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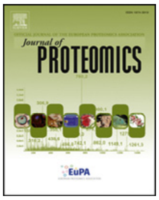

Combined venomics, venom gland transcriptomics, bioactivities, and antivenomics of two *Bothrops jararaca* populations from geographic isolated regions within the Brazilian Atlantic rainforest☆

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ABSTRACT

Bothrops jararaca is a slender and semi-arboreal medically relevant pit viper species endemic to tropical and subtropical forests in southern Brazil, Paraguay, and northern Argentina (Misiones). Within its geographic range, it is often abundant and is an important cause of snakebite. Although no subspecies are currently recognized, geographic analyses have revealed the existence of two well-supported *B. jararaca* clades that diverged during the Pliocene ~3.8 Mya and currently display a southeastern (SE) and a southern (S) Atlantic rainforest (*Mata Atlântica*) distribution. The spectrum, geographic variability, and ontogenetic changes of the venom proteomes of snakes from these two *B. jararaca* phylogroups were investigated applying a combined venom gland transcriptomic and venomic analysis. Comparisons of the venom proteomes and transcriptomes of *B. jararaca* from the SE and S geographic regions revealed notable interpopulational variability that may be due to the different levels of population-specific transcriptional regulation, including, in the case of the southern population, a marked ontogenetic venom compositional change involving the upregulation of the myotoxic PLA_2 homolog, bothropstoxin-I. This population-specific marker can be used to estimate the proportion of venom from the southern population present in the *B. jararaca* venom pool used for the Brazilian *soro antibotrópico* (SAB) antivenom production. On the other hand, the southeastern population-specific D49-PLA_2 molecules, BinTX-I and BinTX-II, lend support to the notion that the mainland ancestor of *Bothrops insularis* was originated within the same population that gave rise to the current SE *B. jararaca* phylogroup, and that this insular species endemic to Queimada Grande Island (Brazil) expresses a pedomorphic venom phenotype. Mirroring their compositional divergence, the two geographic *B. jararaca* venom pools showed distinct bioactivity profiles. However, the SAB antivenom manufactured in Vital Brazil Institute neutralized the lethal effect of both venoms to a similar extent. In addition, immobilized SAB antivenom immunocaptured most of the venom components of the venoms of both *B. jararaca* populations, but did not show immunoreactivity against vasoactive peptides. The Costa Rican bothropic–crotalic–lachesic (BCL) antivenom showed the same lack of reactivity against vasoactive peptides but, in addition, was less efficient immunocapturing PI- and PIII-SVMPs from the SE venom, and bothropstoxin-I, a CRISP molecule, and a D49-PLA_2 from the venom of the southern *B. jararaca* phylogroup.

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The remarkable paraspecificity exhibited by the Brazilian and the Costa Rican antivenoms indicates large immunoreactive epitope conservation across the natural history of *Bothrops*, a genus that has its roots in the middle Miocene.

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1. Introduction

Bothrops jararaca (Wied-Neuwied, 1824) is a well-studied semi-arboreal venomous pit viper species endemic to southern Brazil (S Bahia, Espirito Santo, Rio de Janeiro, Minas Gerais, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul), northeastern Paraguay, and northern Argentina (Misiones), where it occurs in deciduous tropical forests and semitropical upland forests, from near sea level to over 1000 m altitude [1–4] (Fig. 1). *B. jararaca* preys mainly on small vertebrates and exhibits an ontogenetic shift in feeding habit: adult snakes prey mainly on medium-size rodents, whereas food items of juvenile snakes consist mainly of frogs [2]. The specific name, jararaca, is derived from the Tupi words *yarará* and *ca*, which mean "large snake". *B. jararaca* is a slender lancehead that can grow to a maximum total length of 160 cm, although the average length is considerable less [1,2]. *B. jararaca* is abundant in many parts of its broad range of distribution and represents an important cause of snakebite, particularly in heavily populated areas of southeastern Brazil, where it is responsible for most accidental envenomings [5–8]. On average, *B. jararaca* produces about 25 mg, with a maximum of 300 mg, of highly toxic venom, whose median lethal dose (LD_{50}) for mice is 1.4 mg/kg intraperitoneal (1.2 mg/kg intravenous, 3 mg/kg subcutaneous) [9]. In humans, the typical clinical picture of envenoming includes local swelling, petechiae, bruising and blistering of the affected limb, spontaneous systemic bleeding in various organs, subconjunctival hemorrhage and clotting disturbances [8,10,11]. The systemic symptoms can potentially be fatal and may involve hemostatic disorders, intracranial hemorrhage, shock and renal failure [8,10,11].

From a natural history perspective, venom represents an adaptive trophic trait in the evolution of advanced snakes [12–14]. A deep understanding of the composition of venoms and of the principles governing the evolution of venomous systems is of applied importance for exploring the enormous potential of venoms as sources of chemical and pharmacological novelty, but also to fight the dire consequences of snakebite envenomings. In this regard, the last decade has witnessed the development of techniques and strategies for assessing the toxin composition of snake venoms, "venomics", directly (through proteomic-centered approaches) and indirectly (via venom gland transcriptomic and

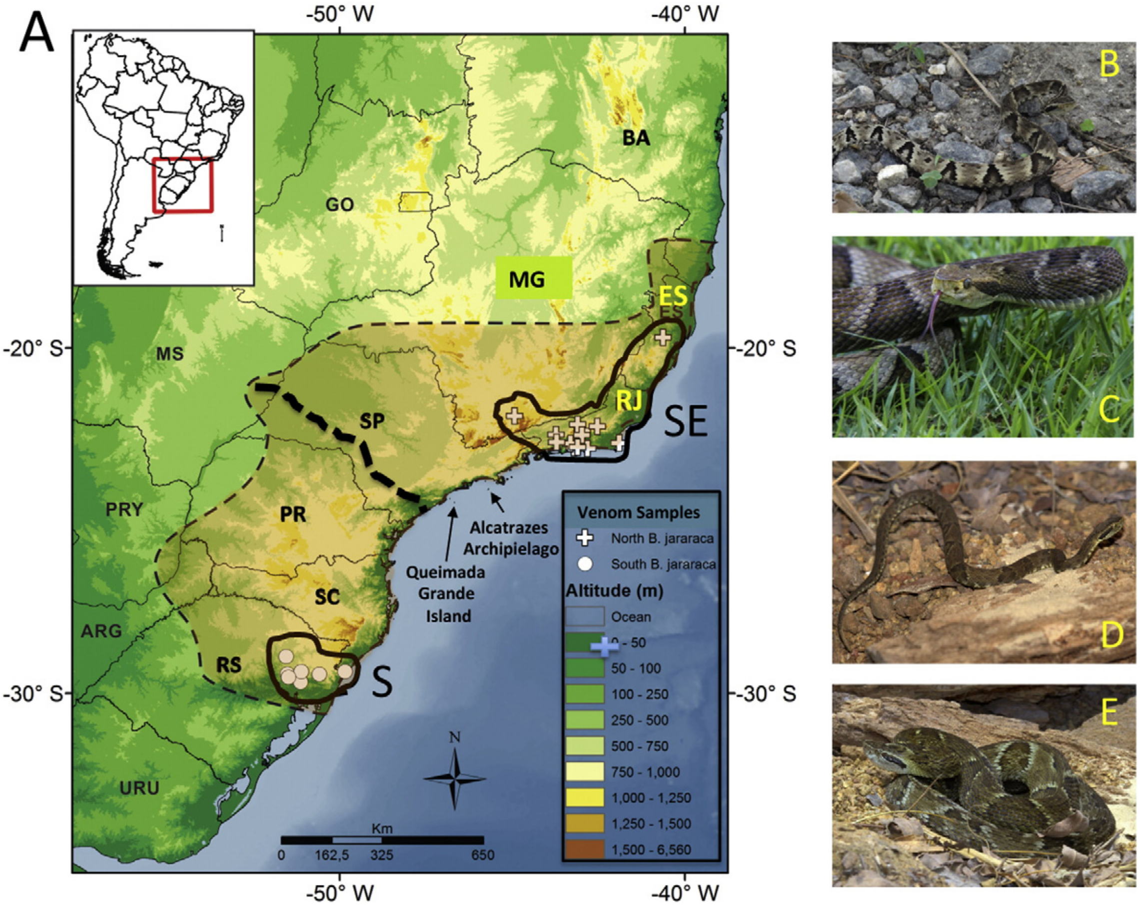

Fig. 1. Geographic distribution of the *B. jararaca* populations investigated. Panel A, physical map of Brazil showing the collection sites of the southeastern (SE) and the southern (S) populations within the Atlantic forest. The number of specimens milked in each locality is indicated in the Materials and methods section. The range of *B. jararaca* in Southern Brazil, including the States of Bahia (BA); Espirito Santo (ES); Rio de Janeiro (RJ); Minas Gerais (MG); São Paulo (SP); Paraná (PR); Santa Catarina (SC); and Rio Grande do Sul (RS) is highlighted in pale orange. The broken line indicates the main genetic barrier between the SE and the S *B. jararaca* phylogroups [3]. Panels B and C, respectively, pictures of juvenile (© Tyelli Ramos) and adult (© Claudio Machado) specimens of *B. jararaca* from the SE population. Panels D and E, respectively, pictures of juvenile and adult specimens of *B. jararaca* from the S population (© Sergio Bavaresco).