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Shedding a light on composition, toxicity and potential therapeutic strategies of internationally relevant ophidotoxins

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Abstract

Among the four-thousand species of snakes currently known to taxonomists, around a fifth of them developed a peculiar hunting tactic based on injecting a toxic cocktail of compounds, generally referred to as “venom”, inside of their prey. This deadly mixture, produced in peculiar glands known as venom or Duvernoy's glands depending on the family, is introduced inside the prey through fangs, specifically designed teeth allowing the venom to reach deep into the victim.

Although the principal idea behind this strategy is common to all venomous snakes, differences begin to emerge once things such as anatomy, venom composition and biological effect of specific ophidotoxins in different species are taken into account.

In this report, such differences are taken into account and discussed, along with providing some relevant information on the current state of venomous snakes' taxonomy, anatomy and biology. A small focus will be dedicated, in particular, to species of national and international relevance, namely the Meadow Viper (V*ipera ursinii*) and the American Rattlesnake (*Crotalus adamanteus* and other *Crotalus spp.*), which will be thoroughly discussed focusing also on an often-neglected aspect, namely venomous snakes' conservation.

Knowing the threat that these venoms pose to both animal and human health is extremely important, and equally as important is knowing what to do and not to do in cases of envenomation. For this reason, the final chapter will be dedicated to discussing myths and proper practices of first aid. This part may be of particular interest for keepers and other professional figures engaging in direct and indirect contact with these animals.

By touching on all these different topics, I aim to sensitize and educate the reader into understanding that, although these animals can be and will be dangerous if provoked, they are as worthy of comprehension and respect as any other living species.

1. Introduction

1.1 Venomous snakes Taxonomy: a complex matter

According to the last report from the Reptile Database (Uetz P et al., 2021), taxonomists and biologists have currently discovered and classified 4073 species of snakes, part of the "Serpentes" or "Ophidia" suborder. Of these, the majority belongs to the superfamily Colubroidea, sometimes referred to as the "advanced snakes" superfamily.

In any case, a general agreement regarding the most well-known families and subfamilies of venomous snakes can be found. These include, but are not limited to the Elapidae family, including popular species such as the King Cobra (*Ophiophagus hannah*) and the Common Coral Snake (*Micrurus fulvius*); the Viperidae family, under which are classified the extremely well known subfamilies of Viperinae and Crotalinae, including species such as the Meadow Viper (*Vipera ursinii*) and the Eastern Diamondback Rattlesnake (*Crotalus adamanteus*) respectively; and the Atractaspididae subfamily, a smaller and rather obscure group of exclusively venomous snakes found in the subsaharan parts of Africa and in some regions of the Middle East (R. Alexander Pyron et al., 2011).

All of these, due to morphological similarities in their venom apparatus and dentition, are collectively referred to as "Front-fanged colubrids" (FFC). (Scott A. Weinstein et al., 2013)

However, what most experts agree upon is the fact that other, less significant and more uncommon species of venomous snakes, now collectively grouped as Non-front-fanged colubrids (NFFC), are found in other families, namely the "Colubridae" family (Underwood G., 1979). The validity and status of this "artificial family" has, however, been the center of a long and heated discussion ever since its creation in 1758 (Scott A. Weinstein et al., 2013). For this reason, herpetological taxonomists are currently revising, discussing and improving on this front, in an ever-changing environment.

In the end, although no definitive and reliable number regarding how many of these species are actually venomous, some sources put the number at around 30-40% (Randy Powell, 2005), while some minor and generally unreferenced sources put the number somewhere in the range between 600-700. This unreliability, according to some notable herpetologists, such as Wolfgang Wüster (2021) and Garth Underwood (1979), can be explained via the general disinterest in the peculiarity of this taxonomical effort, the consequent lack of data and the previously presented disagreement between those involved in it.

1.2 Front-fanged Colubrids and Non-front-fanged Colubrids: anatomical differences

Taxonomy is not the only conflicting science regarding venomous snakes. Indeed, since the discovery, classification and inspection of the so called “Non-front-fanged Colubrids”, the group of venomous snakes included in the Culubridae family, the fields of anatomy and biology have also been re-evaluated and re-considered by scientists worldwide.

On a strictly biological level, both Front-fanged Colubrids and Non-front-fanged Colubrids share the evolutionary capability of producing venom, a mixture of multiple different toxic compounds which is actively injected inside of the victim (Jared C. et al., 2021). A stricter definition of venom, and different types of venom compositions will be discussed in later chapters of the report.

However, differences begin to emerge between these two groups once the anatomy and basic physiology of the respective Venom Delivery Systems (VDS) are considered.

1.2.1 Duvernoy's glands and Venom glands

As early as the 18th century, the main glands connected to the oral cavity of colubrids, namely the labial, temporomandibular, rictal, sublingual, premaxillary, accessory, supralabial (Leonardo de Oliveira et al., 2022) and “parotid”, had been identified. However, following histological studies performed by Aaron M. Taub (1967) on some Non-front-fanged Colubrids and other members of the Colubridae family, “no justification in retaining the term "parotid" for the ophidian gland complex to which it had formerly been applied” was detected. Instead, he proposed to rename such gland as “Duvernoy's gland”, homaging the first man to recognize the structure as distinct.

Subsequent histological studies (Taub, 1967), carried on a wider pool of NFFC species that same year on the now-called Duvernoy’s glands, provided essential insight on the morphology of the organ. “Located in the post-ocular region, behind and juxtaposed to the supralabial gland” (Leonardo de Oliveira et al., 2016), the Duvernoy’s glands appear to be made of a series of tubules, able to collect the secretions produced by cuboidal serous cells, which comprise most of the organ’s cytology. These tubules converge into a primary duct, whose lumen is lined by mucous epithelium, eventually leading to a "terminal vestibule", an epithelial fold surrounding the rear fangs, along which the venomous secretions will flow. Externally, the gland is enveloped in a capsule of connective tissue (Taub, 1967 ; Leonardo de Oliveira et al., 2016). As noted by both, interspecific differences in morphology and histology are present, while intraspecific consistency is rather high.

Although described as homologous (Jackson Timothy, 2017), some minor, yet extremely significant differences are observed once the anatomical features of the Duvernoy's glands are compared to the

Venom Delivery System and glands of Front-fanged Colubrids. Generally speaking, the VDS of most FFC is composed of four universal structures (Kochva E, 1987), namely the main gland, where the venom synthesis is observed, the primary duct, which is involved both in the storage and in the flowing of the secretions, the accessory gland, an organ unique to FFC, connected to the primary duct through an isthmus, and finally the secondary duct, from where the venom will flow out (Mackessy et al., 2006). Just like in NFFC, the most represented cellular type in the venom glands of FFC is a secretory epithelium, but at least six other cellular types have been detected inside of the organ (Mackessy et al., 1991). The type and role of these cells will be discussed in relation to venom storage and self-toxicity prevention in later chapters of this report. Moreover, as per the anatomical features of the Venom Delivery System of NFFC, significant changes in morphology and histology are observed at the interspecific level (Mackessy et al., 2006).

The venom glands, unlike Duvernoy's glands, are characterized by the presence of bigger ducts and a considerably larger lumen (sometimes referred to as the "main grand lumen") (Mackessy, 2022), allowing species possessing such organ to store greater amounts of venom. In bigger snakes, that volume can be as big as 1-2ml (Mackessy, 2022). As in NFFC, the VDS of FFC is enveloped in a capsule of connective tissue, but unlike them, the capsule is also the site of attachment of some muscle fibers, namely the "Compressor Glandulae" muscle (Mackessy et al., 2006). The presence of this muscle allows FFC to drastically increase the pressure at which the venom is injected (Young, B.A. et al., 2001), improving the absorption and distribution times inside of the envenomated prey. As mentioned before, in NFFC, "no direct insertion of adductor muscles or other specialized muscles on the gland" (Mackessy, 2022) can be found, with venom being "presumably expelled from the gland by the compression of the gland against the skin as the jaw adductor muscle contracts" (Mackessy, 2022).

1.2.2 Front-fanged Colubrids and Non-front-fanged Colubrids: dentition

Glands aside, the most prominent difference between the two groups of venomous colubrids, on the basis of which they are divided, is found in the placement and morphology of fangs, a specialized subset of teeth allowing venom to flow from the site of production/storage into of the oral cavity.

Based on both placement and morphology, four different types of snake dentitions are defined (Herrera Y, Fuentes-Retamal S et al., 2022). Non-caniculated, ridged and beveled teeth (Leonardo de Oliveira et al., 2016), located on the posterior part of the maxillary bone are referred to as "opisthoglyphous". These are typically encountered in NFFC. Caniculated tubular fangs, located in the anterior part of the maxilla, are referred to as "proteroglyphous" if fixed in position (as in the case of most Elapids), while they are referred to as "solenoglyphous" if hinged, and able to fold

back inside of the oral cavity (as in the case of Viperids) (S.A.M. Kularatne et al., 2014). The last type of dentition is defined by its lack of specialization, and is thus referred to as "aglyphous".

Although the marked differences in morphology and position seem to indicate different developmental origins, some recent studies (Vonk F. Et al., 2008) carried out on ophidian embryos revealed that the fangs of FFC share a common origin with the ones of NFFC, with both deriving from a specialized odontogenic dental lamina, indicating that these different classes may be more closely related than previously expected

2. Snake venom

2.1 Definitions

Snake venoms, sometimes referred to as "ophidotoxins", are commonly defined along the lines of "biochemical arsenals containing mixtures of bioactive compounds that consist of salts, small molecules, and proteins and peptides" (Schendel Vanessa et al., 2019). However, this seemingly whole-rounded definition is not completely accepted. In particular, Kenneth V. Kardong (1996), notes how the term "venom" is generally used, as in the previous excerpt, as a synonym for "toxic", two words which hold a biologically distinct meaning. He continues to state his point by mentioning that venom is a biological term, used to define how "it is actually used by the snake in its natural environment", while a toxin is defined only by its pharmacological properties of inducing a negative reaction in the body of the prey. The researcher concludes by ironically mentioning how even components isolated from human saliva can be considered as "toxic", while reminding everyone of the absurdity of defining humans as "venomous".

Other publications, such as the one from Kevin Arbuckle (2017), aim "to generate a definition that is sensible, meaningful, and consistent" by encompassing both the toxicological and the biological aspects of the matter. To achieve this, Kevin Arbuckle expands on previous research by Nelsen et al. (2014) and Fry et al. (2009) to list six main attributes used to define venoms. These are:

1. The venom is produced or stored in a specialized structure
2. There is a specialized delivery system used to transfer the venom to another organism
3. The venom is transferred via an injury
4. The venom is actively (as opposed to passively) transferred to another organism
5. The venom functions in predation, defense and some instances of intraspecific competition
6. The venom contains molecules (“toxins”) which interfere with physiological or biochemical processes in another organism

Condensing all of these "requirements" into a definition, Kevin Arbuckle finally defines a venom as "a biological substance produced by an organism that contains molecules (“toxins”) which interfere with physiological or biochemical processes in another organism, which has evolved in the venomous organism to provide a benefit to itself once introduced to the other organism. The venom is produced and/or stored in a specialized structure and actively transferred to another organism through an injury by means of a specialized delivery system".

2.2 Biological and Ecological roles of venom

As most of the current research on snake toxins has been focusing on the clinical aspects of envenomation, only few niche publications on the biological and ecological role of venom have been released. This seeming lack of interest can easily be explained once some of the devastating effects that toxic compounds can have on the human body are taken into account. In this context, "ecological relations of whole venoms are often considered little more than a sidenote" (Arbuckle K., 2017). However, they are crucial to understand the role and evolution of venoms in a wider ecological landscape.

The ability to produce toxins is a trait which has evolved numerous times in nature (Arbuckle K., 2017). This fact alone can give us an idea of just how useful possessing such a weapon really is. However, since venom systems are so diffused, not only in clades such as snakes, some common biological and ecological trends can be observed and discussed. Such trends include venom being used as a predation method and as a form of chemical defense, along with less discussed yet crucial interactions such as venom resistance and venom optimization.

2.2.1 Venom used as a Predation tool

According to Endler (1986), predation can be described and divided by dissecting it into five consecutive steps, namely detection, identification, approach, subjugation, and consumption. Out of these five steps, venom clearly plays its most important role during subjugation, allowing predators to rely on other means other than sheer physicality. However, subjugation in itself is less of an "act" and more of an "art", as predators need to take into account a lot of different things to perform it as efficiently as possible.

The first and most important thing for a predator using venom to keep in mind is the fact that producing such a weapon is, metabolically speaking, an extremely expensive task. A study conducted by McCue and Mason (2006) measured the increase in metabolic rate in the first few days of venom synthesis after extraction in three different snake species, revealing an increase of around 11%. Considering that a complete regeneration of the venom takes around 22-41 days (Oron and Bdolah, 1973), it becomes clear just how energetically expensive such a process can become. This result throughly explains both why the ratio between venom produced and body mass is so low (around 0,5%) (Mirtschin et al., 2006) and why dietary shifts or shifts in hunting tactics which remove the need for venom are closely connected to secondary loss of venom production (Li et al., 2005). Kevin Arbuckle (2017) also notes that, as venoms are so expensive to synthesize, it would be

evolutionarily disadvantageous for predators employing such a tactic to use more than what is needed to incapacitate the prey.

This concept, which sees the predator as conscious about the amount of venom to deploy, is ecologically referred to as "The venom optimization hypothesis". This is still not a completely well understood matter, but some reviews, such as the one by David Morgenstern & Glenn F. King (2013), are currently aiming to shed a light on this extremely curious chapter in ecology, one which could also have some medical significance. This review, encompassing three apparently distinct taxa (snakes, spiders and scorpions), led to some surprising conclusions. Focusing on snakes, but keeping in mind that results are common between the three, scientists found:

A. Snakes may not necessarily kill their preys by using their venom, which is instead used to paralyze/incapacitate. Constriction may be used after, reducing the needed amount of venom

B. Venom may not be used every time the Venom Delivery System is deployed, as in the case of "dry bites". However, dry bites are only ever used defensively, and never offensively

C. The amount of venom used is tailored to the size of the prey. This correlation is, however, not linear, but "thresholds" have been identified in multiple species

D. Different preys induce different hunting behaviors and different degrees of venom release in the predator. The degree of resistance mounted also has an effect on the amount of released venom

E. The available reservoir of venom directly affects the choice of prey, the level of approachability and the number of evasive behaviors once disturbed

All of these points seem to converge toward a "behaviorally controlled venom expulsion that is both prey and predator dependent" (David Morgenstern & Glenn F. King, 2013), allowing venomous animals to use such a weapon as efficiently as possible, also taking into account its metabolic cost. However, once the venom composition (topic of a later chapter) is taken into account, some doubts begin to arise. As David Morgenstern & Glenn F. King (2013) put it, venoms are "unnecessarily rich, almost lavish, in their biochemical complexity", while also being characterized by an "extensive functional redundancy" (also described by Bryan G. Fry et al., 2009).
And although some research suggests that animals are able to modulate their venom composition (Cascardi J. et al., 1999; Yahel-Niv A. et al., 1979), we currently lack a definitive understanding of such a mechanism. As for now, biochemical modulation of venom composition remains a debated topic in the scope of the venom optimization hypothesis.

2.2.2 Venom used as a Defensive tool

Aside from predation, most venomous animals, including snakes, are able and known to use their secretions also as a form of chemical defense. However, taking into account once again the metabolic limitations imposed by the synthesis of the toxic compounds, such animals may instead choose to defend themselves without deploying their chemical arsenal. In such cases, they may deliver what are commonly referred to as "dry bites", "venomous snakebites with no or negligible venom injection, characterized by a lack of clinical manifestations of envenoming" (Russel F.E., 1980).

A recent review article (Manuela B Pucca et al., 2020), set out to investigate some of the possible causes leading to dry bites, along with providing some data regarding the incidence of such phenomenon. Among the possible factors responsible for dry bites the team identified a number of "Snake-related" factors, such as trauma and infection to the glands, lack of available venom for injection and finally, but most importantly, venom metering, of which we have already discussed in the previous sub-chapter. Venom metering (or optimization), is especially significant once we consider that the study was able to detect an incidence of dry bites in the range of around 20-50%, a percentage which is far too high to be the result of "faulty venom deliveries" (Manuela B Pucca et al., 2020) alone.

Such variability in dry bites incidence can be easily explained once factors such as the difficulty and lack of uniformity in detecting a dry bite, intra- and inter- specific behavioral differences, site of injection, …, are taken into account. However, as mentioned earlier, there is strong evidence suggesting that this is a recurring behavior among venomous snakes.

In any case, venomous snakes will only bite as a last resort, instead retreating and avoiding confrontation if not absolutely necessary. Snakes thus defend themselves only when an unavoidable threat (such as capture, handling and, unfortunately, accidental stepping) is perceived, and will always try to ward off potential dangers by signaling (such as in the case of the Rattlesnake) or hiding.

2.3 The physiology of ophidotoxins: synthesis and storage

From a purely technical point of view, venom glands are capable of what can be considered some of the most dangerous, precise and difficult tasks in nature. Indeed, these small organs are not only able to synthesize extremely toxic compounds, compounds which could theoretically be considered toxic to the "host" itself, but are also able to maintain such unstable compounds readily available

and active for extremely prolonged periods of time. In the words of Stephen P. Mackessy and Louise M. Baxter (2006), " A paradoxical task of the venom gland of snakes is the synthesis and storage of an instantly available suite of toxins to immobilize prey and the protection of the snake against its own venom components".

2.3.1 Synthesis

As mentioned in the previous chapter, venoms' toxins are among the most energetically demanding molecules to synthesize, with increases in metabolic activity reaching around 11% (McCue and Mason, 2006). Such a sharp increase in metabolism can be observed all throughout the resynthesis process, which can take between 16 (Stephen P. Mackessy, Louise M. Baxter, 2006) and 41 days (Oron and Bdolah, 1973). A recent study conducted by Perry B.W et al. (2020) finally set out to shed a light on and describe the physiological demands experienced by glandular cells during synthesis of toxic compounds.

Results from this study show that "Following the depletion of stored venom […], the snake venom gland exhibits rapid and high-magnitude upregulation of venom gene transcription, venom protein production and processing, and secretion of venom components into the gland lumen". Indeed, scientists were able to detect that, one day after venom extraction, cells from the venom glands presented a level of gene expression roughly 5000-15000 higher compared to cells of "non-venom tissues", with the number of gene involved easily reaching into the thousands (around 7000). Associated with such a level of gene expression, proliferation of the rough endoplasmic reticulum can also be detected by microscopic examination, indicating that the degree of protein synthesis inside of the cell is increasing (Mackessy, 1991).

Another interesting find from Perry B.W et al. study revolve around the increased activation of stress responses by cells involved in the synthesis of toxic compounds. Such a physiologically demanding process increases the probability of cellular damage, hence why a number of "failsafe" stress responses are expressed by involved cells. "These include the endoplasmic reticulum stress response and unfolded protein response pathways, which are associated with mitigating cellular stress caused by misfolded proteins and high demands for protein processing" (Perry B.W et al., 2020). At the same time, "multiple pathways and URMs (upstream regulatory molecules) related to cellular growth and proliferation, cell cycle regulation and tumor suppression are also inferred to be activated during venom regulation" (Perry B.W et al., 2020). Moreover, further analysis of these pathways led to the conclusion that stress responses are proportionally higher in cells from venom glands compared to "non-venom tissues" even when no synthesis is occurring, indicating a higher baseline activity (Perry B.W et al., 2020).

Histologically speaking, synthesis of toxic compound is associated with what can be described as a "cellular cycle" involving the serous secretory epithelial cells and the "mitochondria-rich cells" of the venom glands (Mackessy et al., 2006). During such cycle, cells change their shape from cuboidal to columnar as synthesis of compounds begins, before turning back to cuboidal once completed.

Other scientists, instead of analyzing the morphological and biochemical changes associated with secretion, have shifted their aim toward establishing how venom synthesis is initiated. A study carried out by Yamanouye N. et al. (1997) was able to prove a connection between sympathetic stimulation and synthesis of toxic compounds, indicating that such stimulus is necessary in order for secretions to be produced. In particular, they were able to block venom production and the aforementioned "cellular cycle" via the administration of reserpine, an alkaloid able to reduce the activity of the nervous system. On the same reserpine-treated patients, the cellular cycle was later reestablished using adrenergic agonists such as isoprenaline or phenylephrine, although protein secretion was only achieved by phenylephrine triggering α-adrenoreceptors.

2.3.2 Storage

However, venom production presents some challenges even after the toxic compounds have synthesized. Indeed, after secretion, one of the biggest challenges left for this organ to overcome becomes that of storing the produced toxins. As mentioned before, two main things have to be taken into account for storing:

1. Preventing a phenomena of self-toxicity
2. Making sure that toxins remain effective even after a long time

Snakes, as all other venomous animals, have developed peculiar countermeasures to avoid complications, while still maintaining the usefulness of their venom.

In the same study by Stephen P. Mackessy and Louise M. Baxter (2006) mentioned earlier in the chapter, the two scientists also compared the histological architecture of the venom delivery system of a snake with that its stomach, detecting some incredible similarities. In particular, they noticed how both in the stomach and in the accessory gland, a clear and common cellular organization is present, with secretory cells occupying the most cranial (for the stomach)/proximal (for the accessory gland) aspects of the respective organs, replaced in the most caudal/distal aspects by mucous-secreting cells.

This spatial distribution led the two scientists to formulate an hypothesis according to which "The sequentially homologous arrangement of the cell types in the rattlesnake accessory gland suggests that venom components from the main gland could become activated by proximally located cell secretions of the accessory gland, requiring mucus secretion to protect the distal parts of the venom gland apparatus from the hydrolytic effects of venom". However, as mentioned by Mackessy and Baxter themselves soon after, no proof has yet been discovered with detection methods such as electrophoresis (to detect the addition of peptides or proteins) and reversed phase high-pressure liquid chromatography (RP-HPLC).

Aside from this theory, which mostly aims at understanding the role of the accessory gland, the main accepted explanation as for why snakes are immune to their venom relies on the biological activity of a type of cell, the "mitochondria-rich cell", briefly mentioned earlier in the context of the "cellular cycle" taking place in the venom glands during venom synthesis. These cells, which constitute around 2% of the glands' epithelial cells population (Mackessy, 1991), are involved in the crucial process of venom acidification (Mackessy et al., 2006 ; Perry et al., 2020), a process made possible thanks to the presence, on the membrane of mitochondria-rich cells, of at least six different types of Vacuolar-ATPases (Mackessy, 1991 ; Perry et al., 2020), four of which have been found to be significantly over-expressed during venom synthesis (Perry et al., 2020). Acidification allows to lower the pH of the venom to around 5.4 (Mackessy et al., 2006), a level at which the enzymatic activity of crucial venom components such as metalloproteases, phospholipases A2, …, (which will be discussed more in depth later on) is significantly reduced (Mackessy et al., 2006 ; 2022). Enzymatic activity is restored once these compounds are injected into the preys, shifting the pH into a more alkaline range. Thus, "acidification of stored venom by the mitochondria- rich cells is a primary mechanism that allows storage of potentially dangerous and unstable venom components in an inactive state that is readily and instantaneously reversed upon injection, permitting long-term storage and on-demand deployment of a potent biological weapon" (Mackessy et al., 2006). Acidification is, although extremely effective, insufficient to completely inactivate the venom (Mackessy et al., 2006). For this reason other mechanisms, such as the synthesis of tripeptide inhibitors, the secretion of citrate for metal-ion chelation and of zymogen precursors (Mackessy et al., 2006 ; Grams et al., 1993 ; Odell et al., 1998) is necessary to avoid autolysis.

As mentioned at the beginning of the sub-chapter, self-toxicity, which may also come from intraspecific agonistic interactions, is a real danger and something to which venomous animals had to evolve countermeasures for. These include modifications to binding sites targeted by toxins

(Takacs et al. 2001) as well as circulating antibodies able to attach and neutralize eventual "stray" toxins (Straight et al. 1976).

2.4 Main venom components and their pathophysiological effects

As mentioned in previous chapters, venoms are extremely diverse and complex mixtures of toxic compounds, constituted mostly by proteins and peptides (90-95%), but also by salts and other small molecules (Sanhajariya Suchaya et al., 2018 ; Oliveira A L et al., 2022). The sheer amount of compounds present inside of venom, along with the extreme level of variability and differentiation between different taxonomical families, has made studying the composition of venom an extremely demanding and time consuming task. However, the ever-so relevant clinical interest posed by envenomation, along with the development of faster and more precise -omics techniques, has allowed scientists to quickly expand our knowledge regarding these fearful compounds and their effects on the organism (Junqueira-de-Azevedo et al., 2016).

Deciphering how venoms interact with the organism at the macro- and microscopic levels requires precise knowledge on the structure and targets of the most important toxic compounds. This knowledge becomes even more important once the number differential targets, along with the complex web of interactions between molecules is taken into account. Indeed, even simpler molecules like phospholipases may target numerous structures, resulting in diverse toxic outcomes (Sampat G. H. et al., 2023 ; R Manjunatha Kini, 2003), as well as interact synergistically with other compounds, such as Three-Finger Toxins (Bittenbinder M.A. et al., 2024). For these reasons, some information regarding structure and site of interaction will be provided before delving into the main pathophysiological effects.

Venoms are mostly composed by proteins and polypeptides, which account for around 90-95% of whole venom (Sanhajariya Suchaya et al., 2018) and for most of the toxic effects. Proteins and polypeptides extracted from snake venom are, depending on their size and ability to catalyze reactions, formally divided into enzymes, characterized by higher molecular weights and possessing catalytic properties, and toxins (or non-enzymes), characterized by lower molecular weights (>30kDa) and no catalytic properties (Chippaux J. P., 2006 ; Mohamed Abd et al., 2019).

Being enzymes, members of this first class of compounds are able to "cause a biological transformation without themselves being modified, which allows them to carry on reacting as long

as they are present in the organism" (Chippaux J. P., 2006). From a clinical point of view, this implies that toxic effects provoked by such molecules are going to increase in magnitude with time, in a form of time-dependent toxicity (Chippaux J. P., 2006). Toxins, on the other hand, mostly depend "on the proportion of the introduced quantity of toxin to that of the corresponding receptors", a form of toxicity referred to as dose-dependent (Chippaux J. P., 2006).

Although hundreds if not thousands of such compounds exist, characterization of both enzymes and toxins extracted from a great number of species (179) of different families led Tasoulis T. and his team (2023) to discover that they could all be grouped in 42 different families, 18 of which could only be detected in less than 5% of all considered species. A further classification was made by the team based on factors such as the percentage of species possessing such protein family and the percentage to which such family contributes inside of a species' whole venom. Based on this second classification, four categories were established, namely the "dominant" protein families (4), the "secondary" protein families (6), the "minor" protein families (12) and the "rare" protein families (18). Following this classification, the main families are:

2.4.1 Phospholipases A_2 (PLA_2)

PLA_2s are a class of lipolytic enzymes able to break the ester bonds at the sn-2 position of glycerophospholipids (Kang T. S. et al., 2011 ; Sampat G. H. et al., 2023). Based on their morphology, amino acid sequence and other factors such as expression, fourteen different classes of this enzyme are recognized (Sampat G. H. et al., 2023). Out of these, four of them, namely those from groups I, II, V and X are recognized as being part of the secretory PLA_2s, which can also be identified inside of snake venoms. A further distinction exists based on "disulfide bonding pattern, three-dimensional structure, and amino acid sequence" (Sampat G. H. et al., 2023), dividing the snake-PLA_2s into Group I and Group II, respectively found in the venom of Elapids and Viperids. (R Manjunatha Kini, 2003 ; Kang T. S. et al., 2011 ; Sampat G. H. et al., 2023).

Due to the ubiquitous distribution of their catalytic targets, PLA_2s possess an almost unlimited amount of binding sites, thus being able to exhibit a wide variety of pharmacological effects (R Manjunatha Kini, 2003), including cytotoxic and neurotoxic effects (Chippaux J. P., 2006 ; Gutiérrez J. et al., 2017 ; Tasoulis T. et al., 2023). Most of the time, these enzymes are able to carry out their biological action by themselves, but as mentioned before, some of them "express their pharmacological effects at full potency only when they form a complex with other protein factor(s)" (R Manjunatha Kini, 2003 ; Bittenbinder M.A. et al., 2024). In any case, all PLA_2s apart from a small group, referred to as $iPLA_2$, or Ca_{2+}-independent PLA_2, require calcium ions to be present and

to bind to an allosteric site in order to work (R Manjunatha Kini, 2003 ; Oliveira A L et al., 2022 ; Sampat G. H. et al., 2023).

PLA_2 homologues, sometimes defined as "PLA_2-like proteins", have also been described in the literature (Oliveira A L et al., 2022). These molecules have seemingly lost their calcium cofactor, in turn losing enzymatic ability (Oliveira A L et al., 2022). Despite the lack of enzymatic activities, myotoxicity has been demonstrated (Gutiérrez J. et al., 2017 ; Oliveira A L et al., 2022).

Discussed by Sampat G. H. and its team (2023), the pathophysiological effects of PLA_2s are extremely varied, inducing toxic effects on multiple target tissues and through multiple pathways. According to their extremely through review, as well as the one from M. A. Bittenbinder's team (2024) PLA_2s:

I. Affect the fluidity of biological membranes, reducing structural integrity, altering permeability and inducing ionic imbalances. A common target of PLA_2s is the myocytes' sarcolemma, explaining the myotoxic nature of these compounds. Furthermore, the uncontrolled influx of ions, and calcium in particular, leads to hyper contraction, further increasing mechanical stress to the tissues. The increased amounts of released myoglobin from damaged myocytes have also been associated with acute renal toxicity

II. Are able to induce the release of acetylcholine from presynaptic neurons, as well as inhibiting the acetylcholine uptake transporters, leading to the suppression of neuromuscular junction activity through vesicle depletion and receptor desensitization. Thus, paralysis and neurotoxicity is achieved

III. Interact with blood clotting factors and procoagulant phospholipids, leading to a severe hemostatic dyshomeostasis

IV. Lead to the release of free fatty acids (namely arachidonic acid), lysophospholipids and other pro-inflammatory mediators, promoting an increased inflammatory response, further mobilizing and spreading venom compounds

V. Release Reactive Oxygen Species (ROS) as a result of their catalytic process, inducing oxidative stress and triggering biological pathways leading to cellular death

VI. Are able to damage the DNA and to induce apoptosis

2.4.2 Snake venom metalloprotease (SVMP)

Snake venom metalloproteases (or "metalloproteinases") are a group of generally smaller proteins (20-100 kDa) (Kang T. S. et al., 2011), found in the venom of both Viperids and Elapids, although concentrations and detection frequencies are much higher in the Viperidae family (34% across all viper species) (Tasoulis T. et al., 2023). Depending on the structure, type and number of catalytic domains, SVMPs are divided in three main groups, namely P-I, P-II and P-III, from simplest to more complex (Kang T. S. et al., 2011; Oliveira A L et al., 2022 ; Tasoulis T. et al., 2023).

Enzymes from the P-III group are considered the "largest, more ancient and most complex enzymes" (Oliveira A L et al., 2022), as they retain all of the family's domains, which have been evolutionarily lost in P-Is and P-IIs (Oliveira A L et al., 2022 ; Tasoulis T. et al., 2023). Domains include the "Metalloproteinase" or "M" domain, which is the catalytic site, the "Disintegrin" or "D" domain and the "Cysteine-rich" or "C" domain (Chippaux J. P., 2006 ; Oliveira A L et al., 2022 ; Kang T. S. et al., 2011). The M domain is the catalytic site which is able to catabolize the lysis of the polypeptide chain. Although the reaction is not completely understood, scientists have determined that Zinc ions play a crucial role in the process (Lingott T. et al., 2009 ; Kang T. S. et al., 2011 ; Oliveira A L et al., 2022). Some other sources seem to agree on the fact that the M domain may also be involved in the recognition of the binding sites (Oliveira A L et al., 2022), which mostly consist of collagen fibers, such as those in the basement membrane of the endothelium, and coagulation factors (Chippaux J. P., 2006 ; Kang T. S. et al., 2011 ; Oliveira A L et al., 2022 ; Tasoulis T. et al., 2023).

On the other hand, neither the C or the D domain possess any catalytic activity, instead being involved in processes of site recognition and structural integrity (Chippaux J. P., 2006 ; Kang T. S. et al., 2011 ; Oliveira A L et al., 2022 ; Tasoulis T. et al., 2023). Moreover, the D site is, as the name may allude, able to interact and interfere with integrins, membrane molecular receptors involved in processes such as platelet aggregation (Gutiérrez J. et al., 2017).

All of the properties possessed by SVMP give venoms incredible toxic capabilities. However, unlike PLA_2s, SVMPs are generally described as being "indirectly cytotoxic", as they do not harm cells directly (Bittenbinder M.A. et al., 2024). According to reviews carried out by two teams directed by Gutiérrez J. (2017) and Bittenbinder M.A. (2024) on the toxic effects of SVMPs, these molecules are able to:

I. Affect the structural integrity of blood vessels by hydrolyzing crucial components of the basement membrane, namely collagen IV fibers and hyaluronic acid, as well as components of cell-cell junctions. Weakening of the basement membrane is soon followed by extravasation of

blood into the extracellular matrix as endothelial cells lose their mechanical stability. Along with blood, toxic compounds also spread deeper and gain access to the tissues, in a case of synergistic action between toxic compounds

II. The resulting hemorrhages have been linked with poor blood perfusions and ischemia, leading to yet another form of indirect cytotoxicity. Severe hemorrhages have also been associated with hypovolemic cardiovascular shock

III. Induce epidermal necrosis and the formation of blisters by acting at the level of the dermal–epidermal interface

IV. Induce the lysis and weakening of the glomerular basement membrane, facilitating the risk of renal thrombosis

2.4.3 Three-finger toxins (3FTx)

3FTx are a group of short-chain proteins (6-9 kDa, or 57 to 74 amino acids) (Chippaux J. P., 2006 ; Bittenbinder M.A. et al., 2024) possessing no catalytic activity. These molecules have been detected as part of the toxic composition of all Elapids, where they account for around 51-52% of the entire venom (Oliveira A L et al., 2022 ; Tasoulis T. et al., 2023), while being almost entirely absent in the venom of Viperids, having been detected in only five species (Tasoulis T. et al., 2023).
Their bizarre yet iconic name comes from their morphology, which consists of "three β-stranded loops (fingers), extending from a globular central core" (Tasoulis T. et al., 2023). 3FTx are further classified on a basis of size into short or long (Chippaux J. P., 2006).

3FTx are able, thanks to their relatively small size and to their spatial conformation, to antagonize a number of receptors, namely acetylcholine and muscarinic receptors, by competing with the ligands (Chippaux J. P., 2006 ; Oliveira A L et al., 2022 ; Tasoulis T. et al., 2023). The effect is thus highly neurotoxic. Some particular 3FTx, instead of possessing neurotoxic effects, are instead cytotoxic. Although no clear explanation as how toxicity is induced to cells, scientists believe that such toxins are either able to unspecifically bind to the membrane, while also inducing the formation of pores, or to induce cytolysis by rupturing lysosomes after internalization by target cells (Bittenbinder M.A. et al., 2024). Both of these theories, as Bittenbinder states in its review, are supported from the fact that an "hydrophobic patch" remains in cytotoxic 3FTx, not present in neurotoxic ones. Moreover, as mentioned earlier, this mechanism may also be possible thanks to the synergistic action of 3FTx and PLA_2s, as the latter may facilitate the cytoplasmic penetration of the former by acting on the plasma membrane's permeability (Bittenbinder M.A. et al., 2024).

2.4.4 Snake Venom serine protease (SVSP)

As the SVMPs mentioned earlier, SVSPs are a family of hemotoxic glycoproteins able to catalyze the lysis of polypeptidic chains (Kang T. S. et al., 2011 ; Tasoulis T. et al., 2023). Comparable in size with metalloproteinases (26-67 kDa) (Oliveira A L et al., 2022), these enzymes are classified, on the basis of their structure, into six "clans", further subdivided into families by amino acid homology and functional similarity (Tasoulis T. et al., 2023). Functional similarity plays a crucial role in characterization as, even with chain homology of around 50-70%, "SVSPs display high specificity toward distinct macromolecular substrates" (Oliveira A L et al., 2022 ; Tasoulis T. et al., 2023). These enzymes are extremely common, as are most hemotoxic compounds, to the venom of Viperids, where they have been detected in all currently sequenced species (Tasoulis T. et al., 2023). Hemotoxic effects arise from the capability of SVSPs to target specific clotting factors and other components of the blood coagulation cascade, inducing severe dyshomeostasis (Chippaux J. P., 2006 ; Kang T. S. et al., 2011 ; Gutiérrez J. et al., 2017 ; Tasoulis T. et al., 2023). Of exceptional importance is the ability of SVSPs to mimic biological activities of "native" enzymes such as thrombin, as well as their fibrinogenolytic activity, two pro-coagulant processes resulting in severe toxicity (Chippaux J. P., 2006 ; Kang T. S. et al., 2011 ; Gutiérrez J. et al., 2017 ; Oliveira A L et al., 2022 ; Tasoulis T. et al., 2023).

2.4.5 Other families

Although most venom compositions can be described using the four macro-families we have just discussed, at least other 32 minor classes of compounds can also be isolated and described (Tasoulis T. et al., 2023). Although equally as interesting, these are generally less abundant and less frequently detected during sequencing, hence why some will only be briefly mentioned, along with their major pathophysiological effects, in Table 1.

	Major effects
L-Amino Acid Oxidase	Hematic dyshomeostasis and apoptosis induction
Cysteine-rich secretory proteins	Increased vascular permeability and pro-inflammatory effects
Disintegrins	Integrin–ligand interactions inhibition, cell adhesion disruption
C-type lectin	Platelet aggregation and other hemotoxic effects, increased vascular permeability, nephrotoxicity …
Phosphodiesterase	Hypotension, venom diffusion facilitation
Acetylcholinesterase	Parasympathetic signaling interruption

Table 1:
Summary of the most significant out of the secondary, rare and minor venom protein families, along with their major pathophysiological effects.

(Oliveira A L et al., 2022 ; Tasoulis T. et al., 2023)

2.5 From toxins to drugs: the medicinal potential of snake venom

Toxic components present inside of snake venoms have the ability to negatively interact with many different targets and pathways inside of the organism. However, the extreme selectivity and efficiency of these "toxic" compounds has led scientist to investigate possible pharmaceutical and therapeutic applications. Snake venom has always been part of traditional medicine, homeopathy and folklore, especially in Asian and African countries. However, with the development of more rigorous scientific methods, isolation and identification techniques, as well with in vivo and in vitro testing, more and more compounds have been discovered and "converted" from toxins to drugs. Examples include:

A. Captopril® is an anti-hypertensive drug developed from a peptide found inside the venom of *Botbrops jararaca*. This peptide is part of a group known as the bradykinin potentiating factors (BPFs), able to antagonize the Angiotensin-Converting-Enzyme (ACE), allowing bradykinin, a powerful vasodilator, to avoid catabolism. (Chippeaux, 2006 ; Mohamed Abd et al., 2019 ; Oliveira A L et al., 2022).

B. Aggrastat® (Tirofiban) is an anticoagulant drug used to treat acute coronary syndrome. It is based on echistatin, a disintegrin found in the venom of *Echis carinatus*. This compound is able to outcompete fibrinogen at its integrin binding site on the platelets, preventing the final step in aggregation to be carried out correctly (Chippeaux, 2006 ; Mohamed Abd et al., 2019 ; Oliveira A L et al., 2022).

C. Integrilin® (Eptifibatide) is another anticoagulant drug based on a disintegrin found in the venom of *Sistrurus miliarius barbourin*. Its mechanism of action is the same as the one from Tirofiban®. (Chippeaux, 2006 ; Mohamed Abd et al., 2019 ; Oliveira A L et al., 2022).

D. Crotamine is a cell-penetrating protein extracted from the venom of *Crotalus durissus*. This toxin is able to induce cytotoxicity by rupturing lysosomes, a mechanism described in 3FTx. Its extreme selectivity for highly metabolic cells has made it a candidate as an anti-tumoral agent, with in vivo experiments showing promising results also as an anti-nociceptive and anti-inflammatory drug (Campeiro, J. D. et al., 2018 ; Moreira, L. A. et al., 2021). However, further testing is still needed before a formal approval. (Chippeaux, 2006 ; Mohamed Abd et al., 2019 ; Oliveira A L et al., 2022

3. Venomous species of national and international relevance

After shedding a light on the generalities of the anatomy, physiology and chemistry of these feared and misunderstood animals, as well as presenting some of the ways in which venoms can be used for therapeutic purposes, this chapter will revolve around the description of the ecology of two venomous snake species, relevant at the national and international level. Understanding how these animals fit into the broader ecosystem, as well as the possible interactions with humans is necessary to educate people and to teach them to respect species which are almost unanimously feared and disliked. This goal becomes ever-so important once we consider that declines in population are commonly observed in snake species. The species taken into account will be the Meadow Viper (V*ipera ursinii*) and the American Rattlesnake (*Crotalus adamanteus* and other *Crotalus spp*.). 3.1 Meadow Viper (V*ipera ursinii*)

3.1 Meadow Viper (*Vipera ursinii)*

The Meadow viper (*Vipera ursinii*, Bonaparte, 1835) is the smallest viper out of the fourteen currently known in Europe, and one of the only four venomous snake species found in the Italian territory (Franchini M. et al., 2017 ; Di Nicola M. R. et al., 2021), where it inhabits most of the Apennine massifs.

3.1.1 Diet and distribution

Commonly found in alpine pastures at around 1400-1900 m (Filippi E. et al., 2004), the *Vipera ursinii* is usually detected in bushes and other secluded spaces, from where it awaits for preys.
Diet in the *Vipera ursinii* mostly consists of invertebrates, generally from the *Orthoptera* order (crickets, grasshoppers…), which account for roughly 76-90% of the diet composition (Agrimi U. et al., 1992 ; Filippi E. et al., 2004). However, smaller mammals (such as *Chionomys nivalis*) have also been identified as suitable preys from collected fecal and gastrointestinal samples (Agrimi U. et al., 1992 ; Filippi E. et al., 2004).

Diet homology had been hypothesized, in other species of vipers, as the main factor behind habitat (and in particular altitude) partitioning (Lapini L.,1983 ; Filippi E. et al., 2004), with species having similar diets inhabiting different areas, heights or microhabitats. However, even with diets as diverse as the ones by *Vipera ursinii* (mostly insectivore) and *Vipera aspis* (mostly based on mammals), partitioning was observed and documented (Filippi E. et al., 2004), further opening the discussion as per why such a phenomenon should occur.

3.1.2 Toxicity and danger for humans

Viper encounters which end in successful envenomation are extremely rare, as proved by Laszlo Krecsak and his team (2011) in a review on the clinical picture of envenomation from *vipera ursinii*. Indeed, the team was only able to identify 64 confirmed cases of envenomation by *vipera ursinii* between 1970-2010 in both Hungary and Romania, two European countries in which the presence of this species is extremely well-known, established and studied.

Results from this review indicate that "envenomings generally display mild and negligible local symptoms only, which spontaneously resolve, without any medical treatment in 48–72 h". Symptoms include numbness, pain and swelling at the site of injection, as well as minor hemorrhages and local edema. In very rare cases vesicles and necrosis have also been described, but these can mainly be associated with unprofessional tools venom extractors and incorrect first-aid practices such as local incisions. Other symptoms displayed include nausea, breathing difficulties and paleness, once again associated with external causes such as psychological distress. Unlike other viper species, anaphylaxis was never recorded, even in susceptible individuals. In any case, no lethality or correlation between the site of envenomation and symptoms has ever been detected in humans. Treatment is, unlike what is recommended for other species, performed without relying on any anti-venom, instead focusing on symptomatic and supportive treatment only.

Such negligible symptoms can somewhat be explained the venom composition is taken into account. Indeed, in a proteomics study conducted by Lang Balija, Maja et al. (2020), the venom from *vipera ursinii* was found to be made of just seven main protein families, namely SVMPs, SVSPs, $sPLA_2$s and some other minor compounds. Furthermore, a higher toxicity toward crickets was also demonstrated in the same article, with crickets having a "Mass-normalized LD_{50}" (described by the team as "the average LD_{50} (in μg)/average body mass of experimental animal (in g)") five times smaller than that of rats, once again leading to the conclusion that this species' venom is tailored toward their trophic niche.
Venom composition aside, important to consider during human envenomation are also the size of the animal's fangs (2-3mm), which prevent deeper injections and access to major vessels, as well as the minute amount of injected venom, which has been estimated at around 1-4 mg (Laszlo Krecsak et al., 2011 ; Lang Balija, Maja et al., 2020)

3.1.3 Conservation

Snakes are among the most feared species by humans, and partially for understandable reasons. The widespread and radical hate for these animals, along with the possible risk of envenomation, have thus far shed a grim and dark light on these fundamentally misunderstood animals. For this reason,

spreading awareness and avoiding misinformation regarding these animals is of fundamental importance, especially once the conservation status of some is taken into account. Indeed, the *vipera ursinii* is currently included in the Appendix I from CITES (EEC regulation no. 3143/887, Commission of 19 October 1987), as well as being classified from the International Union for the Conservation of Nature (IUCN) as "Vulnerable", a ranking that may only worsen if the attitude toward these species isn't changed.

3.2 Eastern Diamondback Rattlesnake (*Crotalus adamanteus)*

The Eastern Diamondback Rattlesnake (*Crotalus adamanteus,* Palisot de Beauvois, 1799) is among the most popular venomous species in the North American continent, where it can be found in the Coastal Plain of the southeastern United States. These animals are notorious for their size, measuring between 1.3-1.5 meters on average and upward of 2.4 meters in the absolute biggest individuals recorded, making them the biggest species in the whole *Crotalus* genus.

3.2.1 Diet and distribution

Individuals of *Crotalus adamanteus* are commonly observed in habitats such as flat woods, grass marshes and swamp forests, common in the American South-East (Florida, North and South Carolina), where most of the species' range is located (Timmerman, 1995).

Their diet is mainly composed of rats, mice and rabbits, which the *crotalus adamanteus* captures by ambushing. Although these animals are known to spend hours to days in striking position, they are also known to change hunting spot rather frequently. This appears extremely clear from Timmerman's (1995) study on the home range, habitat use and behavior of *crotalus adamanteus*, a study which led to the discovery that these animals manage home ranges of up to 400 ha (4 km^2).

3.2.2 Toxicity and danger for humans

The fame surrounding these beautiful animals is, as per most venomous species, the result of fear and exaggeration. Nonetheless, it should be noted that, as per all venomous species, humans are never bitten on purpose, with bites only being recorded as retaliatory after disturbance, catching or handling. Rattlesnakes are extremely tame, and rarely display signs of aggression, as noted by Timmerman's (1995), states that "in only 9 of my 743 visits to diamondback locations was the rattling behavior elicited", with no striking attempts by crotalids ever been recorded during his three-year research, even when accidentally stepped upon. However, unlike in the case of the

previously discussed *vipera orsinii*, fear is a much more grounded and reasonable sentiment once the effects of envenomation are taken into account.

The genus *Crotalus* offers, as stated by Kocholaty W.F. and his team (1971), some of the biggest toxic variability. This thesis is supported by the fact that species in such genus are known to inhabit extremely diverse and far apart habitats, each containing different prey types, thus requiring some evolutionary adaptations to thrive (Deshwal A. et al., 2021). In the words of Phan Phuc in his *Review of Rattlesnake venom* (2023) "high variability in habitat type, altitude, associated diet types, and extensive geographical range allows rattlesnakes to have a high variability in their venom composition".

In the review, based on publications performing proteomic and transcriptomic investigations, Phan Phuc and his team go on to describe the composition of rattlesnakes' venoms, proved to be made, across the entire *Crotalus* genus, by a staggering 63 families and subfamilies. As made clear in the previous chapter, most of these components play a minimal role, and are found in very few species or in very small quantities. Indeed, the main compounds making up the venom of crotalids include SVSPs, PLA_2s, SVMPs (mostly from the P I and P III families), L-Amino Acid Oxidases, C-Type Lectins, Disintegrins and Cysteine-rich secretory proteins, which we have already discussed as being among the most widespread and common families observable during venom analysis.

Envenomation from a *Crotalus adamanteus* will lead to a severe inflammatory reaction in the bitten area as the SVSPs, PLA_2s, SVMPs begin to act on the tissues, as well as bradykinins are released in high concentrations (Chippaux J. P., 2006). Rapid swelling due to the edematous buildup of fluids is also commonly observed (Chippaux J. P., 2006). As enzymes progress their lytic process, muscle and skin necrosis occurs, as well as extravasation, hemorrhages and other hematic disturbances (Chippaux J. P., 2006 ; Patel, Virat, et al., 2023 ; Phan Phuc et al., 2023). Thrombocytopenia and ischemia are also very commonly observed (Patel, Virat, et al., 2023).

Treatment is, unlike in the case of *vipera ursinii*, necessary, as consequences for envenomation may be serious and often life-threatening. Fortunately, treatment is widely available in areas where these species are endemic, commonly found in the form of "Polyvalent crotalid antivenin", a horse serum derivative (Offerman et al., 2001). Due to its equine origin, antivenin has often been described as extremely allergenic (Jurkovich et al., 1988). Offerman et al. (2001) put this belief to the test in a retrospective study involving 11 years of hospital clinical care involving rattlesnake bites. Results show that, although some sort of adverse reaction was observed in 19% of the cases, this was limited to rashes and urticaria. At the same time, "no deaths, amputations, or permanent disability

from snakebite occurred in the patients receiving antivenin" (Offerman et al., 2001), indicating the effectiveness of this procedure.

3.2.4 Conservation

Although the IUCN currently puts the *Crotalus adamanteus* inside the "Least Concern" category, herpetologists working with these animals have noticed a decline in numbers. Indeed, "until recently, the diamondback was very common throughout most of Florida but, although it is still occasionally seen, does not appear to be as abundant" (Timmerman, 1995). The situation becomes even more concerning once we discover that this once widespread species is now considered extinct in Louisiana, as well as a "Species of Special Concern" in both North and South Carolina (Timmerman, 1995 ; Martin, William et al., 2000 ; Kevin M. Stohlgren et al., 2015). The main reasons behind this steady decline are to be found in the loss of suitable habitat, as well as the indiscriminate killing (Timmerman, 1995 ; Martin, William et al., 2000 ; Kevin M. Stohlgren et al., 2015). Timmerman (1995), comments on this by stating that "there remains in the South, as elsewhere, a lingering prejudice against rattlesnakes which continues to lead to their destruction", a crude yet disheartening statements indicating just how important it is to educate ourselves in order to understand and cohabit with these misunderstood animals.

4. Venomous snakes bites: First aid

Although globally represented, snake envenomations are among the most neglected life-threatening "diseases", with over 100000 people losing their lives each year and with around 400000 people being left maimed or disabled to some degree (Avau B. et al., 2016 ; Gutiérrez J. et al., 2017 ; Manuela B Pucca et al., 2020). As we have discussed in the previous chapters, most bites and envenomations are the result of accidental interactions, especially common in more rural areas (Avau B. et al., 2016 ; Gutiérrez J. et al., 2017 ; Manuela B Pucca et al., 2020) of the world, with estimates stating that around ten out of every 100000 people are bitten (Avau B. et al., 2016).
The fact that most accidents take place in more secluded spaces, where medical intervention is slower and oftentimes more precarious, tremendously raises the need for adequate first aid measures. These should in no way replace proper treatment, which should be immediately sought, instead allowing the involved party to partially stabilize the situation and delay toxic effects until medical attention is reached.

4.1 First aid: Do and Don'ts

From popular media to fiction books, misconceptions about first aid following envenomation are plenty and rooted extremely deep in our collective knowledge ("venom sucking" being the most notable example). However, researchers proved that these "hollywoodian" methods are not only ineffective, but also dangerous, oftentimes leading to further damage and worsening of health conditions. For this reason, this subchapter will revolve around discussing the best and worst first aid practices, to follow (or not to follow) in these emergency situations. Information have been uniformly collected from reviews published by experts such as Chippaux (2006), Avau B. et al., (2016), Gutiérrez J. et al. (2017), Jennifer P.C. et al. (2018), Godpower C Michael et al. (2018) and Matteo R. Di Nicola et al. (2021).

A. Snake Identification

   Recognizing the biting species is an extremely important yet rarely mentioned step. However, such a procedure could mean the difference between life or death in more serious scenarios. Knowing the "offending" species will speed up procedures such as antivenin choice and administration, as well as prepare the medical staff to take onto the symptoms. For this reason, experts such as Findlay E. Russell (1967) suggest to "make every effort to identify the snake before treatment", suggesting even to go as far as to kill the animal to present the body to experts for recognition. This last point is, considering also what we mentioned in the last chapter, completely excessive other than dangerous, and should be instead exchanged for

photographic proof or a precise description. This is, however, one of the most overlooked, forgotten and overall difficult steps to achieve. This is completely understandable, as the shock from envenomation would lead even experts to feel overwhelmed and scared.

B. Calming the envenomated individual

Experts and others all seem to agree on the fact that calming down and reassuring the victim of envenomation is a crucial step in providing first aid. Calming down allows venom to flow slower and to be dispersed less in the organism, preventing systemic toxicity to arise as quickly. Moreover, it improves collaboration and allows the other steps to be carried out more quickly, safely and efficiently.

C. Wound incision and Venom extraction

These are some of the most commonly known steps in first aid situations involving envenomation. However, both of these techniques have been proved as inefficient if not outright dangerous. Wound incision has been proved to somewhat reduce swelling, but both the risk of developing a hemorrhagic syndrome, as well as the amount of antivenin necessary proved to be equal for people with or without incisions. Furthermore, the risk of introducing pathogens further increases if non-sterile tools are used.

Venom extraction has also been proved to be essentially useless, producing no discernible difference taking into the account the amount of antivenin necessary or the risk of death/ disability. Moreover, extraction through direct sucking may lead to further problems, although related more to the contact with bodily fluids than to secondary envenomation.

Compared to these two methods, a simple process of wound cleaning is to be highly preferred.

D. Immobilization and Pressure application

Compared to the previous point, these are the actual most important steps to follow when providing primary care after envenomation. Applying pressure through bandages, cloths and elastic wraps, as well as immobilizing the bitten part by using a splint (which may be made by wood sticks, metal pieces…) has been shown to delay the systemic spread of the venom, and has been experimentally and statistically linked with higher survival percentages. It has to be considered, however, that locally necrotizing venoms will have more severe consequences with these procedures, hence why it is not suggested in 100% of the cases. However, it may still be a lifesaving procedure. Of fundamental importance before immobilizing the site of envenomation is to remove any rings, watches, bracelets or similar elements which may cut off circulation following the inevitable swelling of the area.

E. Tourniquet

Tourniquets are commonly used tools during primary care, but can be the source of major complications. As these tools block lymphatic, venous and arterial circulation, they are extremely good at preventing the spread of the venom. However, tourniquets are also responsible for increasing the risk of ischemia and gangrene, as well as concentrating the effects of the venom. Lastly, tourniquets show their most dangerous side once removed, as releasing them may lead to sepsis or embolism, as well as reperfusion syndrome.

F. Anti-inflammatory and analgesics

Lastly, experts agree on the fact that anti-inflammatory drugs and analgesics can be given to bite victims to temporarily manage symptoms such as pain and swelling. However, it should be noted that no NSAIDs (and especially Aspirin) as these are known to alter hemodynamic properties, worsening eventual hemorrhagic syndromes. Thus, only opioids or corticosteroids should be used, but a common agreement is found stating that although possible, no drugs should be administered by untrained personnel.

5. Conclusions

Venomous snakes are extremely beautiful and complex species which have carved a very unique ecological niche for themselves. They have evolved what can be considered one of nature's most fearsome, deadly and yet fascinating weapons, which has allowed them to thrive in almost every place on earth. However, with the overlapping between these animals' habitats and human activities, encounters and aggressive interactions have become more and more common. It goes without saying, after shedding a light on aspects such as the pathophysiology of envenomation, that these animals absolutely can be and will become dangerous if underestimated or provoked, leading to life-threatening or life-ending consequences in the worst scenarios.

Snake bites remain one of the most underrated and overlooked sources of disease, especially in less developed countries, where they are the cause of tens of thousands of deaths each year. Rigorous scientific research and laboratory testing toward the development of effective antivenins is thus still a urgent necessity, and a goal to reach in the near future. However, although the danger is real, venomous snakes remain fundamentally misunderstood and overly feared. As we have seen many times over the course of this review, these animals never strike on purpose, instead relying on their precious venom only when deemed strictly necessary.

Education is thus necessary not only to comprehend what the actual risks are, as well as to learn how to act in such situations, but also to understand that danger is not a justifiable excuse for hate. Understanding this point should allow us to rethink our point of view, to challenge the justifiable yet irrational fear that surrounds these so-called "monsters" which prevents us from appreciating their role in the wider ecological landscape.

Education is also important to understand that the fear and hate surrounding these animals has led them to become victims of unjustified and unregulated killing, which has led, as in the cases of the *vipera ursinii* and the *crotalus adamanteus*, to significant declines in once thriving populations. These are urgent situations, and although some work has been carried out in the right direction, there is still a lot to do.

Lastly, education is also important to recognize that components from the venom we are terrified by can be turned from toxins to drugs, transforming from harmful agents of terror to extremely reliable and efficient therapeutics.

This sort of education is still in its infancy, but one small step at the time, if not appreciation, at least tolerance should be taught.

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