A brief review on the natural history, venomics and the medical importance of bushmaster (Lachesis) pit viper snakes

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\textbf{ABSTRACT}

Snakes of the genus \textit{Lachesis}, commonly known as bushmasters, are the largest venomous snakes in the Americas. Because these snakes have their habitats in areas of remote forests they are difficult to find, and consequently there are few studies of \textit{Lachesis} taxa in their natural ecosystems. Bushmasters are distributed in tropical forest areas of South and Central America. In Brazil they can be found in the Amazon Rainforest and the Atlantic Forest. Despite the low incidence of cases, laquetic envenoming causes severe permanent sequelae due to the high amount of inoculated venom. These accidents are characterized by local pain, hemorrhage and myonecrosis that can be confused with bothropic envenomings. However, victims of \textit{Lachesis} bites develop symptoms characteristic of \textit{Lachesis} envenoming, known as vagal syndrome. An important message of this bibliographic synthesis exercise is that, despite having the proteomic profiles of all the taxa of the genus available, very few structure-function correlation studies have been carried out. Therefore the motivation for this review was to fill a gap in the literature on the genus \textit{Lachesis}, about which there is no recent review. Here we discuss data scattered in a number of original articles published in specialized journals, spanning the evolutionary history and extant phylogeographic distribution of the bushmasters, their venom composition and diet, as well as the pathophysiology of their bites to humans and the biological activities and possible biotechnological applicability of their venom toxins.

1. Overview of genus \textit{Lachesis}

Genus \textit{Lachesis} (Daudin, 1803) (Viperidae: Crotalinae), commonly called in Brazil “Surucucu-pico-de-jaca” and in other countries “Bushmaster”. With the largest known specimens reaching 3.05–3.36 m (Bellairs, 1969), 3.35 m (Ditmars, 1937), 3.5 m (Abalos, 1977), 4.27 m (Dunn, 1951), and 4.5 m (Hoge and Lancini, 1962), bushmasters comprise the longest snakes in the Western Hemisphere, and the longest vipers (Viperidae: Crotalidae) in the world. \textit{Lachesis} taxa are the only oviparous species (Fig. 1B) among New World vipers (Campbell and Lamar, 2004; McDiarmid et al., 1999). They lay up to 20 eggs to which the female provides parental care curling up in the eggs to protect them (Ditmars, 1910; Mole, 1924) (Fig. 1A). It has also been reported that females remain with eggs until hatching, and that males stay close to females for some time after mating (Emesly, 1977).

The four nominal species within \textit{Lachesis} are nocturnal terrestrial venomous pit vipers found in primary and secondary forested areas of Central and South America and on the island of Trinidad (Campbell and Lamar, 2004; McDiarmid et al., 1999; Zamudio and Greene, 1997). The Central American bushmaster, \textit{L. stenophrys} (Cope, 1975), is endemic to the Caribbean coast of Central America; the black-headed bushmaster, \textit{L. melanochela} (Solorzano and Cerdas, 1986) has an extremely limited distribution restricted to the Corcovado. National Park along the Pacific coast of southwestern Costa Rica, and possibly in the extreme western

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part of Panama. The Chocoan bushmaster *L. acrochorda* (Garcia, 1896) ranges in both the Atlantic and Pacific versants of western Panama and into northwestern Colombia, on the Atlantic coast where it extends southward into the Cauca and Magdalena river Valleys, and along the Pacific versant of Colombia into northwestern Ecuador. Among the two subspecies of *L. muta* (Linnaeus, 1766), the nominal subspecies, the South American bushmaster (*L. muta muta*), can be found in South America in the equatorial forests east of the Andes and the island of Trinidad, and the Atlantic Forest bushmaster, *L. muta rhomboeata* (Wied-Neuwied, 1824) inhabits Coastal forests of southeastern Brazil, from southern Rio Grande do Norte to Rio de Janeiro (Fig. 2).

The Central and South American forms diverged 18–6 Mya, perhaps due to the uplifting of the Andes, whereas the two Central American subspecies may have diverged 11–4 Mya with the uprisings of the Cordillera de Talamanca that separates them today (Zamudio and Greene, 1997). The split between Central American *L. melanochephalus* and *L. stenophrys* is estimated to have taken place 11–4 Mya, and differentiation among the South American lineages happened only 800,000 years ago (Fernandes et al., 2004; Zamudio and Greene, 1997) (Fig. 3).

Bushmaster can live long. There are records of a male of *L. m. muta* that lived for more than 16 years in captivity at the Fort Worth Zoo and a specimen of *L. stenophrys* that lasted 31 years and 7 months at the Atlanta Zoo (Slavens and Slavens, 2000). Campbell and Lamar (2004) also mention an *L. stenophrys* from Costa Rica, kept for almost 30 years in Europe. However, there are no data on longevity in its natural ecosystem.

Bushmasters prey primarily on small mammals, such as rodents and marsupials (Beebe, 1946; Cunha and Nascimento, 1993; Martins and Oliveira, 1998; Medem, 1969; Mole, 1924), but also hedgehog (Fountain, 1902), birds and amphibians (Carrillo de Espinosa, 1970). Although the specimens examined were adults, the prey was relatively small. Juvenile snakes (and some adults) maintain control when attacking prey, and an epicantic fold protects the eyes from possible damage during predation. Juvenile individuals may have the tip of the tail bright orange or yellow, but tail movements to attract prey (“tail luring”) has not been reported (Ripa, 2001).

*Lachesis*, daughter of Erebus (Darkness) and Nyx (Night), is a godness who in the Greek mythology assigned individual destinies to mortals at birth. This epithet given to the homonymous genus may refer to the feeling that during an encounter with these imposing snakes one’s own fate is momentarily at the snake’s will. However, human envenomings by *Lachesis* taxa are infrequent as these snakes do not exhibit aggressive behavior, inhabit shelters in fallen trees, burrows and excavations of rodents or in rocky caves of remote forest area (Fonseca, 1949), where contact with man is scarce (Souza, 2007). *Lachesis* have nocturnal habits, remaining throughout the day in a state of torpor. Only in the breeding season, males are on day alert and ready for combat. In addition, venom lethality is weak compared to those of some other vipers (Bolanos, 1972; da Silva et al., 2020). Brown (1973) quote the following LD50 values of *L. m. muta* venom for mice: 1.5 mg/kg (intravenous), 1.6–6.2 mg/kg (intraperitoneal) and 6.0 mg/kg (subcutaneous). Nevertheless, human envenomings can be rather severe due to the large venom yield (200–411 mg) (Brown, 1973; Malaque and França, 2003).

Venomics studies have been conducted on all species and subspecies within genus *Lachesis* (Madrigal et al., 2012; Pla et al., 2013; Sanz et al., 2008). Comparison of their venom proteomes provided an overview of the geographic and ontogenetic variation of the toxic arsenal across genus *Lachesis*. Hence, notwithstanding minor qualitative and quantitative differences, the venom arsenals of *L. melanochephalus* and *L. acrochorda* are broadly similar between themselves and also closely mirror those of adult *L. stenophrys* and *L. muta* venoms. On the other hand, the toxin composition of *L. stenophrys* venom undergoes ontogenetic changes, which involve changes in the concentration of vasoactive peptides and serine proteinases, which steadily decrease from birth to adulthood, and age-dependent biosynthesis of Gal-lectin and snake venom metalloproteinases (SVMPs). The net result is a shift from a bradykinin-potentiating and C-type natriuretic peptide (BPP/C-NP)-rich and serine proteinase-rich venom in newborns and 2-years-old juveniles to a (PI > PII) SVMP-rich venom in adults (Madrigal et al., 2012). The venom of newborn *L. stenophrys* has lower toxicity to mice than venom from older conspecific snakes (Gutiérrez et al., 1990). However, bites by a 10 to 14-day-old and a 2-month-old *L. stenophrys* specimens produced substantial toxicity to humans, which similar to an adult bite completely overwhelmed the 80 kg victim within 30 min (Ripa, 2003). The venomics analysis of neonate and juvenile *L. stenophrys* suggests that the high content of vasoactive peptides and serine proteinases may be responsible for the high toxicity of newborn venom in humans. On the other hand, the high similarity of their venom proteomes is mirrored by a high immunological conservation across the genus (Pla et al., 2013). A corollary of this fortunate circumstance is that antivenoms generated against venom mixtures containing any *Lachesis* spp. venom may exhibit paraspecific protection against (Daltry et al., 1996; Davies and Arbuckle, 2019) the toxic activities of venoms from any other congeneric species (Madrigal et al., 2017).

Adaptations result from selective pressures on both morphological and molecular phenotypic traits that maximize the organism’s fitness in local environments, e.g., the snake foraging success on preferred prey. Venom is an intrinsically ecological trophic trait crucial for the foraging success of the organisms that produce it. Hence, functional evolution of venoms is intimately linked to the ecology and dietary habits of the

Fig. 1. *Lachesis* spp in different behaviors. (A) Parental care of a *L. stenophrys* female along with the eggs (Corrales et al., 2014) (B) A neonate of *L. acrochorda* that just hatched (Daniel Fuentes and Corrales, 2016). Photos kindly provided by Greivin Corrales.
venomous organisms. A still small but increasing number of studies support the idea that snake venom evolution is driven by diet-related selection pressures leading to local adaptations (Barlow et al., 2009; Barua and Mikheyev, 2019; Daltry et al., 1996; Davies and Arbuckle, 2019; Jackson et al., 2004; Smiley-Walters et al., 2019). Consequently, the changes in toxic characteristics of venom that occur during the development of *L. stenophrys* (Madrigal et al., 2012), should be rationalized in the context of its use by the venomous predator. Optimal foraging theory predicts that juvenile gape-limited predators should feed efficiently in order to compete with adults for food (Schoener,
Serotherapy and clinical manifestations of laquetic accidents.

Table 1

| ACTIVITY                | CLASSIFICATION AND INITIAL CLINICAL EVALUATION |
|-------------------------|-----------------------------------------------|
| Serotherapy No. of ampoules | Moderate | Severe |
|                         | 10       | 20     |
| Route of administration | Endovenous | Endovenous |
| Acute inflammatory      | Endothelial injury and necrosis at the bite site. | Release of inflammatory mediators. |
| Coagulant               | Blood coagulability                           |                                 |
| Hemorrhagic             | Bleeding in the bite region (ecchymosis) and at distance (gingival, hematuria). | |
| Vagal neurotoxic        | Cholinergic stimulation (vomiting, abdominal pain, diarrhea, hypotension, shock). | |

(Source: adapted from Brazil, 2019)

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venomous snake, and B. atrox appeared to be the most feared snake species. The authors interpreted that the high incidence, severity, and mortality of B. atrox bites and the severity and mortality of L. muta bites represented the factors that contributed to these species being perceived as the most feared and venomous snakes, respectively. This villagers’ perception reflects the fact that although encounters with human are infrequent owing to their little aggressive behavior and elusive ecological habits, envenomings by Lachesis taxa are characterized by the high amounts of venom inoculated, (200–411 mg) (Brown, 1973; Malaque and França, 2003), which makes bite by bushmasters serious and result in high rates of permanent sequelae and mortality (Oliveira et al., 2002; Sánchez et al., 1992; Tanus Jorge et al., 1997).

The symptoms of envenomations caused by Lachesis taxa can be similar to those observed in bothropic envenomings, i.e. local pain, edema, hemorrhage and myonecrosis. Distinct features of bites by Lachesis taxa are agonizing burning-throbbing local pain and edema, within the first few minutes after the bite, followed within the next 15–20 min by a “vagal symptomatology” characterized by profuse sweating, abdominal colic, nausea, recurrent vomiting, watery diarrhea, diastolic and systolic hypotension, sinus bradycardia, uncoordinated march, lapses of consciousness (Silva-Haad, 1982; Warrell, 2004). The vagal syndrome, may assists in the differential diagnosis between laquetic and bothropic accidents (de Lima and Junior, 2015; 2004). The laquetic accidents may assists in the differential diagnosis between laquetic and bothropic accidents (de Lima and Junior, 2015; Tanus Jorge et al., 1997). Laquetic accidents are classified according to their clinical manifestations as i) Mild: mild or absent edema and mild or absent hemorrhagic manifestations. Absence of vagal manifestations; ii) Moderate: with evident edema and discrete hemorrhagic manifestations at a distance (gingivorrhagia, epistaxis). Absence of vagal manifestations; and iii) Severe: presence of severe edema and systemic manifestations such as profuse hemorrhage. Presence of vagal manifestations (diarrhea, bradycardia, hypotension or shock) (Souza et al., 2020) (Table 1). In Brazil, treatment of laquetic envenoming is based on the severity of the accident, administering when necessary the Brazilian Soro Antibotrópico-Laquéctico (SABL) intravenously (Brasil, 2001; Pardal et al., 2007).

Toxicovenomics refers to the screening of individual venom fractions of a toxin-resolved chromatographic profile for specific toxic activities (Lauridsen et al., 2016; Lomonte and Calvete, 2017). Genus Lachesis meets these criteria for what we can consider it a prospective model system to define knowledge-based omics strategies (Calvete et al., 2018, 2014) for the production of next-generation pan-generic antivenoms.

3. Bioactive components of Lachesis venom

Lachesis m. muta venom gland transcriptomic analysis (Junqueira-de-Azevedo et al., 2006) and proteomic analyses of the venoms of all the nominal species and subspecies within genus Lachesis: L. m. muta, (Sanz et al., 2008); L. m. rhombeata (Pla et al., 2013); L. stenophys, L. acrochorda, and L. melanocophala (Madrigal et al., 2012), have provided a genus-wide insight into the overall complexity of their toxic arsenals. Bushmaster venoms comprise a relatively conserved set of proteins and peptides, belonging to just a few major (i.e. relative abundances between 10 and 30%) of the total venom proteome:
bradykinin-potentiating peptides; snake venom serine proteinases; PIII-and PI-snake venom metalloproteinases; and PLAs2 and minor (L-aminoacid oxidase; galactose-specific lectin; vascular endothelial growth factor; cysteine-rich secretory protein; phospholipase B; and hyaluronidase) toxin families (Fig. 2 and Fig. 4). Pairwise comparisons showed overall similarities of 33–51% across the different conspecific venom proteomes (Pla et al., 2013), with PLA2 showing the greatest interspecific variability (Sanz et al., 2008). Despite the wealth of low-resolution proteomics and biochemical information available (Table 2) (Bregge-Silva et al., 2012; Damico et al., 2005), the timing and relevance of individual venom proteins and overall venom proteome divergence in the mechanisms of adaptation to local ecosystems and speculation of the Bushmasters remains elusive.

Table 2 lists molecules characterized in Lachesis venoms. Most of these proteins are myotoxins, neurotoxins, anticoagulant and antithrombotic PLAs5, thrombin-like, gyroxin and kallikrein serine proteinases, and hemorrhagic PII-metalloproteinases, including L. m. mato LHF-I and II and a new P-1 class metalloproteinase (Lmr-MP) isolated from the venom of L. m. rhombéa venom (Cordeiro et al., 2019). Other toxins include serine proteinase (LmrSP-4), type C lectin (LmrLEC-1) (Wiesel et al., 2019) and bradykinin-potentiating peptides (Pinheiro-Júnior et al., 2018). Furthermore, there is little known about the biological roles of these proteins and peptides in the mechanisms underlying Lachesis envenoming. Furthermore, there are very few superficial investigations on the biotechnological potential of Lachesis venom components, with biological activities mainly restricted to hypertensive effect (Diniz et al., 1992; Pinheiro-Júnior et al., 2018), thrombolytic activity (Agero et al., 2007; Ruçavado et al., 1999; Sánchez et al., 1991), antimicrobial activity against methicillin-resistant strains of Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa (Diniz-Sousa et al., 2018), and antiparasitic activity against Leishmania braziliensis (Bregge-Silva et al., 2012).

4. Concluding remarks and perspectives

Bushmaster are important components of tropical ecosystems. However, our understanding of the details of their natural history, ecology and dietary habits of Lachesis taxa in the wild is scarce and limited to only some species of the genus (Campbell and Lamar, 2004; Ripa, 2001; Savage, 2002; Solórzano, 2004; Zamudio and Greene, 1997), and a good number of reports are derived from observations of specimens in captivity (Boyer et al., 2007; Chacón y Valverde, 2004; Corrales et al., 2014; Ripa, 1994; Souza, 2007). This is, in part, due to the challenge of observing these snakes in their natural habitats in remote tropical moist forested areas where, except during breeding activities, bushmasters are solitary crepuscular or nocturnal predators (Campbell and Lamar, 2004). The limited work conducted on bushmaster species suggests these snakes are dependent on several specific resources and are thus particularly susceptible to disturbance. Hence, due to habitat destruction, the population density of Lachesis has decreased worryingly to the point that L. m. mato rhombea, which exhibits the broadest range in the Amazon region of Brazil and surrounding countries, is listed as vulnerable according to the IUCN Red List of Threatened Species criteria and considered endangered throughout its range (IUCN, 2000). Habitat loss, pollution and poaching are the main reasons for its decline. At the other extreme, the Atlantic bushmaster L. m. rhombea, a species endemic to Brazil, has a very restricted distribution that is becoming increasingly fragmented through deforestation for using the cleared areas for agriculture and human settlements (Martins and Marques, 2000). Another species, the black-headed bushmaster L. melanocephala, inhabits also a very small geographic range, including tropical, premontane and montane humid, very humid, and pluvial forests. The limited work that has been conducted on this bushmaster species suggests that it is particularly susceptible to disturbance (Solórzano, 2004). Due to its restricted geographic range and habitat specificity, the severe and continued loss of lowland and mid-elevation forests throughout its range pose serious threats to the persistence of the endemic black-headed bushmaster in Costa Rica and Panamá (González-Mayá et al., 2014). The paucity of information on L. melanocephala, which is due in large part to its cryptic nature spending much time underground and its occurrence at low densities, prevents any effective conservation actions for this species.

Omic technologies, including comparative genomics across the reptile phylogeny along with paleogeographic niche reconstruction, can provide important insights into the ecological factor and evolutionary pressures that shaped the explosive diversification of many species-rich clades, including caenophidian snakes, in the wake of the Cretaceous-Paleogene (K-Pg) Mass Extinction 66 Mya, when a massive asteroid struck the Earth, brought a calamitous end to the reign of dinosaurs, and account for the loss of 75 percent of known species (Alvarez et al., 1980; Gulick et al., 2019). The K-Pg global mass extinction event left numerous ecological niches vacant creating new ecological opportunities (Peng et al., 2017; Hsiang et al., 2015; Motti et al., 2015; Pyron and Burbrink, 2012; Skipwith et al., 2019). Although some aspects of the phylogey of some clades within the medically important snake families Viperidae, Elapidae, and Colubridae are still under dispute, in general, their phylogenetic relationships and time of divergence between lineages are well supported from fossil and molecular (nuclear and mitochondrial gene) information (Allen et al., 2016; Pyron et al., 2013; Reeder et al., 2015; Zaher et al., 2019). Venom emerged as a key evolutionary innovation that underpinned the explosive radiation of caenophidian snakes. A reliable phylogeny is key to establishing the evolutionary trends that have shaped the patterns of venom across the speciation of a clade of snakes.

An increasing trend in venom analysis is the identification of evolutionary trends across whole genera, taxonomic clades, and phylogenetic families. The overall picture, rather than the individual venom proteomes, provides hints for reconstructing the origin of evolutionary trends (Calvet, 2017, 2013). Lachesis represents one of the few snake genus for which the venom proteomes of all its species have been unveiled. Mapping the pattern of present-day venom variability into a phylogenetic and biogeographic framework may lay the foundation for understanding the evolutionary trends that have shaped the venomic landscape across the clade. In addition, the genome also encodes traces from both functionally-failed recombinations and those that passed the natural selection filter and contributed to the functional genome of the species (Kwon et al., 2016; Li et al., 2018; Lind et al., 2019; Pasquesi et al., 2018; Peng et al., 2020; Perry et al., 2018; Reyes-Velasco et al., 2020; Schield et al., 2019). The development of comparative genomics in the last 20 years has taught us that no lineage can be studied genomically in isolation from related lineages. However, despite the genomes of birds and nonavian reptiles will not only uncover a treasure trove of biological information to reconstruct the evolution of venomous reptiles and their venom genes, but are also critical for understanding genome evolution in mammals and amniotes generally (Janes et al., 2010; Tollis et al., 2018, 2014), snake genome sequencing is in its infancy. Squamates exhibit some of the most extreme and fascinating biological adaptations among vertebrates (Shanley et al., 2014). However, genomic resources are currently only available for a handful of squamous reptiles (Allford et al., 2011; Castoe et al., 2013; Giorgianni et al., 2020; Green et al., 2014; Kerkkamp et al., 2016; Li et al., 2018; Lind et al., 2019; Peng et al., 2020; Schield et al., 2019; Shibata et al., 2018; Suryamohan et al., 2020; Tollis et al., 2014; Ultec-Agote et al., 2014; Vonk et al., 2013; Wang et al., 2019). Comparative Squamata genomics (genomics, transcriptomics, and proteomics) will play a fundamental role in filling this gap and addressing the connection between genome evolution and the present-day adaptive phenotype for fitness related traits (Cenik et al., 2015; Drukewitz and von Reumont, 2019; Eckalbar et al., 2013; Hajirasouliha and Tilgner, 2019).

The wide spectrum of pathological and pathophysiological manifestations of snake envenomings, due to the concerted actions of the unpredicted venom variability across the phylogeny and distribution
Table 2
Main components characterized in venoms of the genus Lachesis.

| NAME             | SPECIE        | PROTEIN/PEPTIDE         | MOLECULAR MASS (kDa) | BIOLOGICAL PROPERTIES                                      | THERAPEUTIC POTENTIAL               | REFERENCES                                      |
|------------------|---------------|-------------------------|----------------------|-------------------------------------------------------------|-------------------------------------|-------------------------------------------------|
| LmrBPP9          | L. m. rhombeata | Bradykinin-potentiating peptides (BPP) | 1.08                 | Inhibit ACE activity in vitro                               | Hypotensive effect                  | Pinheiro-Júnior et al. (2018)                   |
| Lmr-MP           | L. m. rhombeata | Metalloproteinase        | 22.85                | Proteolytic activity on synthetic substrate of human kallikrein | –                                   | Cordeiro et al. (2018)                         |
| LmrSP-4          | L. m. rhombeata | Serine proteinase        | 28.19                | –                                                            | –                                   | Wiezell et al. (2019)                          |
| LmrLEC-1         | L. m. rhombeata | C-type lectin            | –14                  | –                                                            | –                                   | Wiezell et al. (2019)                          |
| LmuTX            | L. m. mura     | PLA2, Lys-49             | 13.8                 | Cytotoxicity on C2C12 cells differentiated in myotubes       | Antibacterial activity              | Diniz-Souza et al. (2018)                     |
| LmLAAO           | L. m. mura     | L-amino acid oxidase     | 60                   | Without myotoxic activities, hemorrhagic and edematogenic    | Cytotoxic activity on AGS and MCF-7 cells | Breggo-Silva et al. (2012)                     |
| Stenoxobin       | L. stenophrys  | Serine proteinase (thrombin-like) | 37                   | α and β-fibrinogenolytic                                     | –                                   | Aragon-Ortiz and Guibenek (1993)              |
| LaPA-1           | L. stenophrys  | PLA2, Asp49              | 13.87                | –                                                            | –                                   | De Athis et al. (2008)                        |
| LMTX-I           | L. m. mura     | Basic PLA2, Asp49        | 14.24                | Phospholipase activity on synthetic substrates; edema; myotoxicity; neurotoxicity | –                                   | Damico et al. (2008, 2006, 2005)              |
| LMTX-II          | L. m. mura     | Basic PLA2, Asp49        | 14.18                | Phospholipase activity on synthetic substrates               | –                                   | Damico et al. (2005)                          |
| LmrTX            | L. m. rhombeata | Basic PLA2, Asp49        | 14.27                | Anticoagulant and antithrombotic activities                  | –                                   | Damico et al. (2012)                          |
| Lmr-PLA2         | L. m. mura     | Acidic PLA2, Asp49       | 13.97                | Inhibition of platelet aggregation                           | –                                   | Cordeiro et al. (2015)                        |
| LV-Ka            | L. m. mura     | Serine proteinase (calicreina-simile) | 33                   | Plasminogen activation                                       | –                                   | Felicori et al. (2003)                        |
| LM-PLA2-I        | L. m. mura     | Acidic PLA2, Asp49       | –                    | Myotoxicity                                                  | –                                   | Fuly et al. (2000)                            |
| LM-PLA2-II       | L. m. mura     | Acidic PLA2, Asp49       | 18                   | Myotoxicity; inhibition of platelet aggregation; edematogenic activity. | –                                   | Fuly et al. (2003, 2002)                     |
| TLE-B            | L. m. mura     | Serine proteinase        | 44                   | α and β-fibrinogenolytic                                     | –                                   | Magalhaes et al. (2003)                       |
| TLB-P            | L. m. mura     | Serine proteinase        | 43                   | α and β-fibrinogenolytic                                     | –                                   | Magalhaes et al. (2003)                       |
| Mutalisin I (LHF-II) | L. m. mura     | Metalloproteinase        | 100                  | α and β-fibrinogenolytic; high hemorrhagic activity; caseinolytic activity. | –                                   | Sanchez et al., 1995, 1987                    |
| Mutalisin II (LHF-II) | L. m. mura     | Metalloproteinase        | 22                   | Degradation of laminin, fibronectin and type IV collagen; edematogenic; low hemorrhagic activity. | Thrombolytic effect                | Agero et al., 2007; Rucavado et al., 1999; Sanchez et al., 1991 |
| Mut IIa          | L. m. mura     | Mutalisin isoform II     | ≈23                  | α and β-fibrinogenolytic; proteolytic activity on dimethylcasein. | –                                   | Sanchez et al. (2003)                        |
| Mut IIb          | L. m. mura     | Mutalisin isoform II     | ≈23                  | α and β-fibrinogenolytic; proteolytic activity on dimethylcasein. | –                                   | Sánchez et al. (2003)                        |
| Hyaluronidase    | L. m. rhombeata | Hyaluronidase            | 60                   | –                                                            | –                                   | Wiezell et al. (2015)                         |
| PLB              | L. m. rhombeata | Phospholipase B          | –                    | –                                                            | –                                   | Wiezell et al. (2015)                         |
| LMR-47           | L. m. mura     | Serine proteinase (gyroxin) | 47                   | α - fibrinogenolytic                                         | –                                   | Aguiar et al. (1996)                          |
| Protein similar to lectin | L. stenophrys | Lactin                   | 16.2                 | Hemagglutination                                             | –                                   | (Aragon-Ortiz et al., 1996; Aragon-Ortiz et al., 1989) |
| Lachesia         | L. m. mura     | Disintegrin              | –                    | Inhibition of platelet aggregation; binding to integrin αββ3 | –                                   | Scarborough et al. (1993)                     |
| LSF              | L. stenophrys  | Metalloproteinase        | 24                   | –                                                            | –                                   | Leonardi et al. (1999)                        |
| Serine proteinase| L. m. mura     | Serine proteinase        | 45                   | –                                                            | –                                   | Magalhaes et al. (1997)                       |
| Kininogenin      | L. m. mura     | Serine proteinase        | 29.7                 | –                                                            | Hypotensive effect                  | Diniz et al. (1992)                          |
| Glyoxin          | L. m. mura     | Serine proteinase        | ≈60                  | High lethality                                              | –                                   | da Silva et al. (1989)                        |
| Thrombin-like    | L. m. mura     | Serine proteinase        | ≈41.47               | Fibrinogenolytic                                            | –                                   | Silveira et al. (1989)                        |
| Serine proteinase| L. m. mura     | Serine proteinase        | 40                   | α and β-fibrinogenolytic                                     | –                                   | Yarleque et al. (1989)                        |
| Arginine esterase| L. m. mura     | –                       | ≈30                  | Arginyl esterase activity                                   | –                                   | Silva et al. (1985)                          |
| BIP              | L. m. mura     | Bradykinin inhibitor     | 1.06                 | Inhibitory activity on bradykinin via B2 receptors           | –                                   | Graham et al. (2005)                         |
| jPLIs            | L. m. mura     | –                       | 36.5                 | –                                                            | –                                   | Lima et al. (2011)                           |
| LNF1 and LNF2    | L. m. mura     | Gamma-type inhibitors    | ≈20                  | –                                                            | –                                   | Forstes-Dias et al. (2003)                    |
|                 | L. m. mura     | PLA2s                   | –                    | –                                                            | –                                   | Magalhaes et al. (1993)                       |

(continued on next page)
range of extant snakes, represents a great challenge for the development, and preclinical evaluation of the efficacy of antivenoms. From a biotechnological stand point, this goal requires knowing the phylogenetically-based, well-characterized venoms of present-day snakes, identifying their key medically-important molecules in the context of a human envenoming, and assessing the specific and para-specific efficacy of current anti-venoms against different medically relevant snake venoms. For the case of the genus *Lachesis*, the high conservation of the overall composition of Central and South American bushmaster venoms provides the ground for rationalizing the “Lachesis syndrome” documented in envenomings by different species of this wide-ranging genus. From an evolutionary ecology perspective legitimate human snake envenomings result from defensive bites inflicted by sympatric venomous snakes when snake and human have a fortuitous encounter in their shared natural environment that blows the snake’s alarms. In this context, knowledge gained on the natural history and organismal physiology of medically important snakes, particularly those that prey on mammals, have conceptually and translational venomics (Calvete, 2019).

Clearly, we believe that it is not an exaggeration to conclude that our field, molecular toxicology, has a very exciting future ahead. We trust this short review on the bushmasters may help to put this genus of medically and ecologically relevant snakes in the spotlight of near future basic and applied developments.

Credit author statement

Rafaela Diniz-Sousa: Conceptualization, Writing, Data curation and Formal analysis, Jeanne do N. Moraes: Writing, Data curation and Formal analysis, Tainara M. Rodrigues-da-Silva: Writing, Data curation and Formal analysis, Cláudia S. Oliveira: Writing, Cleópatra A. da S. Caldeira: Conceptualization, Writing, Data curation and Formal analysis, Editing.

Ethical statement

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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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Table 2 (continued)

| NAME                      | SPECIE | PROTEIN/PEPTIDE DESCRIPTION | MOLECULAR MASS (kDa) | BIOLOGICAL PROPERTIES | THERAPEUTIC POTENTIAL | REFERENCES |
|---------------------------|--------|-----------------------------|----------------------|-----------------------|------------------------|------------|
| Thrombin-like/ | *L. m. muta* | Serine proteinase | 33                  | Plasminogen activation | –                      | (Sanchez et al., 2006, 2009) |
| analogous to | Lm-BBP 1 to 5 and | BBPs and C-type | 1 to 2.2             | –                     | –                      | Soares et al. (2005) |
| gyroxin | Lm-CNp | natriuretic peptides | 1 to 2.2             | –                     | –                      |            |
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