]C[57.0215]SYILNK	HexNAc(5)Hex(5)Fuc(1)NeuAc(1) %	R	Y	189.00	197.00	9.00	3.00	1.25E+08	1.34E+08	1.51	1.43	1.47
	Amine oxidase	TNCSYILNK	TN[2553.9099]C[57.0215]SYILNK	HexNAc(5)Hex(5)Fuc(1)NeuAc(2) %	R	Y	189.00	197.00	9.00	2,3	1.56E+08	2.23E+08	1.88	2.37	2.13
A0A1I9KNR6	Metalloproteinase of class P-III	YNNNSTAIR	YNN[2018.7085]NSTAIR	HexNAc(3)Hex(6)Fuc(1)NeuAc(1) %	K	T	219.00	227.00	9.00	2,3	7.98E+07	1.18E+08	0.96	1.25	1.11
R4NNL0	Zinc metalloproteinase-	FSNCSQEEHR	FSN[2100.7615]C[57.0215]SQEEHR	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) %	R	K	375.00	384.00	10.00	3.00	1.42E+08	1.36E+08	1.71	1.45	1.58

Table S12. The identified N-glycopeptides in the glycoproteome from *V. b. berus* (*Vbb*) snake venom .

	Zinc metalloproteinase-	FSNCSQEEHRK	FSN[2100.7615]C[57.0215]SQEEHRK	HexNAc(5)Hex(4)Fuc(1)NeuAc(1) %	R	Y	375.00	385.00	11.00	3.00	2.83E+07	3.54E+07	0.34	0.38	0.36
A0A0B4U9L8	Zinc metalloproteinase-	YTGNFTAIR	YTGN[1216.4229]FTAIR	HexNAc(2)Hex(5) % 1216.4229	K	T	218.00	226.00	9.00	2.00	5.48E+07	4.48E+07	0.66	0.48	0.57
V5TBK6	Zinc metalloproteinase-	KYTGNSTAIR	KYTGN[1216.4229]STAIR	HexNAc(2)Hex(5) % 1216.4229	R	T	216.00	225.00	10.00	3.00	0.00E+00	3.03E+07	0.00	0.32	0.16
	Zinc metalloproteinase-	KYTGNSTAIR	KYTGN[2018.7085]STAIR	HexNAc(3)Hex(6)Fuc(1)NeuAc(1) %	R	T	216.00	225.00	10.00	3.00	4.23E+07	5.24E+07	0.51	0.56	0.53
	Zinc metalloproteinase-	YTGNSTAIR	YTGN[1216.4229]STAIR	HexNAc(2)Hex(5) % 1216.4229	K	T	217.00	225.00	9.00	2,3	8.21E+08	9.16E+08	9.93	9.74	9.83
	Zinc metalloproteinase-	YTGNSTAIR	YTGN[1378.4757]STAIR	HexNAc(2)Hex(6) % 1378.4757	K	T	217.00	225.00	9.00	2.00	5.06E+07	4.33E+07	0.61	0.46	0.54

Table S12. The identified N-glycopeptides in the glycoproteome from *V. b. berus* (*Vbb*) snake venom .

	Zinc metalloproteinase-	YTGNSTAIR	YTGN[1856.6556]STAIR	HexNAc(3)Hex(5)Fuc(1)NeuAc(1) %	K	T	217.00	225.00	9.00	3.00	1.32E+08	1.37E+08	1.60	1.46	1.53
	Zinc metalloproteinase-	YTGNSTAIR	YTGN[2018.7085]STAIR	HexNAc(3)Hex(6)Fuc(1)NeuAc(1) %	K	T	217.00	225.00	9.00	2,3	6.56E+08	6.92E+08	7.93	7.36	7.64
A3QVN3	Hyaluronidase-1	YIVNVTTAAK	YIVN[1216.4229]VTTAAK	HexNAc(2)Hex(5) % 1216.4229	R	L	354.00	363.00	10.00	2.00	7.56E+07	1.22E+08	0.91	1.30	1.11
	Hyaluronidase-1	GHFFHGIIPQNESLTK	GHFFHGIIPQN[1216.4229]ESLTK	HexNAc(2)Hex(5) % 1216.4229	R	H	93.00	108.00	16.00	3,4	3.43E+07	4.37E+07	0.42	0.46	0.44
W8EFS0	Snake venom 5'-nucleotida	TIVYLNGTTQACR	TIVYLN[1216.4229]GTTQAC[57.0215]R	HexNAc(2)Hex(5) % 1216.4229	K	F	176.00	188.00	13.00	2,3	3.34E+08	3.02E+08	4.05	3.21	3.63

Table S13. A list of the identified *N*-glycans for *M. l. obtusa (Ml) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

Peak	Composition	m/z meas.	z	m/z calc.	Rt [min]	Δ m/z	Score	IntCov. [%]	FragCov. [%]	Core Fucosylated	Bisecting	Glycan Type	Area_exp1	Area_exp2	Rel. Abn 1	Rel. Abn 2	Average	Std. Dev.
G1	Hex3HexNAc5-proc	870.3828	2	870.3770	21.51	-0.0058	82.82	96.39	96.39		+	Bisecting, Complex	1.35	0.82	0.09	0.13	0.11	0.02
G2	Hex5HexNAc2-proc	727.8170	2	727.8108	22.72	-0.0063	86.69	83.10	83.10			High-mannose	7.74	4.17	0.54	0.65	0.59	0.05
G3	Hex4HexNAc5-proc	951.4090	2	951.4034	25.20	-0.0056	md	md	md		+	Bisecting, Complex	1.23	0.85	0.09	0.13	0.11	0.02
G4	Hex4HexNAc5NeuAc1-proc	1096.9588	2	1096.9511	28.36	-0.0077	47.90	43.48	43.48		+	Bisecting, Complex	23.40	11.80	1.64	1.83	1.74	0.09

Table S13. A list of the identified *N*-glycans for *M. l. obtusa (Ml) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

	Hex4HexNAc4NeuAc1-proc	995.4171	2	995.4114	28.60	-0.0057	41.59	37.01	37.01			Complex						
G5	Hex4HexNAc5NeuAc1dHex1-proc	780.3276	3	780.3225	29.79	-0.0051	59.16	40.66	40.66	+	+	Bisecting, Complex	15.59	8.06	1.09	1.25	1.17	0.08
G6	Hex4HexNAc5NeuAc1dHex1-proc	780.3283	3	780.3225	31.30	-0.0058	md	md	md	+	+	Bisecting, Complex	10.11	4.23	0.71	0.66	0.68	0.03
G7	Hex5HexNAc5NeuAc1-proc	785.6609	3	785.6541	31.65	-0.0068	83.25	77.46	77.46		+	Bisecting, Complex	13.90	7.69	0.98	1.19	1.08	0.11
G8	Hex5HexNAc5NeuAc1-proc	785.6603	3	785.6541	32.60	-0.0062	md	md	md		+	Bisecting, Complex	10.51	4.93	0.74	0.77	0.75	0.01
G9	Hex5HexNAc5NeuAc1dHex1-proc	834.3456	3	834.3401	33.12	-0.0055	59.15	38.32	38.32	+	+	Bisecting, Complex	42.65	19.84	2.99	3.08	3.03	0.04

Table S13. A list of the identified *N*-glycans for *M. l. obtusa (Ml) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G10	Hex5HexNAc5NeuAc1dHex1-proc	834.3464	3	834.3401	34.00	-0.0063	md	md	md			Bisecting, Complex	38.87	18.78	2.73	2.91	2.82	0.09
G11	Hex5HexNAc4NeuAc2-proc	814.9991	3	814.9928	34.86	-0.0063	80.26	67.47	67.47			Complex	7.88	2.75	0.55	0.43	0.49	0.06
G12	Hex5HexNAc5NeuAc2-proc	882.6925	3	882.6859	35.22	-0.0066	69.56	56.43	56.43		+	Bisecting, Complex	196.11	93.32	13.76	14.47	14.12	0.36
G13	Hex5HexNAc4NeuAc2dHex1-proc	863.6853	3	863.6788	36.09	-0.0066	56.27	37.69	37.69	+		Complex	3.63	1.62	0.25	0.25	0.25	0.00
G14	Hex5HexNAc5NeuAc2dHex1-proc	931.7131	3	931.7081	36.38	-0.0050	65.11	44.10	44.10	+	+	Bisecting, Complex	561.89	258.87	39.42	40.15	39.78	0.36
G15	Hex6HexNAc5NeuAc2dHex1-proc	985.7310	3	985.7257	38.34	-0.0053	39.70	20.09	20.09	+		Complex	13.66	7.72	0.96	1.20	1.08	0.12

Table S13. A list of the identified *N*-glycans for *M. l. obtusa (Ml) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G16	Hex6HexNAc5NeuAc3-proc	1034.0772	3	1034.0716	39.31	-0.0057	41.43	30.38	30.38			Complex	12.21	6.36	0.86	0.99	0.92	0.07
G17	Hex6HexNAc5NeuAc3dHex1-proc	1082.7629	3	1082.7575	40.26	-0.0054	30.85	24.95	24.95	+		Complex	24.86	14.71	1.74	2.28	2.01	0.27
	Hex6HexNAc6NeuAc2-proc	1004.4030	3	1004.3967	40.50	-0.0063	53.32	33.93	33.93			Complex						
G18	Hex6HexNAc6NeuAc2dHex1-proc	1053.0891	3	1053.0826	41.60	-0.0065	md	md	md	+		Complex	16.25	7.97	1.14	1.24	1.19	0.05
G19	Hex6HexNAc6NeuAc2dHex2-proc	826.3292	4	826.3262	41.90	-0.0030	md	md	md	+		Complex	8.80	3.51	0.62	0.54	0.58	0.04
G20	Hex6HexNAc6NeuAc2dHex2-proc	826.3291	4	826.3262	42.70	-0.0029	md	md	md	+		Complex	47.57	22.23	3.34	3.45	3.39	0.06

Table S13. A list of the identified *N*-glycans for *M. l. obtusa (Ml) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G21	Hex6HexNAc6NeuAc2dHex3-proc	862.8436	4	862.8408	43.60	-0.0028	md	md	md	+		Complex	137.43	64.69	9.64	10.03	9.84	0.20
G22	Hex7HexNAc7NeuAc3dHex1-proc	954.3776	4	954.3729	46.34	-0.0048	28.94	16.49	16.49			Complex	47.95	21.61	3.36	3.35	3.36	0.01
G23	Hex7HexNAc6NeuAc4-proc	939.8668	4	939.8624	47.91	-0.0044	39.15	15.33	15.33			Complex	25.93	8.74	1.82	1.36	1.59	0.23
G24	Hex7HexNAc7NeuAc4dHex1-proc	1027.1517	4	1027.1467	48.34	-0.0050	35.79	15.56	15.56			Complex	155.84	49.50	10.93	7.68	9.30	1.63

Table S14. A list of the identified *N*-glycans for *M. xanthina (Mx) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

Peak	Composition	m/z meas.	z	m/z calc.	Rt [min]	Δ m/z	Score	IntCov. [%]	FragCov. [%]	Core Fucosylated	Bisecting	Glycan Type	Area_exp1	Area_exp2	Rel. Abn 1	Rel. Abn 2	Average	Std. Dev.
G1	Hex5HexNAc2-proc	727.8172	2	727.8108	22.27	-0.0064	91.1	97	97			High-mannose	10.89	9.07	0.93	1.21	1.07	0.14
G2	Hex4HexNAc4dHex1-proc	922.9011	2	922.8927	24.90	-0.0084	md	md	md			Complex	3.98	3.65	0.34	0.49	0.41	0.04
G3	Hex5HexNAc4-proc	930.8981	2	930.8901	27.53	-0.0080	93.9	107	107			Complex	2.47	2.18	0.21	0.29	0.25	0.02
G4	Hex5HexNAc4dHex1-proc	1003.9274	2	1003.9191	29.20	-0.0083	70.9	57	57	+		Complex	59.97	48.23	5.12	6.43	5.78	0.33

Table S14. A list of the identified *N*-glycans for *M. xanthina (Mx) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

	Hex4HexNAc5NeuAc1dHex1-proc	780.3288	3	780.3225	29.45	-0.0063	64.0	43	43	+		Complex						
G5	Hex5HexNAc5dHex1-proc	737.3151	3	737.3083	30.17	-0.0068	93.9	91	91	+	+	Bisecting, Complex	22.49	18.71	1.92	2.49	2.21	0.14
G6	Hex5HexNAc4NeuAc1-proc	717.9683	3	717.9610	30.89	-0.0073	81.7	83	83			Complex	8.73	4.31	0.75	0.57	0.66	0.04
G7	Hex5HexNAc5NeuAc1-proc	785.6616	3	785.6541	31.48	-0.0075	79.8	75	75		+	Bisecting, Complex	6.67	4.09	0.57	0.55	0.56	0.01
G8	Hex5HexNAc4NeuAc1dHex1-proc	766.6535	3	766.6470	32.26	-0.0065	68.6	49	49	+		Complex	30.43	20.46	2.60	2.73	2.66	0.03
G9	Hex5HexNAc5NeuAc1dHex1-proc	834.3466	3	834.3401	32.65	-0.0065	63.2	49	49	+	+	Bisecting, Complex	68.50	42.99	5.85	5.73	5.79	0.03

Table S14. A list of the identified *N*-glycans for *M. xanthina (Mx) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G10	Hex5HexNAc5NeuAc1dHex1-proc	834.3479	3	834.3401	32.70	-0.0078	md	md	md	+	+	Bisecting, Complex	114.74	73.28	9.80	9.77	9.79	0.01
G11	Hex5HexNAc5NeuAc2-proc	882.6937	3	882.6859	34.89	-0.0078	67.7	54	54		+	Bisecting, Complex	12.87	6.93	1.10	0.92	1.01	0.04
G12	Hex6HexNAc5dHex1-proc	791.3333	3	791.3259	35.24	-0.0074	89.5	88	88	+		Complex	13.94	13.00	1.19	1.73	1.46	0.14
G13	Hex5HexNAc4NeuAc2dHex1-proc	863.6864	3	863.6788	35.65	-0.0077	62.0	51	51	+		Complex	5.52	1.88	0.47	0.25	0.36	0.06
G14	Hex5HexNAc5NeuAc2dHex1-proc	931.3805	3	931.3719	35.90	-0.0086	40.1	33	33	+		Complex	333.60	205.10	28.51	27.34	27.93	0.29
G15	Hex6HexNAc5NeuAc1dHex1-proc	888.3655	3	888.3577	37.26	-0.0078	55.6	47	47	+		Complex	9.73	6.29	0.83	0.84	0.84	0.00

Table S14. A list of the identified *N*-glycans for *M. xanthina (Mx) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G16	Hex6HexNAc5NeuAc1dHex1-proc	888.3655	3	888.3577	38.10	-0.0078	md	md	md	+		Complex	36.87	22.05	3.15	2.94	3.05	0.05
G17	Hex6HexNAc6dHex3-proc	956.3941	3	956.3909	38.38	-0.0032	29.3	18	18			Complex	4.84	2.62	0.41	0.35	0.38	0.02
G18	Hex6HexNAc6dHex3-proc	956.3941	3	956.3909	39.10	-0.0032	md	md	md			Complex	12.11	7.71	1.03	1.03	1.03	0.00
G19	Hex6HexNAc5NeuAc2dHex1-proc	985.7324	3	985.7257	40.20	-0.0067	25.1	17	17	+		Complex	56.31	37.02	4.81	4.93	4.87	0.03
G20	Hex6HexNAc5NeuAc2dHex1-proc	985.7324	3	985.7257	40.60	-0.0067	md	md	md	+		Complex	6.61	4.10	0.56	0.55	0.56	0.00
G21	Hex6HexNAc6NeuAc2dHex1-proc	1053.4262	3	1053.4188	41.10	-0.0073	41.0	26	26	+		Complex	49.46	29.52	4.23	3.94	4.08	0.07

Table S14. A list of the identified *N*-glycans for *M. xanthina (Mx) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G22	Hex7HexNAc6NeuAc1dHex1-proc	1010.0770	3	1010.0684	41.81	-0.0085	48.2	28	28	+		Complex	18.20	10.84	1.56	1.44	1.50	0.03
G23	Hex6HexNAc6NeuAc3dHex1-proc	863.0969	4	863.0898	42.30	-0.0071	34.8	21	21	+		Complex	11.28	5.53	0.96	0.74	0.85	0.06
	Hex7HexNAc7dHex3-proc	1078.1062	3	1078.1017	42.44	-0.0045	39.4	19	19	+		Complex						
G24	Hex6HexNAc5NeuAc3dHex1-proc	1082.4310	3	1082.4213	43.00	-0.0097	md	md	md			Complex	17.26	10.70	1.48	1.43	1.45	0.01
G25	Hex6HexNAc6NeuAc3dHex1-proc	863.0969	4	863.0898	43.20	-0.0071	md	md	md	+		Complex	37.23	21.09	3.18	2.81	3.00	0.09
G26	Hex7HexNAc6NeuAc2dHex1-proc	1107.4433	3	1107.4364	43.91	-0.0069	36.4	19	19			Complex	53.83	29.09	4.60	3.88	4.24	0.18

Table S14. A list of the identified *N*-glycans for *M. xanthina (Mx) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G27	Hex7HexNAc6NeuAc3-proc	867.0965	4	867.0885	45.42	-0.0080	36.3	27	27			Complex	7.22	4.46	0.62	0.59	0.61	0.01
G28	Hex7HexNAc6NeuAc3dHex1-proc	903.6103	4	903.6030	46.03	-0.0073	33.6	26	26			Complex	83.62	49.64	7.15	6.62	6.88	0.13
G29	Hex7HexNAc6NeuAc4-proc	939.8700	4	939.8624	47.68	-0.0076	51.8	55	55			Complex	9.16	6.02	0.78	0.80	0.79	0.01
G30	Hex7HexNAc6NeuAc4dHex1-proc	976.3837	4	976.3769	48.24	-0.0068	39.8	22	22			Complex	61.64	49.52	5.27	6.60	5.93	0.33

Table S15. A list of the identified *N*-glycans for *V. a. ammodytes (Vaa) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

Peak	Composition	m/z meas.	z	m/z calc.	Rt [min]	Δ m/z	Score	IntCov. [%]	FragCov. [%]	Core Fucosylated	Bisecting	Glycan Type	Area_exp1	Area_exp2	Rel. Abn 1	Rel. Abn 2	Average	Std. Dev.

Table S15. A list of the identified *N*-glycans for *V. a. ammodytes (Vaa) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G1	Hex5HexNAc2-proc	727.8207	2	727.8108	22.34	-0.0099	87.52	85.92	85.92			High-mannose	6.41	6.96	0.49	0.39	0.44	0.05
G2	Hex4HexNAc3dHex1-proc	821.3652	2	821.3530	23.06	-0.0122	71.35	57.38	57.38			Complex	4.18	3.88	0.32	0.22	0.27	0.05
	Hex3HexNAc5dHex1-proc	943.4191	2	943.4060	23.11	-0.0131	59.76	58.39	58.39	+	+	Bisecting, Complex						
G3	Hex4HexNAc3NeuAc1-proc	893.8840	2	893.8717	25.34	-0.0123	68.62	51.92	51.92	+		Complex	3.40	4.39	0.26	0.24	0.25	0.01
G4	Hex4HexNAc3NeuAc1dHex1-proc	966.9128	2	966.9007	26.24	-0.0121	51.33	30.00	30.00	+		Complex	23.28	23.90	1.79	1.33	1.56	0.23
	Hex4HexNAc5dHex1-proc	1024.4441	2	1024.4324	26.55	-0.0117	71.91	58.55	58.55	+	+	Bisecting, Complex						

Table S15. A list of the identified *N*-glycans for *V. a. ammodytes (Vaa) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G5	Hex4HexNAc5NeuAc1-proc	1096.9651	2	1096.9511	28.03	-0.0140	44.89	38.59	38.59		+	Bisecting, Complex	23.13	26.89	1.78	1.50	1.64	0.14
G6	Hex5HexNAc3NeuAc1-proc	974.9121	2	974.8982	29.11	-0.0139	70.43	54.74	54.74			Complex	62.63	64.44	4.81	3.58	4.20	0.61
	Hex4HexNAc5NeuAc1dHex1-proc	780.3335	3	780.3225	29.33	-0.0110	69.39	53.48	53.48	+	+	Bisecting, Complex						
G7	Hex5HexNAc5dHex1-proc	1105.4739	2	1105.4588	29.20	-0.0151	md	md	md	+		Complex	6.22	8.89	0.48	0.49	0.49	0.01
G8	Hex4HexNAc5NeuAc1dHex1-proc	780.3335	3	780.3225	30.70	-0.0110	md	md	md	+	+	Bisecting, Complex	62.20	67.67	4.78	3.76	4.27	0.51
G9	Hex5HexNAc4NeuAc1dHex1-proc	766.6567	3	766.6470	32.50	-0.0097	md	md	md	+		Complex	32.23	38.92	2.47	2.16	2.32	0.16

Table S15. A list of the identified *N*-glycans for *V. a. ammodytes (Vaa) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G10	Hex5HexNAc5NeuAc1dHex1-proc	834.3514	3	834.3401	32.77	-0.0113	md	md	md	+	+	Bisecting, Complex	91.10	102.72	6.99	5.71	6.35	0.64
G11	Hex5HexNAc5NeuAc1dHex1-proc	834.3499	3	834.3401	33.70	-0.0098	md	md	md	+	+	Bisecting, Complex	49.48	55.28	3.80	3.07	3.44	0.36
G12	Hex5HexNAc4NeuAc2-proc	815.0008	3	814.9928	34.43	-0.0080	85.89	86.75	86.75			Complex	45.55	68.23	3.50	3.79	3.65	0.15
G13	Hex5HexNAc4NeuAc2dHex1-proc	863.6908	3	863.6788	35.64	-0.0120	59.33	42.91	42.91	+		Complex	629.05	860.57	48.29	47.85	48.07	0.22
G14	Hex5HexNAc5NeuAc2dHex1-proc	931.3857	3	931.3719	36.00	-0.0138	37.51	31.79	31.79	+		Complex	48.61	5.56	3.73	0.31	2.02	1.71
G15	Hex6HexNAc5NeuAc2dHex1-proc	985.7357	3	985.7257	38.14	-0.0099	17.68	13.41	13.41	+		Complex	36.52	67.81	2.80	3.77	3.29	0.48

Table S15. A list of the identified *N*-glycans for *V. a. ammodytes (Vaa) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G16	Hex6HexNAc5NeuAc3-proc	1033.7486	3	1033.7353	42.07	-0.0133	47.66	33.08	33.08			Complex	3.81	7.89	0.29	0.44	0.37	0.07
G17	Hex6HexNAc5NeuAc3dHex1-proc	1082.4344	3	1082.4213	42.57	-0.0131	36.21	20.77	20.77	+		Complex	144.34	263.12	11.08	14.63	12.86	1.77
G18	Hex7HexNAc6NeuAc3dHex1-proc	903.6129	4	903.6030	46.21	-0.0099	20.00	15.26	15.26	+		Complex	12.79	30.36	0.98	1.69	1.34	0.35
G19	Hex7HexNAc6NeuAc4dHex1-proc	976.3878	4	976.3769	48.28	-0.0109	31.64	17.65	17.65			Complex	17.63	91.10	1.35	5.06	3.21	1.86

Table S16. A list of the identified *N*-glycans for *V. a. montandoni (Vam) snake venom.* Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

Peak	Composition	m/z meas.	z	m/z calc.	Rt [min]	Δ m/z	Score	IntCov. [%]	FragCov. [%]	Core Fucosylated	Bisecting	Glycan Type	Area_exp1	Area_exp2	Rel. Abn 1	Rel. Abn 2	Average	Std. Dev.

Table S16. A list of the identified *N*-glycans for *V. a. montandoni (Vam) snake venom.* Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G1	Hex3HexNAc2dHex1-proc	638.7942	2	638.7869	14.93	-0.0073	69.2	53	53	+		Complex	13.63	18.26	2.52	2.20	2.36	0.16
G2	Hex3HexNAc3dHex1-proc	740.3353	2	740.3266	17.99	-0.0087	68.8	55	55	+		Complex	18.73	28.23	3.47	3.40	3.43	0.03
G3	Hex4HexNAc3-proc	748.3318	2	748.3240	20.20	-0.0078	83.0	79	79			Complex	1.62	2.11	0.30	0.25	0.28	0.02
G4	Hex3HexNAc4dHex1-proc	841.8755	2	841.8663	20.87	-0.0093	66.4	56	56	+		Complex	7.51	13.21	1.39	1.59	1.49	0.10
G5	Hex4HexNAc3dHex1-proc	821.3617	2	821.3530	22.15	-0.0087	74.4	63	63	+		Complex	23.64	41.12	4.38	4.95	4.66	0.29
	Hex5HexNAc2-proc	727.8193	2	727.8108	22.29	-0.0085	84.9	78	78			High-mannose						

Table S16. A list of the identified *N*-glycans for *V. a. montandoni (Vam) snake venom.* Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G6	Hex4HexNAc3dHex1-proc	821.3616	2	821.3530	22.80	-0.0086				+		Complex	1.83	4.31	0.34	0.52	0.43	0.09
G7	Hex4HexNAc4dHex1-proc	922.9033	2	922.8927	24.76	-0.0106	68.9	58	58	+		Complex	29.00	51.25	5.37	6.17	5.77	0.40
G8	Hex4HexNAc4dHex1-proc	922.9022	2	922.8927	25.30	-0.0095				+		Complex	18.00	31.41	3.33	3.78	3.56	0.22
G9	Hex4HexNAc3NeuAc1dHex1-proc	966.9109	2	966.9007	26.12	-0.0102	62.1	44	44	+		Complex	6.68	10.89	1.24	1.31	1.27	0.04
G10	Hex6HexNAc2-proc	808.8459	2	808.8372	26.73	-0.0087	108.7	135	135			High-mannose	2.09	3.25	0.39	0.39	0.39	0.00
G11	Hex5HexNAc4-proc	930.9000	2	930.8901	27.35	-0.0099	81.9	79	79			Complex	10.79	17.72	2.00	2.13	2.07	0.07

Table S16. A list of the identified *N*-glycans for *V. a. montandoni (Vam) snake venom.* Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G12	Hex4HexNAc4NeuAc1dHex1-proc	1068.4517	2	1068.4404	28.50	-0.0113				+		Complex	10.01	15.14	1.85	1.82	1.84	0.02
G13	Hex5HexNAc4dHex1-proc	1003.9298	2	1003.9191	29.04	-0.0107	70.9	60	60			Complex	193.66	296.63	35.86	35.72	35.79	0.07
G14	Hex5HexNAc4NeuAc1-proc	717.9695	3	717.9610	30.73	-0.0085	79.0	68	68			Complex	12.83	18.98	2.38	2.28	2.33	0.05
G15	Hex5HexNAc4NeuAc1dHex1-proc	766.6557	3	766.6470	32.15	-0.0087	57.7	38	38	+		Complex	100.18	146.02	18.55	17.58	18.07	0.48
G16	Hex6HexNAc5dHex1-proc	791.3347	3	791.3259	34.48	-0.0088	73.5	60	60	+		Complex	9.92	13.99	1.84	1.68	1.76	0.08
G17	Hex6HexNAc5dHex1-proc	791.3347	3	791.3259	35.30	-0.0088				+		Complex	8.51	10.74	1.58	1.29	1.43	0.14

Table S16. A list of the identified *N*-glycans for *V. a. montandoni (Vam) snake venom.* Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G18	Hex5HexNAc4NeuAc2dHex1-proc	863.6889	3	863.6788	35.58	-0.0101	58.5	41	41	+		Complex	34.88	52.44	6.46	6.32	6.39	0.07
G19	Hex6HexNAc5NeuAc1dHex1-proc	888.3676	3	888.3577	37.15	-0.0099	46.1	39	39	+		Complex	6.71	9.77	1.24	1.18	1.21	0.03
G20	Hex6HexNAc5NeuAc1dHex1-proc	888.3676	3	888.3577	37.80	-0.0099						Complex	6.72	9.69	1.24	1.17	1.21	0.04
G21	Hex6HexNAc5NeuAc2dHex1-proc	985.7352	3	985.7257	39.84	-0.0095	28.9	23	23			Complex	10.82	14.85	2.00	1.79	1.90	0.11
G22	Hex7HexNAc6NeuAc1dHex1-proc	1010.0811	3	1010.0684	41.86	-0.0127	26.6	13	13			Complex	3.18	5.66	0.59	0.68	0.63	0.05
G23	Hex6HexNAc5NeuAc3dHex1-proc	1082.4342	3	1082.4213	42.70	-0.0129						Complex	3.72	5.29	0.69	0.64	0.66	0.03

Table S16. A list of the identified *N*-glycans for *V. a. montandoni (Vam) snake venom.* Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G24	Hex7HexNAc6NeuAc2dHex1-proc	1107.1141	3	1107.1002	43.90	-0.0139						Complex	3.07	6.05	0.57	0.73	0.65	0.08
G25	Hex7HexNAc6NeuAc3dHex1-proc	1204.1479	3	1204.1320	46.00	-0.0159						Complex	2.24	3.44	0.41	0.41	0.41	0.00

Table S17. A list of the identified *N*-glycans for *V. b. berus (Vbb) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

Peak	Composition	m/z meas.	z	m/z calc.	Rt [min]	Δ m/z	Score	IntCov. [%]	FragCov. [%]	Core Fucosylated	Bisecting	Glycan Type	Area_exp1	Area_exp2	Rel. Abn 1	Rel. Abn 2	Average	Std. Dev.
G1	Hex3HexNAc2dHex1-proc	638.7982	2	638.7869	14.55	-0.0113	74.76	64.47	64.47	+		Complex	35.77	4.38	0.26	0.52	0.39	0.13
G2	Hex3HexNAc2dHex1-proc	740.3367	2	740.3266	18.00	-0.0101	md	md	md	+		Complex	89.04	8.79	0.65	1.04	0.85	0.19

Table S17. A list of the identified *N*-glycans for *V. b. berus (Vbb) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G3	Hex4HexNAc3-proc	748.3333	2	748.3240	20.01	-0.0093	65.96	72.86	72.86			Complex	32.93	1.94	0.24	0.23	0.24	0.01
G4	Hex3HexNAc4dHex1-proc	841.8759	2	841.8663	20.27	-0.0096	55.81	52.05	52.05	+	+	Bisecting, Complex	34.89	2.69	0.26	0.32	0.29	0.03
	Hex3HexNAc5-proc	870.3870	2	870.3770	20.56	-0.0100	67.48	75.90	75.90			Complex						
G5	Hex4HexNAc3dHex1-proc	821.3622	2	821.3530	21.92	-0.0092	72.35	65.57	65.57	+		Complex	68.68	6.08	0.50	0.72	0.61	0.11
	Hex5HexNAc2-proc	727.8192	2	727.8108	21.92	-0.0084	81.87	86.30	86.30			High-mannose						
G6	Hex3HexNAc5dHex1-proc	943.4157	2	943.4060	22.51	-0.0097	89.02	99.27	99.27	+	+	Bisecting, Complex	323.26	25.58	2.37	3.02	2.70	0.32

Table S17. A list of the identified *N*-glycans for *V. b. berus (Vbb) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G7	Hex4HexNAc4dHex1-proc	922.9037	2	922.8927	24.20	-0.0110	44.71	37.50	37.50	+		Complex	118.99	7.64	0.87	0.90	0.89	0.01
	Hex4HexNAc5-proc	951.4148	2	951.4034	24.29	-0.0114	90.33	105.26	105.26		+	Bisecting, Complex						
	Hex3HexNAc6dHex1-proc	1044.9583	2	1044.9456	24.39	-0.0127	68.92	90.07	90.07	+		Complex						
	Hex5HexNAc3-proc	829.3603	2	829.3505	24.68	-0.0098	62.67	58.18	58.18			Complex						
G8	Hex4HexNAc3dHex2-proc	894.3918	2	894.3819	25.62	-0.0098	62.46	48.77	48.77	+		Complex	562.25	40.65	4.13	4.80	4.46	0.34
	Hex4HexNAc5dHex1-proc	1024.4416	2	1024.4324	26.05	-0.0092	67.83	71.50	71.50	+	+	Bisecting, Complex						

Table S17. A list of the identified *N*-glycans for *V. b. berus (Vbb) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

0.11		0.15	0.18		0.72
2.07		1.81	1.84		4.53
2.18		1.96	2.01		5.25
1.96		1.66	1.66		3.82
18.49		16.58	17.06		44.49
267.24		225.82	226.49		519.82
Complex	Complex	Complex	Complex	Bisecting, Complex	Complex
				+	
+		+	+	+	
24.39	45.76	md	48.85	70.27	md
24.39	45.76	md	48.85	70.27	md
35.05	56.39	md	67.68	76.04	md
-0.0108	-0.0101	-0.0108	-0.0121	-0.0144	-0.0121
27.76	27.96	28.70	29.46	29.82	30.00
995.9216	902.3794	995.9216	1097.4613	1105.4588	1097.4613
2	2	2	2	2	2
995.9324	902.3895	995.9324	1097.4734	1105.4732	1097.4734
Hex4HexNAc4dHex2-proc	Hex5HexNAc3dHex1-proc	Hex4HexNAc4dHex2-proc	Hex4HexNAc5dHex2-proc	Hex5HexNAc5dHex1-proc	Hex4HexNAc5dHex2-proc
G9		G10	G11		G12

Table S17. A list of the identified *N*-glycans for *V. b. berus (Vbb) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G13	Hex6HexNAc4dHex1-proc	1084.9571	2	1084.9455	31.35	-0.0116	48.28	46.89	46.89			Complex	147.44	10.47	1.08	1.24	1.16	0.08
	Hex5HexNAc6-proc	756.3251	3	756.3154	31.76	-0.0097	75.31	127.92	127.92			Complex						
	Hex5HexNAc4dHex2-proc	1076.9612	2	1076.9480	31.90	-0.0131	50.12	34.47	34.47			Complex						
	Hex5HexNAc5dHex1-proc	1105.4732	2	1105.4588	31.70	-0.0144	nd	nd	nd			Complex						
G14	Hex4HexNAc6dHex2-proc	799.6792	3	799.6698	32.50	-0.0094	59.73	52.83	52.83	+		Complex	298.32	20.42	2.19	2.41	2.30	0.11
	Hex6HexNAc3dHex1-proc	983.4173	2	983.4058	32.20	-0.0115	63.45	51.55	51.55	+		Complex						

Table S17. A list of the identified *N*-glycans for *V. b. berus (Vbb) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

	Hex4HexNAc5NeuAc1dHex2-proc	829.0184	3	829.0084	32.20	-0.0099	58.84	44.65	44.65	+		Complex						
	Hex5HexNAc6dHex1-proc	1207.5241	2	1207.5028	32.51	-0.0213	32.30	37.25	37.25	+		Complex						
	Hex5HexNAc5NeuAc1dHex1-proc	834.3496	3	834.3401	32.65	-0.0095	40.36	34.24	34.24			Complex						
	Hex5HexNAc5NeuAc1-proc	785.6658	3	785.6541	32.79	-0.0117	77.26	81.22	81.22			Complex						
G15	Hex5HexNAc5dHex2-proc	786.0021	3	785.9942	32.88	-0.0079	65.65	47.76	47.76	+	+	Bisecting, Complex	790.43	49.89	5.80	5.89	5.85	0.05
G16	Hex5HexNAc6dHex2-proc	853.6991	3	853.6874	33.80	-0.0117	47.10	34.35	34.35	+		Complex	218.80	13.57	1.61	1.60	1.60	0.00

Table S17. A list of the identified *N*-glycans for *V. b. berus (Vbb) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G17	Hex5HexNAc5dHex2-proc	786.0021	3	785.9942	34.80	-0.0079	65.65	47.76	47.76	+	+	Bisecting, Complex	204.51	14.33	1.50	1.69	1.60	0.10
G18	Hex5HexNAc4dHex3-proc	766.9974	3	766.9871	34.99	-0.0103	25.82	45.22	45.22	+		Complex	1121.48	81.18	8.23	9.59	8.91	0.68
	Hex5HexNAc5NeuAc2-proc	883.0378	3	883.0221	35.55	-0.0157	47.38	38.69	38.69			Complex						
G19	Hex5HexNAc5dHex3-proc	834.6903	3	834.6802	35.97	-0.0101	72.28	55.91	55.91	+	+	Bisecting, Complex	2050.66	130.46	15.05	15.41	15.23	0.18
G20	Hex5HexNAc5dHex3-proc	834.6903	3	834.6802	36.90	-0.0101	72.28	55.91	55.91	+	+	Bisecting, Complex	193.88	13.97	1.42	1.65	1.54	0.11
G21	Hex6HexNAc6dHex1-proc	1288.0414	2	1288.0249	37.77	-0.0165	40.02	40.63	40.63	+		Complex	119.19	6.16	0.87	0.73	0.80	0.07

Table S17. A list of the identified *N*-glycans for *V. b. berus (Vbb) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

	Hex5HexNAc5dHex3-proc	834.6903	3	834.6802	37.90	-0.0101	72.28	55.91	55.91	+	+	Bisecting, Complex						
	Hex5HexNAc4NeuAc1dHex3-proc	864.0305	3	864.0189	37.82	-0.0116	44.33	28.03	28.03	+		Complex						
G22	Hex5HexNAc5NeuAc2dHex1-proc	931.7248	3	931.7081	38.01	-0.0167	29.37	26.15	26.15	+		Complex	204.17	12.16	1.50	1.44	1.47	0.03
G23	Hex5HexNAc5NeuAc2dHex1-proc	931.7248	3	931.7081	38.01	-0.0167	29.37	26.15	26.15	+		Complex	749.94	44.00	5.50	5.20	5.35	0.15
G24	Hex6HexNAc6dHex3-proc	956.7390	3	956.7272	39.45	-0.0118	31.79	23.05	23.05	+		Complex	182.89	13.04	1.34	1.54	1.44	0.10
G25	Hex6HexNAc6dHex3-proc	956.7390	3	956.7272	39.45	-0.0118	31.79	23.05	23.05	+		Complex	996.11	48.25	7.31	5.70	6.51	0.81

Table S17. A list of the identified *N*-glycans for *V. b. berus (Vbb) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G26	Hex6HexNAc5NeuAc1dHex2-proc	937.0593	3	937.0437	41.93	-0.0157	34.51	20.48	20.48	+		Complex	860.72	50.34	6.32	5.95	6.13	0.19
	Hex7HexNAc6dHex2-proc	962.0714	3	962.0588	42.92	-0.0126	41.94	38.14	38.14	+		Complex						
	Hex4HexNAc3NeuAc1dHex1-proc	967.4117	2	967.4050	42.98	-0.0067	57.85	45.29	45.29	+		Complex						
G27	Hex6HexNAc6dHex4-proc	1005.0919	3	1005.0769	43.40	-0.0150	nd	nd	nd			Complex	491.98	28.62	3.61	3.38	3.50	0.12
G28	Hex7HexNAc7dHex3-proc	1078.4557	3	1078.4379	43.82	-0.0178	53.75	38.23	38.23	+		Complex	440.61	25.58	3.23	3.02	3.13	0.11
	Hex7HexNAc7dHex4-proc	1126.8008	3	1126.7876	43.68	-0.0131	53.47	52.48	52.48	+		Complex						

Table S17. A list of the identified *N*-glycans for *V. b. berus (Vbb) snake venom*. Hex = hexose; HexNAc = N-acetylhexosamine; Fuc = fucose; NAc = N-acetylhexosamine; NeuAc = N-acetylneuraminic acid.

G29	Hex8HexNAc8dHex1-proc	1102.4602	3	1102.4405	43.77	-0.0197	29.62	37.80	37.80	+		Complex	157.84	5.65	1.16	0.67	0.91	0.25
G30	Hex7HexNAc6dHex4-proc	1059.1093	3	1059.0945	45.40	-0.0148	28.50	22.87	22.87	+		Complex	254.81	12.33	1.87	1.46	1.66	0.21
G31	Hex7HexNAc7dHex4-proc	1126.8063	3	1126.7876	46.00	-0.0187	nd	nd	nd	+		Complex	547.70	26.04	4.02	3.08	3.55	0.47
G32	Hex7HexNAc6dHex5-proc	1107.7993	3	1107.7805	47.50	-0.0188	nd	nd	nd	+		Complex	828.91	37.42	6.08	4.42	5.25	0.83
G33	Hex7HexNAc6dHex4NeuAc1-proc	1204.8328	3	1204.8123	48.90	-0.0205	nd	nd	nd	+		Complex	141.12	5.57	1.04	0.66	0.85	0.19
G34	Hex7HexNAc7dHex4NeuAc1-proc	1272.5282	3	1272.5054	49.40	-0.0228	nd	nd	nd	+		Complex	117.58	2.88	0.86	0.34	0.60	0.26

8. Appendix II: Supporting Information Tables

Part I

Table S18. MI venom Replicates in the mass range of 500 Da- 5 kDa.								
M1.1	**M1.2**	**M1.3**	**MI2.1**	**MI2.2**	**MI2.3**	**MI3.1**	**MI3.2**	**MI3.3**
m/z	**m/z**	**m/z**	**m/z**	**m/z**	**m/z**	**m/z**	**m/z**	**m/z**
524.05	524.04	524.07		524.03		524.04		
558.21	558.20	558.24	558.17		558.18		558.19	558.20
568.08	568.07	568.10	568.04	568.05	568.07	568.06	568.06	568.07
586.26	586.26	586.28	586.22	586.23	586.23	586.23	586.24	586.25
608.11	608.10	608.14	608.09				608.12	608.10
628.35	628.34		628.29				628.32	628.34
644.01	644.01	644.05	643.98	643.98	643.99	643.99	644.00	644.00
650.04	650.04	650.06	650.00	650.01	650.02	650.02	650.02	650.02
656.07			656.02	656.03		656.04	656.05	656.05
666.03	666.02	666.04	665.98	666.00	666.01	666.00	666.01	666.00
703.48	703.48		703.44	703.44	703.45		703.46	703.46
855.16	855.15	855.19	855.11	855.12	855.13	855.14	855.14	855.14
861.18	861.17	861.20	861.13	861.14	861.16	861.16	861.16	861.16
877.16	877.15	877.17	877.10	877.12	877.14	877.14	877.14	877.14
1060.23	1060.23		1060.19					
1066.26	1066.26		1066.21	1066.22	1066.24	1066.25	1066.24	1066.24
	1168.82	1168.84		1168.78	1168.82	1168.80		1168.81
	1349.93	1349.95	1349.87	1349.88	1349.94	1349.92	1349.92	1349.92
		1365.93	1365.85	1365.86	1365.92	1365.89	1365.90	1365.90
		1716.32	1716.22		1716.32		1716.29	
	1810.40	1810.42	1810.32	1810.32	1810.44		1810.40	1810.40
	4392.13	4392.19			4393.25		4392.15	

Table S19. The most abundant 10 protein peaks of the analyzed venoms in the mass range of 5 kDa to 60 kDa

a)Ml				b)Mx				c)Vaa				d)Vam				e)Vbb			
m/z	**Area**	**Xnormalized**	**Relative abundance**	**m/z**	**Area**	**Xnormalized**	**Relative abundance**	**m/z**	**Area**	**Xnormalized**	**Relative abundance**	**m/z**	**Area**	**Xnormalized**	**Relative abundance**	**m/z**	**Area**	**Xnormalized**	**Relative abundance**
5000.00	0.00	0.00	0.00	5000.00	0.00	0.00	0.00	5000.00	0.00	0.00	0.00	5000.00	0.00	0.00	0.00	5000.00	0.00	0.00	0.00
6801.577	1.19E+06	43.51	8.303061	6824.32	1.35E+06	46.32	13.23781	6748.874	7.57E+05	24.95	3.484581	6781.645	3.68E+06	46.60	14.38133	6811.88	1.26E+06	10.56	2.918693
6881.766	35238	0.00	0	6904.018	610267	20.63	5.895367	7141.492	19647	0.00	0	6913.372	1.66E+06	20.88	6.444971	6898.928	5.25E+05	44.41	12.27674
7011.615	858060	30.89	5.894315	7025.566	18058	0.00	0	6816.537	1.40E+06	46.64	6.513193	6957.014	656414	8.18	2.524983	7069.876	8.76E+05	7.30	2.017029
7075.725	498000	17.37	3.315012	7199.003	229533	7.37	2.105208	6897.78	1.16E+06	38.58	5.388424	7100.541	11812	0.00	0	7147.578	15796	0.00	0

4.992815	7.935657	4.053489	9.93658	27.64512	3.774064	3.9461
18.06	28.71	14.66	35.94	100.00	13.65	14.27
2.15E+06	3.40E+06	1.74E+06	4.25E+06	1.18E+07	1.63E+06	1.70E+06
7409.297	7566.39	13542.87	13674.78	13850.49	13951.05	13992.45
1.576939	30.86337	4.137697	11.38336	6.113547	2.929888	2.000998
5.11	100.00	13.41	36.88	19.81	9.49	6.48
414388	7.89E+06	1.07E+06	2.92E+06	1.57E+06	759782	522646
7355.211	13614.79	13799.09	13879.64	13974.51	14025.69	14105.52
4.261421	9.147303	9.362354	7.133117	13.96556	11.90513	9.91309
30.51	65.50	67.04	51.08	100.00	85.25	70.98
921466	1.96E+06	2.00E+06	1.53E+06	2.98E+06	2.54E+06	2.12E+06
6936.972	7426.083	13546	13614.24	13684.6	13847.3	13929.64
2.474892	28.58069	3.62018	1.53551	16.47413	1.934442	2.795031
8.66	100.00	12.67	5.37	57.64	6.77	9.78
266669	2.89E+06	381717	172305	1.67E+06	212379	298828
7461.5	13703.31	13763.9	13806.24	13860.31	14019.49	14457.09
3.417236	3.969637	19.08357	3.57613	11.8933	12.85407	5.781281
17.91	20.80	100.00	18.74	62.32	67.36	30.29
512270	589383	2.70E+06	534451	1.70E+06	1.83E+06	842281
7081.487	7340.307	13654.61	13801.46	14077.66	14202.76	14595.66

29000.00	0.00	0.00	0.00	29000.00	0.00	0.00	0.00	29000.00	0.00	0.00		29000.00	0.00	0.00	0.00	29000.00	0.00	0.00	0.00
	sum X Nor	524.01			sum X Nor	349.89			sum X Nor	716.05			sum X Nor	324.01			sum X Nor	361.73	
	Xmax	2.70E+06			Xmax	2.89E+06			Xmax	2.98E+06			Xmax	7.89E+06			Xmax	1.18E+07	
	Xmin	35238			Xmin	18058			Xmin	19647			Xmin	11812			Xmin	15796	

Table S20. The most abundant 10 peptide peaks of the analyzed venoms in the mass range of 500 Da to 5 kDa

a)Ml				b)Mx				c)Vaa				d)Vam				e)Vbb			
m/z	Intensity	Xnormalized	Relative abundance	m/z	Intensity	Xnormalized	Relative abundance	m/z	Intensity	Xnormalized	Relative abundance	m/z	Intensity	Xnormalized	Relative abundance	m/z	Intensity	Xnormalized	Relative abundance

Table S20. The most abundant 10 peptide peaks of the analyzed venoms in the mass range of 500 Da to 5 kDa

500.00		0.00	0.00	500.00		0.00	0.00	500.00		0.00	0.00	500.00		0.00	0.00	500.00		0.00	0.00
506.12	690692.00	15.36	4.28	506.08	277840.00	21.38	14.21	506.12	672691.00	13.77	11.94	506.11	282467.00	12.52	4.53	506.10	403694.00	6.35	4.52
522.08	331906.00	7.09	1.98	524.05	664463.00	52.05	34.59	522.09	356322.00	7.20	6.25	524.10	949604.00	42.50	15.37	522.08	221919.00	3.39	2.41
524.10	2530372.00	57.76	16.10	526.07	209788.00	15.98	10.62	524.11	2859849.00	59.18	51.33	526.12	205320.00	9.05	3.27	524.09	1870062.00	30.28	21.53
526.11	615242.00	13.62	3.80	550.03	176667.00	13.35	8.88	526.12	591402.00	12.08	10.48	568.09	1734467.00	77.76	28.12	526.10	387742.00	6.09	4.33
550.08	441511.00	9.62	2.68	568.04	1269012.00	100.00	66.47	550.09	436123.00	8.86	7.68	630.14	192143.00	8.46	3.06	550.07	270340.00	4.18	2.97

Table S20. The most abundant 10 peptide peaks of the analyzed venoms in the mass range of 500 Da to 5 kDa

568.08	628.04	643.97	665.98	854.98	5000.00
4362736.00	212612.00	633976.00	215493.00	263013.00	
100.00	4.34	14.05	4.41	5.50	0.00
27.87	1.21	3.92	1.23	1.53	0.00
643.92	649.95	665.91	854.93	4021.27	5000.00
350336.00	194223.00	255789.00	230401.00	230474.00	
27.13	14.75	19.63	17.62	17.62	0.00
18.03	9.80	13.05	11.71	11.71	0.00
568.09	723.29	851.36	1072.47	1144.50	5000.00
4825580.00	331261.00	342038.00	356399.00	3241438.00	
100.00	6.68	6.91	7.20	67.11	0.00
86.72	5.79	5.99	6.25	58.20	0.00
656.03	665.97	876.98	1144.52	1159.48	5000.00
420818.00	710608.00	332297.00	594203.00	2229329.00	
18.74	31.76	14.76	26.53	100.00	0.00
6.78	11.49	5.34	9.59	36.17	0.00
568.07	587.07	643.97	1144.49	3872.34	5000.00
3289628.00	127259.00	315207.00	6143064.00	136943.00	
53.44	1.84	4.91	100.00	2.00	0.00
38.00	1.31	3.49	71.10	1.42	0.00

Table S21. Ml, Mx, Vaa, Vbb, and Vam venoms proteins and peptides mass peaks distribution at the mass range of 500 Da to 30 kDa .						
	(0.5-5) kDa	(5-6) kDa	(7-9) kDa	(12-13) kDa	(13-15) kDa	(20-30) kDa
Ml	100 peaks	14 peaks	35 peaks		47 peaks	4 peaks
Mx	91 peaks	17peaks	31peaks	6peaks	46peaks	
Vaa	100 peaks	4 peaks	42 peaks	13 peaks	34 peaks	7 peaks
Vam	73 peaks	19 peaks	30 peaks		51 peaks	peaks
Vbb	83 peaks		31 peaks	4 peaks	20 peaks	44peaks
WA	94 peaks	8 peaks	46 peaks	2 peaks	33 peaks	11 peaks

Table S22a. SDEV values of the matched peptides by mass m/z and their corresponding relative abundance among the five venoms.											
Ml		**Mx**		**Vaa**		**Vam**		**Vbb**			
m/z	**Relative abundance (Normalized Area)**	**m/z**	**Relative abundance (Normalized Area)**	**m/z**	**Relative abundance (Normalized Area)**	**m/z**	**Relative abundance (Normalized Area)**	**m/z**	**Relative abundance (Normalized Area)**	**SDEV (m/z)**	**SDEV (%)**
568.113	1.50664	568.146	0.96076	568.154	4.58737	568.135	0.1611	568.148	0.00496	0.016177	1.859765
586.294	4.08359	586.324	0.0105	586.325	0	586.316	0	586.327	0.41474	0.013627	1.787592
644.036	7.91481	644.073	1.46915	644.075	2.76895	644.062	0.67473	644.075	2.34561	0.016664	2.845231
855.133	2.9923	855.185	0.55804	855.193	0.8643			855.19	1.72915	0.028359	1.090218
1060.18	1.93416	1060.25	0.2276	1060.25	0.38724			1060.25	0.21628	0.034856	0.832228
1066.2	0.70634	1066.27	0	1066.27	0.40241	1066.25	1.01179	1066.27	0.70244	0.030939	0.382147

Table S22b. SDEV values of the unmatched peptides by mass m/z. (2kDa-60kDa)									
Ml		**Mx**		**Vaa**		**Vam**		**Vbb**	
m/z	**Relative abundance %**	**m/z**	**Relative abundance %**	**m/z**	**Relative abundance %**	**m/z**	**Relative abundance %**	**m/z**	**Relative abundance %**
2062.764	0	2011.456	3.282078	2206.925	13.14419	3163.179	1.827591	3049.082	5.87463
2210.992	12.0336	2371.936	0	2667.992	0	3234.208	2.652804	3120.112	2.284069
2310.903	10.45628	2667.964	4.029484	2777.195	6.878416	3720.432	5.020048	3234.145	0
2323.916	11.30408	2854.071	4.092791	3857.456	5.558916	3790.491	0	3856.738	7.900012
2342.89	13.0675	3953.31	16.668	3871.414	11.37509	3793.313	12.35256	3872.343	43.30908
2356.867	7.298398	4005.386	9.253154	3928.477	12.75723	3857.489	32.97939	3927.583	3.487863
4391.27	9.214895	4021.274	33.64724	3942.446	21.43314	3928.516	45.16761	3943.376	34.34573
6801.577	8.303061	6824.32	13.23781	6748.874	3.484581	6781.645	14.38133	6811.88	2.918693
6881.766	0	6904.018	5.895367	7141.492	0	6913.372	6.444971	6898.928	12.27674
7011.615	5.894315	7025.566	0	6816.537	6.513193	6957.014	2.524983	7069.876	2.017029
7075.725	3.315012	7199.003	2.105208	6897.78	5.388424	7100.541	0	7147.578	0
7081.487	3.417236	7461.5	2.474892	6936.972	4.261421	7355.211	1.576939	7409.297	4.992815
7340.307	3.969637	13703.31	28.58069	7426.083	9.147303	13614.79	30.86337	7566.39	7.935657
13654.61	19.08357	13763.9	3.62018	13546	9.362354	13799.09	4.137697	13542.87	4.053489
13801.46	3.57613	13806.24	1.53551	13614.24	7.133117	13879.64	11.38336	13674.78	9.93658
14077.66	11.8933	13860.31	16.47413	13684.6	13.96556	13974.51	6.113547	13850.49	27.64512
14202.76	12.85407	14019.49	1.934442	13847.3	11.90513	14025.69	2.929888	13951.05	3.774064
14595.66	5.781281	14457.09	2.795031	13929.64	9.91309	14105.52	2.000998	13992.45	3.9461
29000	0	29000	0	29000		29000	0	29000	0

Table S22c. SDEV values of the matched peptides by mass m/z.					
Ml	**Mx**	**Vaa**	**Vam**	**Vbb**	**STDEV**
506.117	506.079	506.123	506.113	506.104	0.016392
522.084	522.034	522.088	522.087	522.075	0.021274
524.099	524.054	524.106	524.104	524.09	0.020539
526.113	526.068	526.118	526.119	526.103	0.020781
550.081	550.033	550.085	550.086	550.069	0.021335
568.08	568.037	568.086	568.088	568.072	0.020262
643.971	643.924	644.008		643.972	0.029875
650.016	649.945	650.012	650.036	650.01	0.030943
665.976	665.914	665.95	665.968	665.96	0.021602
681.924	681.882	681.941		681.918	0.021852
713.115	713.051	713.116	713.127	713.103	0.029398
854.983	854.931	855	855.001	854.98	0.025532
870.947	870.899	870.96		870.943	0.022963
	1072.44	1072.47	1072.49	1072.47	0.019738
		1144.5	1144.52	1144.49	0.014526
1899.87	1899.83	1899.85			0.021197

Table S23a. A list of the identified proteins from the crude venom of Ml.

	m/z	Intensit y	Relativ	Protein Family	MS/MS Fragme ntation	Accessi	Protein species	Protein Family		score
1	557.281	21849	6.59140	Snake venom metalloprotein ase m12b family	YFVE	P0C8I7	Factor X-activator 1 heavy chain (Fragment)	Snake venom metalloproteinase m12b family	26%	10
2	568.113	5055	1.52499	Snake venom metalloprotein ase	S.RETY.Q	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase	11%	7
3	586.294	13530	4.08173	Bradykinin-potentiating peptides (BPPs)	QRWP.S	P0DKZ8	Bradykinin-potentiating peptide	Bradykinin-potentiating peptides (BPPs)	57%	16
4	627.262	18053	5.44623	snake three-finger toxin family	AYITC	P0DMV	7.2 kDa cytotoxin RVV-7 (Fragment)	snake three-finger toxin family	25%	8
5	644.036	26130	7.88289	Bradykinin-potentiating peptides (BPPs)	EEGGRP.P	P0C7S1	Bradykinin-potentiating peptide 2	Bradykinin-potentiating peptides (BPPs)	60%	14
6	649.301	57867	17.4573	Snake venom serine protease	ECNIN	P86531	Vipera russelli proteinase RVV-V homolog 2 (Fragment)	Snake venom serine protease	33%	9

7	665.316	26369	7.95500	Snake venom metalloproteinase m12b family	EQFNK	P86536	Coagulation factor X-activating enzyme heavy chain (Fragment)	Snake venom metalloproteinase m12b family	26%	8
8	702.837	14927	4.50317		QLPSVCG		Peptide matches not assigned to protein hits			
9	832.728	15642	4.71887	Snake venom metalloproteinase m12b family	AFNGNYF	P0C8I7	Factor X-activator 1 heavy chain (Fragment)	Snake venom metalloproteinase m12b family	46%	9
10	838.753	16057	4.84407	Snake venom metalloproteinase m12b family	SRERYQ	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase m12b family	16%	4
11	855.133	9941	2.99900		SCSTCNIK		Peptide matches not assigned to protein hits			
12	860.779	23901	7.21045	Procougulant protein	FNGNYFV	P0C8I7	Factor X-activator 1 heavy chain (Fragment)	Procougulant protein	46%	5
13	876.782	16780	5.06219	Snake venom metalloproteinase m12b family	VSVSPAFNG	P0C8I7	Factor X-activator 1 heavy chain (Fragment)	Snake venom metalloproteinase m12b family	60%	9

14	1044.05	12048	3.63464		IYTHIARGL		Peptide matches not assigned to protein hits			
15	1050.07	5778	1.74310	Snake venom serine protease	VGGDECNINE	P86531	Vipera russelli proteinase RVV-V homolog 2 (Fragment)	Snake venom serine protease	66%	9
16	1060.18	6461	1.94915	Procougulant protein	P.AFNGNYFVE	P0C8I7	Factor X-activator 1 heavy chain (Fragment)	Procougulant protein	60%	12
17	1066.2	2423	0.73097	Disintigrin family	S.VSSHYCTGR.S	Q1JRG	RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	20%	6
18	1082.05	12162	3.66903	Admalysin	SCKLTRGSAC	A0A7L4	Disintegrin and metalloproteinase domain-containing protein 5 (Fragment)	Admalysin	12%	5
19	1145.94	100	0.03016	Disintigrin family	V.YPKKVTVLPT.G	A0A6G5	Disintegrin (Fragment)	Disintigrin family	12%	4
20	1271.17	9162	2.76399		WIQGMGHITGTA		Peptide matches not assigned to protein hits			

21	1277.19	7544	2.27587	Phospholipase A2	QFRNLPVGSCR	A0A6G5	Phospholipase A2 1 (Fragment)	Phospholipase A2	21%	2
22	2000.53	1965	0.59280	Thrombin-like snake venom serine protease	IGGAKCNINEHRSIVLL Y	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	51%	2
23	2185.08	1938	0.58465		DLNKL GVATV AKGVC GACKK PI		Peptide matches not assigned to protein hits			
24	2195.11	2294	0.69205		KQLLL QTHEIS RLQER NS		Peptide matches not assigned to protein hits			
25	2195.66	1474	0.44467	snake three-finger toxin family	LKCNKLVPLFYKTCPA GKN	P0C5H4	Cytotoxin drCT-1 (Fragment)	snake three-finger toxin family	95%	10
26	3988.8	2027	0.61150	Disintegrins	TTGPCCRQCKLKPAG TTCWRTSVSSHYCTG RSCE	Q1JRG	RTS-containing short disintegrin ML-G3 (Fragment)	Disintegrins	79%	2
		331477								

Table S23b. A list of the identified proteins from the crude venom of Mx snake.

	m/z	**Intensity**	**Relativ**	Protein Family	Matched peptide / MS/MS Fragmentation	Accessi	Protein species	Protein Family	Protein	score
1	534.975	124418	13.9555	Snake venom metalloproteinase m12b family	SPAFN	P0C8I7	Factor X-activator 1 heavy chain (Fragment)	Snake venom metalloproteinase m12b family	33%	11
2	538.978	30665	3.43958	growth factor family	EVYE	P0CJ33	Snake venom vascular endothelial growth factor toxin IC1 (Fragment)	growth factor family	30%	11
3	560.104	23989	2.69075	Uncharacterized protein	LTITL	V8N3R4	Uncharacterized protein	Uncharacterized protein	45%	13
4	568.146	9962	1.11740	Snake venom metalloproteinase	S.RETY.Q	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase	11%	7
5	570.894	288540	32.3644	venom metalloproteinase m12b family	NDYC	P0C6A5	Disintegrin VA6	venom metalloproteinase m12b family	6%	4
6	586.324	1904	0.21356	Bradykinin-potentiating peptides (BPPs)	QRWP.S	P0DKZ8	Bradykinin-potentiating peptide	Bradykinin-potentiating peptides (BPPs)	57%	15

7	593.932	23463	2.63175	Snake venom serine protease	GGDECN	P86531	Vipera russelli proteinase RVV-V homolog 2 (Fragment)	Snake venom serine protease	40%	11
8	607.849	11021	1.23618				Peptide matches not assigned to protein hits			3
9	644.073	14273	1.60095	Bradykinin-potentiating peptides (BPPs)	EEGGRP.P	P0C7S1	Bradykinin-potentiating peptide 2	Bradykinin-potentiating peptides (BPPs)	60%	14
10	651.738	23737	2.66249		LIVIPP		Peptide matches not assigned to protein hits			5
11	679.793	21602	2.42301		XXXXCN		Peptide matches not assigned to protein hits			1
12	723.693	176821	19.8333	Snake venom metalloproteinase m12b family	YQKALT	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase m12b family	16%	7
13	834.39	18005	2.01955	Snake venom metalloproteinase m12b family	TFIELVI	P86536	Coagulation factor X-activating enzyme heavy chain (Fragment)	Snake venom metalloproteinase m12b family	36%	10

14	855.185	6547	0.73435		LEENYTS		Peptide matches not assigned to protein hits			
15	912.353	28418	3.18754	Snake venom phospholipase A2	LFQFARM	P18999	Phospholipase A2 (Fragment)	Snake venom phospholipase A2	31%	9
16	1060.25	3745	0.42006	Procougulant protein	P.AFNGNYFVE	P0C8I7	Factor X-activator 1 heavy chain (Fragment)	Procougulant protein	60%	12
17	1066.27	1815	0.20358		PLHNAASYGH		Peptide matches not assigned to protein hits			
18	1133.24	45992	5.15875		FDPCGGGDPVL		Peptide matches not assigned to protein hits			
19	1145.29	1883	0.21120	metaloproteinase inhibitor	H.HHHHHGVGGGGG.G	P0C7K5	Poly-His-poly-Gly peptide 1	metaloproteinase inhibitor	50%	10
20	1155.2	19867	2.22840	Disintigrin family	GRSCECPSYP		RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	23%	6

21	2320.79	2223	0.24934	phospholipase A2 family	RCEKMVCECDQKAAS CFQK	A0A6G5	Phospholipase A2 1 (Fragment)	phospholipase A2 family	37%	6
22	2656.93	2380	0.26695	Disintigrin family	CTTGPCCRQCKLKPA GTTCWRTSV	Q1JRG	RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	55%	7
23	3258.38	2944	0.33021	Disintigrin family	TGPCCRQCKLKPAGT TCWRTSVSSHYC		RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	62%	8
24	3341.48	7319	0.82094		MVSHGFTSSALFCLANTS YERMHTRILVL		Peptide matches not assigned to protein hits			
		891533								
Table S23c. A list of the identified proteins from the crude venom of Vaa snake.										
	m/z	**Intensit y**	**Relativ**	Protein Family	Matched peptide / MS/MS Fragmentation	Accessi	Protein species	Protein Family	protein	score
1	524.114	5045	1.80480	Snake venom phospholipase A2 (PLA2)	FARM	P18999	Phospholipase A2 (Fragment)	Snake venom phospholipase A2 (PLA2)	18%	8

2	526.132	1836	0.65681	Bradykinin-potentiating peptide	EAIPP		Bradykinin-potentiating peptide	Bradykinin-potentiating peptide	45%	13
3	568.154	12371	4.42561	Snake venom metalloprotein ase	S.RETY.Q	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase	11%	7
4	586.325	880	0.31481	Bradykinin-potentiating peptides (BPPs)	QRWP.S	P0DKZ8	Bradykinin-potentiating peptide	Bradykinin-potentiating peptides (BPPs)	57%	15
5	644.075	7816	2.79610	Bradykinin-potentiating peptides (BPPs)	EEGGRP.P	P0C7S2	Bradykinin-potentiating peptide 3	Bradykinin-potentiating peptides (BPPs)	60%	14
6	723.473	6443	2.30492	Snake venom serine protease	NINEHP	P86532	Vipera russelli proteinase RVV-V homolog 4 (Fragment)	Snake venom serine protease	37%	8
7	851.629	4676	1.67279		QGIVSYGK		Peptide matches not assigned to protein hits			
8	855.193	3045	1.08932		GLAYDEGM		Peptide matches not assigned to protein hits			

10	1060.25	1850	0.66182	Procoaugulant protein	P.AFNGNYFVE	P0C8I7	Factor X-activator 1 heavy chain (Fragment)	Procoaugulant protein	60%	12
11	1066.27	1888	0.67541		PLHNAASYGH		Peptide matches not assigned to protein hits			
9	1072.77	5731	2.05021	phospholipase A2 family	RNLPVGSCRA	A0A6G5	Phospholipase A2 1 (Fragment)	phospholipase A2 family	19%	2
12	1144.84	54486	19.4918	Snake venom serine protease	F.FFNVTTQKC.E	A0A6G5	Serine protease inhibitor 7 (Fragment)	Snake venom serine protease	14%	4
13	1159.77	3097	1.10792	Snake venom serine protease	ECNINEHPF	P86532	Vipera russelli proteinase RVV-V homolog 4 (Fragment)	Snake venom serine protease	56%	8
14	1176.78	2913	1.04209	Snake venom metalloproteinase m12b family	NKTFIELVIV	P86536	Coagulation factor X-activating enzyme heavy chain (Fragment)	Snake venom metalloproteinase m12b family	52%	7
15	1892.6	2288	0.81851		RGSDT DAPDT VTGSC LLQ		Peptide matches not assigned to protein hits			

16	1915.59	1919	0.68650		RGALV PCQTD CLHDG NC		Peptide matches not assigned to protein hits			
17	2030.43	4526	1.61913	Adamalysin	KDVEKVKLRLIEIANYV	A0A6G5	Disintegrin and metalloproteinase domain-containing protein 7 (Fragment)	Adamalysin	12%	2
18	2068.32	8683	3.10626	Snake venom metalloprotein ase m12b family	KYSSIFMSPILSNPPILY	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase m12b family	50%	2
19	2265.21	4868	1.74148	Disintigrin family	TTGPCCRQCKLKPAG TTCWR		RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	46%	1
20	2384.09	5833	2.08670		AVEVT WDYGS SIPSPY LRDND		Peptide matches not assigned to protein hits			
21	2396.02	11455	4.09792		RDLINV TSSAD DTLDS FGEWR		Peptide matches not assigned to protein hits			
22	2542.78	13470	4.81876	Thrombin-like snake venom serine protease	VIGGAKCNINEHRSIVL LYSSRL	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	65%	2

23	2705.51	6286	2.24875		CRCGYNGKHCQYNVNECLSQPCK		Peptide matches not assigned to protein hits			
24	2721.47	5924	2.11925	Snake venom metalloproteinase m12b family	VTKYSSIFMSPILSNPPILYFSDC	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase m12b family	66%	2
25	2775.51	18090	6.47153		GTSCENRICECDKAAAICFRQNLN		Peptide matches not assigned to protein hits			
26	2798.48	4870	1.74219	Thrombin-like snake venom serine protease	CNINEHRSIVLLYSSRLFGHTLIN	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	68%	2
27	2863.24	10657	3.81244	Disintigrin family	TTGPCCRQCKLKPAGTTCWRTSVSSH		RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	60%	1
28	2978.01	23151	8.28205		DACLGDSGGPLVCQADGAWFVAGIVSWGD		Peptide matches not assigned to protein hits			
29	3245.43	6711	2.40079		VSWGDLCGLSNRPGVYTRVSFYQDWIQT		Peptide matches not assigned to protein hits			

30	3445.07	6907	2.47091		DIVSPAVCGNYLVELGED CDCGSPRDCQNPC		Peptide matches not assigned to protein hits			
31	3517.97	19819	7.09006		DCPIMTKQCISLFGSRATV AEDSCFQENQKG		Peptide matches not assigned to protein hits			
32	3673.65	4899	1.75257		EKEDEAPKMCGVTQTNW ESDEPIKKASQLNLT		Peptide matches not assigned to protein hits			
33	3849.26	7099	2.53960		CGLFLECTDPCCNATTCK LVPGAQCATGQPCCHQC		Peptide matches not assigned to protein hits			
		279532								

Table S23d. A list of the identified proteins from the crude venom of Vam snake.

	m/z	Intensity	Relativ	Protein Family	Matched peptide / MS/MS Fragmentation	Accessi	Protein species	Protein Family	Protein	score
1	568.135	3847	0.37932	Snake venom metalloproteinase	S.RETY.Q	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase	11%	7

2	586.316	2306	0.22737	Bradykinin-potentiating peptides (BPPs)	QRWP.S	P0DKZ8	Bradykinin-potentiating peptide	Bradykinin-potentiating peptides (BPPs)	57%	15
3	644.062	8760	0.86375	Bradykinin-potentiating peptides (BPPs)	EEGGRP.P	P0C7S3	Bradykinin-potentiating peptide 4	Bradykinin-potentiating peptides (BPPs)	60%	14
4	655.315	104398	10.2938		ALPKGAV		Peptide matches not assigned to protein hits			
5	750.029	22341	2.20286	growth factor family	VYERIA		Snake venom vascular endothelial growth factor toxin IC1 (Fragment)	growth factor family	46%	10
6	855.171	12759	1.25806	Snake venom serine protease	I.NEHPFLV	P86532	Vipera russelli proteinase RVV-V homolog 4 (Fragment)	Snake venom serine protease	43%	11
7	860.756	25591	2.52332		ARPLHPVA		Peptide matches not assigned to protein hits			
8	1066.25	11984	1.18164		CAQMAGGGWW		Peptide matches not assigned to protein hits			

9	1072.55	22804	2.24852		PLLLFSLVGL		Peptide matches not assigned to protein hits			
10	1144.82	3516	0.34668	Snake venom serine proteas e	F.FFNVTTQKC.E	A0A6G5	Serine protease inhibitor 7 (Fragment)	Snake venom serine protease	14%	4
11	1159.62	410510	40.4771		NVLKDIQMPC		Peptide matches not assigned to protein hits			
12	1181.61	65764	6.48446		MASTLSHQLGH		Peptide matches not assigned to protein hits			
13	3006.69	14230	1.40310	Disintigr in family	TCWRTSVSSHYCTGR SCECPSYPGN		RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	58%	1
14	3044.6	16291	1.60632	Thrombi n-like snake venom serine proteas	VIGGAKCNINEHRSIVL LYSSRLFGHT	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	77%	3
15	3115.25	17082	1.68432	Thrombi n-like snake venom serine proteas	GGAKCNINEHRSIVLL YSSRLFGHTLIN	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	80%	3

16	3158.01	23147	2.28234	Disintigrin family	GPCCRQCKLKPAGTT CWRTSVSSHYC		RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	60%	1
17	3228.65	27581	2.71954	Thrombin-like snake venom serine proteas	IGGAKCNINEHRSIVLL YSSRLFGHTLIN	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	82%	3
18	3285.37	18203	1.79485	Thrombin-like snake venom serine proteas	IGGAKCNINEHRSIVLL YSSRLFGHTLIN	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	82%	3
19	3355.97	21315	2.10170	Thrombin-like snake venom serine proteas	IGGAKCNINEHRSIVLL YSSRLFGHTLINK	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	85%	4
20	3711.82	26912	2.65358	Thrombin-like snake venom serine proteas	GGAKCNINEHRSIVLL YSSRLFGHTLINKEWV	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	91%	5
21	3781.38	15097	1.48859		KGCNPKLAIYSYSFQRGN IVCGRNNGCLRTICEC		Peptide matches not assigned to protein hits			
22	3784.22	37576	3.70507		KGCNPKLAIYSYSFQRGN IVCGRNNGCLRTICEC		Peptide matches not assigned to protein hits			

23	3787.07	7479	0.73744		GLIQDYCKSYLLVASVMA HELGHNLGMEHDDGNC		Peptide matches not assigned to protein hits			
24	3847.9	40769	4.01990	Disintigr in family	GPCCRQCKLKPAGTT CWRTSVSSHYCTGRS CE	Q1JRG	RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	74%	2
25	3918.41	53915	5.31613		SALFLLEHQANISARTQD GETALQLAIKNQLPLVVD		Peptide matches not assigned to protein hits			
		1014177								

Table S23e. A list of the identified proteins from the crude venom of Vbb snake.

	m/z	Intensit y	Relativ	Protein Family	Matche d peptide / MS/MS Fragme ntation	Accessi	Protein species	Protein Family	Protein	Score
1	520.772	10814	1.46933	Snake venom metalloprotein ase m12b family	VSPAF	P0C8I7	Factor X-activator 1 heavy chain (Fragment)	Snake venom metalloproteinase m12b family	33%	9
2	568.148	2353	0.31970	Snake venom metalloprotein ase	S.RETY.Q	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase	11%	7

3	576.489	2321	0.31536	snake three-finger toxin family	CNKLV		Cytotoxin drCT-1 (Fragment)	snake three-finger toxin family	25%	8
4	586.327	4998	0.67909	Bradykinin-potentiating peptides (BPPs)	QRWP.S	P0DKZ8	Bradykinin-potentiating peptide	Bradykinin-potentiating peptides (BPPs)	57%	15
5	644.075	17461	2.37247	Bradykinin-potentiating peptides (BPPs)	EEGGRP.P	P0C7S4	Bradykinin-potentiating peptide 5	Bradykinin-potentiating peptides (BPPs)	60%	14
6	721.058	6441	0.87515	Snake venom serine protease	ECNINE	P86531	Vipera russelli proteinase RVV-V homolog 2 (Fragment)	Snake venom serine protease	40%	9
7	855.19	13482	1.83184		GLAYDEGM		Peptide matches not assigned to protein hits			
8	1060.25	3717	0.50504		SSFFLPTFD		Peptide matches not assigned to protein hits			
9	1066.27	6855	0.93140	Disintigrin family	S.VSSHYCTGR.S	Q1JRG	RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	20%	6

10	1071.83	26011	3.53419	Snake venom serine protease	ECNINEHR	P86531	Vipera russelli proteinase RVV-V homolog 2 (Fragment)	Snake venom serine protease	53%	8
11	1144.84	35687	4.84890	Snake venom serine protease	F.FFNVTTQKC.E	A0A6G5	Serine protease inhibitor 7 (Fragment)	Snake venom serine protease	14%	4
12	1160.13	20762	2.82099		PLGVCRDLCK		Peptide matches not assigned to protein hits			
13	1166.13	28799	3.91300	Snake venom phospholipase A2	EMIVKMTGKE	P0DKX	Neutral phospholipase A2 RVV-PFIIc' (Fragment)	Snake venom phospholipase A2	52%	7
14	1176.17	8421	1.14418		PGQAANRTFKS		Peptide matches not assigned to protein hits			
15	1182.14	16750	2.27587	Snake venom metalloprotein ase m12b family	MSPILSNPPIL	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase m12b family	30%	3
16	1188.18	6426	0.87312	phospholipase A2 family	CEKMVCECDQ	A0A6G5	Phospholipase A2 1 (Fragment)	phospholipase A2 family	19%	2

17	1204.19	4416	0.60001	Disintigrin family	CRQCKLKPAGT	Q1JRG	RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	25%	2
18	1257.44	4571	0.62107		IATGLYPESHGL		Peptide matches not assigned to protein hits			
19	1331.52	3563	0.48411	Snake venom serine protease	GGDECNINEHRS	P86531	Vipera russelli proteinase RVV-V homolog 2 (Fragment)	Snake venom serine protease	80%	10
20	2054.13	5037	0.68439	Snake venom metalloprotein ase m12b family	ATSEQFNKTFIELVIVV D	P86536	Coagulation factor X-activating enzyme heavy chain (Fragment)	Snake venom metalloproteinase m12b family	94%	10
21	2680.28	5257	0.71428	Snake venom metalloprotein ase m12b family	TKYSSIFMSPILSNPPIL YFSDC	P0DJ44	Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase m12b family	63%	2
22	2750.82	4641	0.63058	Snake venom metalloprotein ase m12b family	MVTKYSSIFMSPILSNP PILYFSD		Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	Snake venom metalloproteinase m12b family	66%	2
23	2760.72	7149	0.97135		KLKPAGTTCWRTSVSSH YCTGRSCE		Peptide matches not assigned to protein hits			

24	2831.22	4087	0.55531	Thrombin-like snake venom serine protease	GGAKCNINEHRSIVLL YSSRLFGHT	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	71%	3
25	2872.95	9902	1.34541	Thrombin-like snake venom serine protease	KCNINEHRSIVLLYSSR LFGHTLI	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	68%	2
26	2943.41	9207	1.25098	Thrombin-like snake venom serine protease	VIGGAKCNINEHRSIVL LYSSRLFGH	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	74%	3
27	2999.99	5390	0.73235	Thrombin-like snake venom serine protease	IGGAKCNINEHRSIVLL YSSRLFGHTL	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	77%	3
28	3042.76	54763	7.44081		LIKYNACVNATDKWAFTP LHEAAQKGR		Peptide matches not assigned to protein hits			
29	3113.16	34388	4.67240		PAKSRTLCAGVPRRRIGS CLGDSGGPLIC		Peptide matches not assigned to protein hits			
30	3141.14	9932	1.34949	Thrombin-like snake venom serine protease	NINEHRSIVLLYSSRLF GHTLINKEW	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	74%	3

31	3155.76	31380	4.26369	Thrombin-like snake venom serine protease	VIGGAKCNINEHRSIVL LYSSRLFGHTL	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	80%	3
32	3226.12	19679	2.67384		CEEGFTGPTCNETEKGP CHPNPCKNQGEC		Peptide matches not assigned to protein hits			
33	3380.66	12845	1.74528	Thrombin-like snake venom serine protease	VIGGA KCNINE HRSIVL LYSSR LFGHT LIN	P18692	Thrombin-like enzyme cerastobin (Fragment)	Thrombin-like snake venom serine protease	85%	4
34	3608.17	5172	0.70273	Disintigrin family	CTTGPCCRQCKLKPA GTTCWRTSVSSHYCT GR	Q1JRG	RTS-containing short disintegrin ML-G3 (Fragment)	Disintigrin family	74%	2
35	3841.57	27712	3.76531	phospholipase A2 family	RDRCEKMVCECDQKA ASCFQKHLFSYNPQF		Phospholipase A2 1 (Fragment)	phospholipase A2 family	58%	1
36	3854.67	6095	0.82814		LHVCGGSLISSQWVVTAA HCFDGPLVTSKYRVHLG		Peptide matches not assigned to protein hits			
37	3857.17	139949	19.0153		TTAAKICSRVLCRKNGRC VRKHSDSNAFLHLFPE		Peptide matches not assigned to protein hits			

38	3911.67	18263	2.48144		MINKKTGTFGLLSYVYYGCYCGLGGKGKPQDATDRC	Peptide matches not assigned to protein hits			
39	3927.28	101285	13.7619		GIRNYHLHTISHCNCDNRFRDCLRNLNDTISN	Peptide matches not assigned to protein hits			
		735981							

Table S24. List of the shared protein by mass among Viperidae venom species.										
m/z	Protein Family/ Subtypes	peptide	Accession code	coverag	Score	Relative abundance %				
						Ml	Mx	Vaa	Va	Vbb
(a) Matched peptides found in all venom										
568.141	snake venom metalloprotease (SVMP)/Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	RETY	P0DJ44	11%	7	1.50664	0.96076	4.58737	0.1611	0.00496

586.319	Bradykinin-potentiating peptides (BPPs)/Bradykinin-potentiating peptide	QRWP	P0DKZ8	57%	16	4.08359	0.0105	0.01	0.01	0.41474
644.066	Bradykinin-potentiating peptides (BPPs)/Bradykinin-potentiating peptide	EEGGRP	P0C7S1	60%	14	7.91481	1.46915	2.76895	0.67473	2.34561
855.177	Peptide matches not assigned to protein hits	SCSTCNIK				2.99230				
	Peptide matches not assigned to protein hits	LEENYTS					0.55804			
	Peptide matches not assigned to protein hits	GLAYDEGM						0.8643		1.72915
1060.27	Snake venom procoagulant proteins (Procoagulant)/Factor X-activator 1 heavy chain (Fragment)	AFNGNYFVE	P0C8I7	60%	12	1.93415	0.2276	0.38724		
	Peptide matches not assigned to protein hits	SSFFLPTFD								0.21628

1066.25	Disintigrin (DIS)/RTS-containing short disintegrin ML-G3 (Fragment)	VSSHYCTGR	Q1JRG7	20%	6		0.01		1.01179	
	Peptide matches not assigned to protein hits	PLHNAASYGH				0.70634		0.40241		
	Peptide matches not assigned to protein hits	CAQMAGGGWW								0.70244
(b) Matched peptides of venom found in tow or three of the venom										
1144.82	snake venom serine protease (SVSP)/Serine protease inhibitor 7 (Fragment)	FFNVTTQKC	A0A6G5ZW02	14%	4			21.4003	0.1265	5.16932
526.128	Bradykinin-potentiating peptides (BPPs)/Bradykinin-potentiating peptide	EAIPP	P0C7K3	45%	13			0.38165		
560.154	Uncharacterized protein	LTITL	V8N3R4	45%	13	2.61494				
570.937	Disintigrin (DIS), /Disintegrin VA6	NDYC	P0C6A5	6%	4		33.813			

	Three-Finger Toxins protein (3Ftoxin)/Cytotoxin 1f (Fragment)	YKTC	P85429	26%	11					
723.583	snake venom metalloprotease (SVMP)/Zinc metalloproteinase-disintegrin-like VaH1 (Fragments)	YQKALT	P0DJ44	16%	7		20.6382			
	snake venom serine protease (SVSP)/Vipera russelli proteinase RVV-V homolog 4 (Fragment)	NINEHP	P86532	37%	8			2.22083		
834.423	Snake venom procoagulant proteins (Procoagulant)/Coagulation factor X-activating enzyme heavy chain (Fragment)	TFIELVI	P86536	36%	10		1.90926			
	Peptide matches not assigned to protein hits	DPAKPYGS								
860.767	Snake venom procoagulant proteins (Procoagulant)/Factor X-activator 1 heavy chain (Fragment)	FNGNYFV	P0C8I7	46%	5	7.23705			2.43433	
	Peptide matches not assigned to protein hits	ARPLHPVA								

1072.38	phospholipase A2 family (PLA2)/Phospholipase A2 1 (Fragment)	RNLPVGSCRA	A0A6G5ZUF5	19%	2			1.93659		
	Peptide matches not assigned to protein hits	PLLLFSLVGL							2.14296	
	snake venom serine protease (SVSP)/Vipera russelli proteinase RVV-V homolog 2 (Fragment)	ECNINEHR	P86531	53%	8					3.67024
1145.61	Disintigrin (DIS)/Disintegrin (Fragment)	YPKKVTVLPT	A0A6G5ZVR7	12%	4	0.01				
	snake venom metalloprotease (SVMP)/Poly-His-poly-Gly peptide 1	HHHHHGVGGGGG	P0C7K5	50%	10		0.00802			
1176.47	Snake venom procoagulant proteins (Procoagulant)/Coagulation factor X-activating enzyme heavy chain (Fragment)	NKTFIELVIV	P86536	52%	7			0.8116		
	Peptide matches not assigned to protein hits	PGQAANRTFKS								0.94506

Appendix III: Article

‘’Composition Characterization of Various Viperidae Snake Venoms Using MS-based Proteomics, *N*-glycoproteomics and N-glycomics’’