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REVIEW

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Differences between poison and venom: An attempt at an integrative biological approach

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Abstract
We discuss the use of the terms venom and poison, in the context of integrative biology, with particular emphasis on behaviour and natural history. Our purpose is to reach a broad scientific audience, especially that dedicated to zoology. The meaning of the two terms is reviewed from the secretory perspective, mainly focussed on the reptiles and amphibians. We justify the use of the two words, based on biological and behavioural differences.

KEYWORDS
amphibians, poison, reptiles, snakes, toads, Venom

1 | INTRODUCTION

In scientific terms, the most usual definition for harmful animal toxins classify them into two categories: venoms, the toxins that are injected into the victim by an action of the venomous species, and poisons, the toxins that are delivered as the result of an action by the victim. However, the scarce scientific literature on the subject shows that, despite several attempts, in practice, there is no consensus among theoretical and naturalistic biologists regarding the use of both terms. As a result, among laypeople and even scientists, the concept of venom and poison remains controversial. In this text, we intend to discuss their use in a context of integrative biology, with particular emphasis on behaviour and natural history. Our purpose is to reach a broad scientific audience, especially that of the zoological area, in which confusion about the meaning of venom and poison is recurrent. As this is a complex topic, involving thousands of species of living beings, we intend to make a small exploratory foray, on the morphological and behavioural systems associated with selected venomous/poisonous species. We then review the meaning of the two terms from the secretory perspective, based mainly on the secretion systems of reptiles and amphibians. Finally, we justify the use of the two words, based on consistent morphological and behavioural evidence.

2 | THE CONVENTIONAL DEFINITION OF VENOM AND POISON

From a biological perspective, it seems that venom and poison are different, although both are composed of toxic substances that, depending on the concentration, have the potential to kill or cause damage to health (Nelsen et al., 2014). In general, both can act by injection or absorption through mucous membranes or the intact skin. However, only venoms are produced by living beings, generally in glands, and used in defence or attack. Poisons are also secreted by living beings, although they can also have an artificial or mineral origin (Nelsen et al., 2014; Osterhoudt, 2006). Both venoms and poisons contain toxins, "a substance produced by a living organism that is capable of causing dose-dependent pathophysiological injury to itself or another living organism; sometimes called a biotoxin or biological toxin" (Nelsen et al., 2014).

Most dictionaries of the English language show a real synonymisation between the terms venom and poison. The most substantial entries refer exclusively to poison, with most of the examples based on literary terms instead of science. However, among the entries in dictionaries more devoted to scientific dissemination, or among journalistic articles of scientific nature, we notice differences between the terms, even though somewhat superficial (Groove, 2002). In such texts, venom is defined as a substance produced in glands of snakes, spiders, scorpions, centipedes, wasps, bees, etc., and endowed of means of injecting toxic substances into the aggressor or prey through stings or teeth, which can be solid, grooved or canaliculated (Figure 1). Conversely, poison, in general, is used solely for defence and refers to substances produced by animals like amphibians (Jared et al., 2009; Mailho-Fontana et al., 2014, 2018; Regis-Alves et al., 2017; Toledo & Jared, 1995). They are deprived of injection mechanisms, depending exclusively on the own action of the aggressor (Figure 2). Poisons in animals also come from glands (or are stored in reservoirs), acting mainly via mucosa, especially the oral mucosa, or directly through the intact skin.

Taking into account the existence of other types of toxin production and delivery mechanisms that do not perfectly fit the definitions of venom or poison, Nelsen et al. (2014) introduced the term toxungen to define toxic biological secretions delivered to the body surface of the victim without an accompanying wound. The authors also clarify that "whereas poison delivery is essentially passive and relies primarily on the actions of the victim to introduce the toxins, toxungen delivery depends on actions taken by the toxic organism". This category includes, for example, the fire salamander (*Salamandra salamandra*), spitting snakes (*Naja* spp. and *Hemachatus haemachatus*), the Texas horned lizard (*Phrynosoma cornutum*) and also invertebrates such as bombardier beetles (family Carabidae) (Nelsen et al., 2014).

For a more in-depth understanding of this theme, we recommend the substantial papers of Arbuckle (2015), Nelsen et al. (2014) and Osterhoudt (2006) that using different approaches provide information based on areas that we will not consider in this paper.

FIGURE 1 Venom apparatus of *Lachesis muta* employed in active defence. Note the yellowish venom oozing from the caniculated teeth

3 | ANTHROPOCENTRIC (MIS) INTERPRETATION OF THE USE OF VENOMS AND POISONS BY ANIMALS

Venomous and poisonous animals use their toxic substances primarily for feeding or defence. Snakes, amphibians, arachnids, and other animals are considered a threat to public health (both for humans and human pets) because they can cause envenomation. However, such accidents are not an attack in the strict sense of the term as in most occasions the animals only defend themselves, in response to what they interpret as a threat, making use of available weapons developed along with their evolutionary history. Of course, the use of toxins as a defence without prior warning, sometimes with lethal consequences, frontally contradicts human interpretation. As a result, the animal defensive instinct is most times conceived as an insidious behaviour, directly reaching human concepts of good and evil. Here, fits a clear definition of aggressor based not only on human concepts but also on the natural reactions of venomous and poisonous animals when facing aggression. Aggressors can be classified into two biological categories: the potential aggressor, usually a predator, and the presumed aggressor, consisting of any living being that inadvertently crosses the limits of security of the animal, setting off its defensive (venomous or poisoning) system. Snakes, arachnids and some many other animals that occasionally envenomate humans belong to the second category.

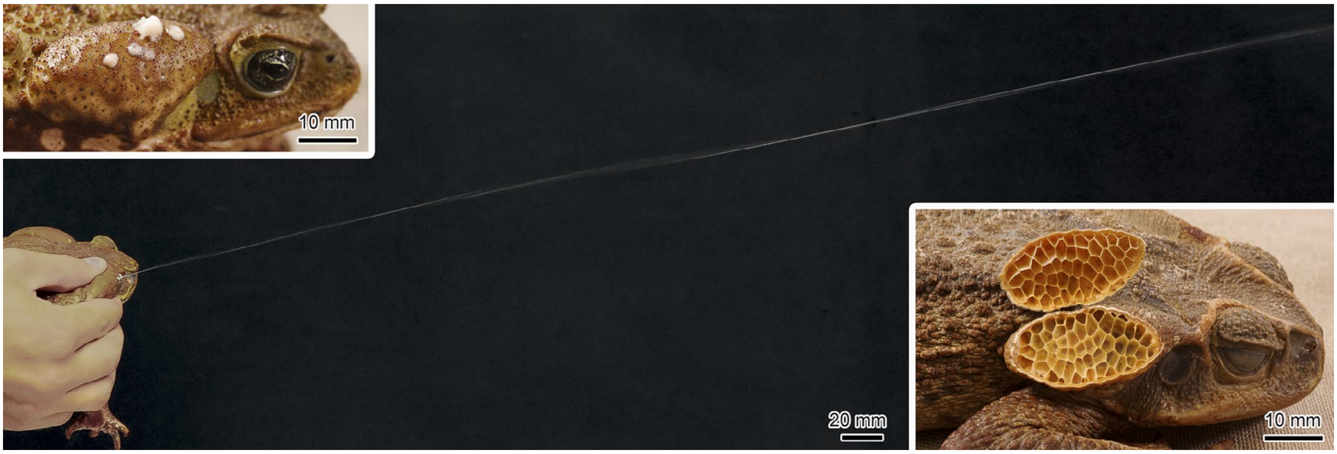

FIGURE 2 The poisonous toad *Rhinella icterica* and its passive chemical defence. Manual mechanical pressure upon the parotoid macrogland simulates the attack of a predator. Note the abrupt release of poison in the form of a potent jet when the parotoid gland is compressed. The upper insert illustrates the presence of poison drops on the parotoid surface after poison release. The lower insert shows the honeycomb-like internal architecture of the parotoid, where each alveolus accommodates a large poison gland

4 | ACTIVE AND PASSIVE DEFENCES

In general, basically, two types of animal defensive behaviour can be defined: active defence, performed as a response to attacks promoted by aggressors, generally combative and using physical force with the aid of teeth or claws; and passive defence, based in non-reactive defensive strategies, exclusively dependent on an attack by the potential or supposed aggressor.

In nature, active defence is much more widespread. Most animal species (including *Homo sapiens sapiens*) tend to respond to attacks by counterattacking. Conversely, animals exist that rely on passive behaviour, such as the porcupine or the hedgehog that, when attacked, remain static, trusting that the aggressor will mechanically self-harm with their spines. In particular, defence with spines is usually very efficient, causing injuries that are often severe or even lethal. Other animals use shells (such as turtles or armadillos) as a form of passive defence, creating physical barriers and hindering the aggressor's action.

On the other hand, roughly speaking the physical advantages of animal active or passive defence can be many times accompanied by the use of chemical compounds, venoms or poisons, composing active or passive modes of chemical defence.

5 | VENOM AND ACTIVE CHEMICAL DEFENCE

Venomous animals, in general, rely on active chemical defence. Snakes, especially viperids, actively defend themselves through an abrupt and effective injection of venom. Regardless of the real intention of the potential (or presumed) aggressor, a simple threat is sufficient to provoke a strike, with the snake widely opening the mouth and exposing and positioning forward the prominent pair of fangs, ready to inject the venom. Simultaneously with the strike, through a fast, momentary sting (and not a prolonged bite), the snake voluntarily compresses the venom glands using specific surrounding muscles, causing the immediate expulsion of the venom through the fangs (Figure 3).

6 | POISON AND PASSIVE CHEMICAL DEFENCE

Poisonous animals exhibit passive chemical defence that, in general, is not well understood. Among vertebrates, unlike aggressive snakes, in amphibians the chemical arsenal is primarily directed towards defence. When amphibians are bitten, the oral mucosa of the predator (or aggressor) comes into contact with the secretion produced by the skin poison glands (Toledo & Jared, 1995). At the moment of the aggression, amphibians, through body postures, such as increasing the body volume or offering to the aggressor body regions with a high concentration of poison glands, favour poison to be expelled (Jared et al., 2009; Regis-Alves et al., 2017). Furthermore, it is already well known that amphibian poison is not only directed to predators or aggressors but also serves as a defence against microorganisms and desiccation (Duellman & Trueb, 1994; Hillman et al., 2010; Toledo & Jared, 1995). Two basic types of skin glands are present in these animals, the poison (or granular) glands and the mucous glands (Figure 4), both located in the dermis, despite their epidermal origin (Toledo & Jared, 1995). At least among salamanders and newts,

FIGURE 3 The viperid snake *Bothrops jararacussu* and the venomous apparatus used for chemical active defence, visible when the animal opens the mouth to project the fangs. Note in the lower scheme the presence of the compressor muscle of the venom gland and the duct connecting the gland to the innoculatory fang. Original of Augusto Esteves, extracted from Canter (2013). Serpentes, Arte & Ciência. (1st Ed.) Instituto Butantan, São Paulo

(Order Caudata), the toxic compounds are present in the poison and the mucous glands and are used against possible predators (Mailho-Fontana et al., 2019). In the three amphibian orders, including Gymnophiona (caecilians), the mucous glands, besides acting in breathing, may also produce bactericidal and fungicidal agents (Sawaya, 1940). This hypothesis may explain why despite the body surface of amphibians being permanently moist and mucous, and therefore in theory providing a good culture medium for microorganisms, under natural conditions these animals have healthy skin, with a notorious ability to rapidly regenerate in case of harm (Brockes & Kumar, 2008; Godwin & Rosenthal, 2014; Tanaka & Reddien, 2011).

7 | DISADVANTAGES OF USING VENOM AS A DEFENCE

The great variety in nature of defensive modes through the introduction of toxic substances into aggressors or predators is the result of independent evolutionary investments in the various groups of living beings, acting in different systems of production and discharge of venoms or poisons (Arbuckle, 2015; Nelsen et al., 2014). Viperids, for example are endowed with an injection system of high precision that, together with the organs used in environmental perception, was primarily moulded to their dietary needs. Most viperid species are lurking predators, waiting motionless for their prey to approach. Therefore, their energy expenditure in feeding is minimal: a precise and straightforward strike is enough to promote venom injection into the prey, guaranteeing its immediate immobilisation and availability for easy swallowing. Venom occasionally used in defence seems to represent a physiological disadvantage for the snake that will necessarily go through a period without being able to feed, waiting for the venom glands to refill. None or little venom in the glands means no chance of immediate and complete prey immobilisation. This fact seems to be directly related to the use by the snakes of auditive or visual warnings directed to the aggressor or predator, for example, rattling among rattlesnakes and exhibition of vibrant colours among the coral snakes, trying to avoid defensive attacks (Owings et al., 2002) and, in most cases, promptly escaping from the danger. Likewise, some other snakes (such as species of genus *Bothrops*), when threatened, rhythmically hit the tail tip on the litter covering the forest floor (Araújo & Martins, 2006) producing sounds very similar to those coming from the rattlesnakes' rattle.

8 | ENTRANCE VIAS FOR VENOM AND POISON INTO THE ORGANISM

When considering the behavioural characteristics related to active and passive modes of defence, it is essential to discuss how venom and poison access into aggressors' organism occurs. Here are other fundamental and little-explored differences between the two terms. Because animals with active defence, in general, make use of injections applied to the aggressor's body, venoms usually reach the organism through the bloodstream. In the case of humans, statistics

FIGURE 4 Cutaneous glands of anuran amphibians. The dermis (d) presents mucous glands (m) and poison glands (g) (or granular glands). Also note the epidermis (e), which is poorly cornified. Histological section stained with haematoxylin–eosin. Species: *Brachycephalus ephipium*

show that the most common parts of the body affected by venomous animals are the feet, legs and hands (Gold et al., 2002; Gras et al., 2012; Kitchens, 1987). Spiders and scorpions can be stepped on or somehow compressed against the body when a person is putting on clothes or shoes or is laying on a bed. On the other hand, animal poison always penetrates the organism through mucous surfaces, in particular the oral mucosa. Taking amphibians as an example, among which most reports involve toads, poisoning always occurs when they are snapped up by the aggressor (Toledo & Jared, 1995).

9 | VENOM E VENOMOUS GLANDS

A distinctive characteristic of venoms and poisons is the type of glandular system responsible for their production. Venomous animals due to their active behaviour need a fast injection system, endowed with well-developed muscles surrounding the venom glands, perfectly synchronised with the strike or sting to expel the venom at the exact moment the fangs or stings are introduced into the victim (Mackessy & Baxter, 2006; Mackessy, 2010; Soliman et al., 2013; Weinstein et al., 2010;). The venom glands, in this case, count with a reservoir compartment (or glandular lumen) (Figure 5) which is supplied continuously by the secretory product of the surrounding cells (Mackessy & Baxter, 2006; Soliman et al., 2013; Weinstein et al., 2010). For venomous animals, the most convenient time for feeding is precise when the venom glands are full, guaranteeing efficient predation.

10 | MORPHOLOGY OF AMPHIBIAN POISONOUS GLANDS

In amphibians in general, due to the passive mode of defence, their skin poison glands are devoid of muscles, relying only on a thin monolayer of myoepithelial cells surrounding the secretory epithelium (Delfino et al., 2001). In most species, the myoepithelium stimulates a relatively slow discharge of secretion to the body surface. In case of a threat, some species can release the skin secretion on the body surface, mainly on the dorsum (Toledo & Jared, 1995). Another possible function for the myoepithelial layer is the venom homogenisation within the gland through slow and gentle contractions (Toledo & Jared, 1995). In the case of toads (species belonging to family Bufonidae), the defence mostly relies on the dorsal parotoid macroglands, in which poison extrusion at the moment of the threat is not immediate and depends on the external action of the aggressor (Jared et al., 2009). In this case, poison release needs the energy of a bite that develops a function similar to that of the muscles surrounding the venom glands of snakes, arachnids, etc. In other words, the force of a bite provides the sufficient energy to trigger the glandular system, which extrudes the poison usually in the form of jets, similar to "shots" directed to the oral mucosa of the predator/aggressor (Regis-Alves et al., 2017) (Figure 2). Conversely to the immediate action of venom, amphibian poison needs to cross the oral mucosa to invade the organism, acting in relatively slow action. If a dog bites a toad, a case that is quite common in urban peripheries (Regis-Alves et al., 2017) where remnants of forest persist, usually the intense cleaning of the oral mucosa is sufficient to prevent the poison from reaching the bloodstream. However, under natural conditions of predation or attack, the toxins present in toads' poison, especially those with cardiotoxic effects, must succeed to penetrate the mucosa and reach the bloodstream (Mailho-Fontana et al., 2018), causing envenomation with high potential of lethality.

Just as the morphology of snake venom glands was shaped by evolution to play an active function, amphibian poison gland morphology was moulded to function passively. In anuran amphibians (toads, frogs and tree frogs), the most successful order of amphibians with 7,245 extant species (Frost, 2020), these glands are microscopic and constituted by a sole syncytial cytoplasmic mass, with numerous nuclei and devoid of internal membranes (Delfino et al., 2001; Toledo & Jared, 1995) (Figure 6). The syncytial arrangement of anuran glands appears to be a well-derived condition with significant secretory advantages. The poison produced in the

FIGURE 5 Structural characteristics of snake venom glands. The epithelial cells (arrows) secrete the venom (V) via exocytosis, which accumulates in the wide glandular lumen (L), occupying most of the glandular volume. Histological section stained with haematoxylin–eosin Species: *Bothrops jararaca*

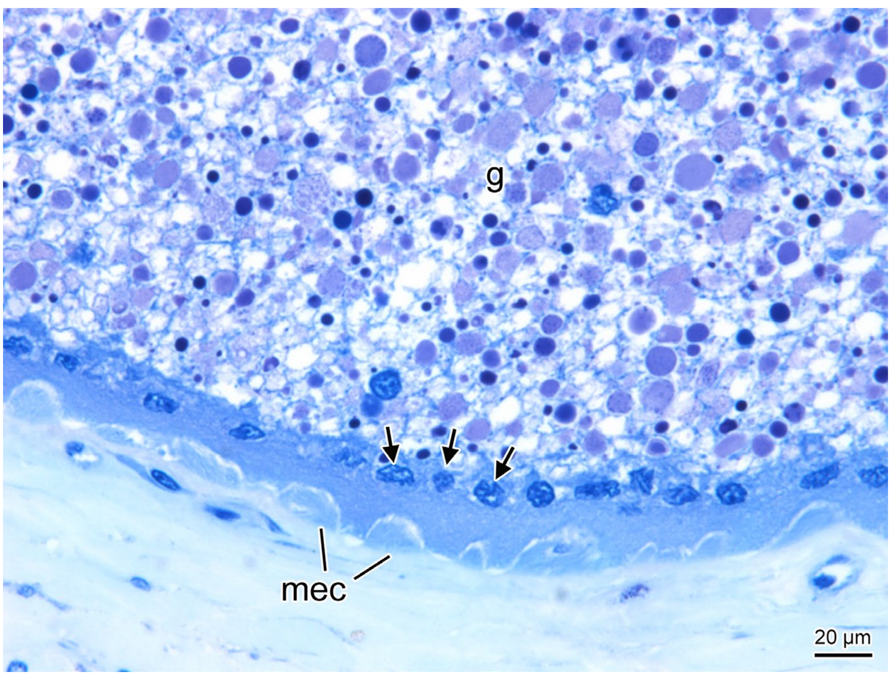

FIGURE 6 Structural characteristics of anuran poison glands. The figure shows part of a poison gland (g) constituted by a single multinucleated cell (or syncytium) full of heterogeneous secretion granules. It is surrounded by myoepithelial cells (mec), with characteristics similar to smooth muscle fibres. The arrows point to the various nuclei in peripheral position. Histological section stained with toluidine blue-fuchsin. Species: *Rhinella marina*

peripheral cytoplasm of the syncytium is gradually transferred to the glandular interior, without much energy expenditure, and, when necessary, is discharged in bulk directly to the exterior. Unlikely, the venom in the glands of snakes needs to cross cell membranes to reach the glandular lumen and, to be discharged, is dependent on an inoculatory apparatus. Particularly in Bufonidae (toads) and Phyllomedusinae (leaf frogs), their evolutionary histories led the poison glands to accumulate and hypertrophy in strategic positions of the body, forming the aforementioned parotoid macroglands. Their dorsal position, behind the eyes, is favourable to poison aggressors when biting from the front. Such accumulations consist of dozens or hundreds of glands assembled (Toledo & Jared, 1995) (Figure 2), each gland taking the form of a small compartment, or an alveolus, full of poison (Antoniazzi et al., 2013). The same happens with the tibial macroglands of some toads, located on the hind legs, in case the animal is attacked from behind (Mailho-Fontana et al., 2017, 2018). The term "macrogland" was proposed by our group (Toledo & Jared, 1995), in opposition to isolated poison glands scattered throughout the body skin. When observed in transversal sections, the macroglands show an internal structure resembling a honeycomb (Figure 2), with each alveolus framed by dermal collagen walls, which can resist the mechanical force exerted by the aggressor's bite (Jared et al., 2009; Mailho-Fontana et al., 2018; Regis-Alves et al., 2017). Each alveolus contains a syncytial gland with a duct communicating the gland to the skin surface through a duct (Figure 7). The epithelial walls of the ducts are very thick, functioning as plugs, similar to bottle stoppers (Jared et al., 2009; Regis-Alves et al., 2017). When a bite reaches the parotoid, its potential energy spreads throughout the interior of the alveoli but is restrained by the resistant collagen walls (Jared et al., 2009; Regis-Alves et al., 2017). If the pressure reaches the maximum, the plugs, which are the most fragile alveoli region, are forced to disrupt, thereby liberating the pressurised poison that squirts in the form of a long-range jets. In general, several alveoli are affected in one bite, generating a toxic (and even lethal) spray composed of many jets.

Thus, despite their microscopic size, the plugs play a fundamental role in the toad's strategy of passive defence, particularly in the macroglands. The plugs are composed of keratinocytes, the same epithelial cells covering the whole body (Jared et al., 2009). Along with the evolution of toad defensive system, the plugs must have passed through gradual thickening to balance the force exerted by average aggressor's bite, so that they can function in precise synchronism for poison discharge on the aggressor's oral mucosa (Mailho-Fontana et al., 2014; Regis-Alves et al., 2017). Right after they have been disrupted, the plugs start regeneration, concomitantly with the process of alveolar reorganisation (Jared et al., 2014). The disclosure of the morphology and functioning of this elaborated process is a result of research carried out by our group at Butantan Institute, based on fieldwork and laboratory experiments (Jared et al., 2009; Mailho-Fontana et al., 2014; Regis-Alves et al., 2017).

11 | KOMODO DRAGON AND GILA MONSTER

It is worth emphasising that current definitions of venom and poison do not necessarily predict the immediate or accelerated death of prey or aggressors (Jackson et al., 2017).

FIGURE 7 Structural characteristics of the parotoid macrogland of a toad. The dermis (d) models the honeycomb-like structure, accommodating the poison glands. Each poison gland (g) has a duct blocked by an epithelial plug (pl) that serves as a "bottle stopper". Each duct is surrounded by mucous glands (m). Histological section stained with haematoxylin–eosin. Species: *Rhinella jimi*

This fact is evident through the observation of the predatory method of Komodo dragons (*Varanus komodoensis*) that bite their prey (that can be as large as buffalos) and patiently wait for the slow action of their oral venom until they die (Fry et al., 2009). Similar to Gila monsters (*Heloderma* sp), the venom injection system of Komodo dragons does not rely on modified inoculating teeth such as those found in viperids and other snakes (Mackessy, 2010; Weinstein et al., 2010). The venom of these lizards is produced in glands located at the base of the lower jaw teeth, and it penetrates the prey body through injuries caused by bites. To stimulate the venom to be released by the glands and for maximum efficiency in venom inoculation, the lizard not only bites but also holds the prey with its teeth and chews it for some time (Fry et al., 2010). It is clear that such an inoculatory system, considered as basal, is very different to the much-improved system present in modern viperid snakes, especially those possessing solenoglyphous dentition, characterised by the presence of large glands, surrounded by muscles and connected to hollow teeth.

12 | POISON AS MEANS OF AGGRESSOR'S LEARNING

Going back to the differences between venom and poison (and between activity and passivity), active animals seem to use venom, attacking the supposed aggressor, mainly for their individual protection. In contrast, poisonous animals seem to have a type of "social" defence, aiming at the protection of the whole population of a certain species. Beyond the protection of individuals, the social defence objectives that, through experiencing the harmful effects of the poison via attempts at predation or aggression, the predator/aggressor ends up learning that a specific prey (or victim) is dangerous and should be avoided. For example, cases of dogs attacking toads are quite common. Such attacks seem to be motivated mostly by pure curiosity and not for the intention of causing harm to the toad. Observing many cases attended at Butantan Institute coming from the outskirts of São Paulo city, we conclude that there are far more deaths of toads than dogs, with dogs most times learning to avoid playing with toads (Regis-Alves et al., 2017). Some research has been already done around this theme, observing the aggressive behaviour of birds on anuran amphibians. In one experiment, owls were offered specimens of the leopard frog *Eupemphix nattereri*, animals having a pair of macroglands on the lower back in the form of large and prominent black circular spots. The owls ventured into predation but ingested only two frogs, refusing to prey on the subsequent offerings, since they felt the harmful effects of the poison, a condition verified through the occurrence of vomiting and faeces on their perches (Sazima & Caramaschi, 1986). The data indicate that the owls learned that animals with such pattern on their backs should be avoided since they experienced the effects of the poison expelled by the macroglands in their digestive system and possibly also in their nervous system. Similar events probably happen with many other amphibians, including toads, since they are large animals living in different areas and can be easily recognised and avoided by aggressors that may have previously experienced their poison.

The learning dynamic seems also to be at the basis of the display of exuberant warning colours by many amphibians and snakes. This is the case, for example, of the poison dart frogs (Dendrobatidae), among which highly lethal skin toxins can be found. Associated with these frogs, there are even cases of mimicry, where harmless species mimic the warning colours as a protection against predators (Prates et al., 2012). In relation to venomous animals, the same dynamic is also observed, especially in relation to the coral snakes, from which several harmless or mildly venomous colubrid snakes are mimics (Greene & McDiarmid, 1981).

13 | CHEMICAL COMPOSITION OF VENOMS AND POISONS

Considering snakes and amphibians, the chemical composition of venoms and poisons have differences. In general, venoms are mainly composed of proteins and/or peptides, usually containing a small variety of other molecule classes. Such composition is probably related to venom function, directed to kill and ingest prey, in case of feeding (Kang et al., 2011), and, to cause physiological harm or even death of the predator/aggressor, in case of defence. On the other hand, the composition of poisons seems to have a much wider variety of molecules, particularly among amphibians. Amphibian poison glands secrete steroids, alkaloids, biogenic amines, peptides, proteins, mucopolysaccharides, and many other compounds yet to be identified (Clarke, 1997; Toledo & Jared, 1995). This fact is directly related to the passive use of poison and mucus in defence and, quite possibly, in other vital functions that remain unknown. Amphibians defend themselves not only from predators but also from microorganisms; besides, they make use of skin secretions for reproduction (as pheromones, for example) and water balance, among other functions (Duellman & Trueb, 1994; Hillman et al., 2010; Raaymakers et al., 2017; Toledo & Jared, 1993, 1995). Since each amphibian group or even individual species is very specific concerning the microhabitats they colonise, they present a remarkable variety in skin chemical production, which may have evolved in parallel to the peculiar ecological needs of each species. It is quite possible that, out of the 8,200 amphibian species recognised today (Frost, 2020), at least 8,200 different compounds may exist, some already identified, but the vast majority still unknown. From this assertion, it is quite easy to deduce the rich potential of this vertebrate group for the identification of new bioactive compounds, with a high perspective of serving as new drugs and pharmaceutical substitutes. Unfortunately, this potential tends to disappear due to the rapid escalation of life extinction the planet is experiencing as a consequence of human activities, reaching the delicate amphibians in a particular aggressive manner.

14 | ACTIVE AND PASSIVE DEFENSIVE SYSTEMS: ADVANTAGES AND DISADVANTAGES

Conjecturing about the superiority or inferiority of active or passive defensive systems is an unfounded task. Ruled by the evolutionary principles of parsimony, both systems are equally efficient for the adaptation of each group. Viperids present a very sophisticated improvement in their active system of venom injection, with a quick response by immediate emptying of their venom glands, followed by an extended period used for prey digestion, when they cannot count on venom. Toads, on the other hand, serve as examples of maximum improvement among amphibians of the passive defensive system, represented by their macroglands, particularly the parotoids. Parotoids cover a quite extensive dorsal area of the animal and are composed of dozens and even hundreds of glandular alveoli, each one endowed with a plug. Toads can be attacked several times but remaining with intact alveoli, provided with poison ready to squirt in case of new attacks and able to shoot the poison directly on the oral mucosa of the predator/aggressor (Mailho-Fontana et al., 2014; Regis-Alves et al., 2017). At the first contact with the mucosa, an unpalatable taste predominates, repelling the aggressor. In case it insists on snapping the amphibian again, other poison discharges will be available.

15 | UNDEFINED SITUATIONS REGARDING THE USE OF THE TERMS VENOM AND POISON

Following the traditional definitions treated above, cases exist in which animal defensive systems can be considered unclear concerning the presence of venom or poison. Toads of genus *Rhaebo* (particularly *R. guttatus*), distributed throughout the Amazon and Central America, are a suitable example. When threatened, they show typical behaviour of active defence. As an exception among toads, they voluntarily squirt poison from their parotoids, directing the jets upward, possibly aiming to reach larger visually oriented predators (such as mammals or birds) (Jared et al., 2011; Mailho-Fontana et al., 2014). In the case of humans, the poison can reach the face, at distances of about two metres. On one occasion, during fieldwork, while recording this peculiar behaviour, poison jets from an individual of *R. guttatus* hit the lens of our camera. This behaviour is accomplished by stereotyped body postures and sudden movements of the front legs in a coordinated manner, which compresses one of the parotoids. Anatomical and histological examination showed that, compared with other toads, morphological differences exist in the parotoid completion, including the epithelial plugs, which may explain the possibility of active defence (Jared et al., 2011; Mailho-Fontana et al., 2014). Despite not being as lethal as that of *Rhinella,* which is the most common toad genus in South and Central America, the poison of *R. guttatus* is more inflammatory, showing great potential to act via the mucosa (Mailho-Fontana et al., 2014).

The urodele amphibian *Salamandra salamandra*, with a wide European distribution, is another case that may fall into the category of animals with active defence. As *R. guttatus*, this salamander also manages, without applying any external pressure, to voluntary squirt the poison through muscle contractions (Brodie & Smatresk, 1990). Nevertheless, unlike *Rhaebo*, even counting with parotoid macroglands, the poison comes from dorsal wart-like glandular accumulations, symmetrically located along the midline of the body. This salamander, similar to *R. guttatus*, appears to have control over the poison squirt process, since the poison jets can originate from different parts of the dorsal line, reaching distances of up to two metres.

Among snakes of the Colubridae family, which are considered non-venomous, the Asian species *Rhabdophis tigrinus* stands out, feeding mainly on toads and, exceptionally, being considered a poisonous animal of passive defence. This snake has a peculiar structure known as nuchal gland, located in the dorsal region of the neck (the nape), where the poison sequestered from ingested toads is stored (Hutchinson et al., 2007). When disturbed by predators/aggressors, the snake tends to raise the head, exposing the nape, similarly to the behaviour observed on toads that expose one of the parotoids to confront the aggressor. As in the parotoids, the strategic position of the nuchal gland favours the snake to be snapped precisely at the right place aiming at poisoning the aggressor.

16 | VENOMOUS AMPHIBIANS

Among Amphibia certain groups escape the usual classification as poisonous and can be considered truly venomous animals since they have means of injecting toxins as defence, even if rudimentary when comparing to snakes. This is the case of the so-called casque-headed tree frogs. Our research group has already studied two of these species, *Corythomantis greeningi*, endemic to the Brazilian semi-arid region, and *Aparasphenodon brunoi*. Despite living in Atlantic Rainforest, this latter species inhabits "restingas", defined as dry areas in between the forest and the sea, covered with characteristic vegetation growing in a sandy and saline soil that suffers from marine influence (Jared et al., 2015). Like most members of the casque-headed group, the two species have a typical flat head, whose dermis is mostly substituted by mineralised tissue, constituting a cranial coosification. The behaviour of phragmosis also characterises these animals, which can enter backwards in holes, such as tree holes, cracks or bromeliad axils (common in "restinga" areas, as is the case of *A. brunoi*), closing the aperture with their heads that serve as lids. When the frogs are in phragmosis, while the head remains exposed to the environment, the body is protected inside the orifice, enabling them to avoid desiccation (de Andrade & Abe, 1997; Jared et al., 2005). *Corythomantis greeningi* and *A. brunoi* show a rigid dermal bone in the head skin shaped in the form of spines that cross the dermis in direction to the epidermis, surrounded by many poison glands. If these tree frogs are grabbed in the hand palm, they usually headbutt, causing the spines to pierce the skin and simultaneously press the dermal glands, injecting their poison (Jared et al., 2015). Such behaviour must reproduce what happens when they are bitten by a predator, who may suffer from the poisoned injuries provoked by the spines inside its mouth. Moreover, this extraordinary injection mechanism must be useful while the animals remain in phragmosis, protecting them not only from desiccation but also deterring predatory attempts. We found that the poison of the two tree frogs shows high toxicity, with LD50 much higher than that of *Bothrops jararaca*, the snake responsible for most of the ophidic accidents in Brazil (Jared et al., 2015).

Among salamanders, similar atypical active venomous systems of defence occur, particularly within species of the Asian genera *Tylototriton* and *Echinotriton* and of the European genus *Pleurodeles* (Nowak & Brodie, 1978; Brodie et al., 1984; Heiss et al., 2010). These amphibians have several small circular gland accumulations in the skin distributed in a pair of rows, one on each side of the dorsal region, each one coinciding with the internal tips of the ribs (Brodie et al., 1984; Heiss et al., 2010; Nowak & Brodie, 1978). When the salamanders are threatened, they compress the body so that each rib tip, functioning as a spine, trespass the skin, crossing through the correspondent macrogland. The ribs are then transformed into a series of poisoned spears, ready to be introduced into the oral mucosa of the aggressor. Such a mechanism is much similar to that represented by the head spines of the casque-headed tree frogs.

Another interesting system involving the use of toxins was recently discovered in caecilians. These amphibians are gathered in the order Gymnophiona and are popularly known as blind-snakes due to their serpentiform body, fossorial habits and reduced eyes. As in the other amphibian orders (Anura and Caudata), they are characterised by the presence of cutaneous poison and mucous glands in the whole body. In a recent study carried out with caecilian amphibians, we have shown in the entire group the existence of a different type of gland located in a row along the upper and lower jaws, clearly connected to the base of the teeth through glandular ducts (Mailho-Fontana et al., 2020). The embryological analysis demonstrated that such glands originate from the same tissue that gives origin to the snake venom glands. That means that in both caecilians and snakes, these are dental glands, coming from the dental tissue that also gives origin to the teeth. When a caecilian bites a prey to feed, an intense sticky secretion is released around the lips. This secretion has shown to contain not only mucus to facilitate swallowing but also bioactive compounds, such as phospholipase A_2 enzymes, which are common in venoms of several animals, including snakes, bees and wasps (Mailho-Fontana et al., 2020). As caecilians date from the Jurassic period, around 250 million years ago (Pyron, 2011; San Mauro, 2010), it is possible to infer that their dental glands constitute an adaptive convergence with those of snakes, that appeared some 150 million years after, in the Middle Jurassic-Lower Cretaceous (Caldwell et al., 2015). The discovery of oral (or dental) glands in caecilians indicate that these amphibians form a unique representative group of vertebrates carrying venomous characteristics used for active predation (and probably defence) and, at the same time, being poisonous, passively defending themselves from predators through skin poison glands spread on the whole body and specially concentrated in the tail (Jared et al., 2018).

17 | LINGUISTIC PROBLEMATICS: POISON AND VENOM *VERSUS VENENO* AND *PONZOÑA/PEÇONHA*

In an attempt to add new perspectives to the long discussion about the differences between *venomous* and *poisonous* animals, it is worth remembering that the equivalent terms in Iberian languages, Portuguese and Spanish, make things even more problematic. Hundreds of million people use these two languages, both in Europe and in the Americas, mainly in tropical or semi-tropical countries with a rich fauna of reptiles, amphibians and arachnids, groups of animals that most use venoms and poisons in defence. Curiously, in these Iberian languages, the use of the two terms is exactly the opposite of the English language. The terms *peçonha* (used in *animais peçonhentos*) in Portuguese and *ponzoña* (used in *animales ponzoñosos*) in Spanish precisely mean venom. On the other hand, *veneno*, both in Spanish and Portuguese, means poison. Over the past few decades, with the global influence of the English language, mainly in the scientific areas, inaccurate translations of books, articles and television series and reports, dealing with biological areas, have added further confusion to the scenario. On the other hand, in the Iberian languages, besides the terms lacking good biological clarification, they are very often misused, according to their etymological standards. The origin of the inversion of meaning is an open discussion: it could have occurred, either in the Iberian languages, or, more likely, in the modern consolidation of French and English. Such confusion might be untangled by delving into linguistic research, looking for the meanings of poison and venom in other languages close to English and French, and comparing them to those close to Portuguese and Spanish. However, considering the essential Latin origin of both languages, Spanish and Portuguese may have maintained the most relative meaning to the original terms. According to the Webster International Dictionary (Groove, 2002), the term poison comes from Middle English *poysoun*: *potion*, poisonous drink, which comes from the *Old French poison*, originally from the Latin *potio* (*potionis*), from *potare*: to drink. On the other hand, the same dictionary states that the term venom comes from *Old French* and *Middle English venim*, originated from Latin *venenum*, a term that in several dictionaries shows very few acceptations, most times meaning a poison.

Another point to add to this presumably endless discussion is that the word poison is often associated with the mystical *potion* traditionally prepared by wizards and witches and generally associated with doing evil. Poison, taken from the popular use, would be much more adequate than the word venom, mainly referring to snakes. Moreover, the meaning of venom is more generalist and does not necessarily refer to animals. Accordingly, Oswaldo Vital Brazil, a renowned Brazilian pharmacologist, stated that "the word poison has a much more restricted meaning than venom, and can even be considered as a type of venom" (Brazil, 1982).

Just as an illustration, it is worth mentioning an excerpt from the historical translation into English of Don Quijote de la Mancha, by Miguel de Cervantes, made by John Ormsby, in 1885. This translation is one out of many others that use the term poison, instead of venom (Part 1, chapter 14), when translated from the original Spanish *ponzoña*:

> As the viper, though it kills with it, does not deserve to be blamed for the **poison** it carries, as it is a gift of nature, neither do I deserve reproach for being beautiful.

This sentence is part of the speech of the shepherd Marcela, accused of being responsible for the suicide of shepherd Grisóstomo since she summarily refused his love. In a time when science was still very incipient, the text calls attention not only to the translation of the term venom to poison but also for its rationality. It treats the term venom as a natural characteristic, suitable for the modern ecological and evolutionary conception, and not as a "choice" of the serpent of doing evil, so prevalent at Cervantes' time. The shepherd defends in her speech that the characteristic of being venomous is inherent to the animal and is part of its biological completion, as well as her unparalleled beauty.

Finally, it is interesting to quote Shakespeare's own words, in his play "As you like it" Act 2, Scene 1, using the same term appearing in Ormsby's translation, coincidentally appropriating the same Latin etymological meaning used in Portuguese and in Spanish:

> Sweet are the uses of adversity, Which, like the toad, ugly and **venomous**, Wears yet a precious jewel in his head.

18 | FINAL CONSIDERATIONS

This work tries to demonstrate that behind the definition of the terms poison and venom, there is more than a simple linguistic concern. Apart from the conventional and merely functional interpretation, based on the form of transferring the toxic secretion from the producing organism to the recipient organism, we argue that elements based on biology, behaviour and natural history offer support to the terms poison and venom. We propose an integrative vision, taking into account important variables that hitherto remained unnoticed. First, it is worth to consider the type of chemical defensive behaviour of the organism, whether passive or active. It seems clear that venomous organisms, use the toxic secretion primarily for predation (or attack), but can also use it in case they are attacked (even if inadvertently), characterising an active defence. Differently, poisonous organisms, most times, make use of their toxins only for defence (against predators and, in some cases, also against microorganisms).

Moreover, venomous animals inject the toxins into their victims, directly reaching blood circulation. In the case of poisonous animals, on the other hand, the first contact of the poison is usually via mucosa (mainly the oral mucosa), or through the skin of the victim. Another point to consider is that the glands of active or passive organisms seem to present anatomical and morphological differences. In organisms of active defence, the glands communicate with teeth or stings and are surrounded by muscles acting on the rapid expulsion of the venom through an injection. In contrast, in organisms of passive defence (as amphibians) the action promoted by the aggression of the aggressor, through bites, for example, provides sufficient energy to provoke ejection or release of the poison that first reaches mucous membranes or the skin, indirectly accessing the blood circulation. Another aspect that differentiates venom and poison is chemical composition. Venoms, which are mainly used for predation by active injection, seem to have been selected by evolution to provide organisms with substances aiming at paralysing or killing their prey and are usually composed of few classes of compounds. Poisons, in contrast, are not used for predation and usually present an extensive variety of substances, also used for other purposes, besides defence. As already highlighted along with this work, the poison, in general, is not lethal, despite being able to inflict in the victim enormous physiological and morphological discomfort, including intense inflammation and pain. Thus, poisoning can serve as a pedagogical factor, contributing to collective defence within a species or a group of similar organisms, stimulating aggressor's "learning" through painful memories and discouraging possible further attacks (Herzig et al., 2020).

Regarding the term toxungen (Nelsen et al., 2014), its use seems difficult within the context of passive and active defences. Its use appears to better apply to organisms that, despite having passive defence, can actively eject poison, for example, skunks, toads of genus *Rhaebo*, and salamanders (*Salamandra salamandra*). The black-necked spitting snakes (*Naja nigricollis*) represent an animal of typical active defence, producing a very lethal venom able of rapidly detaining prey. In this case, the term toxungen to designate the venom would be debatable, since these snakes, although being able to defend themselves through venom spitting, continue using the typical active defensive mechanism through bites.

Finally, we entered in a relatively unexplored area, analysing venom and poison from the linguistic perspective, showing that in Portuguese and Spanish, languages very close to Latin, the two terms have opposite meanings in relation to English. Thus, venom means "peçonha", in Portuguese and "ponzoña" in Spanish; and poison means "veneno", both in Portuguese and Spanish. It is important to keep in mind that the speakers of these two languages represent hundreds of millions of people mainly living in regions where the richest fauna and flora in the world are located, with hundreds of species of venomous ("ponzoñosos" and "peçonhentos") or poisonous ("venenosos") organisms. We believe that this fact further strengthens the confusion between the terms worldwide. The broad influence of the English language, particularly in science education, but also in wildlife documentaries, often results in erroneous translations without a scientific or grammatical basis and plays a fundamental role in the maintenance of this debate.

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