AFTER THREE DECADES, THE REDISCOVERY OF *BOTHROPS BILINEATUS* (SQUAMATA, VIPERIDAE) IN ESPÍRITO SANTO STATE, SOUTHEASTERN BRAZIL

APÓS TRÊS DÉCADAS, A REDESCOBERTA DE *BOTHROPS BILINEATUS* (SQUAMATA, VIPERIDAE) NO ESTADO DO ESPÍRITO SANTO, SUDESTE DO BRASIL

Rafael Scherrer Mathielo[1], Bryan da Cunha Martins[1*], Lucas Aosf[2], Thiago Marcial de Castro[1] & Alexander Tamanini Mônico[3]

[1]*Independent researcher, Vitória, Espírito Santo, Brazil.*
[2]*Instituto de Manejo da Biodiversidade (IMBio), Guaratinguetá, São Paulo, Brazil.*
[3]*Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil.*
[*]*Correspondence:* bryancmartins@hotmail.com

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Resumo.— O gênero *Bothrops* apresenta ampla diversidade ecológica e distribuição neotropical, incluindo espécies raras ou pouco abundantes. *Bothrops bilineatus,* apesar de possuir ampla distribuição geográfica, é considerada incomum e, no Espírito Santo, encontra-se criticamente ameaçada. Após mais de três décadas sem registros oficiais no estado, este estudo documenta a redescoberta da espécie na Área de Proteção Ambiental Pedra do Elefante, em Nova Venécia. Em 2024, foram registrados um indivíduo adulto, observado em duas ocasiões, e um indivíduo juvenil, indicando possível viabilidade reprodutiva local. Os registros ocorreram em floresta bem preservada, corroborando a dependência da espécie por ambientes florestais conservados. Os resultados destacam a importância da APA da Pedra do Elefante como último refúgio conhecido da espécie no estado e reforçam a necessidade de inventários direcionados e ações de conservação.

Palavras chave.— Conservação, jararaca-verde, Mata Atlântica, répteis, serpentes.

Abstract.— The genus *Bothrops* exhibits wide ecological diversity and a Neotropical distribution, including rare or low-abundance species. *Bothrops bilineatus,* despite its broad geographic range, is considered uncommon and is critically endangered in the state of Espírito Santo, Brazil. After more than three decades without official records in the state, this study documents the rediscovery of the species in the Pedra do Elefante Environmental Protection Area, municipality of Nova Venécia. In 2024, one adult individual was recorded on two occasions and one juvenile individual was observed, indicating possible local reproductive viability. The records were obtained in well-preserved forest, corroborating the species' dependence on conserved forest environments. These findings highlight the importance of the Pedra do Elefante EPA as the only known remaining refuge for the species in Espírito Santo state and reinforce the need for targeted inventories and conservation actions.

Keywords.— Atlantic foest, conservation, reptiles, snakes, two-striped forest pitviper.

The genus *Bothrops* Wagler, 1824 comprises 48 species distributed throughout the Neotropics, 30 of them occurring in Brazil (Nogueira et al., 2019; Cavalcante et al., 2022; Uetz et al., 2026). These species can be terrestrial (e.g., *B. neuwiedi* species group; Carrasco et al., 2012; 2019); arboreal (e.g., *B. taeniatus* species group; Martins et al., 2002; Dal Vechio et al., 2018), or semi-arboreal (e.g., *B. jararaca*; Sazima, 1992). They occupy a wide range of habitats, such as dense forests (e.g., *B. jararacussu*; Muscat et al., 2021), open areas (e.g., *B. alternatus* species group; Araújo & Martins, 2006), anthropized environments (e.g., *Bothrops atrox*; Bernarde, 2014), and even islands (e.g., *B. insularis* and *B. sazimai*; Duarte et al., 1995; Barbo et al., 2016).

Some species are considered rare because of the difficulty of recording individuals in the field work (e.g., *B. brazili* and *B. taeniatus*; Campbell & Lamar, 2004), although the concept of rarity is broader than that, involving occupancy, environmental filtering, range size, and movement (Crisfield et al., 2024) as well

as evolutionary histories that favor camouflage patterns and cryptic habits (Gaston & Curnutt, 1998). Furthermore, others may be considered rare in a specific environment/biome, and common in others. A great example is *B. bilineatus*, a Viperidae species distributed throughout Amazonia and parts of Atlantic forests – from Rio de Janeiro to Alagoas – with a single record for Caatinga in Ceará state (Nogueira et al., 2019; Cavalcante et al., 2022). In Amazonia, it occurs as *B. bilineatus smaragdinus*, whereas in the Atlantic forests it is represented by *B. bilineatus bilineatus*. Despite having this widespread geographic range, occurring in Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela, Guyana, French Guiana, and Suriname (Nogueira et al., 2019), *B. bilineatus* is considered truly uncommon throughout its distribution when analyzed inventories and studies involving snakes' diversity and abundance (Cunha & Nascimento, 1993; Bernarde et al., 2021), except for the Alto Juruá region in the Brazilian Amazonia (Acre state), where this species is reported as common (Turci et al., 2009; Silva et al., 2020). Although this species is not very abundant in most of its occurrence area, it is not classified as threatened in Brazil according to ICMBio red list (ICMBio/MMA, 2018). However, due to the low occupation in Espírito Santo state, the extinction risk is higher (Crisfield et al., 2024) and the species is considered critically endangered (IEMA, 2022).

The green jararaca *B. bilineatus* is an arboreal species with predominantly nocturnal habits, only occasionally observed active during the morning (Dal Vechio et al., 2018; Fonseca et al., 2021). However, most of the day these species stay resting on the vegetation, and could not be easily noticed, once its green color provides camouflage (Turci et al., 2009; Fonseca et al., 2021).

Many historical records of *B. bilineatus* have been made in the state of Espírito Santo (e.g., municipalities of Aracruz, Baixo Guandu, Colatina, Conceição da Barra, Fundão, Ibiraçu, João Neiva, Linhares, Santa Leopoldina, Santa Teresa and Vitória) (Nogueira et al., 2019). In most of these areas, the environment has undergone significant alterations of their original characteristics, resulting from uncontrolled urban expansion, real estate speculation, and deforestation associated with the advancement of agricultural and livestock activities (Menezes et al., 2014; Rosado et al., 2014). Due to the higher sensitivity of this species to anthropized environments, and the fact that it has not been recorded in these municipalities in recent years, according to the last inventories (e.g., Tonini et al., 2010; Cozer et al., 2020; Pereira-Ribeiro et al., 2022), it is feasible that it no longer occurs in most of these areas.

Over the last three decades, no records of *Bothrops bilineatus* have been made in Espírito Santo state. The last one (and official) occurred in the Conceição da Barra municipality in 1992 (ZUEC-REP 1674) (Nogueira et al., 2019). Since then, a few informal records have emerged, the most reliable of which was made in 2017 by the botanist Anderson Alves-Araújo at Área de Proteção Ambiental da Pedra do Elefante (hereafter, APA da Pedra do Elefante, Fig. 1), which provides a photograph as evidence (Anderson Alves-Araújo, pers. comm.). Given the relevance of this record, we decided to focus our efforts on the surroundings



Figura 1. Vista aérea da APA da Pedra do Elefante, município de Nova Venécia, noroeste do estado do Espírito Santo, Brasil. Foto: Rafael Scherrer Mathielo.

Figure 1. Aerial view of APA da Pedra do Elefante, Nova Venécia municipality, northwest of Espírito Santo state, Brazil. Photo: Rafael Scherrer Mathielo.

of the APA da Pedra do Elefante. We conducted fieldwork sporadically to find the species.

There were three expeditions in total, without any pre-established sampling design. The first expedition took place on December 14th, 2023, during which we surveyed the plots where the observation had been previously reported. However, we were unsuccessful. The second expedition on January 10th, 2024 (approximately 19:00 h), we recorded an adult individual of *B. bilineatus* (total body length [TBL] about 1000 mm) (Fig. 2A) at APA da Pedra do Elefante, in Nova Venécia municipality, northwest of Espírito Santo state (APA centroid at 18.764334° S, 40.457540° W, WGS 84; ca. 270 m a.s.l.). One month later, on February 11th, 2024 (approximately 23:00 h), we revisited the area and observed the same individual, in the same place, undergoing the process of shedding its skin (Fig. 2B). We confirmed that it is the same individual through (i) size, (ii) coloration of the post-ocular scales and (iii) a scar on the supralabial scale (Fig. 2). The individual was perched approximately 80 cm above ground.

The next day, 12 February 2025 (approximately 15:00 h) we revisited the trail and found a juvenile individual of the species (Fig. 3), approximately 70 meters from the local where the previous individual was located. The juvenile had a TBL of about 300 mm and was perched approximately 60 cm above the ground. Grego et al. (2012) recorded the reproduction of *B. bilineatus* providing data on the size of neonates (255–270 mm TBL). Bernarde et al. (2021) recorded individuals measuring 250mm and considered them young. Finally, da Fonseca et al. (2021) considered individuals with a total length ≤ 300 mm to be juvenile. Considering that the recorded specimen presents an estimated total body length (TBL) close to the upper limit described for neonates in Grego et al. (2012) and also within the range of individuals regarded as young/juveniles by Bernarde et al. (2021) and da Fonseca et al. (2021) we therefore classify it as a juvenile. Although the northern and northwestern regions of the state of Espírito Santo suffer severe impacts associated with monocultures and high deforestation rates, they still retain ecological relevance, considering that Espírito Santo has only a brief history of studies involving its herpetofauna, and that the northern portion is still regarded as a sampling gap (Bérnils et al., 2014). Occasional surveys, such as the one presented in this study and the case reported by Mathielo et al. (2024), which rediscovered two other rare snake species for the state (*Drymoluber brazili* and *Apostolepis longicaudata*), highlight the need for inventories that encompass this region.

Our record raises the discussion about the possibility that the species may still occur in other areas within Espírito Santo state. In the Municipality of Conceição da Barra (record made in 1992), there are some forest remnants (e.g., Floresta Nacional do Rio Preto) that may still harbor this species less than 100 km from our record. However, no records have been made in the region since that date.

Some other areas that require attention regarding the possible occurrence of the species include the municipalities of Aracruz, Ibiraçu, Fundão, Linhares, and Santa Teresa, where the species has historical records and where the following protected areas are located: Parque Natural Municipal do Aricanga (Aracruz and Ibiraçu), Parque Municipal Goiapaba-Açu (Fundão), Reserva Biológica de Sooretama (Linhares), Reserva Biológica Augusto Ruschi and Estação Biológica de Santa Lúcia (Santa Teresa). Focused field surveys could help to verify whether the species still occurs in these areas.

Although APAs are protected areas with few restrictions regarding human occupation and land use (Bruner et al., 2004), it is undeniable that they contribute to the preservation of biodiversity. The APA da Pedra do Elefante remains the only known remaining forest area that shelters the green jararaca in the Espírito Santo state. The fact that this area is surrounded by pasture and plantations, as well as industries and cities, is something to worry about. However, even though *B. bilineatus* is susceptible to human-modified environments, it seems to resist well in the interior of the forest. Furthermore, in the APA da Pedra do Elefante, we found three of the four continental species of the genus *Bothrops* occurring in the Espírito Santo state (e.g., *B. jararaca*, *B. leucurus* and *B. bilineatus*) which demonstrates a great capacity for environmental support. Also, Bergallo et al. (2000) concluded that in Rio de Janeiro state, *B. bilineatus* has a strong dependence on well-preserved slope forests, our record from APA da Pedra do Elefante corroborates their statement, as it was obtained in an inselberg formation with well-preserved forests along its slopes.

We observed the presence of some forest fragments surrounding the APA da Pedra do Elefante, which suggests the possibility of *B. bilineatus* occurring beyond the APA boundaries. However, the occurrence of additional records, as well as the potential existence of a metapopulation, remain hypotheses that require further investigation. We have evidences that the population is still reproductively viable (juvenile record), but we cannot determine whether it is through crossbreeding or parthenogenesis (such as some individuals of *B. atrox* species group; Cubides-Cubillos et al., 2020), since we have not handled the individuals found to determine their sex or collect genetic data as they represent an endangered species. Future studies



Figura 2. Indivíduo adulto de *Bothrops bilineatus* observado em (A) 10 de janeiro de 2024 e em (B) 11 de fevereiro de 2024 na APA da Pedra do Elefante, município de Nova Venécia, noroeste do estado do Espírito Santo, Brasil. Fotos: (A) Thiago Marcial de Castro, (B) Rafael Scherrer Mathielo.

Figure 2. Adult individual of *Bothrops bilineatus* observed on (A) January 10th, 2024 and (B) February 11th, 2024 at APA da Pedra do Elefante, Nova Venécia municipality, northwest of Espírito Santo state, Brazil. Photos: (A) Thiago Marcial de Castro, (B) Rafael Scherrer Mathielo.



Figura 3. Indivíduo juvenil de *Bothrops bilineatus* observado em 12 de fevereiro de 2024 na APA da Pedra do Elefante, município de Nova Venécia, noroeste do estado do Espírito Santo, Brasil. Foto: Rafael Scherrer Mathielo.

Figure 3. Juvenile individual of *Bothrops bilineatus* observed on February 12th, 2024 at APA da Pedra do Elefante, Nova Venécia municipality, northwest of Espírito Santo state, Brazil. Photo: Rafael Scherrer Mathielo.

need to be conducted to assess the population's status, verify the existence of a metapopulation and the genetic diversity of this population, to establish plans to ensure its continued existence in the area.

Finally, the collaboration between researchers from different fields (e.g., botanists, ornithologists and mammalogists) is fundamental to expanding the possibilities of species records. For example, the species *Lachesis rhombeata*, whose last record in the state of Espírito Santo was made by a mastozoologist in 2007 (Bérnils et al., 2014). These collaborations enhance the coverage area as multiple researchers of these areas are surveying their group on Espírito Santo State.

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Note: We did not manually handle the individuals found. We have decided not to disclose the exact geographic coordinates of the record described here to prevent snake collectors from searching for the specimens.

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