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# South American snake venoms with abundant neurotoxic components. Composition and toxicological properties. A literature review

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ABSTRACT

In South America there are three snake genera with predominantly neurotoxic venoms: *Crotalus, Micrurus* and *Hydrophis*, which include nine species/subspecies, 97 species and a single marine species, respectively. Although accidents with neurotoxic venoms are less frequent than those with anticoagulant, cytotoxic or necrotic venoms (e.g. from *Bothrops*), they are of major public health importance. Venoms from genus *Crotalus* have been extensively studied, while data on the venoms from the other two genera are very limited, especially for *Hydrophis*.

The venoms of North and South American *Crotalus* species show biochemical and physiopathological differences. The former species cause bothrops-like envenomation symptoms, while the latter mainly have neurotoxic and myotoxic effects, leading to respiratory paralysis and, occasionally, renal failure by myoglobinuria and death, often with no local lesions. *Micrurus* and *Hydrophis* also cause neurotoxic envenomations.

Many studies have isolated, identified and characterized new enzymes and toxins, thus expanding the knowledge of snake venom composition.

The present review summarizes the currently available information on neurotoxic venoms from South American snakes, with a focus on protein composition and toxicological properties. It also includes some comments concerning potential medical applications of elapid and crotalic toxins.

## 1. Introduction

South American snake venoms with abundant neurotoxic components are present in species belonging to the *Crotalus* (Viperidae family), *Micrurus* and *Hydrophis* (Elapidae family) genera, which are commonly called rattlesnakes, coral snakes and sea snakes, respectively (de Oliveira, 2009; Gutierrez, 2011; Brischoux et al., 2016). In South America, *Crotalus* is composed of three species (**Table 1**) with *Crotalus durissus* being the most abundant (**Figure 1.A, 1.B, 1.C and 1.D**). It includes nine subspecies distributed throughout South America to northern Argentina (Martino et al., 1979; Acosta de Perez et al., 1997; Pinho and Pereira, 2001; de Oliveira, 2009; Gutierrez, 2011; Giraudo et al., 2014; Nori et al., 2014; Araújo et al., 2016; Garcia Denegri et al., 2019).

Among the South American genera of family Elapidae, *Micrurus* (figures 1.E to 1.M) comprises about 97 species/subspecies (Da Silva and Stites, 1999; Campbell and Lamar, 2004; Olamendi et al., 2008; Correa-Netto et al., 2011; Nori et al., 2014; Tanaka et al., 2016; Garcia Denegri et al., 2019) (**Table 2**), while *Hydrophis* is represented by *Hydrophis platurus* (yellow-bellied sea snake, **Figure 1.N**). The latter, which is the only South American species of the rare subfamily Hydrophiinae (**Table 2**), is occasionally found off the North Pacific and Caribbean Sea coasts (Campbell and Lamar, 2004; Brischoux et al.,

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**Table 1**
Distribution of South American species and subspecies of rattlesnakes.

| Family | Subfamily | Genus | Specie | Subspecie | Distribution |
|---|---|---|---|---|---|
| Viperidae | Crotalinae | *Crotalus* | *C. unicolor* | | Ven. |
| | | | *C. vegrandis* | | Ven. |
| | | | | | Gua. |
| | | | | *C.d. durissus* | Gua. |
| | | | | *C.d. cascavella* | Bra. |
| | | | | *C.d. collilineatus* | Bra. |
| | | | | *C.d. cumanensis* | Col, Gua, Ven. |
| | | | *C. durissus* | *C.d. drynas* | Bra, Gua. |
| | | | | *C.d. marajoensis* | Bra. |
| | | | | *C.d. runuima* | Bra, Gua. |
| | | | | *C.d. terrificus* | Arg, Bol, Bra, Par, Per, Uru. |
| | | | | *C.d. trigonicus* | Bra, Gua. |

**Reference**: Arg: Argentina, Bol: Bolivia, Bra: Brazil, Col: Colombia, Gua: Guayana, Par: Paraguay, Per: Peru, Uru: Uruguay, Ven: Venezuela (Uetz, 2010)

2016; Durban, Sasa and Calvete, 2018).

Although accidents caused by the above-mentioned snakes are less frequent than those by genus *Bothrops* and *Lachesis*, they are of major public health importance. Indeed, their venoms have myotoxic and neurotoxic effects, which may be highly lethal as in the case of *Crotalus durissus terrificus* (*C.d.t.*) (Bucaretchi et al., 2002). About 30% of snakebites in Latin America occur in South America, mainly due to vipers of genus *Bothrops*, followed by *Crotalus* species and, to a lesser extent, *Micrurus* species (less than 1%) (Kasturiratne et al., 2008; de Roodt et al., 2013; Gutierrez et al., 2011, 2018; Tanaka et al., 2016; García Denegri et al., 2019). There are only a few cases involving sea snakes.

In Latin America, as in other parts of the world, the number of snakebites is underestimated because of the incomplete information available in health centers. Bearing this in mind, the number of registered deaths in Latin America ranged from 540 to 2300 in 2007 (Kasturiratne et al., 2008), while in South America average annual mortality was about 257 deaths for the period 1980-2015 (Chippaux, 2017).

The large amount of information on bothropic envenomation is most likely attributed to a higher frequency of snakebites by *Bothrops*, while *Crotalus* venom has been thoroughly investigated for more than 70 years (Neumann and Habermann, 1955; Hendon and Fraenkel-Contrat 1971; Canziani, 1984; Araújo et al., 2016; Gutierrez, 2018). The isolation and characterization of toxins from the venom of South American *Crotalus* indicate that it is composed of crotoxin (CTX, a group II phospholipase $A_2$, $sPLA_2$), constituting over 50% of the mass venom; thrombin-like enzymes (TLE), with serine protease structure; L-amino acid oxidases (LAAOs) and crotamine (CTM), among others (Alexander et al, 1988; Faure and Bon, 1988; Aird et al., 1989; Francischetti et al., 1997; Georgieva et al., 2010).

In turn, the main toxins found in the South American genera *Micrurus* and *Hydrophis* are three-finger α-neurotoxins (3FT-α) and group I $sPLA_2$ (Lomonte et al., 2016; Aird et al., 2017; Dashevsky and Fry, 2018). They induce acute neurotoxicity, leading to respiratory paralysis and death (Kitchens and Van Mierop, 1987; Gutierrez et al., 2009; Corrêa-Netto et al., 2011; Zornetta et al., 2012; Foo et al., 2019). Experimental studies with *Micrurus* venoms reported myotoxic effects in mice (de Roodt et al., 2012) but only a few human cases have been documented compatible with the experimental myotoxicity observed (Manock et al., 2008; Bucaretchi et al., 2016).

Unlike other types of venoms such as bothropic venoms (e.g., *B. alternatus, B. asper*, or *B. atrox* species) that provoke tissue destruction and a strong inflammatory response due to their proteolytic and cytotoxic components (Teixeira et al., 2009, Gutierrez et al., 2010; 2018), neurotoxic venoms may frequently lead to severe envenomation followed by death. For this reason, these very toxic venoms (at a very low lethal dose -LD-) cannot be inoculated in large quantities, making the immunization process more difficult (Fusco et al., 2015). While *C.d.t.* and *Micrurus* venoms produce little or no local effect, bothropic envenomation causes tissue destruction, necrosis and edema (Ownby, 1982; Azevedo-Marques et al., 1985; Garcia Denegri et al., 2019). In either case (hemotoxic/cytotoxic or neurotoxic venoms), understanding the mechanisms underlying responses is important for different purposes. For example, knowledge of the precise mechanisms involved in local signs of envenomation will contribute to the resolution of the inflammatory process (i.e., formation of scar tissue). Moreover, some neurotoxic components usually induce immunosuppression in certain experimental models, suggesting that better knowledge of their immunological properties is useful to obtain neutralizing antibodies in the serum from hyperimmunized animals (Cardoso and Mota, 1997; Sampaio et al., 2001, 2003; Cruz et al., 2005; Zuliani et al., 2005; Teixeira et al., 2009, Gutierrez et al., 2010; 2018).

Given that available data on neurotoxic venoms are scarce in contrast to bothropic ones, this review focuses on compiling and updating relevant information on South American snake's venoms of public health importance, with abundant neurotoxic components. A description of their composition and toxic activity is presented together with the pharmacology of some components, which may serve as useful tools for future studies of different human diseases. We also addressed the venoms of genus *Micrurus* and *Hydrophis*, for which there is very little information.

This review is based on currently published data from *in vivo* and *in vitro* experiments and includes reports of human envenomations. Finally, we provide data overlooked by previous reviewers, which are compiled in a few pages easily accessible to readers.

## 2. Envenomation by South American snakes

### 2.1. Envenomation by Crotalus species

It is worth noting that the venom of rattlesnakes differs biochemically between South American species (particularly *C.d.t.*) and North and Central American species, preventing the extrapolation of results. North American species have hemotoxic and histotoxic venoms, which usually induce responses similar to those caused by *Bothrops* species. An exception is provided by *Crotalus scutulatus* (Mohave rattlesnake) with highly neurotoxic, type-A venom due to the presence of the Mojave toxin, a crotoxin-related neurotoxin (Clark et al., 1997). The signs and symptoms of envenomation produced by South American *Crotalus* species are shown in **Table 3,** where it can be seen that they are predominantly neurotoxic. Envenomation may lead to respiratory paralysis followed by death, generally without local tissue damage (Gutierrez et al., 2018) but with variable systemic myotoxicity, rhabdomyolysis and coagulopathies (de Sousa et al., 2003; Warrell, 2004; Azevedo Marques et al., 2009). Myotoxic effects are clearly evidenced by high levels of creatine kinase in blood and muscle myonecrosis (Cupo et al., 1988; Vital-Brazil and Fontana, 1993; Ruiz de Torrent et al., 2002; Rangel Santos et al., 2004a). The venoms of South American *Crotalus* species possess group II $sPLA_2$ as the main enzyme, representing the main contributor to overall lethality (Breithaupt, 1976; de Roodt, 2002; Gutierrez et al., 2009; García Denegri et al., 2019).

### 2.2. Envenomation by Micrurus species

Envenomations caused by South American elapids are characterized by pain, absence of local effects and, like their relatives from the Old Continent such as kraits and cobras, predominance of neurotoxicity due to the occurrence of neuromuscular blockade (Vital-Brazil and Barrio,


**Fig. 1.** Some representative species/sub-species of the three genera of South American snakes venoms with abundant neurotoxic components **A**: *Crotalus durissus terrificus*; **B**: *Crotalus durissus;* **C**: *Crotalus durissus ruruima;* **D**: *Crotalus durissus cumanensis;* **E**: *Micrurus altirostris;* **F**: *Micrurus corallinus;* **G**: *Micrurus mipartitus;* **H**: *Micrurus nigrocinctus;* **I**: *Micrurus ibiboboca;* **J**: *Micrurus frontalis;* **K**: *Micrurus spixii;* **L**: *Micrurus surinamensis;* **M**: *Micrurus pyrrhocriptus;* **N**: *Hydrophis platurus.* Photographs retrieved from ArgentíNat, www.argentinat.org except photo **C** from www.flickr.com (Accesed 08 March 2021). Authors: (A) De Angeli Elston, J.G., 2020; (B) Pereira, G. M., 2019; (C) Uwe, K., 2018; (D) Pereira, G. M., 2019; (E) Funes, L., 2011; (F) Mendez, F., 2017; (G) Yánez-Muñoz, M. H., 2017; (H) Acosta Chavez, V., 2012; (I) Silva Lopes, J. R., 2019; (J) Costa, H. C., 2019; (K) Dantas, S., 2017; (L) Sullivan, R., 2013; (M) Herpguy, 1996; (N) Flaxington, 2006.

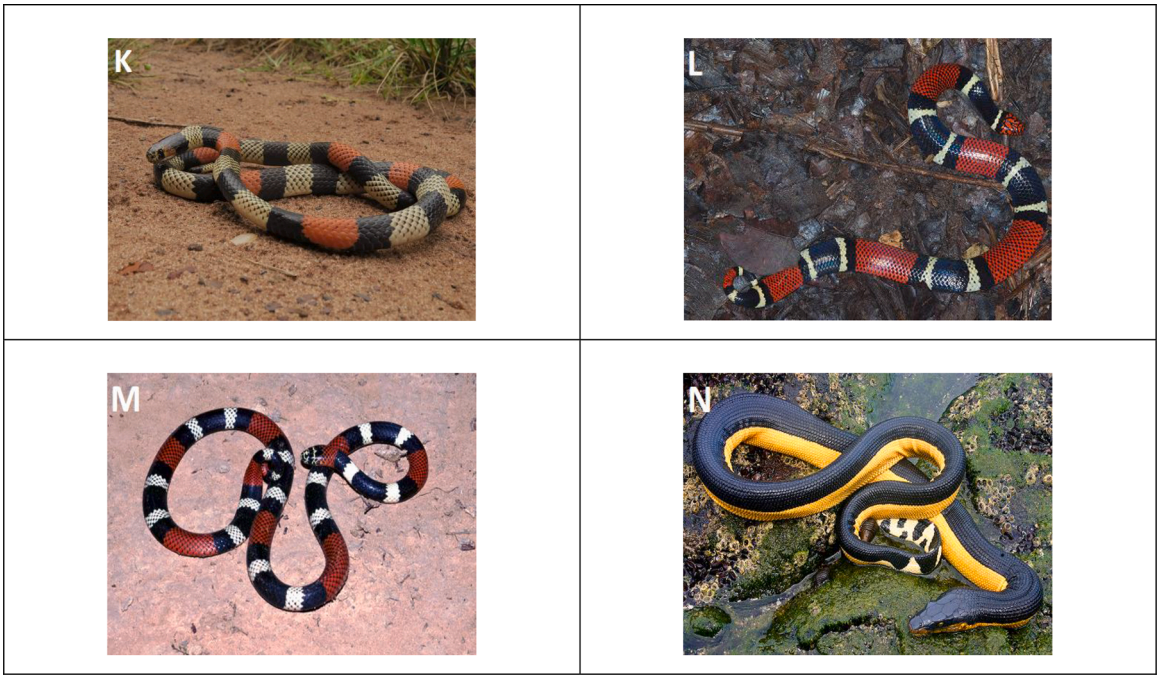


**Fig. 1.** (*continued*).

1950a and Vital-Brazil and Barrio, 1950b; 1987; Ainsworth et al., 2018; de la Rosa et al., 2018; Tan et al., 2019; Trento et al., 2019; Nielsen, Frank and Turchioe, 2019). Neurotoxicity is induced by snake venom α neurotoxins, which belong to the family of three-finger toxins (3-FTxs) and to group I $sPLA_2$ (Dashevsky and Fry, 2018; Gutierrez et al., 2018; Foo et al., 2019). These toxins cause the following signs and symptoms: eyelid ptosis, diplopia, ophthalmoplegia, dysarthria, and eventually respiratory paralysis that may lead to death (**Table 4**) (de Roodt et al. 2013; Bucaretchi et al., 2016). As in the case of *C. durissus*, experimental studies reported that $sPLA_2$ from South American elapids induces myotoxicity and neurotoxicity (Rosso et al., 1996; Dal Belo et al., 2005; Olamendi Portugal et al., 2008; Fernandez et al., 2011; de Roodt et al., 2012; Vergara et al., 2014; Gutierrez et al., 2018). However, myotoxicity is rare in humans (Bucaretchi et al., 2016).

In addition, some of these venoms produced myotoxic and nephrotoxic effects and coagulation disorders under certain experimental conditions (de Roodt et al., 2012; Gutierrez et al., 2018).

### 2.3. Envenomation by Hydrophis species

Although there are very few human cases involving this sea-snake because of its non-aggressive behavior and infrequent encounters, its myotoxic and neurotoxic venom is extremely toxic and lethal (Shin et al., 2003). This is mainly due to the action of the α-neurotoxins acting as antagonists of skeletal muscle nicotinic receptors at the post-synaptic level. The bite is generally slightly painful, but it is followed by muscle tenderness, which persists for several days. In a case reported from Costa Rica, the patient experienced intense pain after the bite, great hypersensitivity, edema and no systemic manifestations (McQuade et al., 2004). In another case report, a patient was diagnosed with Guillain-Barre syndrome (GBS, an acute-onset acquired polyneuropathy) preceded by respiratory or gastrointestinal illness (Hameed et al., 2019). Systemic myotoxicity may cause pain (Tu, 1975). Most envenomations (80%), exhibit classic signs of neurotoxicity such as muscle paralysis, vomiting, abdominal pain, nausea, drowsiness, seizures, headache, restlessness, sweating, and increased body temperature. The severity of neuromuscular toxicity depends on the amount of venom injected. Signs and symptoms usually include cranial nerve palsy, palpebral ptosis, mydriasis, and ophthalmoplegia with blurred vision or diplopia. These are followed by dysphagia, hypersalivation, dysarthria, and tongue disability associated with increased risk of broncho-aspiration. Subsequently, myalgia and spasticity appear in some cervical and dorsal muscles, while tendon reflexes disappear. Finally, respiratory muscle paralysis leads to death (Tu et al., 1975; Lucena et al., 2011). Therefore, in the acute phase death occurs by respiratory paralysis due to the action of neurotoxins (Cañas, Castaño and Castro-Herrera, 2016). In addition, the venom is often responsible of myolysis leading to myoglobinuria, which aggravates the clinical conditions and may cause mortality from renal failure in the final phase.

## 3. Venom composition and toxicological properties

The limited availability of snake venoms is a major limitation in research on toxin composition and characterization. This explains, for example, the smaller number of studies concerning the venoms of South American *Micrurus* and *Hydrophis* snakes compared to those of vipers and even *Micrurus* species.

Venoms are usually obtained by manual pressure or electrical stimulation of the salivary glands, but also from snake venom gland cells cultured *in vitro* (Post et al., 2020). In addition, venom components have been obtained using molecular biology techniques like recombinant DNA with toxin genes and expression in bacteria (de la Rosa et al., 2018). The analysis of snake venoms has improved with technological advances. Many components were isolated and characterized in terms of both structure and physiopathological involvement. In the last decade, mass spectrometry has become a valuable method for proteomics research and this also applies for venomics (Calvete, 2011). As widely known, snake venoms are composed of enzymes, peptides and complex mixtures of inorganic compounds, and their combined action is responsible for the pathological effects of envenomation (de Roodt, 2002; Hayes et al., 2017). It has also been observed that toxins can act synergistically with each other (Faure and Born, 1988; Xiong and Huang, 2018).

Venom composition is variable at the inter- and intraspecific levels. However, the neurotoxic nature of the venom does not change, regardless of intra-specific, seasonal, ontogenetic and geographical

**Table 2**
Distribution of South American species and subspecies of the *Micrurus* and *Hydrophis* genera.

| Family | Subfamily | Genus | Specie | Subspecie | Distribution |
|---|---|---|---|---|---|
| | | | *M. albicinctus* | | Bra, Per. |
| | | | *M. altirostris* | | Arg, Bra, Par, Uru. |
| | | | *M. ancoralis* | | Col, Ecu. |
| | | | | *M.a.ancoralis* | Ecu. |
| | | | | *M.a.jani* | Col. |
| | | | *M. anellatus* | | Bra, Bol, Per. |
| | | | | *M.a.anellatus* | Per. |
| | | | | *M.a.balzanii* | Bol. |
| | | | | *M.a.bolivianus* | Bol. |
| | | | *M. averyi* | | Bra, Gua. |
| | | | *M.balyocoriphus* | | Arg, Par. |
| | | | *M. bocoucorti* | | Ecu, Per. |
| | | | *M. boicora* | | Bra. |
| | | | *M. brasilensis* | | Bra. |
| | | | *M. camilae* | | Col. |
| | | | *M. circinalis* | | Ven. |
| | | | *M. clarki* | | Col. |
| | | | *M. collaris* | | Bra, Gua, Gua F, Sur, Ven. |
| | | | | *M.c.collaris* | Ven. |
| | | | | *M.c.breviventris* | Gua. |
| | | | *M. corallinus* | | Arg, Bra, Par. |
| | | | *M. decoratus* | | Bra. |
| | | | *M. diana* | | Bol, Bra. |
| | | | *M. dissoleucus* | | Col, Ven. |
| | | | | *M.d.dissoleucus* | Col, Ven. |
| | | | | *M.d.dunni* | Col. |
| | | | | *M.d.melanogenys* | Col. |
| | | | | *M.d.nigrirostris* | Col, Ven. |
| | | | *M. diutius* | | Gua, Ven. |
| | | | *M. dumerilli* | | Col, Ecu, Ven. |
| | | | | *M.d.antioquiensis* | Col. |
| | | | | *M.d.carinicaudus* | Col, Ven. |
| | | | | *M.d. colombianus* | Col. |
| | | | | *M.d.dumerilli* | Col. |
| | | | | *M.d.trasandinus* | Col, Ecu. |
| | | | | *M.d.venezuelensis* | Ven. |
| Elapidae | Elapinae | *Micrurus* | *M. filiformis* | | Bra, Col, Per. |
| | | | *M. frontalis* | | Arg, Bra, Par. |
| | | | *M. hemprichii* | | Bol, Bra, Col, Ecu, Gua, Gua F, Sur, Ven. |
| | | | | *M.h.hemprichii* | Col, Gua, Ven. |
| | | | | *M.h.ortoni* | Bol, Bra, Col, Per. |
| | | | *M. ibiboboca* | | Bra. |
| | | | *M. isozonus* | | Bra, Gua, Ven. |
| | | | | *M.i.isozonus* | Col. |
| | | | | *M.i.sendneri* | Col. |
| | | | *M. langsdorffi* | | Bra, Col, Ecu, Per. |
| | | | *M.lemniscatus* | | Arg, Bol, Bra, Col, Ecu, Gua, Gua F, Par, Per, Sur, Ven. |
| | | | | *M.l.lemniscatus* | Bra, Gua. |
| | | | | *M.l.carvalhoi* | Arg, Bra, Par. |
| | | | | *M.l.frontifaciatus* | Bol, Bra. |
| | | | | *M.l.helleri* | Bol, Bra, Col, Ecu, Per, Ven. |
| | | | *M.margaritiferus* | | Per. |
| | | | *M. medemi* | | Col. |
| | | | *M. meridensis* | | Ven. |
| | | | *M. mertensi* | | Ecu, Per. |
| | | | *M. mipartitus* | | Bra, Col. |
| | | | | *M.m.mipartitus* | Bra. |
| | | | | *M.m.anomalus* | Col, Ven. |
| | | | | *M.m.decussatus* | Col, Ecu. |
| | | | | *M.m.popayanansis* | Col. |
| | | | | *M.m.rozei* | Ven. |
| | | | *M. multifasiatus* | | Col. |
| | | | *M. multisculatus* | | Col, Ecu. |
| | | | *M. narducci* | | Bol, Bra, Col, Ecu, Per. |
| | | | | *M.n.narducci* | Bol. |
| | | | | *M.n.melonotus* | Ecu. |
| | | | *M. nattereri* | | Bra, Col, Ven. |
| | | | M.nigrocinctus | | Col. |
| | | | M.obscurus | | Bol, Bra, Col, Per. |
| | | | *M.oligoanellatus* | | Col. |
| | | | *M. ornatissimus* | | Col, Ecu, Per. |
| | | | *M. pacaraimae* | | Bra. |
| | | | *M. paranaensis* | | Bra, Sur. |
| | | | *M. peruvianus* | | Per. |

(*continued on next page*)

**Table 2** (*continued*)

| Family | Subfamily | Genus | Specie | Subspecie | Distribution |
|---|---|---|---|---|---|
| | | | *M. petersi* | | Ecu. |
| | | | *M. potyguara* | | Bra. |
| | | | *M. psyches* | | Bra, Col, Gua F, Sur, Ven. |
| | | | *M.putumayensis* | | Bra, Col, Per, Ven. |
| | | | *M.pyrrhocryptus* | | Arg, Bol, Bra, Par. |
| | | | *M. remotus* | | Bra, Col, Ven. |
| | | | *M. renjifoi* | | Col. |
| | | | *M. sangilensis* | | Col. |
| | | | *M. scutiventris* | | Bra, Col, Ecu, Per. |
| | | | *M. serranus* | | Bol. |
| | | | *M. silviae* | | Arg, Bra, Par. |
| | | | *M. spixii* | | Bol, Bra, Col, Ecu, Ven. |
| | | | *M. spurelli* | | Col. |
| | | | *M.steindachneri* | | Ecu, Per. |
| | | | | *M.s.steindachneri* | Ecu. |
| | | | | *M.s.orcesi* | Ecu. |
| | | | *M. surinamensis* | | Bol, Bra, Col, Ecu, Gua, Gua F, Per. |
| | | | *M. tikuna* | | Bra, Col. |
| | | | *M. tricolor* | | Bra, Par. |
| | | | *M. tschudii* | | Bol, Ecu, Per. |
| | | | | *M.t.tschudii* | Ecu, Per. |
| | | | | *M.t.olssoni* | Ecu, Per. |
| | Hydrophis | *Hydrophiinae* | *H. platurus* | | Pacific coast of Chi, Col, Ecu, Per. |

**Reference**: Arg: Argentina, Bol: Bolivia, Bra: Brazil, Chi: Chile, Col: Colombia, Ecu: Ecuador, Gua: Guayana, Gua F: Guayana Francesa, Par: Paraguay, Per: Peru, Uru: Uruguay, Ven: Venezuela (Uetz, 2010).

differences (Chippaux et al., 1991; Alape-Girón et al., 2008; Lanari et al., 2010, 2014; Calvete et al., 2010; Calvete, 2011).

Below we describe the main neurotoxic components of the venoms from South American snakes and provide a brief mention of those that are less abundant and exhibit other toxic activities (e.g. myotoxic and cytotoxic).

As previously stated, the medical importance of predominantly neurotoxic venoms relies on their lethality. This is supported by different studies carried out with the venom of *C.d.t.* from northern Argentina and southern Brazil (Hendon and Faenkel-Conrat, 1971; Breithaupt, 1976; Sanchez et al., 1992; Ruiz de Torrent et al., 2002, Rangel Santos et al., 2004a; Rodriguez et al., 2012). Many neurotoxins from some northern *Micrurus* species are not lethal to mammals and reptiles but induce paralysis of prey (Benard-Valle et al., 2012). In addition, only a few South American *Micrurus* species (e.g., *M. corallinus* and *M. spixii*) exhibit pre- and post-synaptic neurotoxicity, their venoms being the most potent within this genus (Vital-Brazil, 1987; da Silva and Aird, 2001; Tanaka et al., 2010) and among South American snakes.

**Table 5** shows $LD_{50}$ (in μg / 18-22 g mice, i.p., 48 h) for different representative South American species with non-neurotoxic venoms, such as *Bothrops* species (included for comparative purposes), as well as South American species of *Crotalus* and *Micrurus*. So far, lethality data are not available for *Hydrophis platurus*. Interestingly, the neurotoxic venoms of *Crotalus* and *Micrurus* species show a higher lethality (lower $LD_{50}$ values) than do bothropic ones.

In addition, **Table 5** presents a comparison of myotoxicity scores for genus, species and subspecies based on serum creatine kinase levels and expressed in micrograms of venom inoculated. In the myotoxicity test, groups of four mice were injected i.m. in the right gastrocnemius with venom and plasma creatine kinase levels were quantified 3 h after injection (for detailed information see bibliography). It should be noted that the most lethal venoms of *Crotalus* generally show the highest myotoxic activity.

### 3.1. Venom from Crotalus snakes

The venoms of the South American species and subspecies of the genus *Crotalus* contain enzymes and toxins of predominantly neurotoxic action. Almost all of them comprise $sPLA_2$ enzymes and proteases (serine and, to a lesser extent, metalloproteases), followed by crotamine (CTM), in this case only in some groups from certain regions, C-type lectin-like proteins (CTLLP), L-amino acid oxidases (LAAO) disintegrins, cysteine-rich secretory proteins (CRISPs), and a number of vasoactive peptides, among others (Alexander et al, 1988; Faure and Bon, 1988; Aird et al., 1989; Francischetti et al., 1997; Alape-Girón et al., 2008; de Georgieva et al., 2010; Calvete, 2011; Corrêa-Neto et al., 2011).

The analysis of rattlesnake venoms from Brazil and Argentina (Faure and Bon, 1988; Fusco et al., 2020) revealed the main components and the existence of a large number of homologous proteins. **Figure 2** shows the composition of some South American snake venoms (proteomes). *Bohtrops* venoms possess histotoxic effects due to the large amount of metalloproteinases and serine proteases (and myotoxins in several cases). Except for *C. durissus cumanensis*, the venoms of South American *C. durissus* are primarily composed of Crotoxin (CTX), which is a neurotoxic complex with enzymatic activity. It is composed of a basic subunit with seven intra-disulfide bridges (CTX-B), which belongs to Group II $sPLA_2$ enzymes (D49) with enzymatic activity, and an acid subunit called crotapotin (which means: crotoxin potentiator-inhibitor or CTX-A) deprived of catalytic activity. Both components are linked by intermolecular forces (Canziani et al., 1982, 1985; Six and Dennis, 2000). Furthermore, some CTX components appear as isoforms. Each subunit shows variants and alternative combinations (Raw et al., 1986; Rangel Santos et al., 2004). Likewise, the separation of these components reduces the toxicity of CTX and increases the enzymatic activity of the B subunit (Hendon and Faenkel-Conrat, 1971; Breithaupt, 1976; Hendon and Tu, 1979; Canziani et al., 1983), allowing its use as an immunogen (Kaiser et al., 1986; Kaiser and Middlebrook, 1988; Choumet et al., 1988; Higashi et al., 1989; Dos Santos et al., 1989; Cardoso et al., 2000; Guidolin et al., 2013; Fusco et al., 2015b). Crotalic $sPLA_2$ enzymes hydrolyze phospholipids releasing fatty acids and lysophospholipids due to their intrinsic catalytic activity. This affects the properties of neurons at the presynaptic level of the myoneural junction, causing neurotoxic effects. In addition, $sPLA_2$s bind to muscle fibers where the membrane-bound protein interactions induce myotoxic effects. It is not totally clear where the crotoxin binds to cellular structures to exert the damage. In the axon of *Torpedo marmorata*, CTX binds with high affinity to a 48-kDa protein. As in the case of other venom-derived $sPLA_2$, an individual sample of CTX may contain a number of $sPLA_2$ isoforms. Four isoforms of CTX-A and CTX-B are likely to coexist and the toxicity and stability of CTX depends on the specific CTX-B isoform involved in the formation of the complex. Some CTX-B isoforms can form heterodimers which are inhibited by CTX-A. Nevertheless, it has

**Table 3**
Envenomation and toxicological role of the South American Crotalus venom.

| Clinical observations[a,b,c,d,e] | Toxicological properties of the South American Crotalus venom | | |
|---|---|---|---|
| | Neurotoxic | Myotoxic | Coagulant |
| Neurotoxic, myotoxic and coagulant syndrome. **-Local manifestations:** Minimal local signs characterized by mild erythema, with or without mild edema at the bite site. There is no pain in the bitten area, there may even be hypoesthesia or anesthesia. **- General manifestations:** Patients quickly develop blurred vision, diplopia, anisocoria, decreased visual acuity, eyelid ptosis (myasthenic facies), difficulty speaking, myalgia and darkening of the urine (myoglobinuria). Blood clotting disorders up to incoagulability and general manifestations such as nausea, vomiting, sweating, drowsiness, restlessness or agitation are observed in approximately half of the cases. In severe envenomations, respiratory paralysis and acute kidney failure can occur. Neurological signs and symptoms persist for about 2 weeks, gradually disappearing without apparent sequelae. | By presynaptic action on neuromuscular endings, inhibiting the release of acetylcholine (Ach), and inducing motor paralysis. | Due to lesions in skeletal muscle fibers (rhabdomyolysis) with myoglobinuria (dark urine), and nephrotoxicity with the development of acute renal failure. | Due to the action of the coagulant components (prolongation of the clotting time until blood incoagulability owing to consumption of fibrinogen). In any case, coagulation alterations are not always seen in injured people. |

[a] Ministerio de Salud, 2007
[b] Fundaçao Nacional de Saúde, 1999
[c] Vital-Brazil et al.1972
[d] Azevedo Marques et al., 1985
[e] Cupo et al. 1988.

been proposed that, *in vivo*, the binding of the CTX complex and the release of the CTX-A subunit might allow the formation of the CTX-B dimer leading to the full expression of neurotoxicity (Harris et al., 2013). These alterations are predominant and characteristic of envenomations inflicted by these snakes (van Deenen and de Haas, 1963; Gopalakrishnakone et al., 1984; Hawgood, 1990; Bon, 1997). The neurotoxic action has been postulated to be produced by presynaptic blockade of acetylcholine release due to damage to the membrane and mitochondria of the terminal neuron (Gopalakrishnakone et al., 1984). It could also result from a nonspecific mechanism at the postsynaptic level by sensitization of the acetylcholine receptor, as described in other neurotoxic envenomations (Ranakawa et al., 2013).

**Table 4**
Envenomation and toxicological role of the South American Elapidae venom.

| Clinical observations[a,b,c,d] | Toxicological properties of the South American Elapidae venom |
|---|---|
| Rapid neurotoxic syndrome, usually within the first hour of intoxication and is considered potentially serious. **Local manifestations**: they are minimal, with or without mild pain, little edema, with hypo or anesthesia. **General manifestations**: characterized by neuro-muscular, motor and sensory involvement, with progress from the bite site with paralysis of the facial muscles, pharyngolaryngeal (suffocation crisis, sialorrhea, dysphagia), ocular (palpebral ptosis, myasthenic facies, ophthalmoplegia, anisocoria), intercostals and diaphragm. Paralysis of the respiratory muscles can lead to respiratory failure and death. Coagulation disorders and myoglobinuria have not been clinically described. | Predominantly neurotoxic action due to neurotoxins in the venom. In most species they act at the post-synaptic level, with few exception of *M. corallinus* and *M. dumerili*, which would act at the pre- and post-synaptic level. Neurotoxins block the binding of acetylcholine (Ach) to nicotinic cholinergic receptors on the neuromuscular plaque (postsynaptic level; curare-simile clinical effect). At the pre-synaptic level, they inhibit the release of Ach from nerve endings. |

[a] Ministerio de Salud, 2007
[b] de Roodt et al. 2013
[c] Bucaretchi et al. 2016
[d] Fundaçao Nacional de Saúde, 1999.

On the other hand, some South American specimens of *C. durissus* have a low-molecular-weight basic toxin called CTM, which blocks voltage-dependent sodium channels. CTM is a polypeptide with myotoxic activity (α-myotoxin) (Mebs and Ownby, 1990; Vital-Brazil, 1990b) and high degree of intracellular penetration, capable of interacting with proteoglycans of the extracellular matrix (Matsubara, 2009) and cell membrane lipids; it forms vacuoles and shows myonecrotic activity (Araujo et al., 2016).

Gyroxin is another toxin present in crotalic venoms affecting the central nervous system. It is a heat-labile 33,000-35,000 kDa protein (Vital-Brazil, 1972; Seki et al., 1980) associated with Np-tosyl-l-arginine methyl ester (TAME) esterase activity (Barrabin et al., 1978). The i.v. injection of gyroxin into mice produces temporary episodes characterized by opisthotonos and rotations around the long axis of animals (Barrio, 1961). Later, Alexander et al (1988) demonstrated that the TLE isolated from *C.d.t.* produces the gyroxin syndrome and that this ability is shared by TLE found in other snake venoms. This enzyme promotes coagulation because it acts as a platelet aggregator (Canziani et al., 1982; Prado Franceschi and Antunes, 1989; Maruñak et al., 2004; Serrano and Maroun, 2005; Calvete et al., 2010; Georgieva et al., 2010). Moreover, gyroxin induces hyperexcitation and running followed by tachypnea, immobility, stretching of posterior limbs and grooming behavior in mice (Barros et al., 2011). A study carried out by Ruiz de Torrent et al (2007) showed that the TLE isolated from *C.d.t.* has neurotoxic effects resulting in histopathological changes, ganglioside modifications in some areas of the brain and behavioral alterations in neonatal rats. Changes in ganglioside composition, which is one of the chemical markers of brain maturation, were especially detected in the hypothalamus, hippocampus, and prefrontal cortex. The animals showed a noticeable delay in maturation of the righting reflex, posture and motor response after TLE treatment. Such behavioral alterations were consistent with histological changes found in the cerebellum and

**Table 5**
Different $LD_{50}$ and myotoxicity for representative species of the *Bothrops, Crotalus* and *Micrurus* genera from South America in experimental designs under animal model (mice 18-22 g). Score index: null myotoxicity (-), moderated myotoxicity (+), and high myotoxicity (++).

| Specie/Subspecie | LD 50 (μg/mice) | Miotoxicity (Score index) |
|---|---|---|
| *Micrurus corallinus* | 7(5-27)[a] | +[b] |
| *Micrurus spixii* | 8(6-16)[a] | ++[c] |
| *Micrurus altirrostris* | 9(7-13)[a] | ++[d] |
| *Micrurus nigrocinctus* | 10[e] | +[f] |
| *Micrurus mipartitus* | 9[g] | -[f] |
| *Micrurus isozonus* | 11.3[h] | |
| *Micrurus lemniscatus* | 13(7-22)[a] | +[b] |
| *Micrurus frontalis* | 22(4-29)[a] | +[f] |
| *Micrurus hemprichii* | 47(20-88)[a] | +[b] |
| *Micrurus surinamensis* | 58(43-87)[a] | -[f] |
| *Micrurus ibiboboca* | 76 (67-79)[a] | +[b] |
| *Micrurus spixii spixii* | | +[b] |
| *Micrurus spixii obscurus* | | +[b] |
| *Crotalus durissus terrificus* | 1.7(1.5-2)[g] | ++[i] |
| *Crotalus durissus collineatus* | 1.7(1.4-2.14)[j] | ++[j] |
| *Crotalus durissus cascavella* | 1.6 (1.3-2.0)[j] | ++[j] |
| *Crotalus vegrandis* | 5.3[k] | - |
| *Crotalus durissus cumanensis* | 4.2[l] | +[m] |
| *Crotalus durissus ruruima* | 2.4[n] | ++[o] |
| *Bothrops jararaca* | 32.4 (24.6-42.6)[p] | +[p] |
| *Bothrops asper* | 66.2 (49.5 – 88.6)[q] | +[r] |
| *Bothrops alternatus* | 84.8 ± 43.9[s] | +[t] |
| *Bothrops jararacussu* | 74.5 (54.0e100.9)[u] | +[v] |
| *Bothrops moojeni* | 125.1 (80.3e178.9)[u] | +[t] |

[a] Tanaka et al., 2010
[b] Gutierrez et al., 1992
[c] Terra et al., 2015
[d] Moraes et al., 2003
[e] Cecchini et al., 2005
[f] Gutierrez et al., 1983
[g] Baudou et al., 2017; Fusco et al., 2020
[h] Salazar et al., 2011
[i] Fusco et al., 2015a
[j] Santoro et al., 1999
[k] Aguilar et al., 2001
[l] Pirela de las Salas et al., 2006
[m] Nuñez et al., 2004
[n] Dos-Santos et al., 2005
[o] Dos-Santos et al., 1993
[p] Antunes et al., 2010
[q] Otero et al., 2002
[r] Gutierrez et al.,1984
[s] Lanari et al., 2010
[t] Mamede et al., 2016
[u] Oguiura et al., 2014
[v] Maruñak et al., 2007.

prefrontal cortex of newborn rats, both areas being related to motor activities.

Additionally, these venoms contain C-type lectins (CTLs), which belong to an important group of snake venom-derived hemorrhagic proteins (Koh et al., 2006). Convulxin (Prado Franceschi and Vital-Brazil, 1981) stands out among CTLs. It is a high-molecular-weight protein that induces a powerful platelet activation, producing thrombi and emboli in several capillary nets; these cause hypoxias leading to convulsions and alterations in the circulatory and respiratory systems (Lima et al., 2005). Platelet aggregation is mediated by $Ca^{2+}$-dependent reactions when convulxin binds to GPVI platelet receptors (Jandrot-Perrus et al., 1997; Kanaji et al., 2003).

*Crotalus* venoms also include non-neurotoxic enzymes. The L- amino acid oxidase (LAAO) enzymes catalyze the conversion of L-amino acids into $H_2O_2$ and are responsible for the characteristic yellow color of these venoms because they are bound to riboflavin (Mackessy, 2010). Phosphodiesterases (PDEs) have multiple functions in nucleotide metabolism, though their role is not yet fully understood (Georgieva et al., 2010; Fox, 2013). There are minor components such as the C-type natriuretic peptide (CNP), which was isolated from the venom of *C. durissus cascavella*. It has cardiovascular and renal properties that exert diuretic and hypotensive effects due to increased urinary flow and glomerular filtration rate, and decreased mean arterial pressure (Evangelista et al., 2008). Finally, these venoms also possess ecto-5-nucleotidases, glutaminyl cyclase, and nerve growth factors (García Denegri et al., 2019).

### 3.2. Venom from Micrurus snakes

The venoms of South American elapids have been less studied than those of vipers. As mentioned above, this may be mainly due to the difficulty in finding and maintaining them in captivity and to the small amount of venom extracted. In addition, the rarity of *Micrurus* accidents may discourage research on this topic. Most South American elapid venoms contain more non-enzymatic protein toxins than do crotalic and botropic venoms. *Micrurus* venoms have postsynaptic α-neurotoxins of low molecular weight belonging to the 3-FTxs family and some species show a large amount of group I $sPLA_2$ with presynaptic action (β-neurotoxins) (Alape-Girón et al., 2008; Calvete, 2011; Corrêa-Neto et al., 2011; Lomonte et al., 2016; Gutierrez et al., 2018).

α-Neurotoxins are classified as type I (short-chain), type II (long-chain), and basal or ancestral form. Although the high toxicity of many elapid venoms has been associated with short-chain (60-62 amino acids) (Barber et al. 2013; de la Rosa et al., 2019) and long-chain (66-74 amino acids) α-neurotoxins, their functional sites are not identical (Rusmili et al., 2019). They share several common features, but also have significant differences accounting for the observed variation in their specificities (Servent and Menez, 2001; Hedge et al., 2010). Regardless of the functionality of the type of 3-FTxs (change of amino acids in its primary chain and / or amount of disulfide bridges that it has), it is necessary to consider where these neuronal receptors for these toxins are located since they will not necessarily be toxic in envenoming (Benard-Valle et al., 2012). Proteomic studies developed with venoms from different *Micrurus* species identified 3-FTxs and $sPLA_2$s as the main components (Lomonte et al., 2016). However, 3-FTxs were firstly described for some colubrids (Fry et al., 2003; Xiong and Huang, 2018), they are the group of non-enzymatic toxins most commonly found in the majority of elapids (America, Asia and Africa) and hydrophiids (sea snakes), and their presence was suggested in vipers (Shelke et al., 2002). They constitute approximately 20-95% of the venom of *Micrurus* snakes (Harvey, 1991; Kini, 2002) depending on the species analyzed in proteomic studies (**Figure 2**) (Correa Neto et al., 2011; Durban, Sasa and Calvete, 2018; Sanz et al., 2019a, 2019b). The name of the toxin family derives from the three characteristic β-stranded loops extending from a hydrophobic and globular core, cross-linked by four or five conserved disulfide bridges, (Ménez, 1998; Tsetlin, 1999).

In addition to α-neurotoxins, the 3-FTxs comprise κ-neurotoxins, fasciculins, cardiotoxins / cytotoxins, muscarinic toxins, calciseptine and dendroaspins among others (Xiong and Huang, 2018). The short-chain α-neurotoxins act by binding to cholinergic receptors (nicotinic α and non-α subunits of the acetylcholine receptor) at the neuromuscular junction (Tu and Miller, 1989). They induce neuromuscular blockade, respiratory paralysis and finally death (Vital-Brazil and Barrio, 1950a and Vital-Brazil and Barrio, 1950b; Rosso et al., 1996; Nirthanan, 2004; Dal Belo et al., 2005; Olamendi Portugal et al., 2008; Correa Neto et al., 2011; Fernandez et al., 2011; Vergara et al., 2014; Gutierrez et al., 2018). On one hand, the high degree of intra-genus conservation observed in short-chain α-neurotoxins provides insight into the potential evolutionary processes involved in the composition of elapid venoms (Slowinski et al., 1997; Fry et al., 2003; Lynch, 2007). On the other hand, 3-FTxs share structural folds but vary in their primary structures and spatial conformations, which would have contributed to different target sites: α-neurotoxins, which antagonize muscle nAChR; k-bungarotoxins, which recognize neuronal nicotinic receptors;

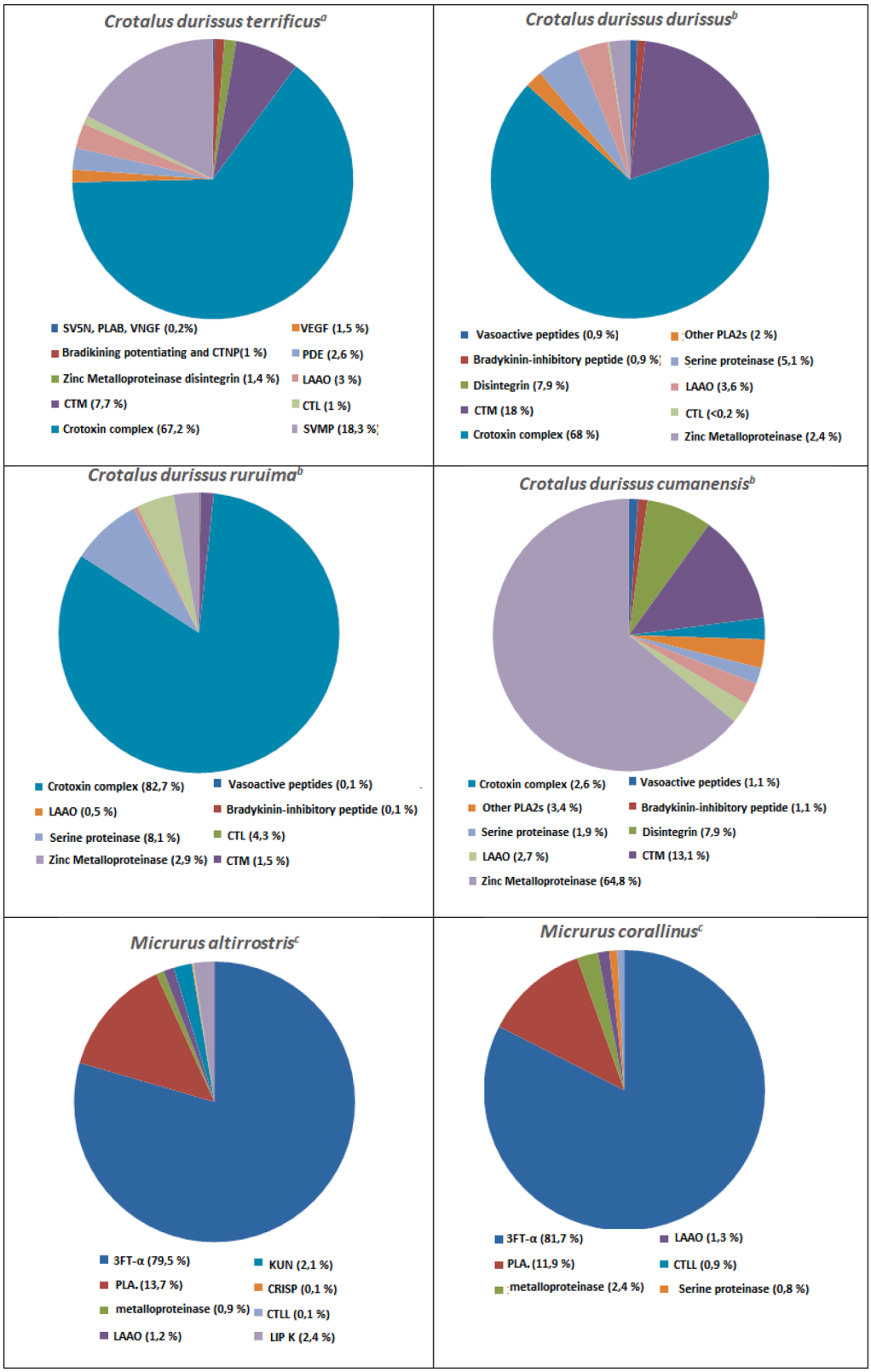


**Fig. 2.** Proteomes of the main and most representative species of *Crotalus, Micrurus, Hydrophis* and *Bothrops* venoms from South America. Generally, a great amount of CTX can be observed in *Crotalus;* SVMP in *Bothrops* and 3 -FTxs and $sPLA_2$ in *Hydrophis* and *Micrurus genera.* SVMP (snake venom metalloproteinase), SVNP (Snake venoms 5 nucleotidase), PLA (phospolipase A), PLA B (phospolipase B), VNGF (venom nerv growth factor), CTNP (C-Type natriuretic peptids), VEGF (Vascular entothelial growth factor), CRISP (cysteine-rich secretory protein), CTM (crotamine), PDE (phosphodiesterases), LAAO (L-aminoacidoxidase), CTL (C-type lectin), CTLL (C-type lectin-like), KUN (Kunitz-type serine protease inhibitor), OHA (ohanin-like), LIP (lysosomal acid lipase A), HYALU (hyaluronidase), ECTON (ecto-59-nucleotidase), GLU TCYC (glutaminyl cyclase). [a]Fusco et al., 2020; [b]Calvete et al., 2010; [c]Lomonte et al., 2016; [d]Durban et al 2018; [e]Sanz et al 2019a; [f]Sanz et al 2019b; [g]Olamendi-Portugal et al., 2018; [h]Sousa et al., 2013; [i] Alape-Girón et al 2008.

muscarinic toxins, with selectivity towards distinct types of muscarinic receptors; fasciculins, that inhibit acetylcholinesterase; calciseptine, that blocks the L-type calcium channels; cardiotoxins / cytotoxins, that exert their toxicity by forming pores in cell membranes; dendroaspins, which are antagonists of various cell-adhesion processes; dendroaspins, which are antagonists of cell-adhesion, and β-adrenoreceptor antagonist β cardiotoxins, and b-cardiotoxins, which bind to b1- and b2-adrenergic receptors (Kini and Doley, 2010). A phylogenetic study carried out by Dashevsky and Fry (2018) based on sequences from *Micrurus* 3-FTxs revealed that α-neurotoxins belong to 8 monophyletic clades and that

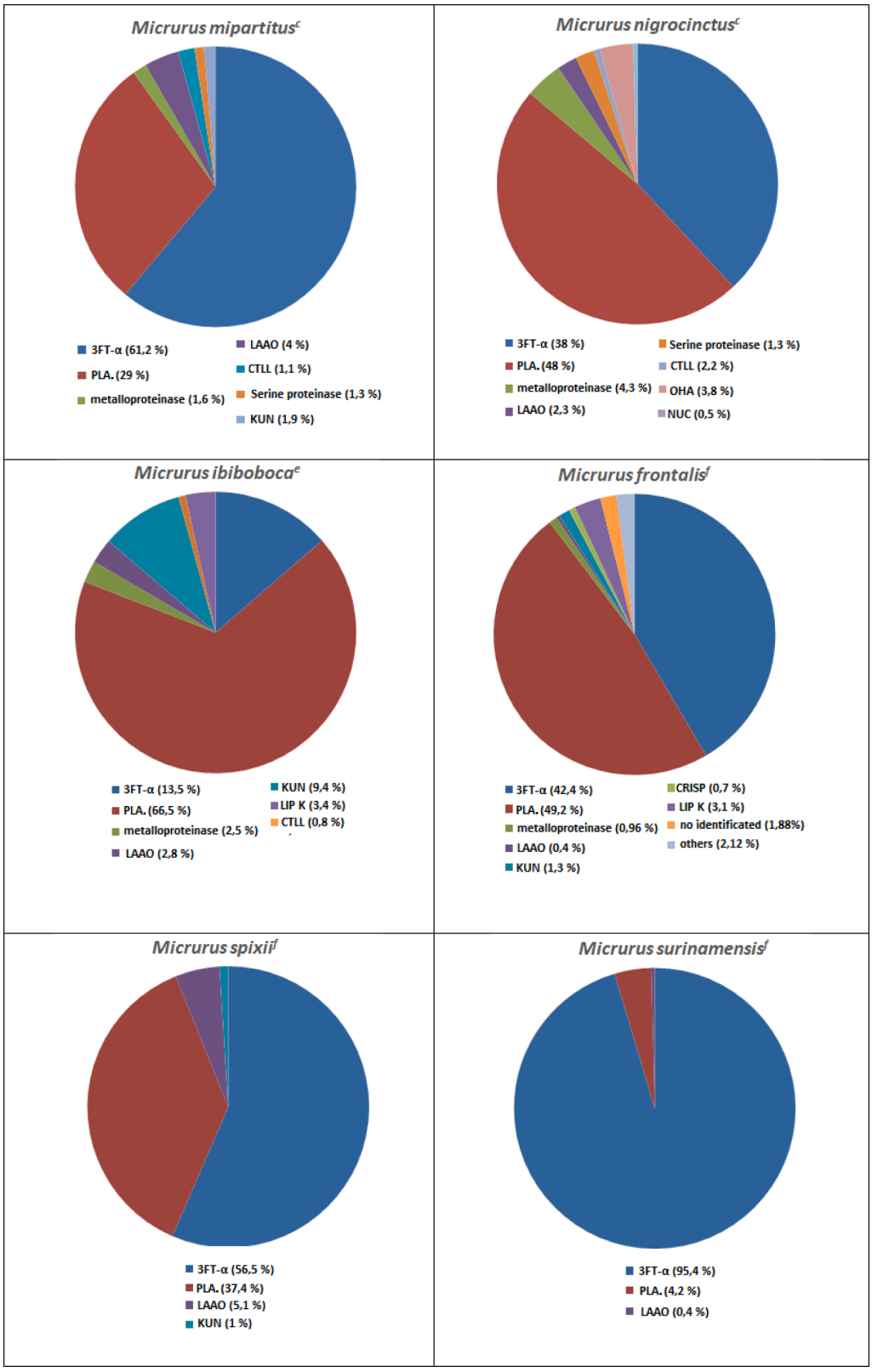


**Fig. 2.** (*continued*).

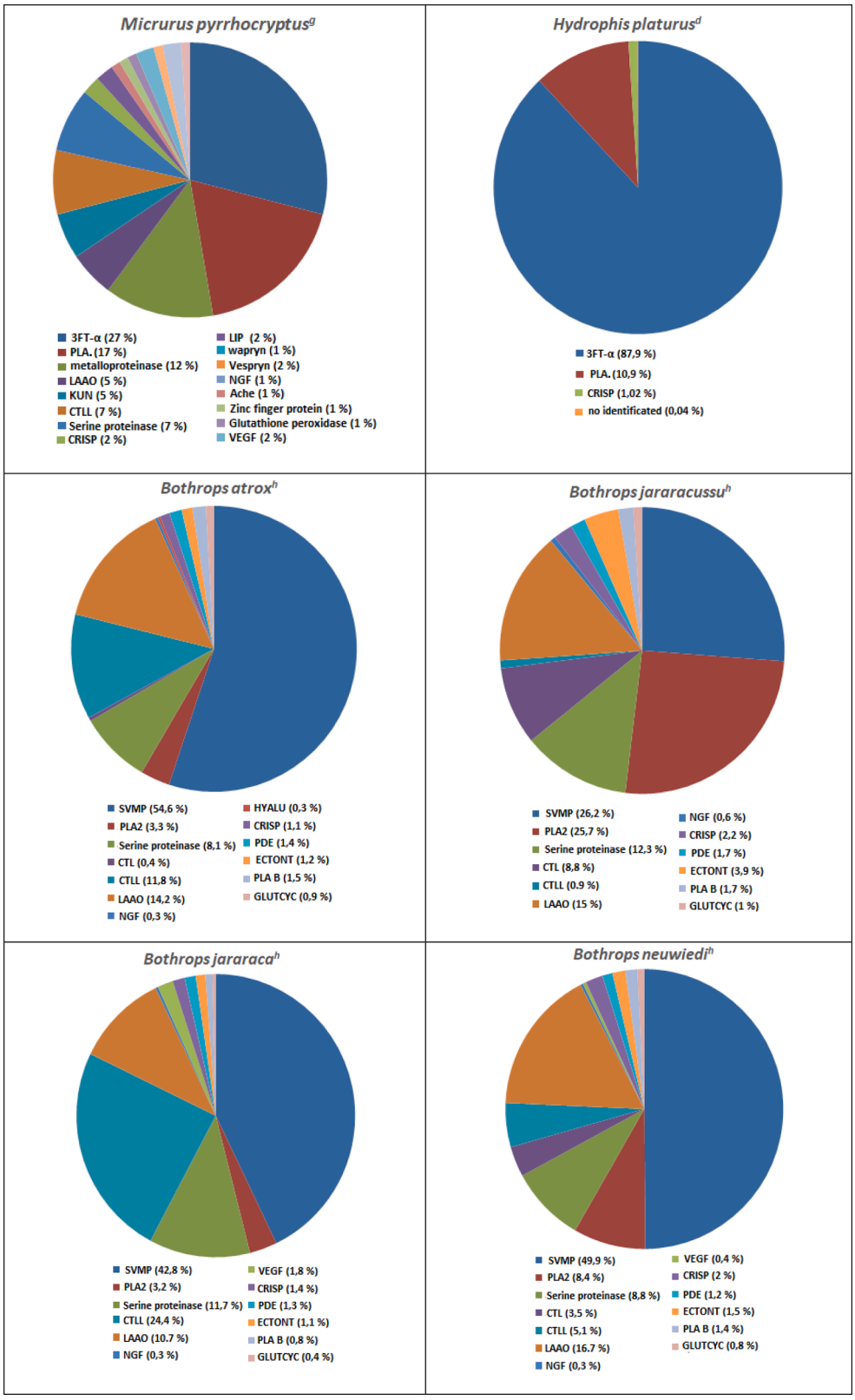


**Fig. 2.** (*continued*).

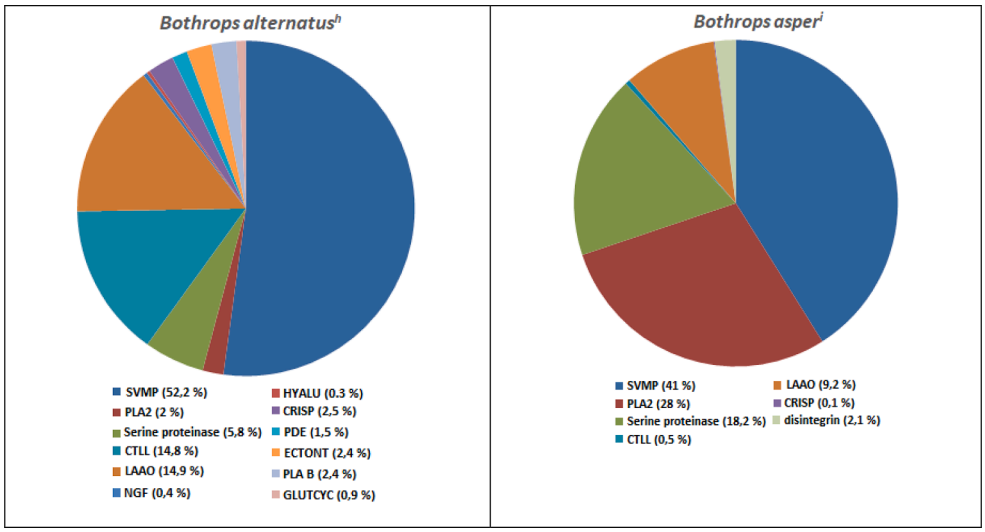


**Fig. 2.** (*continued*).

functional residues would not be well conserved in most of the *Micrurus* toxins. The intra-genus diversity in α-neurotoxin structure may explain the low immunoreactivity of antivenoms against *Micrurus* species (Correa Neto et al., 2011).

On the other hand, the most common enzymes in South American elapids belong to group I sPLA$_2$ of the Phospholipase A$_2$ superfamily, of which some have neurotoxic activity (Six and Dennis, 2000; Lomonte et al., 2016). Proteomic analysis of different *Micrurus* species indicated that this group represents approximately 12-66% of the whole venom (Correa Neto et al., 2011; Lomonte et al., 2016; Aird et al., 2017; Sanz et al., 2019a, 2019b). The neurotoxic mechanisms of action of sPLA$_2$s are not fully characterized (Harris et al., 2013). They could bind to the presynaptic membrane and hydrolyze phospholipids of the outer layer, which alters the structure of the plasma membrane, promoting the exocytosis of neurotransmitters (Rosseto et al., 2008). Then, sPLA$_2$s would enter the neuron and bind to mitochondrial membranes, generating pores that change the shape of axons (Rigoni et al., 2008; Barrientos et al., 2011). Moreover, sPLA$_2$ neurotoxins are known to form complexes with other toxins, such as the sPLA$_2$-KUN complex in the venom of *M. tener* from Texas, USA (Bohlen et al., 2011; Baconguis et al., 2014). These complexes are possibly responsible for pain transmission to immobilize prey. Recently, Sanz et al. (2019b) described for the first time a sPLA$_2$-KUN complex homologous to heterodimeric toxins in the coral snake *Micrurus frontalis*, but its function is not entirely clear. In regard to the non-neurotoxic components, LAAO enzymes, metalloproteinases, phosphomonoesterases, and hyaluronidases have been reported in the venom of *Micrurus* species (Tan and Ponnudrai, 1992; Tanaka et al., 2010; Correa Neto et al., 2011; Vergara et al., 2014).

### 3.3. Venom from Hydrophis snakes

The yellow-bellied *Hydrophis platurus* is the only South American sea snake (family Elapidae, subfamily Hydrophiinae). Its venom is similar to that of *Micrurus* species and contains α-neurotoxins of the 3FTx-α family (e.g. Pelamis toxins α and β) as well as group I sPLA$_2$ and cysteine-rich secretory proteins (CRISP) (Tu et al., 1975; Mori, Ishizaki and Tu, 1989; Durban, Sasa, and Calvete, 2018). Transcriptional analysis of microRNA and mRNA profiles of the venomous glands of *H. platurus* indicated that the venom is mainly composed of 3FTx-α-neurotoxins (88%), followed by sPLA$_2$s (10-11%) and CRISP (1%) (**Figure 2**) (Durban, Sasa and Calvete, 2018). There is no information on the clinical manifestations of envenomation by *H. platurus* (Gutiérrez, 2010) because reports of sea snakebites are very rare. Nevertheless, it is expected that the venom has a neurotoxic effect due to the action of α-neurotoxins, which may provoke flaccid paralysis, systemic myotoxicity and renal failure by myoglobinuria as described after envenomation by other sea snake species (Tu et al., 1989).

## 4. Potential medical applications of crotalic and elapid toxins

### 4.1. Pharmacological applications

Since ancient times, snakes have been associated with the cure of certain diseases (Canziani, 1984). The earliest known relationship between snakes and medicine dates back to ancient Egyptian and Greek civilizations (about 3,000 BC) and concerned the therapeutic properties of snake venoms, including resuscitation. Theriaca, the first important work on toxicology, written by Nikandros of Kolophone in the second century BC, dealt with the action and treatment of poisons and venoms from animals.

The diverse biological functions of snake venom proteins are widely known today. Their enzymes and toxins are of great interest in the field of health sciences and have great potential for the development of new biotherapeutics and as biotechnological tools (Koh et al., 2006; Samy et al., 2017; Duarte et al., 2019). However, *in vitro* results must be interpreted with caution as they may not necessarily lead to therapeutic results.

The first therapeutic drugs based on snake venom components came from the half of XX century (Cooney and Rosemblut, 1975). Clotting factors from snake venoms are used for the treatment of some types of hemorrhage. For example, batroxobin is a coagulant component of bothropic venoms; it is also known as reptilase or hemocoagulase and sold under the brand names Botropase®, Defibrase® and Plateltex® (Bruck and Salem, 1954; Yamanaka et al., 1968; Shenoy et al., 2014; Bordon et al., 2020).

The widely known Captopril®, which was discovered in *Bothrops jararaca* venom in 1975, is the first orally active inhibitor of the AChE enzyme to treat hypertension (Patlak, 2003) (Patlak, 2003). Integrilin® (Eptifibatide) and Aggrastat® (Tirofiban), derived from the venoms of *Sistrurus miliaris* and *Echis carinatus*, respectively, have been approved by the FDA (Tarek Mohamed Abd El-Aziz et al., 2019) as anti-platelet drugs, among other pharmacological functions (Lazarovici et al., 2019). Several new drugs are under clinical evaluation.

Like other snake venoms, those from *Crotalus* and *Micrurus* are a rich source of enzymes and toxins useful for the development of new biotechnological tools with therapeutic applications (Soares, 2012; Ollero et al., 2016; Costa et al., 2018; Muller et al., 2018; Foo et al., 2019; Baudou et al., 2020). Their toxins have gained growing interest as

antitumor agents against different types of cancer and as analgesics, bactericides and regulators of blood pressure. Moreover, they are increasingly being investigated for the treatment of diseases such as Alzheimer's disease, Parkinson's disease, cystic fibrosis and even some forms of schizophrenia (Koh et al., 2006; Khusro et al., 2018).

Compounds isolated from the venom of South American snakes such as CTX of rattlesnakes and 3-FTx α-neurotoxins of elapids show immunomodulatory properties and different patents have been obtained by different research groups (Sampaio et al., 2010; Zhang et al., 2010; Odolczyk et al., 2013; Han et al., 2014; Wang et al., 2014; Faure et al., 2016; Samy et al., 2017; Vulfius et al., 2017; Khusro et al., 2018; Muller et al., 2018; Foo et al., 2019; Tarek Mohamed Abd El-Aziz et al., 2019; Santos et al., 2020).

CTX, which is the first snake-venom toxin to be crystallized (Slotta and Fraenkel-Contat, 1938) has been extensively characterized. It shows immunomodulatory, antibactericidal, antiviral, antitumor and analgesic activities, and also modulates hemostasis and calcium channels in myocytes, among other effects (Sampaio et al., 2010; Han et al., 2014; Zhang et al., 2010; Odolczyk et al., 2013; Wang et al., 2014; Khusro et al., 2018; Araujo et al., 2016). Its antitumor properties were demonstrated *in vivo* with murine models and *in vitro* using cultures of different human tumor cell lines (Corin et al., 1993; Calmette et al., 1993; Rudd et al, 1994; Faure et al., 1993; Rudd et al., 1994; Donato et al., 1996). This enzyme was even tested in a Phase I Clinical trial (Cura et al., 2002).

Recently, $sPLA_2$s from *C.d.t.* were found to have *in vitro* cytotoxic, antiproliferative and pro-apoptotic activities on tumor models of human cancer, including brain glioma, pituitary adenoma and pancreatic, cervical, and esophageal cancer cell lines (Soares et al., 2010; Muller et al., 2018).

CTX is able to trigger the intrinsic pathway of apoptosis (Yan et al., 2006) and its cytotoxicity is mediated by specific interactions with cell-surface receptors associated with damage (Krizaj et al., 2000; Montecucco et al., 2008). On this basis, Muller et al. (2018) proposed the use of CTX in cancer research, but no recent studies have been conducted to test its efficacy.

The role of neurotoxins and myotoxins as cytotoxic agents may sound strange, but it is explained by two mechanisms acting synergistically. One of them is possibly related to the function of CTX subunit A as a "chaperone" molecule, preventing the nonspecific binding of subunit B to membranes (Bon et al., 1979; Habermann and Breithaupt, 1978; Chang and Su, 1981). The other one may be related to the specific binding of CTX to target membranes (Degn et al., 1981; Delot and Bon, 1983), suggesting that subunit A is probably involved in target recognition. CTX would circulate in a non-dissociated form (i.e., as a complex) until it recognizes specific "acceptor sites" on the target membranes. Some of these sites have been identified (Lambeau et al., 1989, 1990). On binding, CTX dissociates into its subunits; subunit B remains bound, whereas the subunit A is released to the medium (Bon et al, 1979; Radvanyi et al., 1979). Thus, subunit A transforms the $PLA_2$ from an unspecific cytotoxin into a self-target toxin compound after binding.

CTX-induced cytotoxic effects appear to be highly selective toward cell lines expressing a high density of epidermal growth factor receptors (Donato et al., 1996), suggesting that epidermal growth factor receptors or a receptor function, play a role in targeting. On binding and subsequent CTX dissociation, the bound subunit B starts a rapid phospholipid hydrolysis around the "acceptor site," a highly localized and/or specialized event as proposed for myotoxic $PLA_2$ (Fletcher et al., 1997; Ownby et al., 1999), which leads to cell death. *In vivo* and *in vitro* studies concerning the action of CTX on different types of tumors have reported different levels of antitumor activity. Growth inhibition was 87% for adenocarcinoma cell lines, 83% for Lewis lung carcinoma, 69% for MX-1 human mammary carcinoma and only 44% for HL-60 leukemia cells, suggesting that CTX has a higher specificity for solid tumors (Newman et al., 1993; Cura et al., 2002; Ye et al., 2011; Hang et al., 2014). In this sense, several mechanisms of antitumoral activity have been proposed, such as phospholipid hydrolysis, apoptosis (Ye et al., 2011; Hang et al., 2014; Muller et al., 2018) and immune-related processes (de Araujo et al., 2019; Freitas et al., 2018).

Based on these data, "*Crotoxin complex as cytotoxic agent*" was patented more than 20 years ago, giving rise to a number of clinical trials of patients with solid tumors refractory to conventional therapies. A daily application for about 30 days reduced the tumor mass by more than 50% in some patients and a complete regression was observed in one of them (Cura et al., 2002). In addition, CTX-B from *C.d.t* venom may have a potential application in cystic fibrosis. Faure et al (2016), using electrophysiological techniques in oocytes, CFTR-HeLa cells and mouse colon tissue demonstrated that this toxin is a new ligand and allosteric modulator of Cystic Fibrosis Transmembrane Regulator (CFTR, a cyclic AMP-regulated chloride channel).

Several experimental studies confirmed the analgesic activity of *C.d.t.* venom in humans (Piccolo et al., 2000, 2004; Nogueira-Netto et al., 2008; Konno et al., 2008; Gutierrez et al., 2008; SantAnna et al., 2019). In rats, CTX had an inhibitory effect on the pain-evoked discharge of neurons in the parafascicular thalamic nucleus or the sciatic nerve (Zhu et al., 2008; Nogueira-Neto et al., 2008), thus exerting an analgesic action on the central nervous system. However, the analgesic mechanisms involved are still under discussion.

The neurotoxic protein CTM from *C.d.t.* venom also showed analgesic capacity in mouse models (Lima et al., 2018), with a potency 30 times higher than that of morphine (Mancin et al., 1998). It is a 5-kDa basic toxin, endowed with a unique biological versatility, acting on sodium channels in the cell membrane, having cell penetration activity and exhibiting selective antitumor activity. Since 2004, Kerkis and collaborators from the Butantan Institute in Brazil have conducted research on the potential use of CTM in clinical oncology (Kerkis et al., 2004, 2010; Nascimento et al., 2007; Hayashi et al., 2008; Pereira et al., 2011; Rádis-Baptista and Kerkis, 2011; Nascimento et al., 2012; Hayashi et al., 2012).

Numerous research groups from North America, South America (Brazilian), Europe and Asia showed that CTM is a promising antifungal drug with low harmful effects on mammalian cells (Yamane et al., 2013). In addition, CTM has proved to be a drug carrier with direct impact on the DNA of proliferating cells (Chen et al., 2012; Rodrigues et al., 2013), to have beneficial effects in the treatment of myasthenia (Hernandez-Oliveira e Silva, 2013), and to inhibit KV channels (Peigneur et al., 2012). Studies addressing the structural characterization of CTM (Ponce Soto et al., 2010; Coronado et al., 2012) have allowed a deep understanding of its mechanism of action at the molecular level. Resistance against most current antibiotics has prompted researchers to investigate the potential use of snake venom-derived peptides as an alternative approach (Samy et al., 2017). The bactericidal effect shown by CTM deserves special consideration, as it has emerged as a new powerful and less toxic natural tool for the rational design of new drugs. CTM and CTM-derived peptides from *C.d.t* venom (10 μg/well) caused marked permeabilization of *Staphylococcus aureus* (Yount et al., 2009) and *Escherichia coli* (at 25-100 μg/ml), emerging as a promising agent with antimicrobial activity (Santamaria et al., 2005). Similar effects have been reported using the isolated subunit CTX-B, which showed bactericidal activity against a wide range of bacterial pathogens depending on the enzymatic activity of $sPLA_2$s (Sampaio et al., 2010).

On the other hand, TLEs are hemotoxic enzymes isolated from *C.d.t.* venom with pharmacological application in hemostatic disorders and coagulopathies. They are currently used in the treatment of vascular thrombosis because of their effect on fibrinogen hydrolysis (Barrabin et al., 1978; Raw et al., 1986; Markland 1998; Soares et al., 2012; Costa et al., 2018). In addition, these enzymes are used as biochemical tools for evaluating changes in hemostasis induced by the venom of different viperids (Duarte et al., 2019).

The LAAO enzymes present in the venoms of South American *Crotalus* species catalyze the oxidation of L-amino acids and release hydrogen peroxide with bactericidal effects. For this reason, these

enzymes are regarded as potential biotechnological tools for the development of new antimicrobial agents (Izidoro et al., 2014). Toxicological properties attributed to LAAOs include apoptosis induction, edema formation, imbalance in platelet aggregation, and they also show antiviral, antibacterial and antiparasitic activities. A new LAAO with therapeutic potential for T-acute lymphoblastic leukemia (ALL) was isolated from the venom of the red-tailed coral snake, *Micrurus mipartitus*. It had pro-apoptotic activity in cell cultures through a $H_2O_2$-mediated signaling pathway dependent mostly on CASPASE-3 pathway (Bedoya-Medina et al., 2019). This enzyme has also been proposed as a potential antibacterial drug against Gram-positive bacteria such as *S. aureus*. However, the action pathways involved in cell death remain to be elucidated (Izidoro et al., 2014; Rey-Suarez et al., 2018).

The neurotoxins of elapids have received much less attention in the field of pharmacology. The $sPLA_2$s were used for the characterization of muscle receptor functions and the identification of nicotinic and muscarinic receptor subtypes in the peripheral and central nervous system (Vulfius et al., 2017; Foo et al., 2019). Recently, a $sPLA_2$ from the venom of *Micrurus lemniscatus* (named Mlx-8) was observed to have affinity for muscarinic acetylcholine receptors (mAChRs). This neurotoxin appeared to inhibit the intracellular signaling pathway linked to activation of mAChRs in rat hippocampus, supporting its use as a pharmacological tool for examining muscarinic cholinergic function (Santos et al., 2020). Foo et al (2019) isolated a 3-FTx called fulditoxin from the venom of North American *M. fulvius*. The structure of fulditoxin revealed four dimers held together by $Ca^{2+}$-dependent bond forming a tetrameric protein complex composed of eight subunits. This complex binds with high affinity to nicotinic acetylcholine receptor (nAChRs), causing postsynaptic neuromuscular blockade. Fulditoxin offers great potential for future studies on the treatment of Alzheimer's disease, Parkinson's disease and even some forms of schizophrenia (Foo et al., 2019).

### 4.2. Immunomodulatory properties

Immunomodulation is another important biological property of snake venom toxins. CTX or $sPLA_2$s from *C.d.t.* could down-modulate the response of the immune system by affecting its cellular and molecular components (Sampaio et al., 2010; Favoretto et al., 2011). These effects were observed both in intoxicated patients and *in vitro* models, in which immunosuppression resulted in a decreased production of specific antibodies (Schaeffer et al., 1988). The *C.d.t.* venom has been investigated for its anti-cancer activities associated with the inflammatory response induced in Ehrlich ascites tumor cells by the release of IL-2, IL-8 and TNF-α; these cytokines presumably produce lymphocyte stimulation and inhibition of tumor growth (da Silva et al., 1996). Moreover, this venom was shown to inhibit phagocytic activities and shift macrophage response toward an anti-inflammatory M2 profile (Sampaio et al., 2006). The anti-inflammatory effect of crotalic toxins is particularly important in patients with chronic arthritis (Liu et al., 2009).

Immunomodulation by CTX-B is also associated with anti-inflammatory processes. It regulates the release of arachidonic acid, a precursor of lipids such as lipoxin and prostaglandins G or E, which are mediators of inflammatory processes by inhibiting the spread of macrophages and phagocytic activity. The catalytic action of CTX also generates different lipid molecules inducing the formation of lipid droplets. These are true cytoplasmic organelles that intervene in inflammatory processes through the synthesis of lipid mediators (Sampaio et al., 2006; Moreira et al., 2008; Guijas et al., 2014; Gianotti et al., 2017, Rodriguez et al., 2020).

Teixeira et al (2020) proposed a down-modulatory action of CTX (40 μg/kg injected subcutaneously) in pro-inflammatory cells of female C57BL/6 mice. In addition, pain relief was observed in MOG35-55-induced experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. This disease produces demyelination of neurons and inflammation that leads to loss of motor and sensory functions, along with chronic pain. The authors reported that, at non-toxic dose, the anti-inflammatory and immunomodulatory effects of CTX delayed the progression of the disease and reduced the symptoms. During disease progression, they observed decrease in the populations of Th-1 and Th-17 lymphocytes and increase in the differentiation of regulatory T cells; inhibition of IFN-γ and GM-CSF production; and reduction in the frequency of macrophages in the microglia and in the number of migrating cells to the central nervous system (Teixeira et al., 2020).

## 5. Conclusion

The present review summarizes current knowledge of the venoms of South American snakes with predominantly neurotoxic components, focusing on protein composition and toxicological properties. The lethality of these venoms depends on their composition. CTX, a toxic complex with enzymatic activity, is the primary component of the venom of genus *Crotalus*, while those from genera *Micrurus* and *Hydrophis* mainly contain α-neurotoxins of the 3-FTx family. Under experimental conditions, these venoms show lower $LD_{50}$ values and a higher toxic potency than do those from genus *Bothrops*. There is extensive information on the venoms of genera *Crotalus* and *Micrurus*, but research has mostly focused on a few species (e.g. *C. durissus* and its subspecies *C. d. terrificus* and *C.d. collilineatus*). Only a few venoms from 97 species/subspecies of South American coral snakes belonging to genus *Micrurus* have been studied in detail. Likewise, very little has been reported concerning the genus *Hydrophis*.

We have compiled updated information, highlighting data on little-known venoms to promote the study of their composition, pathophysiology and pharmacological properties with potential therapeutic application.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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