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Snake venomics of *Lachesis muta rhombeata* and genus-wide antivenomics assessment of the paraspecific immunoreactivity of two antivenoms evidence the high compositional and immunological conservation across *Lachesis*

Davinia Pla[a], *Libia Sanz*[a], *Pedro Molina-Sánchez*[b], *Virginia Zorita*[b], *Marvin Madrigal*[c], *Marietta Flores-Díaz*[c], *Alberto Alape-Girón*[c,d], *Vitelbina Núñez*[e,f], *Vicente Andrés*[b], *José María Gutiérrez*[c], *Juan J. Calvete*[a,g,*]

[a]*Laboratorio de Venómica y Proteinómica Estructural, Instituto de Biomedicina de Valencia, C.S.I.C., Valencia, Spain*
[b]*Laboratory of Molecular and Genetic Cardiovascular Pathophysiology, Department of Epidemiology, Atherothrombosis and Imaging, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain*
[c]*Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica*
[d]*Departamento de Bioquímica, Facultad de Medicina, Universidad de Costa Rica, San José, Costa Rica*
[e]*Programa de Ofidismo/Escorpionismo, Universidad de Antioquia, Medellín, Colombia*
[f]*Escuela de Microbiología, Universidad de Antioquia, Medellín, Colombia*
[g]*Departamento de Biotecnología, Universidad Politécnica de Valencia, Spain*

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ABSTRACT

We report the proteomic analysis of the Atlantic bushmaster, *Lachesis muta rhombeata*, from Brazil. Along with previous characterization of the venom proteomes of *L. stenophrys* (Costa Rica), *L. melanocephala* (Costa Rica), *L. acrochorda* (Colombia), and *L. muta muta* (Bolivia), the present study provides the first overview of the composition and distribution of venom proteins across this wide-ranging genus, and highlights the remarkable similar compositional and pharmacological profiles across *Lachesis* venoms. The paraspecificity of two antivenoms, produced at Instituto Vital Brazil (Brazil) and Instituto Clodomiro Picado (Costa Rica) using different conspecific taxa in the immunization mixtures, was assessed using genus-wide comparative antivenomics. This study confirms that the proteomic similarity among *Lachesis* sp. venoms is mirrored in their high immunological conservation across the genus. The clinical and therapeutic consequences of genus-wide venomics and antivenomics investigations of *Lachesis* venoms are discussed.

Biological significance
The proteomics characterization of *L. m. rhombeata* venom completes the overview of *Lachesis* venom proteomes and confirms the remarkable toxin profile conservation across the five clades of this wide-ranging genus. Genus-wide antivenomics showed that two antivenoms, produced

* *Corresponding author at:* Instituto de Biomedicina de Valencia, C.S.I.C., Jaime Roig 11, 46010 Valencia, Spain. Tel.: +34 96 339 1778; fax: +34 96 369 0800.
E-mail address: jcalvete@ibv.csic.es (J.J. Calvete).

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against *L. stenophrys* or *L. m. rhombeata*, exhibit paraspecificity towards all other congeneric venoms. Our venomics study shows that, despite the broad geographic distribution of the genus, monospecific antivenoms may achieve clinical coverage for any *Lachesis* sp. envenoming.

1. Introduction

Lachesis (Daudin, 1803 [1]) is a wide-ranging genus of venomous pitvipers (collectively called "bushmasters") found in remote forested areas of Central and South America, and on the island of Trinidad [2,3]. It comprises the longest vipers in the world, with adults varying in length from 2 to 2.5 m (the largest known specimen was of 3.65 m). Bushmasters, nocturnal terrestrial predators of small mammals (mainly rodents and marsupials), are unique among New World vipers in laying eggs (about a dozen in an average clutch) rather than giving birth to live young [2,3]. Four nominal species are currently recognized in genus *Lachesis* [2,3]: *L. stenophrys*, from the Caribbean coast of Central America; *L. melanocephala*, which 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 part of Panama; *L. acrochorda* (Chocoan bushmaster), found 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; and *L. muta*, found in South America east of the Andes [2,3]. *Lachesis* is one of the three Fates in Greek mythology. The scientific name *Lachesis muta* means "silent fate" referring to their tail shaking in a similar manner to rattlesnakes, though they lack a rattle. This led to some calling it 'the mute rattlesnake'.

Within *L. muta*, populations distributed in the Guyana Shield are slightly different from those distributed in South of the Amazonian Basin and in the Atlantic rainforest, from the Brazilian state of Ceará to the state of Rio de Janeiro, are often referred to as *L. m. rhombeata* [2]. Zamudio and Greene [4] estimated phylogenetic relationships within the genus *Lachesis* using mitocondrial DNA. The molecular data are consistent with the hypothesis that vicariant geologic events underlie differentiation in these snakes. The results of the phylogenetic analysis combined with statistical data suggested independence between the Central and South American clades, with specimens assigned to *L. melanocephala* being more closely related to those in the Caribbean of Lower Central America (*L. stenophrys*). Based on morphological characters, Fernandes et al. [5] corroborated these findings. Although their analysis did not include a representative sample of *L. acrochorda*, they examined a single individual from Darien (Panama) and proved its closest affinity with *L. m. muta*. Thus, the current view of the phylogenetic relationships of the four nominal species is: [((*L. stenophrys*, *L. melanocephala*), *L. acrochorda*), (*L. muta muta*, *L. muta rhombeata*)] [M. Sasa, unpublished data]. Divergence between South and Central American bushmasters might have occurred 18.0–6.5 Mya, the split between Central American *L. melanocephala* and *L. stenophrys* is estimated to have taken place 11–4 Mya, and differentiation among the South American lineages happened only 300,000 to 800,000 years ago [4].

Human envenomings by *Lachesis* taxa are infrequent but rather severe due to the large venom yield (200–411 mg) [6,7], although venom lethality in mice is weak compared to those of some other vipers [8]. Brown [7] gives the following LD_{50} values of *L. m. muta* venom for mice: 1.5 mg/kg (intravenous), 1.6–6.2 mg/kg (intraperitoneal), 6.0 mg/kg (subcutaneous). A review of reports of 20 cases of bites in humans reliably attributed to *Lachesis* in Costa Rica, French Guiana, Brazil, Colombia and Venezuela confirms the severity of these envenomations, characterized by agonizing burning-throbbing local pain and edema, within the first few minutes after the bite. Mild hemorrhage around the bite site and local blister formation are common. Systemic manifestations are characterized by hemorrhage, coagulopathy, and cardiovascular collaps, and what has been dubbed the "Lachesis syndrome", a series of alterations associated with a "vagal symptomatology", i.e., profuse sweating, abdominal colic, nausea, recurrent vomiting, watery diarrhea, diastolic and systolic hypotension, and sinus bradycardia, together with sensorial disorders (uncoordinated march, lapses of consciousness) and serious hemodynamic alterations within a very short time frame (15–20 min). These signs and symptoms can be considered pathognomonic of envenomations by snakes of the genus [9–14]. The occurrence of intraspecies variability in the biochemical composition and symptomatology after envenomation by snakes from different geographical location and age has long been appreciated by herpetologists and toxinologists, and appears to be a general trend of snake venoms [15]. In envenomings attributed to *L. m. muta* in the French Guiana, Hulin et al. [16] described, in addition to severe pain and local swelling, incoagulable blood, hypofibrinogenemia, and elevated fibrin (ogen) degradation products in the serum. Generally *Lachesis* venom consumes prothrombin and fibrinogen, resulting in a disseminated intravascular coagulation type coagulopathy with increased coagulation and bleeding times.

Comparison of the venom proteome of *L. muta rhombeata*, here reported, with those of the other Central and South American bushmasters [17,18] streamlines the remarkable similar compositional and pharmacological profiles across *Lachesis* venoms. This unusual circumstance for wide-ranging species suggests that ancestral toxin genes might have been evolving under purifying selection since the split of *Lachesis* in the mid-Miocene [4]. Our finding of the high genus-wide intraspecific venom phenotype conservation [18] allowed us to predict that monospecific Lachesic antivenoms may exhibit paraspecificity against the other congeneric species [18]. Here, we confirm the high immunological conservation across genus *Lachesis* using comparative antivenomics to assess the efficacy