

## Mini Review

# An overview of *Bothrops erythromelas* venom

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### Abstract

This review discusses studies on the venom of *Bothrops erythromelas* published over the past 36 years. During this period, many contributions have been made to understand the venomous snake, its venom, and its experimental and clinical effects better. The following chronological overview is based on 29 articles that were published between 1979 and 2015, with emphasis on diverse areas. The complexity of this task demands an integration of multidisciplinary research tools to study toxinology. This science is in need of renewed conceptual and experimental platforms aimed at obtaining a profound understanding of the highly complex pathophysiology of snakebite envenoming and toxins isolated from snakes.

Key-words: *Bothrops erythromelas*. Snake. Venom.

### INTRODUCTION

Venomous and non-venomous snakes are distributed worldwide, especially in tropical and subtropical areas<sup>(1)</sup>. Envenoming is neglected in many countries, which makes it a public health concern<sup>(2) (3) (4)</sup>. Snake venoms are fascinating models for drug design<sup>(1)</sup> and their antidotes are still under development. As a result, research in this area has been in existence since the 19<sup>th</sup> century<sup>(5)</sup>. Three hundred and eighty-six species of snakes have been identified in Brazil, of which 62 are venomous, 32 belong to the family Elapidae, and 30 belong to the family Viperidae<sup>(6)</sup>. *Bothrops erythromelas*, which belongs to the family Viperidae (**Figure 1**), is responsible for most of the snakebite accidents in Northeast Brazil<sup>(7)</sup>.

The species is found in the Caatinga ecoregion, an exclusive Brazilian biome that covers an area of about 850,000km<sup>2</sup> and includes part of the Brazilian States of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia, Maranhão, and Minas Gerais<sup>(7)</sup>.

### OVERVIEW ON *BOTHRUPS ERYTHROMELAS* VENOM

To date, 29 studies have been published on *Bothrops erythromelas*. The first study on this snake dates back to 1979. Nahas et al.<sup>(8)</sup>, compared the coagulating activity of 26 different

*Bothrops* snake venoms. Using a specific clotting system, they showed that *B. erythromelas* venom presents no thrombin-like activity, because it was not able to directly clot fibrinogen. Furthermore, it is speculated that the absence of the thrombin-like activity is due to a fibrinogenolytic effect of the venom.

In 1990, Moura-da-Silva et al.<sup>(9)</sup> evaluated the differences in the distribution of myotoxins from different *Bothrops* species. Briefly, antigens with high myotoxic activities were isolated from *Bothrops jararacussu* venom and their cross reactivities were analyzed by western blotting and enzyme-linked immunosorbent assay using *Bothrops* antivenoms. *Bothrops jararacussu*, *Bothrops moojeni*, *Bothrops neuwiedi*, and *Bothrops pradoi* antivenoms were found to be active against the isolated myotoxins; however, *B. erythromelas* antivenom showed no activity against the antigens. Moreover, *in vivo* myotoxicity studies confirmed the absence of related myotoxins in *B. erythromelas* venom.

In 1991, two related studies were conducted. The first one compared 9 snake venoms from adult *B. erythromelas* females and their offspring<sup>(10)</sup>; this study found that caseinolytic and fibrinolytic activities were lower in venoms from the newborn snakes than in those from their mothers. Although variations were observed in the amidolytic activities of the venoms from most *Bothrops* snakes and their offspring, the venom of *B. erythromelas* showed no amidolytic activity. In a comparative analysis of the coagulating activities of *Bothrops* venoms, *B. erythromelas* venom presented the highest levels of factor X (FX) and prothrombin activators without showing a thrombin-like activity. It was emphasized that the degree of procoagulation in the newborn snakes' venoms is related to the ability to activate

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**FIGURE 1.** Snake: *Bothrops erythromelas*, popularly known as Caatinga lancehead, *jararaca-da-seca*, or *jararaca malha de cascavel*.  
**Source:** <http://www.luar.dcc.ufmg.br>.

prothrombin and FX. However, this procoagulating activity seems to decrease as the newborn snakes grow. Moreover, these researchers demonstrated that the venom of an adult *B. erythromelas* has a higher protein content than that from a newborn snake does.

The second study isolated and compared myotoxins from different species of *Bothrops* by fast protein liquid chromatography and isoelectrofocalization<sup>(11)</sup>. No basic proteins with phospholipase and/or myotoxic activity in the *B. erythromelas* venom was detected in this study. This confirmed the data obtained by Moura-da-Silva et al.<sup>(9)</sup>.

In 1992, Maruyama et al.<sup>(12)</sup> complemented Nahas et al.'s work<sup>(8)</sup> by investigating the enzymatic properties of factor II (FII) and FX activators from *B. erythromelas* venom. They demonstrated that both activators are inhibited by ethylenediaminetetraacetate and 1,10-phenanthroline. They also hypothesized that metalloproteinases with molecular weights of 70-90kDa were present in the venom. These researchers observed that FII activator activity of *B. erythromelas* was approximately 30 times greater than that of *Oxyuranus scutellatus* venom but similar to that of *Daboia russelli* venom. Furthermore, they found that FX activator activity was calcium-dependent and that the venom of *B. erythromelas* contains two hemorrhagic factors and two fibrinolytic enzymes.

Flores et al.<sup>(13)</sup> were the first to study the proinflammatory effects of *B. erythromelas* venom; they demonstrated that *Bothrops erythromelas* and *Bothrops alternatus* venoms induced the migration of neutrophils into the peritoneal cavities of rats. When injected into the peritoneal cavity of rats and into the air pouch, the venom induced migration of the cells to the injection site. This migratory response was thought to occur due to the phospholipase activity of the venom. Moreover, *B. alternatus* venom showed a phospholipase activity that was two times higher than that exhibited by *B.*

*erythromelas* venom. Additionally, *B. erythromelas* venom induced neutrophil recruitment 2-3 times more than that induced *B. alternatus* venom. Moreover, treatment with dexamethasone or nordihydroguaiaretic acid, followed by stimulation with *Bothrops* venom, resulted in a significant reduction in neutrophil migration. This suggests that leukotriene B<sub>4</sub>, which is a lipoxygenase metabolite of an arachidonic acid derivative, acts as a chemotactic mediator. Macrophages are the main source of leukotriene B<sub>4</sub>. They are also the predominantly resident cells in the rat air pouch; therefore, rats injected with thioglycolate and then with *B. erythromelas* or *B. alternatus* venom had more neutrophils in their peritoneal cavities than the control rats had.

The reproductive aspects of some viviparous species from Bahia (Brazil), including *B. erythromelas*, was investigated by Lira-da-Silva et al.<sup>(14)</sup>. The researchers observed that the gestation period of *B. erythromelas* was about 123 days and that parturition occurred preferably in the summer season. Furthermore, the female snakes produced an average of 11 hatchlings/gestation with 16.80-19.20cm.

In 1998, Vasconcelos et al.<sup>(15)</sup> studied the *in vivo* distribution of *B. erythromelas* venom. In that study, the venom was labeled with <sup>131</sup>I and administered subcutaneously to mice. The results showed a higher amount of venom in subcutaneous tissues than in the heart, bladder, brain or diaphragm. This indicates that *B. erythromelas* venom does not target most internal organs; hence, systemic effects of envenomation from *B. erythromelas* would be related to an indirect action of the venom.

In some regions in Brazil, heparin is used for treating bothropic accidents associated with antithropic serum (ABS). In 2001, Boechat et al.<sup>(16)</sup> verified the action of heparin (Liquemine, Roche, Brazil) on the main biological activities (hemorrhagic, *in vitro* and *in vivo* coagulating, edematogenic, phospholipasic, and lethal) of *Bothrops atrox* and *Bothrops erythromelas* venoms. This study verified that heparin (3 and 6IU) was not effective in neutralizing the hemorrhagic and coagulating activities of both venoms. However, heparin, at dose of 6 IU, was capable of neutralizing the edematogenic activity of *B. erythromelas* venom. It also increased the effectiveness of ABS. Moreover, heparin neutralized the phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activity of *B. erythromelas* venom by 28%. Furthermore, heparin was more effective in neutralizing the venom's lethal activity when it was administered with ABS.

Casey et al.<sup>(17)</sup> compared the toxic effects of the venoms from five *Bothrops* snakes (*Bothrops alternatus*, *Bothrops moojeni*, *Bothrops neuwiedi*, *Bothrops jararacussu*, and *Bothrops jararaca*) and a combination of these venoms (AgB). They investigated the ability of polyvalent ABS, produced by Ezequiel Dias Foundation, to recognize and neutralize toxic compounds in the venoms. The results showed that ABS inhibited the toxic effects of each of the five venoms, as well as those of *Bothrops erythromelas*, *Bothrops atrox*, and *Bothrops leucurus* venoms.

Silva et al.<sup>(18)</sup> were the first to conduct a study involving the molecular cloning, purification, and characterization of a prothrombin activator of *B. erythromelas*. Their findings complemented those obtained by Nahas et al.<sup>(8)</sup>, and they

successfully purified the prothrombin activator berythrativase, by single cation-exchange chromatography. They demonstrated that berythrativase makes up approximately 5% of the venom. Additionally, berythrativase presented as a single band protein with a molecular weight of 78kDa under reducing conditions in sodium dodecyl sulfate polyacrylamide gel electrophoresis. This study showed that prothrombin hydrolysis by berythrativase resulted in a fragment pattern similar to that generated by Group A prothrombin activators. The latter convert prothrombin into meizothrombin; however, this occurs independent of the prothrombinase complex but is typical of a metalloproteinase. Furthermore, the enzymatic activity of berythrativase was rapidly inhibited by chelators, such as ethylenediaminetetraacetic acid and o-phenanthroline. It was also observed that after prolonged incubation with berythrativase, the  $\alpha$ -chain of human fibrinogen was slowly digested; however, no effects on the  $\beta$ - or  $\gamma$ -chains were observed. Additionally, the enzyme triggered procoagulating and proinflammatory responses. It also positively regulated the surface expressions of intracellular adhesion molecule-1 and E-selectin on human umbilical vein endothelial cells (HUVECs). Berythrativase is functionally similar to Group A prothrombin activators and its primary structure is related to that of hemorrhagic metalloproteinases from snake venoms. As a result, it does not show a hemorrhagic activity, which is characteristic of other snake venom metalloproteinases.

In 2004, Zamuner et al.<sup>(19)</sup> compared the myotoxic and neurotoxic effects of *Bothrops* venoms (*B. erythromelas*, *B. jararaca*, *B. jararacussu*, *B. moojeni*, and *B. neuwiedi*) and evaluated their neutralization using a commercial antivenom produced by the Institute Vital Brazil (Rio de Janeiro, RJ, Brazil). Although all the venoms were myotoxic when they were assessed by their release of creatine kinase, the *B. erythromelas* venom seemed less active than the other venoms. This was in agreement with the data obtained by Moura-da-Silva et al.<sup>(9)</sup> and Moura-da-Silva et al.<sup>(11)</sup>. However, Zamuner et al.<sup>(19)</sup> indicated that the *B. erythromelas* venom was more lethal than the others were. *In vitro* neurotoxicity studies showed that the venoms were neurotoxic to chick nerve-muscle preparations. A commercial antivenom was used to neutralize the myotoxic effect of the venoms; however, its neuroprotective effect was variable, indicating that the neurotoxic venom component(s) differ among the venoms.

Junqueira-de-Azevedo et al.<sup>(20)</sup> have investigated the *B. erythromelas* snake venom vascular endothelial growth factor (svVEGF), which is an angiogenic protein. The protein seems to be responsible for many of the features of Viperidae envenomation, such as hypotension and increase in vascular permeability, which results in spreading of the venom. In the work by Junqueira-de-Azevedo et al.<sup>(20)</sup>, western blot assays were conducted using mice antisera against svVEGF isolated from *Bothrops insularis*. The results indicated the presence of svVEGFs in *B. erythromelas* venom. The complete sequence of *B. erythromelas* svVEGF cDNA was found to contain 1213 nucleotides. The deduced protein showed an open reading frame of 146 amino acid residues, with an initiation codon (ATG) at

position 205 and a stop codon (TGA) at position 643, which are similar to the respective codons in the *B. insularis* svVEGF sequence.

In 2004, the chromatographic behavior of an acidic PLA<sub>2</sub> isolated from *B. erythromelas* snake venom by size-exclusion chromatography was studied by Aird<sup>(21)</sup>, who observed that the PLA<sub>2</sub> interacted hydrophobically with the matrix resin, which was constituted of agarose and dextran, thereby retaining the protein on the matrix. Additionally, it was highlighted that different buffers at various pHs, as well as organic solvents, such as acetonitrile (30%), could be used to improve chromatographic resolution.

Schattner et al.<sup>(22)</sup> complemented the results reported by Silva et al.<sup>(18)</sup> by evaluating the effects of two P-III snake venom metalloproteinases (SVMPs), berythrativase and jararhagin, isolated from *B. erythromelas* and *B. jararaca*, respectively, on HUVECs. Both SVMPs were capable of stimulating the releases of interleukin-8 (IL-8), nitric oxide, and prostacyclin (PGI<sub>2</sub>) by the HUVECs. Berythrativase also increased the expression of decay-accelerating factor; however, it did not affect the viability of the HUVECs even when it was used at high concentrations. The former effect may be the reason for the hemorrhagic activity of berythrativase.

Grazziotin and Echeverrigaray<sup>(23)</sup> performed a random amplified polymorphic DNA analysis to study the genetic relationships among 11 *Bothrops* species and found that *B. erythromelas* showed genetic relations with *B. moojeni*.

Some animals have a natural resistance to the effects of snake venoms, which in many cases can be explained by the presence of neutralizing factors in the animal's serum<sup>(24) (25)</sup>. Moreover, resistance to the effects of snake venom has been studied in the serum of *Didelphis marsupialis*, a very common opossum in South America<sup>(26) (27) (28) (29)</sup>. In 2005, Martins et al.<sup>(30)</sup> investigated the action of an antithrombotic factor isolated from *D. marsupialis* serum on the renal effects of *B. erythromelas* venom without systemic interference. Their study showed that *B. erythromelas* venom reduced renal perfusion pressure (PP) and renal vascular resistance (RVR). Additionally, the venom decreased glomerular filtration rate (GFR) at 60 minutes and then increased it at 120 minutes after perfusion. Furthermore, the urinary flow (UF) was increased significantly, whereas the tubular transport percentages of sodium (%TNa<sup>+</sup>) and potassium (%K<sup>+</sup>) decreased. They observed that the isolated antithrombotic factor at 10 $\mu$ g/mL blocked the effects of the venom on PP, RVR, %TNa<sup>+</sup>, and %TK<sup>+</sup>. However, it was not effective in reversing the effects of the venom on UF and GFR. At higher concentrations (30 $\mu$ g/mL), the antithrombotic factor was able to reverse all the renal effects induced by the *B. erythromelas* venom. Finally, they concluded that the *B. erythromelas* venom altered all the renal functional parameters that were evaluated. Additionally, the antithrombotic factor inhibited all the renal effects induced by the venom on isolated kidneys from Wistar rats.

In 2006, Pereira et al.<sup>(31)</sup> studied berythrativase and jararhagin to complement the studies conducted by Schattner et al.<sup>(22)</sup> and Silva et al.<sup>(18)</sup>. Pereira et al.<sup>(31)</sup> studied the differences in the biological effects of the two SVMPs on different

hemostatic properties. Next, they characterized the biological effects of the proteins and compared their effects on HUVECs. They evaluated the release and modulation of coagulation and fibrinolytic factors by the cells, as well as the expressions of their related genes. The results showed that berythrativase and jararhagin induced the release of von Willebrand factor but did not modulate its gene expression level. Additionally, berythrativase increased the expression of the tissue factor in the HUVECs. This study concluded that each SVMP acts in a specific manner. Specifically, jararhagin has a preferential local action, while berythrativase is a systemic procoagulating protein that acts on the surfaces of HUVECs.

De Albuquerque Modesto et al.<sup>(32)</sup> isolated a novel acidic phospholipase A<sub>2</sub> with an aspartate at position 49 (PLA<sub>2</sub> Asp-49) from *B. erythromelas* venom, named BE-I-PLA<sub>2</sub>, characterized it, and described its complete sequencing. BE-I-PLA<sub>2</sub> has a molecular weight of 13,649.57Da and a cDNA sequence of 457 base pairs (bp). They incubated BE-I-PLA<sub>2</sub> with platelet-rich plasma and showed that the former has a potent inhibitory effect on aggregation induced by arachidonic acid and collagen but not that by adenosine diphosphate. Moreover, BE-I-PLA<sub>2</sub> showed no binding to/interference with principal platelet receptors. BE-I-PLA<sub>2</sub> was shown to stimulate endothelial cells to release PGI<sub>2</sub> but not nitric oxide, which suggests that BE-I-PLA<sub>2</sub> has a potential antiplatelet activity *in vivo*.

These data are interesting because they highlight that berythrativase, a P-III snake venom metalloproteinases (SVMPs) from *B. erythromelas*, can stimulate endothelial cells to release nitric oxide and PGI<sub>2</sub><sup>(22)</sup>. Therefore, the effects of both toxins contribute to the effects observed after *B. erythromelas* bites.

Moura da Silva et al.<sup>(33)</sup> published another study on metalloproteinases. In that study, the effects of jararhagin, which is a hemorrhagic P-III SVMP, and berythrativase, which is a procoagulant and non-hemorrhagic P-III SVMP, were compared. The results showed that both SVMPs inhibited collagen-induced platelet aggregation. Moreover, the monoclonal antibody MAJar 3, which neutralizes the hemorrhagic effect of *Bothrops* venoms and inhibits the binding of jararhagin to collagen, did not react with berythrativase. Furthermore, the jararhagin-collagen complex retained the catalytic activity of the toxin, as was evidenced by the hydrolysis of fibrin. Moreover, the three-dimensional structures of the metalloproteinases were studied to clarify why the two SVMPs exhibited different effects. The authors pointed out that the subdomain disintegrin located in front of the catalytic domain found in jararhagin is able to mediate the binding of the latter to collagen and react with the monoclonal antibody. The study therefore revealed a novel function of the disintegrin domain during hemorrhage.

Souza et al.<sup>(34)</sup> studied the peptide profiles of *Bothrops* venoms (*B. alternatus*, *B. erythromelas*, *B. insularis*, *B. jararaca*, *B. jararacussu*, *B. leucurus*, and *B. moojeni*) by direct infusion nano-electrospray ionization mass spectrometry (nano-ESI-MS). The data obtained were then subjected to principal component analysis (PCA). The results showed the presence of common peptides among the venoms. However, each venom contained unique taxonomic marker peptides.

Furthermore, this study describes that a bradykinin-potentiating peptide, QGGWPRPGPEIPP, is common to the seven *Bothrops* venoms. QGGWPRPGPEIPP is a specific marker because it is not present in the venom of *Crotalus durissus terrificus* (rattlesnake). The PCA of the peptides showed that the venom of *B. erythromelas* is phylogenetically close to those of *B. jararaca* and *B. insularis*. Additionally, its peptide profile was similar to that of *B. jararaca*. *B. alternatus* is phylogenetically different from the other *Bothrops* species examined in the study, with the order of decreasing proximity being *B. erythromelas*, *B. jararaca*/*B. insularis*, *B. jararacussu*, *B. moojeni*, and *B. leucurus*. This relationship is the same as the one observed in the PCAs of the peptides in the various venoms, which showed that *B. erythromelas* venom is the closest to *B. alternatus* venom but the most different from *B. leucurus* venom. These researchers concluded that fingerprinting using direct infusion nano-ESI-MS in positive ion mode, followed by chemometric analysis provides a rapid means to analyze low-molar-mass peptides in snake venom samples. This provides a reliable mass fingerprinting spectra for venom classification and quality control.

Rocha et al.<sup>(35)</sup> continued the studies conducted by Vasconcelos et al.<sup>(15)</sup> by investigating the plasma pharmacokinetics of *B. erythromelas* venom labeled with <sup>131</sup>I in the presence and absence of an antivenom in mice. In the presence of the antivenom, the percentage radioactivity of *B. erythromelas* in plasma was higher and its elimination half-life was longer than the respective values were in the absence of the antivenom. Thus, the data indicated a redistribution of the venom from the tissues to the vascular compartment associated with the treatment of envenomed mice with anti-venom 15 min after venom injection. The researchers concluded that the pharmacokinetics of *B. erythromelas* venom in the presence of an antivenom follows a modified profile and is likely the result of redistribution of the venom from the peripheral compartment to the central compartment. This may contribute to the understanding and optimization of treatment against envenoming in humans.

Estevão-Costa et al.<sup>(36)</sup> studied phospholipase A<sub>2</sub> inhibitors (PLIs) present in snake serum. Three different structural classes of PLIs ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) were noted from the study. The  $\gamma$  class members are potent inhibitors of PLA<sub>2</sub> and are from the venoms of Viperidae snakes. They further documented the  $\gamma$ PLIs in the venoms of six *Bothrops* snakes (*B. erythromelas*, *B. neuwiedi*, *B. leucurus*, *B. jararacussu*, *B. moojeni*, *B. alternatus*, and *B. jararaca*). The mature proteins possessed 181 amino acid residues following a 19-residue signal peptide, similar to the  $\gamma$ PLIs in the venom of *Crotalus durissus terrificus*. Two of the deduced proteins from *B. erythromelas* and *B. neuwiedi* venoms were considered as exceptions. They showed consistent insertions of 4-amino acid residues in their structures. However, further studies should be conducted on  $\gamma$ PLIs because this class of proteins may be useful in the development of selective inhibitors of secretory PLA<sub>2</sub> from several sources.

Studies on the clinical and epidemiological profiles of snakebites caused by *Bothrops* and *Bothropoides* snakes, including *Bothrops erythromelas*, which are responsible for most of the snakebite accidents in Northeast Brazil<sup>(7)</sup> were

reported only after 2010. Oliveira et al.<sup>(37)</sup> evaluated victims of snakebites who were admitted to the Paraíba Information Centre for Toxicological Assistance and found that, the annual incidence of snakebite accidents was 5.5 for every 100,000 inhabitants. They also found that the lethality rate following such accidents was 0.2%. The accidents were considered mild and the patients were examined about 6 hours after the bite. Most of the patients were rural male workers aged between 11 and 20 years old. The study also showed that the feet and toes were the most affected body parts.

Another research in this area was conducted by Luna et al.<sup>(38)</sup>, who collected blood samples from patients bitten by *B. erythromelas* before and after the patients were administered an antivenom. Humoral response [immunoglobulin M (IgM) production] was analyzed to ascertain the effectiveness of the treatment. In all the blood samples, a protein with a molecular weight of 38kDa was found before and after serum therapy. The results suggested that this protein could be used as a marker to indicate envenomation by *B. erythromelas*.

A study performed by Luna et al.<sup>(39)</sup> showed that, *Bothrops erythromelas* and *Crotalus durissus cascavella* venoms induced high interferon-gamma and IL-6 production in mouse splenocytes. Nitric oxide was significantly produced in the splenocytes only by *B. erythromelas* venom, which also induced a higher rate of cell death than *C. durissus cascavella* venom did. The results of the study showed that *B. erythromelas* and *C. durissus cascavella* venoms induce distinct *in vitro* responses through cytokine and nitric oxide productions. This study complemented the data obtained by Flores et al.<sup>(13)</sup>, which first showed the proinflammatory effects of *B. erythromelas* venom.

In a study by Machado et al.<sup>(40)</sup>, 140 venom samples were collected from 93 different localities and used to investigate specie boundaries. The study aimed to hypothesize the phylogenetic relationships among the venoms based on 1,122bp of *cyt b* and *ND4* from mitochondrial DNA. Additionally, they investigated the patterns and processes involved in the evolutionary history of the group of venoms. The results indicated that *B. neuwiedi* venom is highly monophyletic. However, *Bothrops diporus*, *Bothrops lutzi*, *Bothrops erythromelas*, *Bothrops mattogrossensis*, *Bothrops neuwiedi*, *Bothrops marmoratus*, and *Bothrops pauloensis* were polyphyletic based on their morphologies.

Two recent studies by Jorge et al.<sup>(41)</sup> applied a venomomics approach to define the proteome and geographic variability of venoms of adult *B. erythromelas* from five geographic regions. They found that the five venoms exhibited highly conserved venom proteomes. The overall toxin profile of a snake venom explains the local and systemic effects observed in envenomation. The five venoms showed qualitatively and quantitatively overlapping antivenomic profiles against the antivenoms [antibothropic-crotalic-laquetic, produced by the *Instituto Clodomiro Picado* (San José, Costa Rica); antibothropic, produced by *Instituto Vital Brazil* (Niterói, Brazil)] generated using different bothropic venoms in immunization mixtures. The large immunoreactive epitope conservation across the *Bothrops* genus offers promise for the generation of broad-spectrum bothropic antivenoms.

Another study by Santoro et al.<sup>(42)</sup> investigated the thrombin-like activity of venoms from hybrids born in captivity from the mating of a female *B. erythromelas* and a male *B. neuwiedi*, which are two species whose venoms are known to display ontogenetic variations. They found that the features of venoms from hybrid snakes are genetically controlled during ontogenetic development. Despite the presence of thrombin-like enzyme genes in hybrid snakes, they are silenced during the first six months of life.

## CONCLUSIONS

This overview discusses articles on the venom of *Bothrops erythromelas* that have been published over the past 36 years. During this period (1979-2015), many contributions have enabled a better understanding of the venomous snake, its venom, and its experimental and clinical effects. However, because *B. erythromelas* is the cause of most snakebite accidents in Northeast Brazil, the number of available publications is not sufficient to elucidate the venom's variations and mechanism of action. Further studies must be conducted to improve the knowledge on the venom's components, which can be used in basic science and clinical applications to enhance the effectiveness of treatments. Moreover, the introduction of new methods in proteomics and genomics can lead to the discovery of new compounds. These can serve as research tools or templates for the development of drugs. The application of these sensitive and comprehensive methods allows studying any venom and/or its components possible. As such, the complexities of these tasks demand the integration of multidisciplinary research tools to study toxinology.

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## Conflicts of interest

The authors declare that there are no conflicts of interest.

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