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Original Article

Venomous snakes of medical importance in the Brazilian state of Rio de Janeiro: habitat and taxonomy against ophidism

Serpentes peçonhentas de importância médica no estado do Rio de Janeiro: habitat e taxonomia contra ofidismo

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Abstract

Snakebite envenoming is a major global health problem that kills or disables half a million people in the world's poorest countries. Identifying the biting snake and its habitat use is key to understanding snakebite eco-epidemiology and optimizing its clinical management. To prevent and combat the neglected snakebite disease, we characterize the morphology, geographic distribution, habitat use, and snakebites of medically important venomous snakes in the state of Rio de Janeiro (Brazil). Despite *Philodryas* spp. not being considered of medical importance by the Brazilian Ministry of Health, we also explore their data once the bites may require medical intervention, may cause death, and their consequences are underestimated. Methods: We assessed taxonomy and geographic data from specimens housed in scientific collections, the literature, and the Notifiable Diseases Information System. Our data revealed fragility in the morphological characters recommended to distinguish *Bothrops jararaca* from *B. jararacussu,* identify the subspecies of *Crotalus durissus* and distinguish the species of *Philodryas*. To help identify these species, we present an identification key to the venomous snake species from Rio de Janeiro based on the morphological data collected. We record the genera *Bothrops* and *Micrurus* in all mesoregions of the state. Here, we provide the first record of *C. durissus* in the Serrana region, supporting the hypothesis of geographic expansion of the species in the state. The crotalic antivenom must not be missing in Médio Paraíba, Centro-Sul Fluminense, and Serrana, where the rattlesnake *C. durissus* occurs. *Bothrops bilineatus* and *Lachesis muta* have historical records presented for the first time herein. However, these species are likely endangered or extinct in the state. There were 7,483 snakebites reported between 2001 and 2019, with an annual average of 393.8 cases. The *Bothrops* genus is responsible for the majority of accidents. The highest number of cases occurred in the Serrana region, the largest pole of family agriculture in Rio de Janeiro. We improve the identification of venomous snake species, better delimit their distribution, and update the number of cases of snakebites, thus providing greater precision in the attention to this problem in Rio de Janeiro. We emphasize the importance of clinical studies to test using bothropic-crotalic antivenom and heparin in all mesoregions to treat *B. jararacussu* envenomation; and mechanical ventilation, atropine, and anticholinesterases in the emergency health centers in the Metropolitana and Norte Fluminense regions due to the occurrence of the coral *M. lemniscatus* in these areas.

Keywords: biogeography, Colubridae, Elapidae, tropical neglected diseases, Viperidae.

Resumo

O envenenamento com serpentes de importância médica é um grande problema de saúde global que mata ou incapacita meio milhão de pessoas nos países mais pobres do mundo. A identificação de serpentes de importância médica e aspectos de sua história de vida é fundamental para compreender a eco-epidemiologia dos acidentes ofídicos e otimizar seu manejo clínico. Para prevenir e combater a doença negligenciada do ofidismo, caracterizamos os dados de morfologia, distribuição, uso de habitat e acidentes ofídicos das serpentes peçonhentas de importância médica no estado do Rio de Janeiro, Brasil. Apesar de *Philodryas* spp. não serem consideradas de importância médica pelo Ministério da Saúde do Brasil, também apresentamos seus dados porque suas picadas podem exigir intervenção médica, causar morte e suas consequências são subestimadas. Foram avaliados dados morfológicos e de habitat de espécimes pertencentes a coleções cientificas, literatura e dados do Sistema de Informação de

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Agravos de Notificação. Nossos dados revelaram caracteres morfológicos frágeis recomendados para distinguir *Bothrops jararaca* e *B. jararacussu*, para identificar as subespécies de cascavel *Crotalus durissus* e para distinguir as serpentes cipó *Philodryas* spp. Para auxiliar na identificação dessas espécies apresentamos uma chave de identificação para as mesmas com base nos dados morfológicos coletados. Registramos os gêneros *Bothrops* e *Micrurus* em todas as mesorregiões do estado. Já o soro anticrotálico não pode faltar no Médio Paraíba, Centro-Sul Fluminense e Serrana, onde encontramos a cascavel *Crotalus durissus*. Aqui estendemos o registro da cascavel pela primeira vez para a região Serrana, corroborando a hipótese da expansão da espécie no estado. *Bothrops bilineatus* e *Lachesis muta* possuem registros históricos aqui apresentados pela primeira vez. Essas espécies provavelmente estão ameaçadas ou extintas no estado. Foram 7,483 acidentes entre 2001-2019, com média anual de 393.8. O gênero *Bothrops* é responsável pela maioria dos acidentes. O maior número de casos ocorreu na região Serrana, maior polo da agricultura familiar do Rio de Janeiro. Aperfeiçoamos a identificação das espécies de serpentes de importância médica, delimitamos melhor sua distribuição e atualizamos os acidentes ofídicos, proporcionando maior precisão na atenção ao ofidismo no estado do Rio de Janeiro. Ressaltamos a importância de estudos clínicos para testar o uso de soro antibotrópico-crotálico e heparina em todas as mesorregiões para o tratamento do ofidismo *B. jararacussu* e ventilação mecânica, atropina e anticolinesterásicos nos prontos-socorros das regiões Metropolitana e Norte Fluminense pela ocorrência do coral *M. lemniscatus*.

Palavras-chave: biogeografia, Colubridae, doenças tropicais negligenciadas, Elapidae, Viperidae.

1. Introduction

Snakebites affect about 5.4 million people worldwide, resulting in 130,000 deaths annually (WHO, 2021). Additionally, more than 400.000 people are left with permanent physical and psychological sequelae (Kasturiratne et al., 2008; Williams et al., 2010), significantly impacting their quality of life (Gutiérrez, 2012). Snakebite is a neglected disease in many tropical and subtropical regions, primarily affecting the most deprived and politically impoverished rural communities (Gutiérrez et al., 2006; Gutiérrez et al., 2010; Williams et al., 2010; Gutiérrez et al., 2011; Gutiérrez, 2012; Gutiérrez et al., 2017; Gutiérrez, 2020).

On average, 27,120 snakebite cases are reported annually in Brazil, and these cases are strongly associated with environmental and socioeconomic factors (Lira-da-Silva et al., 2009; Schneider et al., 2021). The state of Rio de Janeiro harbors the following medically important venomous snake species: the lanceheads *Bothrops jararaca*, *B. jararacussu*, *B. fonsecai*, *B. bilineatus*, *B. alternatus*, and *B. neuwiedi*; the coral snakes *Micrurus corallinus*, *M. decoratus*, and *M. lemniscatus*; the rattlesnake *Crotalus durissus*; and the bushmaster *Lachesis muta* (Souza and Machado, 2017). Some bites caused by the genus *Philodryas* have been categorized as mildly dangerous, and one death has been reported (Salomão and Di Bernardo, 1995). The Lichtenstein's green racer (*P. olfersii*) and the Patagonia green racer *P. patagoniensis* also occur in Rio de Janeiro state (Souza and Machado, 2017). Due to the medical attention required in some cases [e.g., Salomão and Di Bernardo (1995)], *Philodryas* have also been considered of medical importance in the past [e.g., Brasil (2001); Puorto and França (2003)], but not anymore (see Brasil, 2023).

Species-specific assistance should be considered against ophidism (Calvete et al., 2009) as a robust taxonomic underpinning is essential to help ensure the replicability of research results (Wüster and Quijada-Mascareñas, 2009). Moreover, defining the geographic boundaries and accessing the species' natural history have implications for species-specific treatment (Madrigal et al., 2017). Field and laboratory observations indicated that "the taxonomic value" of some characters commonly employed to distinguish species of medical importance in Rio de Janeiro State, such as *B. jararaca* and *B. jararacussu*, seem to overlap. During management and curatorship activities in the Instituto Vital Brazil Serpentarium and scientific collection, we found several specimens of *Bothrops* from Rio de Janeiro state non-diagnosable at the species level using the available literature [e.g. Campbell and Lamar (2004)].

Brazil (1901) had already shown that the crotalic antivenom was "much more active" than the bothropic antivenom in treating *B. jararacussu* envenomation. Dos-Santos et al. (1992) showed that the combined use of bothropic and crotalic antivenoms more effectively neutralized the myotoxic, coagulant, and lethal activities of *B. jararacussu* venom than when these antivenoms were used alone. Heparin and commercial bothropic antivenom effectively neutralized the neurotoxic effects of *B. jararacussu* crude venom on the phrenic nerve-diaphragm of mice (Rostelato-Ferreira et al., 2010). Therefore, the use of specific bothropic antivenom in conjunction with a complementary treatment is often discussed to treat *B. jararacussu* envenomation, as some biological activities of the venom observed in mice are not fully neutralized (Correa-Netto et al., 2010; Araujo et al., 2017).

The identification of the subspecies of *Crotalus durissus* remains weak. A better recognition of these subspecies may help determine the composition of the venom, considering not only those crotamine-positive (which are poorly recognized by the antivenom) but also the entire complex set of proteins necessary to produce a more efficient antivenom (Tasima et al., 2020). Finally, coral snake antivenom produced in Brazil using only the venoms of *M. frontalis* and *M. corallinus* neutralized the venoms of *M. frontalis*, *M. corallinus*, and *M. spixii* but not those of *M. altirostris* and *M. lemniscatus* (Tanaka et al., 2010). Considering the need for improving the elapid antivenom [e.g., Ciscotto et al. (2011)], clinical studies have recommended that *M. lemniscatus carvalhoi* envenomation be treated with anticholinesterase drugs as an adjunctive treatment to serum therapy (Coelho et al., 1992; Vital Brazil and Vieira, 1996).

In that regard, morphology-based species identification, geographical maps, and natural history data could help make public health interventions to treat snakebites more specific and efficient [e.g., Geneviève et al. (2018)]. Unfortunately, toxicology and medical science have a long history of paying little attention to the systematics and taxonomy of venomous snakes, leading to difficulties in replicating experimental results and even unnecessary mortality of patients bitten by snakes (Warrell and Arnett, 1976; Wüster, 1996; Warrell, 2008; Visser et al., 2008). For example, an antivenom assessment considers the presence of any venomous snake as a prerequisite for vulnerability; however, different species contribute differently to the risk of envenomation (Longbottom et al., 2018).

Therefore, snakebite cases are also underreported and neglected (Vaiyapuri et al., 2013). For example, in India, deaths from snakebites are about 20 times higher than the official records (Mohapatra et al., 2011). Evidence shows that the same pattern frequently occurs in Brazil (Machado, 2018), making the fight against snakebites even more urgent. At the clinical level, the identity of the biting snake can help healthcare professionals anticipate victims' syndromes and support decision-making when treating the patient (i.e., whether or which type of antivenom to administer). This decision is important because antivenoms are effective against a limited number of venomous snakes and have potentially lethal side effects, such as fatal allergic reactions, and should not be used to treat nonvenomous snakebites (Silva et al., 2016). This is especially important because antivenom vials are scarce and expensive in many countries. Therefore, we aim to facilitate and improve the identification of venomous snake species by mapping and gathering data on habitat use in Rio de Janeiro state. Despite not being considered a snake of medical importance by the Brazilian Ministry of Health, we also updated the database on snakebites and included data on *Philodryas* species because their bites may require medical intervention (Brasil, 2001), may cause death (Salomão and Di Bernardo, 1995), and their consequences are underestimated (Puorto and França, 2003). We expect to improve the identification of snake species by reporting new morphological and habitat use data and detailing their geographic distribution across Rio de Janeiro.

2. Material and Methods

2.1. Study area

The Rio de Janeiro state (in southeastern Brazil) has an area of 43,750,425 km^2, 92 municipalities, and a population of 17,463,349 inhabitants (IBGE, 2022). The population density is 399.16 people per square kilometer, with 15,454,239 people living in urban areas, of which 11,838,752 (74% of the state's total) inhabit the Metropolitana region. More than 1.2 million people are starving, and 22% of the population lives below the poverty line (IBGE, 2010; Neri, 2022). The state lies within the threatened Atlantic Forest biome (Colombo and Joly, 2010). The habitat heterogeneity in the state provides an extraordinary variety of habitats and species (Veloso and Góes-Filho, 1982; INEA, 2011; Martins et al., 2012; Silva and Dereczynski, 2014). Geopolitically, it is subdivided into regions (Baixadas Litonâneas, Centro-Sul Fluminense, Costa Verde, Médio Paraíba, Metropolitana, Nordeste Fluminense, Norte Fluminense, and Serrana), which are used by the government to adopt health policies (IBGE, 2018). This geopolitical division directly influences the distribution of antivenoms, which is based mainly on snakebite data from the Notifiable Diseases Information System (Brasil, 2022).

3. Data Source

3.1. Sampling

We obtained on habitat use and geographic distribution data from 2,123 specimens accessed at the Coleção Científica de Serpentes Instituto Vital Brazil Scientific Collection. We also analyzed historical books from 1940 to 1963 by the medical doctor and herpetologist Dr. Tycho Ottilio de Siqueira Machado found in the library of the Instituto Vital Brazil. We complemented our data using reliable records obtained from the scientific literature (Müller, 1885; Amaral, 1933; Machado, 1944, 1956; Hoge, 1966; Hoogmoed, 1997; Melgarejo, 2003; Bastos et al., 2005; Rocha and Sluys, 2006; Silveira and Evers-Junior, 2007; Silva and Rodrigues, 2008; Bérnils, 2009; Pontes et al., 2009; Martins et al., 2012; Duarte and Menezes, 2013; Vechio-Filho, 2015; Nogueira et al., 2019). Species nomenclature follows Costa and Bernils (2018). We did not include doubtful species records, i.e., those lacking voucher specimens for identification and those with an incomplete occurrence record that made mapping impossible.

3.2. Mapping

Geographic coordinates were obtained from the Instituto Brasileiro de Geografia e Estatística (IBGE, 2018) and gazetteers (USBGN and CidVil). Records lacking precise locality data were georeferenced using municipality centroids. We extracted historical climatic data on temperature, phytophysiognomy, and elevation for each point record using a 30 arc-second spatial resolution from WorldClim version 2.1 for the period between 1970 and 2000 (Fick and Hijmans, 2017) to construct an environmental profile of geographical occurrence for each species. Geographic distribution maps were produced in QGIS version 3.26 (QGIS Development Team, 2020).

3.3. Morphological data

Pholidosis, morphometric, and color pattern data were collected from preserved voucher specimens (Supplementary file I). We examined 202 specimens from 12 species and five genera. Terminology for scale counts and qualitative traits follows Campbell and Lamar (2004) for Viperidae, Thomas (1976) for Colubridae, and Silva Junior et al. (2016) for Elapidae. We measured head width (HW) using a digital caliper to the nearest 0.1 mm. We measured the snout-vent length (SVL) and tail length

(TL) using flexible ruler (Dowling, 1951; Thomas, 1976; Francini et al., 1990). We determined the sex of specimens through a small incision at the base of the tail or by the presence of an everted hemipenis (Yuki, 1994).

3.4. Snakebites in Rio de Janeiro

We obtained data on snakebites in Rio de Janeiro state from 2001 to 2019 by accessing the Notifiable Diseases Information System (Brasil, 2022). We accessed, organized, and computed the data in an electronic spreadsheet and included cases of patients who recovered (with or without sequelae) and patients who died from the snakebites. This study used exclusively public data provided by the Notifiable Diseases Information System (Brasil, 2022).

4. Results

We recorded 2,123 specimens collected in Rio de Janeiro state between 1820 and 2021, with a richness of five genera and 13 species distributed in three families (Figures 1-8).

4.1. Taxonomy

We found that the distinction between *B. jararacussu* and *B. jararaca* and between *P. patagoniensis* and *P. olfersii*

Figure 1. Distribution map (A) and pictures of live specimens of *Bothrops jararaca* showing a range of colour patterns from Niterói (B), Miguel Pereira (C-D), State of Rio de Janeiro (E), Petrópolis (F), siblings with different dorsal colour patterns same mother from Guapimirim (G, H). Photos by Breno Hamdan (B, C, F, G, H), Gustavo Cunha (D, E).

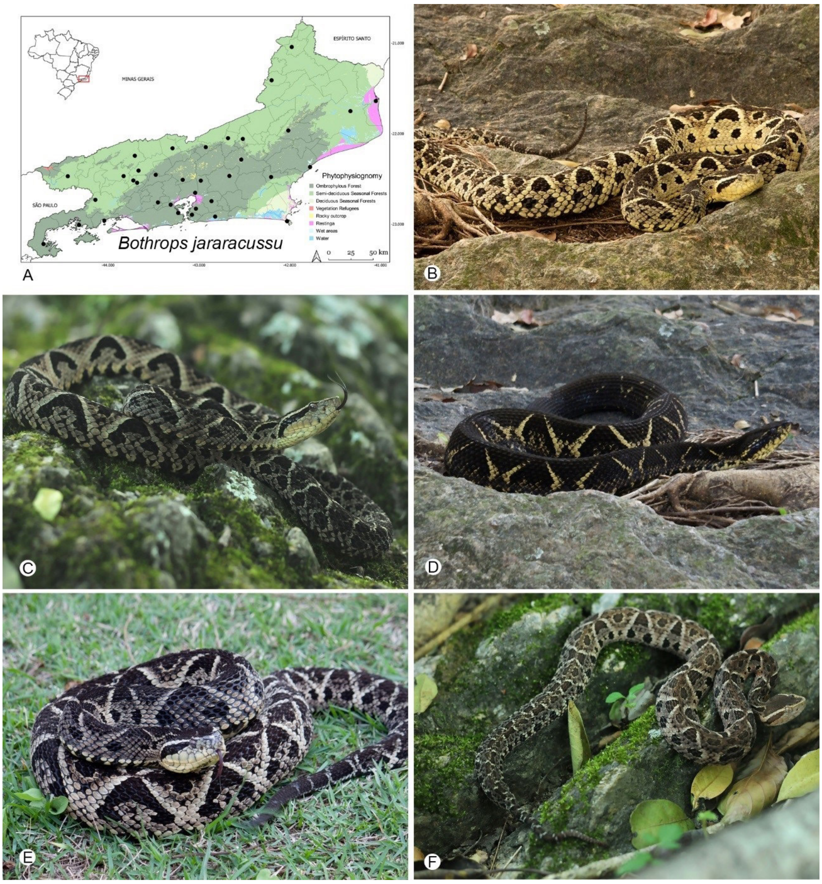

Figure 2. Distribution map (A) and pictures of live specimens of *Bothrops jararacussu* showing different colour patterns from the State of Rio de Janeiro (B, D-E), Cachoeiras de Macacu (C), and Rio de Janeiro (F). Photos by Claudio Machado (B, D, E) and Breno Hamdan (C, F).

was being hampered because of the overlap in the current diagnostic traits, especially in preserved specimens. To help in species identification, we therefore proposed a new combination of characters, presented below in an artificial identification key for medically important snakes in Rio de Janeiro state (Figures 1-8; Supplementary file II).

Bothrops jararacussu has 168-182 ventral scales in males and 174-182 in females (*vs.* 186-214 and 195-210 in male and female of *B. jararaca*, respectively); generally more than five dorsal scales reaching the internasal scales (*vs.* four or less in *B. jararaca*); top of the head dark brown (*vs.* diffuse dark markings in *B. jararaca*); labial color generally uniform (*vs.* generally stained in *B. jararaca*); small circles on the basal part of the lateral triangles (*vs.* only well-designed triangles in *B. jararaca*); triangle blotches with a lighter border around them connecting with the surrounding borders (*vs.* triangle with a lighter border not contacting other triangle blotches) (Figures 1-2).

Crotalus durissus subspecies were impossible to identify due to the large overlap of diagnostic characters available to date. We were also unable to find characters that would allow us to identify the subspecies of *C. durissus*. A few specimens fit the diagnosis of *C. d. terrificus,* most of them showing diagnostic characters for two or more subspecies, such as the presence or absence of contrasting diamond-shaped spots on the last third of the body and short *versus* long white paravertebral lines on the first third of the body (Figure 5).

Philodryas patagoniensis has a brown ventral scale border (*vs* white in *P. olfersii*); a brown head (*vs.* bronze in *P. olfersii*); a blackened dorsal shield border on the first third of the body (*vs.* whitish in *P. olfersii*); the background color of the belly darkening towards the tail (*vs.* uniform in *P. olfersii*); postocular stripe absent (*vs.* present in *P. olfersii*); dorsal background color with black spots (*vs.* uniform in *P. olfersii*) (Figure 7). We had no issue identifying the other species (Figures 4-6).

4.2. Geographic distribution and habitat use

The snake species of medical importance in Rio de Janeiro are widely distributed across all geopolitical regions (Figure 8). We found *Bothrops* (n = 1,594) in Costa Verde, Centro-Sul Fluminense, Metropolitana, Serrana, Médio Paraíba, Noroeste Fluminense, Norte Fluminense, and Baixadas Litorâneas; *Lachesis* (n = 11) in Noroeste Fluminense, Norte Fluminense, Baixadas Litorâneas, Serrana, Centro-Sul Fluminense, and Metropolitana; *Crotalus* (n = 96) in Centro-Sul Fluminense, Costa Verde,

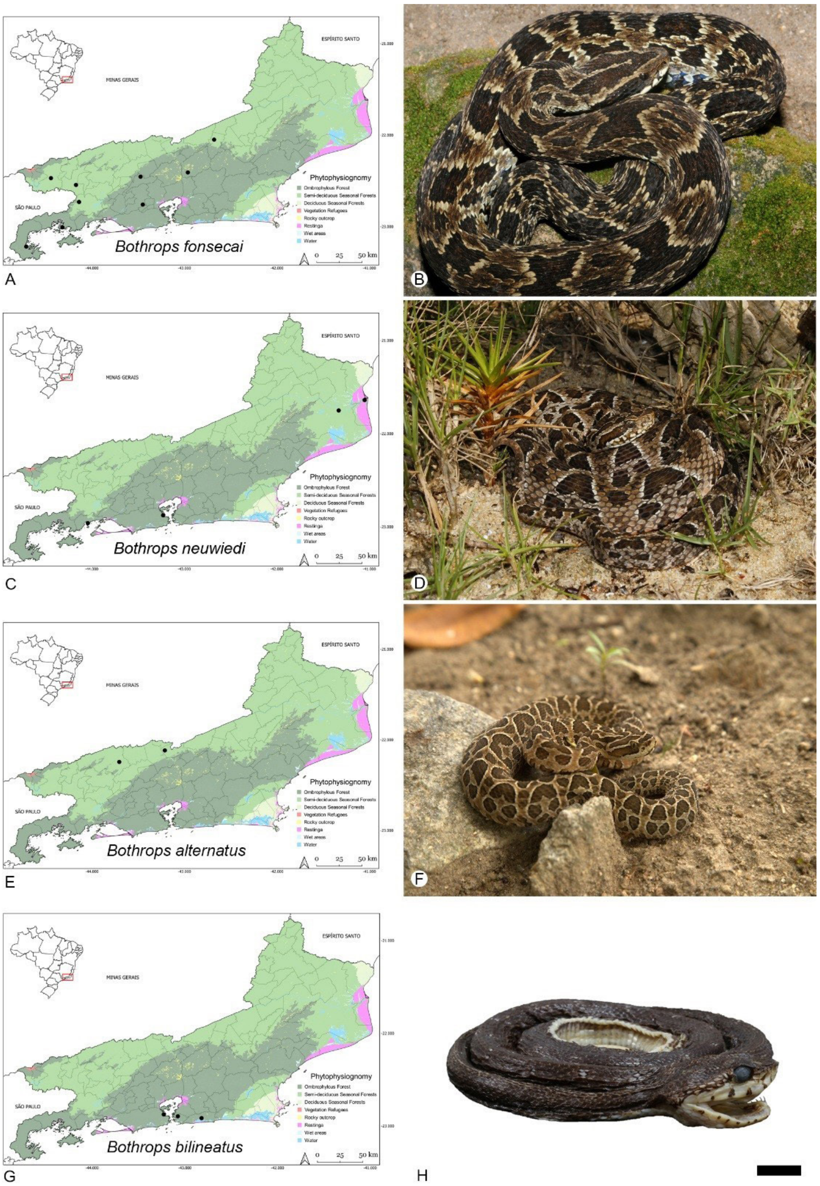

Figure 3. Distribution map (A, C, E, G) and live specimens' pictures of *Bothrops fonsecai* from Nova Friburgo (B); *B. neuwiedi* from São João da Barra (D); *B. alternatus* specimen outside of Rio de Janeiro (F); and a preserved specimen of *B. bilineatus* from Maricá (H). Photos by Breno Hamdan (B), Antonio Bordignon (D), Claudio Machado (F), Gustavo Cunha (H).

Médio Paraíba, Metropolitana, Serrana, and Noroeste Fluminense; *Micrurus* (n = 325) in Costa Verde, Centro-Sul Fluminense, Metropolitana, Serrana, Médio Paraíba, Noroeste Fluminense, Norte Fluminense, and Baixadas Litorâneas; and *Philodryas* (n = 97) in Costa Verde, Centro-Sul Fluminense, Metropolitana, Serrana, Médio Paraíba, Norte Fluminense, and Baixadas Litorâneas.

The species with the most records of unique localities were *B. jararaca* (recorded in 64 municipalities), followed by *B. jararacussu* and *M. corallinus* (recorded in 35 municipalities). We found that the species are widely distributed and habitat generalists (Table 1). However, we found that *B. neuwiedi* and *M. lemniscatus* are restricted to lowland restingas and mangroves (Figures 3-4; Table 1). On the other side, *B. fonsecai* and *M. decoratus* occur in high-altitude rainforest areas (Figures 3-4; Table 1).

We obtained records of *B. bilineatus* in ombrophilous forests, including rare reports for the municipalities of Rio de Janeiro, Niterói, and Maricá (Figure 3), and two records for *B. alternatus* in semi-deciduous seasonal forests the municipalities of Valença and Três Rios (Figure 3; Table 1). We also retrieved nine records of *Lachesis muta* in ombrophilous and semi-deciduous seasonal forests in Santa Maria Madalena, Campo Grande, Itaboraí, Areal, São Fidelis, Cardoso Moreira, Cambuci, Campos dos Goytacazes, and Rio das Ostras (Figure 6).

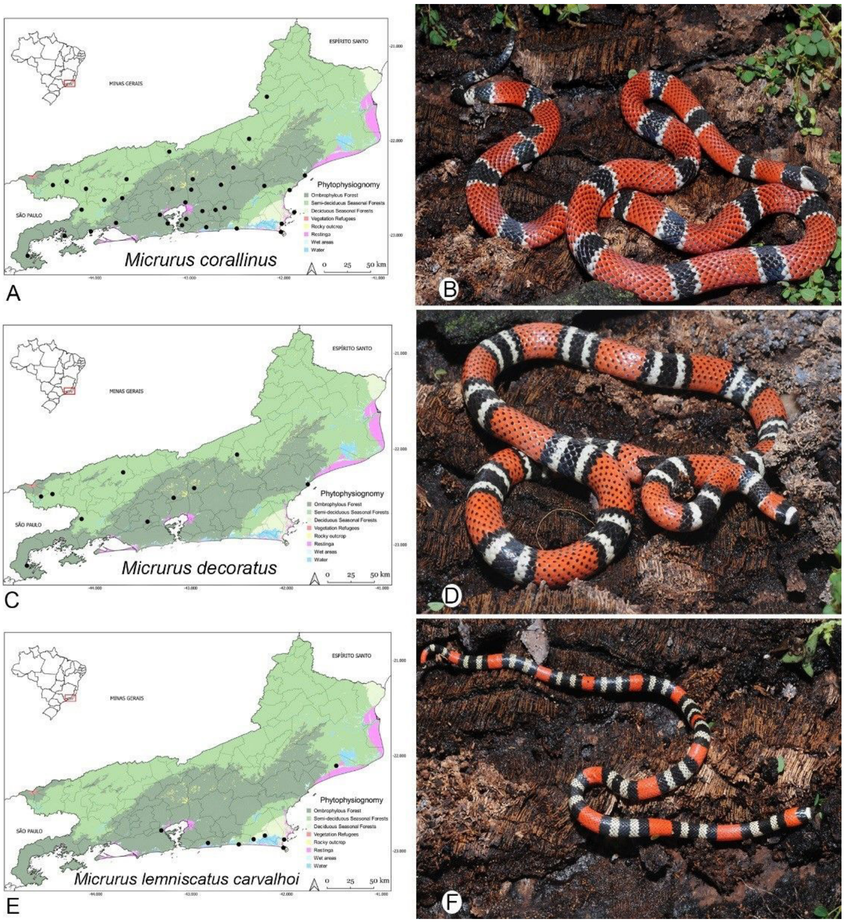

Figure 4. Distribution map (A, C, E) and pictures of live specimens of *Micrurus corallinus* from Cachoeiras de Macacu (B), *M. decoratus* from Petrópolis (D), *M. lemniscatus carvalhoi* from Saquarema (F). Photos by Breno Hamdan (B, D, F).

Table 1. Environmental profile of the habitat use of the venomous snakes of medical importance in the Brazilian state of Rio de Janeiro. Ombrophilous forest (OF); Semi-deciduous seasonal forests (SDSF); Deciduous seasonal forests (DSF); Restinga (RE); Mangrove (MA); Minimum (MIN); maximum (MAX); Average (AVER); Meters (M).

SPECIES	PHYTOPHYSIOGNOMY	ALTITUDE (m) MIN-MAX (AVER)	TEMPERATURE (ºC) MIN-MAX (AVER)
B. bilineata	OF	2-5(4)	20.1-27.9(23.5)
B. fonsecai	SDSF; OF	5-1217(595)	17.1-26.5(21.2)
B. jararaca	SDSF; OF; DSF; MA	2-871(30)	18.2-27.5(22.3)
B. jararacussu	SDSF; OF; DSF; MA	2-846(273)	18.7-27.5(22.6)
B. neuwiedi	OF; MA; SDSF	2-18(10)	21.3-28.5(24.5)
C. durissus	SDSF; OF	5-1648(385)	17.8-28.3(22.3)
M. corallinus	SDSF; OF; RE	2-871(182)	19.3-28.3(23.2)
M. decoratus	SDSF; OF	2-1290(587)	16.7-26.7(21.0)
M. lemniscatus	MA; OF; SDSF; DSF	4-560(80)	19.4-28.1(23.1)
P. olfersii	SDSF; OF; DSF; MA	2-871(257)	18.6-28.3(22.8)
P. patagoniensis	SDSF; OF; DSF; MA	2-871(223)	19.1-27.9(22.9)
L. muta	SDSF; OF	4-615(149)	19.6-29.1(23.8)

Figure 5. Distribution map (A) and pictures of live specimens of *Crotalus durissus* from Itatiaia (B, F), Barra do Piraí (D), Valença (E). Photos by Breno Hamdan (B, C, E, F), Eduardo Vassouras (D).

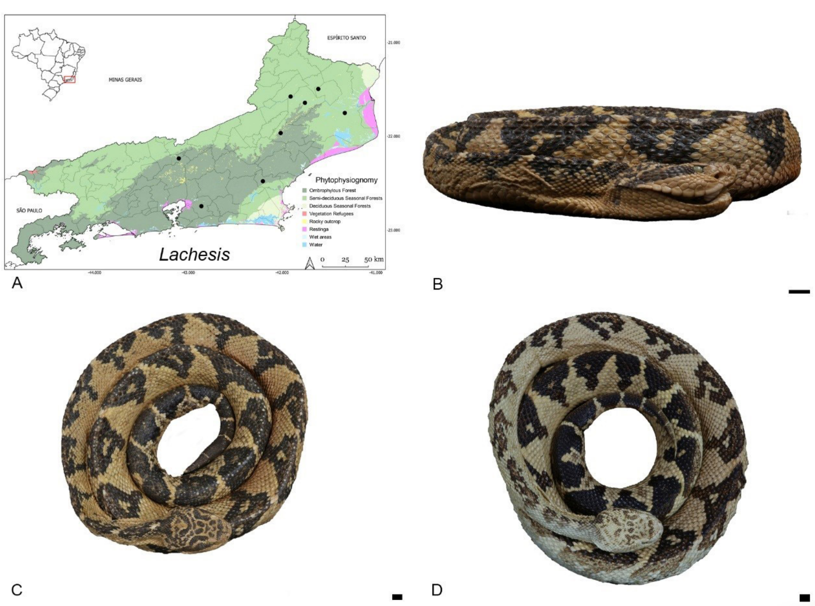

Figure 6. Distribution map (A) and pictures of preserved specimens of *Lachesis muta* from Santa Maria Madalena (B, C), Campos (D). Photos by Gustavo Cunha (B, C, D).

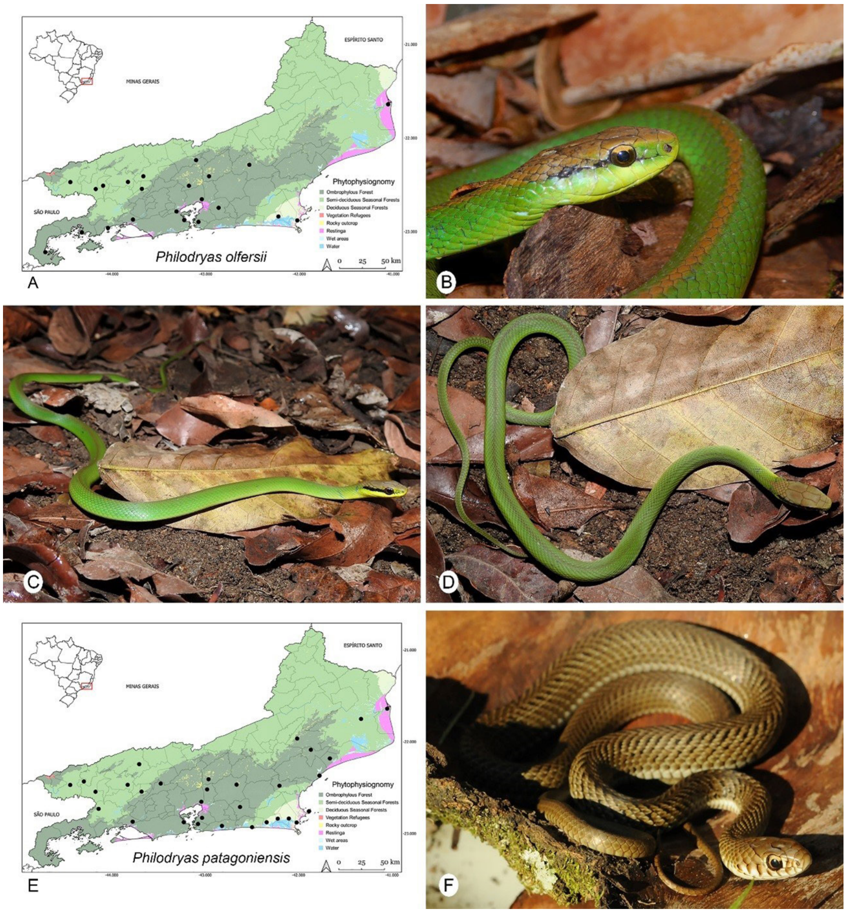

Figure 7. Distribution map (A, E) and pictures of live specimens of *Philodryas olfersii* from Rio de Janeiro (B), Niterói (C, D), an of *P. patagoniensis* from Maricá (F). Photos by Breno Hamdan (B, F), Miguel Relvas (C, D).

4.3. Snakebites in the state of Rio de Janeiro: an update

We recorded 7,483 venomous snakebites in Rio de Janeiro, with an annual average of 393.8 ± 61.5 cases. Most patients (99.7%) recovered, and 0.3% died (Supplementary figure I). The highest number of cases occurred in the Serrana region (n = 2,905), followed by Metropolitana (1,948) and Costa Verde (1,615). The lowest number of cases occurred in the Norte Fluminense region (n = 473) (Supplementary figure II). We found no records of snakebites in the municipalities of Italva, Mesquita, Nilópolis, São João da Barra, and São João do Meriti (Supplementary figure II).

4.4. Identification key to the venomous snakes of medical importance from Rio de Janeiro state

1. Loreal pit present 2
No loreal pit absent...8
2. Tail with rattle...*Crotalus durissus*
Tail with no rattle...3
3. Caudal region with small bristling scales.......*Lachesis muta*
Flat tail tip..4
4. Uniformly green body color pattern.......*Bothrops bilineatus*
Non-green body color pattern...5
5. Prelacunal and second supralabial separate.............6
Prelacunal and second supralabial fused forming the lacunolabial...7
6. Postorbital stripe hook-shaped posteriorly; top of the head with spear-shaped marking; 8-10 intersupraoculars; 51-57 subcaudals in males and 39-49 in females......................................*Bothrops fonsecai*
Postorbital not forming a hook-shaped stripe posteriorly; top of head with no spear-shaped marking; dorsal pattern of dark, bold headphone-shaped blotches; top of head with a range of transverse and longitudinal tan to white markings....................................*Bothrops alternatus*
Postorbital not forming a hook-shaped stripe posteriorly; top of head without spear-shaped marking; top of head not showing a range of transverse and longitudinal tan to white markings......................................*Bothrops neuwiedi*
7. Between 166-182 ventral scales in males and 170-186 in females; more than five scales reaching the internal scales (Supplementary figure III); top of the head generally uniform dark brown; labial color generally uniform; small circles on the basal part of the lateral triangles; triangle blotches with a lighter border around them connecting with the surrounding borders...*Bothrops jararacussu*

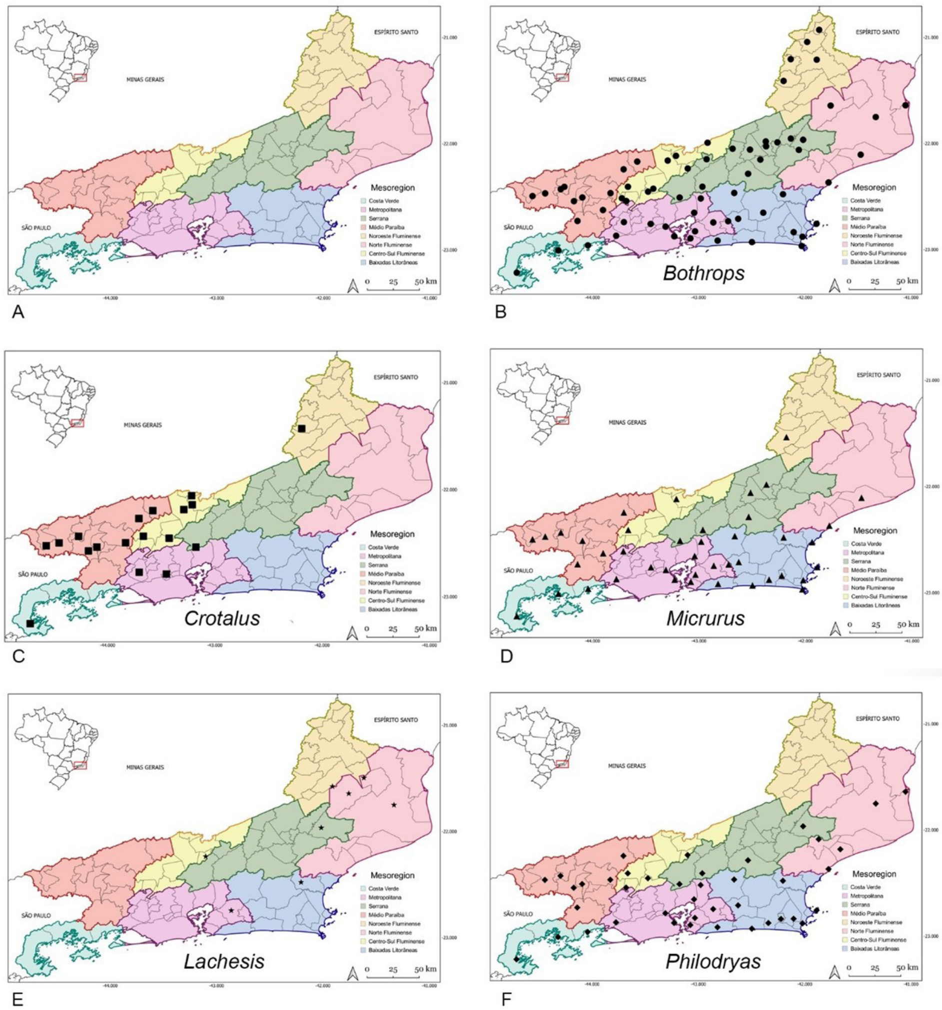

Figure 8. Political mesoregions of Rio de Janeiro state (8A) with a classification following IBGE (2018). Distribution map of *Bothrops* (8B), *Crotalus* (8C), *Micrurus* (8D), *Lachesis*, (8E) and *Philodryas* (8F) genera in Rio de Janeiro State.

Between 195-210 ventral scales in males and 186-214 in females; four or less scales reaching the internal scales (Supplementary figure III); head generally stained dorsally with diffuse dark markings; labial colour generally stained; only well-designed triangle blotches; triangle blotches with a lighter border not contacting other triangle blotches.......……………*Bothrops jararaca*
8. Black, red, and white coral snake pattern.................9
Not a coral snake pattern...
Antiophidic serum is not recommended
9. Black rings arranged in triads, separated by red rings......10
Wide red rings separated by black rings with white borders, blackhead cap not covering parietal tips.........
....*Micrurus corallinus*
10. First black ring sequences are missing in the first ring...*Micrurus decoratus*
First complete black body triad present, black ring on nape usually not covering parietals, 7-17 body triads
....*Micrurus lemniscatus carvalhoi*

5. Discussion

Identifying the causative agent of envenomation and knowing its geographic distribution and habitat use are helpful to understanding human-snake conflict dynamics and providing adequate health facilities (Molesworth et al., 2003; Kasturiratne et al., 2008; Gutiérrez, 2012; Gutiérrez et al., 2017). *Bothrops* and *Micrurus* genera were recorded in all eight Rio de Janeiro mesoregions. Bothropic and elapid antivenoms can be found in antivenom care centers in all eight mesoregions; however, bothropic antivenom is found in 22 centers, while elapid antivenom is found in 13 centers.

Our data revealed a weak combination of characters recommended to distinguish *Bothrops jararaca* from *B. jararacussu,* mainly due to the wide overlap of the diagnostic character "area occupied by interspaces" about 1.5 to 2 times larger than (in *B. jararaca*) or subequal (in *B. jararacussu*) to dorsal blotches (Campbell and Lamar, 2004). Thus, we provide a more apparent distinction between *Bothrops* species from Rio de Janeiro state. *Bothrops jararaca* was the most recorded species in Rio de Janeiro, which agrees with other studies [e.g., Pontes et al. (2009)]. However, we found *B. jararacussu* in all mesoregions, and species-level identification is indicated. Therefore, we recommend that clinical studies with good experimental design be conducted to evaluate the use of bothropic-crotalic antivenom serum and heparin in the treatment of bites by *B. jararacussu* (dos-Santos et al., 1992; Rostelato-Ferreira et al., 2010).

Crotalus durissus is restricted to the mesoregions of Médio Paraíba, Centro-Sul Fluminense, and Serrana, and therefore, crotalic antivenom must not be lacking in these mesoregions. *Crotalus durissus collilineatus* venom is predominantly positive for crotamine, while *C. d. cascavella* is negative and *C. d. terrificus* is mainly negative (Tasima et al., 2020). Considering that the Brazilian crotalic antivenom may be deficient, we recommend that further studies be conducted to better characterize the geographic and morphological boundaries of rattlesnake subspecies in Brazil to thereby help ensure the recommended use of both crotamine-positive and crotamine-negative entire venoms to produce the antivenom (Tasima et al., 2020).

Crotalus durissus population may have first expanded towards the state's coast during the 1950s (Duarte and Menezes, 2013) (Figure 5). The Médio Paraíba region, where the species is now most abundant, housed coffee production during the 19th century and later became a pasture area, which may help explain the expansion of *Crotalus* (Bastos et al., 2005). After all, the significant floods between 1950 and 1967 in the Rio Preto region may have contributed to the dispersal of rattlesnakes in deforested areas of some other municipalities, such as Valença (Bastos et al., 2005). Here, we provide the first record of rattlesnakes in the Serrana mesoregion, supporting the hypothesis of the geographic expansion of the species in the state. Assigning individuals of *C. durissus* to any subspecies is impossible because of the high level of overlap of the following diagnostic traits: absence or presence of contrasting diamond-shaped spots on the last third of the body and short or long paravertebral white lines on the first third of the back [see Harris and Simmons (1976); Amaral (1977)]. A deep taxonomic review to set the *Crotalus durissus* limits is required. Moreover, the epidemiological surveillance by monitoring and evaluating the geographic expansion of the species needs to consider the first records of the species for the Serrana region, which would facilitate interventions against snakebites.

We improved the distinction between live and preserved specimens of *P. olfersii* and *P. patagoniensis* previously reported in the literature [e.g., Peters and Orejas-Miranda (1970); Thomas (1976)]. *Philodryas olfersii* venom has proteolytic, hemorrhagic, fibrinogenolytic, and edematogenic activities (Assakura et al., 1992). Bites by specimens of *Philodryas* may manifest pain, heat, erythema, edema, and ecchymosis and are sometimes treated with bothropic antivenom (Correia et al., 2010; Medeiros et al., 2010). *Philodryas* spp. were recorded in all mesoregions, except the Noroeste Fluminense. Despite the previous occurrence of some severe cases, including one death (Salomão and Di Bernardo, 1995), no further concern is currently necessary.

Given the geographic distribution of *M. lemniscatus*, the antivenom service centers in the coastal, Metropolitana, and Norte Fluminense regions should be equipped with artificial respiration and elapid antivenom. The elapid antivenom produced in Brazil only with *M. frontalis* and *M. corallinus* venoms can neutralize those of *M. frontalis*, *M. corallinus*, and *M. spixii* but not well *M. altirostris* and *M. lemniscatus* (Tanaka et al., 2010). Considering the need for improvement of elapid antivenom, the complementary use of anticholinesterase to treat envenomations by *M. lemniscatus carvalhoi* but not those of *M. corallinus* (Coelho et al., 1992; Vital Brazil and Vieira, 1996) should quickly become part of clinical studies for future uses in snakebite therapy. In severe bites by *M. lemniscatus*, in which the venom acts postsynaptically, anticholinesterases may be useful as an ancillary measure (particularly when antivenom is unavailable or insufficient) and for patients treated with high doses of antivenom but with no improvement of paralysis or with delayed recovery (Bucaretchi et al., 2006). Ciscotto et al. (2011) also highlight the importance of using *M. frontalis* venom to produce heterologous elapid antivenom and suggest replacing *M. corallinus* venom with *M. ibiboboca* venom to enhance antivenom since *M. ibiboboca* antivenom was able to cross-react more efficiently with other venom proteins. Despite these results, additional experimental and clinical studies are needed to identify an eventual deficiency of the Brazilian Coralsnake Antivenom to neutralize *Micrurus* spp. envenoming efficiently. Nevertheless, considering the interspecific variation of the venoms, the discussion to produce *Micrurus* antivenoms (Ciscotto et al., 2011) and that we found *M. corallinus* and *M. lemniscatus* in sympatry, their morphological distinction becomes relevant for species-specific attention.

Lachesis is historically recorded in six Rio de Janeiro mesoregions, with no record in the last 18 years. Therefore, *Lachesis* antivenom is unavailable in the state. Epidemiological data indicate that *Lachesis* is responsible for 4% of snakebites in Brazil (about 1,450 accidents per year), with an average mortality rate of 1.04%, *i.e.*, 15 people per year (Silva et al., 2015). Considering the rarity of *L. muta* in Rio de Janeiro, the question remains whether it is worthwhile for the state of Rio de Janeiro to house *Lachesis* antivenom in some mesoregions where the species may occur.

We surveyed 7,483 records of snakebites in Rio de Janeiro between 2001 and 2019, with an annual average of 393.84, therefore updating the data on snakebites in the state (Machado and Lemos, 2016; Bochner and Struchiner, 2004. The highest number of cases occurred in the Serrana region (n = 2,905), followed by Metropolitana (n = 1,948) and Costa Verde (n = 1,615), and the lowest number of cases occurred in the Norte Fluminense (n = 473) mesoregion (Supplementary figure II). The Serrana mesoregion is the largest pole of family agriculture in Rio de Janeiro and the central pole of olericulture in the state, responsible for supplying the metropolitan region (Moreira et al., 2002). In this region, intensive family farming with little mechanization predominates, which may increase the encounter between humans and snakes. This observation alone should not be considered sufficient to understand the serious problem and the failure to combat snakebites, but it can be a starting point for devising environmental education strategies to mitigate the problem.

It should also be noted that snake populations are affected by multiple factors, such as habitat destruction, decrease or increase in prey populations, changes in climate variables, and the use of agrochemicals (Gutiérrez, 2020). We recommend further epidemiological research to address the causes that preclude the effective combating of snakebites.

We improve the identification of venomous snake species, better delimit their geographic distribution, and update snakebite cases, thus providing greater precision in snakebite care in Brazil. Mapping comprehensive datasets is imperative for understanding human-animal conflict dynamics (such as vulnerability to medically important snakebites) and for providing background information needed to enable adequate health facilities as well as the provision of antivenom and other therapeutic innovations (Molesworth et al., 2003; Kasturiratne et al., 2008; Gutiérrez et al., 2010; Gutiérrez, 2012). The established centers against ophidism must provide antivenom supplies considering the snake genus distribution, epidemiology data on snakebites and professionals with expertise in animal venom. A good relationship between humans and snakes requires public awareness and education (Gouveia et al., 2015). Accordingly, a booklet disseminating our findings is in preparation to be distributed in snakebite care centers in Rio de Janeiro state.

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Supplementary Material

Supplementary material accompanies this paper.

Supplementary figure I. Snakebite in Rio de Janeiro State from 2001-2019 (deaths).

Supplementary figure II. Snakebites per Rio de Janeiro State mesoregions (2001-2019).

Supplementary figure III. Viperidae patterns of head shape and scutellation highlighting the number of scales reaching the internal scales.

Supplementary file I. Voucher specimens morphologically examined from the Coleção Científica de Serpentes Instituto Vital Brazil Scientific Collection (IVB).

Supplementary file II. Tables 1-9 reporting the snakes morphological data.

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