REPTILES & AMPHIBIANS

Anurans of the Ventania Environmental Protection Area, Miracema, Rio de Janeiro, Brazil

Lucas Custodio Lomba da Rocha[1], Tatiely da Silva Candido[1], Érica da Silva Santiago[1], and Elvis Almeida Pereira[2]

[1]Centro Universitário Redentor (UniRedentor), Itaperuna, 28.300-000, Rio de Janeiro, Brazil
[2]Universidade Estadual de Campinas (UNICAMP), Instituto de Biologia, Departamento de Biologia Animal, Laboratório de História Natural de Anfíbios Brasileiros (LaHNAB), Campinas, 13083-862, São Paulo, Brazil (elvisaps@hotmail.com; https://orcid.org/0000-0002-8810-7878)

Abstract.—Wildlife inventories are crucial for understanding biodiversity, guiding conservation efforts, and assessing species' geographic distributions. This study presents the first detailed inventory of anuran species in the Ventania Environmental Protection Area, located in the Municipality of Miracema, State of Rio de Janeiro, Brazil. This area, which contains some of the largest remaining forest fragments in the region, is under intense anthropogenic pressure, including deforestation, fires, and agricultural encroachment. We employed three sampling methods—visual encounter surveys, audio recordings, and pitfall traps, we recorded 31 anuran species, mainly from the Hylidae family (16 species). Notably, *Phasmahyla lisbella*, an endangered species, was recorded within the study area. Our findings underscore the importance of preserving the last forest remnants in the region, which provide vital habitats for both generalist and specialist anuran species. The data contributes to the knowledge of the region's biodiversity and highlights the need for further studies and conservation initiatives to mitigate the impact of human activities on these vulnerable ecosystems.

Amphibian populations are declining globally (Condez 2009) due to factors such as habitat alterations and destruction, climate change, industrial activities, use of chemicals in agriculture, introduction of exotic species, and fungal diseases (Beebee and Griffiths 2005; Pounds et al. 2006; Condez 2009; Scheele et al. 2019; Goessens et al. 2022; Jacinto-Maldonado et al. 2023; Luedtke et al. 2023). Of these, habitat destruction is the most critical, with the Atlantic Forest Ecoregion particularly affected, as only about 10% of its original vegetation remains (Adeodato 2016).

The Atlantic Forest Ecoregion is recognized as a biodiversity hotspot (Moura et al. 2012), with its diverse flora and topography providing a wide range of environments favorable to the development of local biota. Nevertheless, amphibian populations in the Atlantic Forest have declined drastically due to habitat loss and other environmental pressures. Of the 543 documented species, 472 are endemic to this ecoregion (Haddad et al. 2013). Amphibians play vital roles in ecosystem stability and preservation, functioning as both prey and predators (ICMBio 2022). Amphibian communities are widely distributed in terrestrial and aquatic environments and are key indicators of changes in many ecosystems (Heyer et al. 1994; Silvano and Pimenta 2003).

Given the global decline of amphibians and the critical status of the Atlantic Forest, understanding of biodiversity, especially in understudied areas, particularly in remaining forest fragments, is of critical concern. The Ventania Environmental Protection Area (hereafter Ventania EPA), with an area of 6,848.57 ha and spanning 54.84 km and located in one of the last forest remnants in the northwestern region of Rio de Janeiro (Detzel et al. 2023), holds significant biological value for the conservation of species across various taxonomic groups.

In the context of amphibians, the Ventania EPA stands out for hosting newly described species, such as *Phasmahyla lisbella* (see Pereira et al. 2018), which is already listed as endangered by the IUCN (IUCN SSC Amphibian Specialist Group and Instituto Boitatá de Etnobiologia e Conservação da Fauna 2023), underscoring the region's importance as a refuge for the biodiversity of the Atlantic Forest. However, the conservation of this natural heritage faces severe challenges. Much of the local forest is being rapidly degraded by the expansion of cattle ranching, a predominant activity in the region (Detzel et al. 2023). Areas unsuitable for such activities, such as hilltops and steep slopes, still retain forest cover but are highly vulnerable to fires, often triggered by practices such as pasture burning and land clearing. These anthropogenic pressures, coupled with the lack of detailed information about the local biodiversity, highlight an urgent need for studies documenting the amphibian fauna of the

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Ventania EPA and providing critical data for management and conservation actions in a biome that is among the most threatened on the planet.

We herein present an inventory of the anuran fauna of the Ventania EPA with the goals of addressing topics such as taxonomy, geographic distribution, species abundance, community composition, and conservation status, while also identifying potential threats to its continued existence.

Materials and Methods

We conducted our survey in the Ventania EPA, in Miracema, in the Northwest Fluminense Region, State of Rio de Janeiro, Brazil (-21.345383, -42.173602; Fig. 1). This area lies near the Muriaé and Pomba Rivers, two large tributaries of the Paraíba do Sul River Basin, at elevations of 138–856 m between the Mantiqueira and Serra do Mar Mountain Ranges The region's climate is classified as Aw "rainy tropical with dry winter" (Alvares et al. 2014), with an average rainfall of 1,150 mm and a mean annual temperature of 23 ºC (Köppen 1948).

The Ventania EPA relief is rugged, with springs, waterfalls, streams, swamps, and dozens of dams (Fig. 1). The forest formation is classified as seasonal semideciduous (FSS) (Ab'Sáber 2007). Its last fragments are threatened by anthropic actions, primarily related to agriculture and specifically the use of fire to clear pastures.

We conducted surveys between August 2016 and November 2017, dedicating three days per month to field activities for a total of 45 days and 225 sampling hours. We monitored different environments, including dams, swamps, puddles, and both temporary and permanent streams (Fig. 2; Table 1). We used methods described by Heyer et al. (1994) as "Complete Species Inventories," "Visual Encounter Surveys," and "Audio Strip Transects." We installed pitfall traps in some areas (Table 1) to increase sampling efficiency for terrestrial species.

One specimen per species was preserved and deposited in the amphibian collection at the Coleção Zoológica de Referência da Universidade Federal do Mato Grosso do Sul (ZUFMS). Species were identified based on morphological

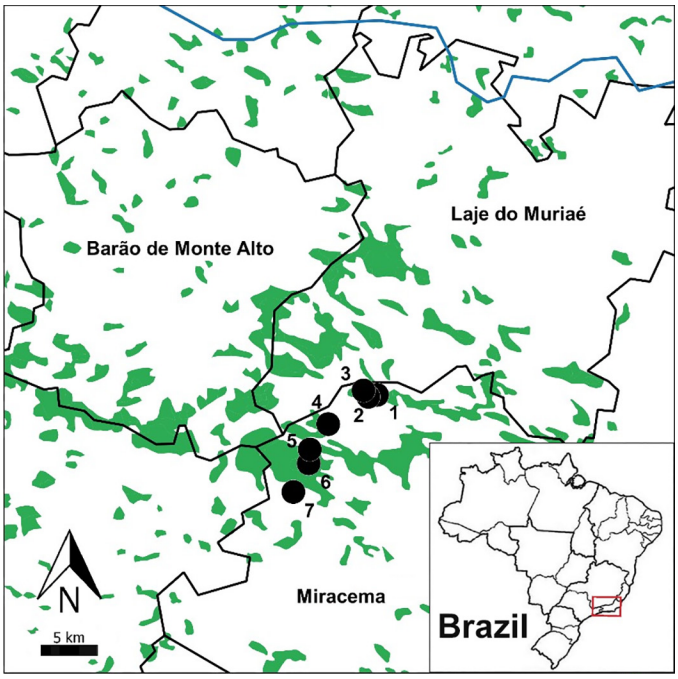

Figure 1. Map of Miracema, Rio de Janeiro, Brazil, showing locations of sampling sites (black dots), fragments of Atlantic Forest (dark green), and the Rio Muriaé (blue line). 1 = Maned Wolf Dam, 2 = Levi Water Tank, 3 = Eucalyptus Lake, 4 = Romilson's Lake, 5 = Cuíca Forest, 6 = Prehensile-tailed Porcupine Forest, 7 = Sloth's Forest.

Figure 2. Habitats at Eucalyptus Lake (left) and Maned Wolf Dam (right) in the Ventania EPA, Miracema, Rio de Janeiro, Brazil. Photographs by Lucas Custodio Lomba da Rocha.

Table 1. Sampling sites in the Ventania EPA, Miracema, Rio de Janeiro, Brazil.

Site	Habitat (coordinates)	Pitfall Trap
1	Maned Wolf Dam (dam without vegetation) (21°21.80'S, 42°13.14'W)	
2	Levi Water Tank (spring with dense vegetation) (21°19.97'S, 42°12.24'W)	X
3	Eucalyptus Lake (reservoir with monoculture of *Pinus* sp.) (21°21.03'S, 42°12.74'W)	
4	Romilson's Lake (series of small reservoirs) (21°19.17'S, 42° 10.94'W)	
5	Cuíca Forest (recovered forest fragment with weir) (21°19.22'S, 42° 11.18'W)	X
6	Prehensile-tailed Porcupine Forest (forest fragment next to marsh) (21°19.06'S, 42°11.31'W)	X
7	Sloth's Forest (recovered forest fragment with streams) (21°20.66'S, 42° 12.72'W)	

and acoustic traits documented in the literature, as well as comparisons with specimens in the collections of the Museu de Zoologia João Moojen (MZUFV) and ZUFMS. We analyzed calls using Raven Pro 1.5 for Windows (Bioacoustics Research Program 2012), analyzing parameters such as call duration, number of notes per call, frequency range (in kHz), and dominant frequency (in kHz). The terminology for the spectral parameters of the calls was adopted from Köhler et al. (2017).

The conservation status of each species was determined using the Red List of Threatened Species by the International Union for Conservation of Nature (IUCN 2023) and the Brazil Red Book of Threatened Species of Fauna (Ministério do Meio Ambiente 2022). Taxonomy was based on the most recent list of Brazilian amphibians (Segalla et al. 2021).

We used Fisher's test in R version 4.3.3 (R Development Core Team 2024) with significance established at $\alpha = 0.05$ to evaluate differences in abundance between species with the highest and lowest number of records. To determine the efficiency of sampling effort, we performed a species accumulation curve, using 1,000 randomizations generated from a presence-absence data matrix for each night of observation. We used the species richness estimator Bootstrap using EstimateS v. 9.0.0 to determine the expected amphibian richness with a 95% confidence interval (CI) (Colwell and Coddington 1994; Colwell 2013).

Results and Discussion

We recorded 31 anuran species in 14 genera and nine families: Bufonidae (1 species), Craugastoridae (1 species), Cycloramphidae (1 species), Hylidae (16 species), Hylodidae (2 species), Leptodactylidae (6 species), Odontophrynidae (1 species), Phyllomedusidae (3 species) (Fig. 3; Table 2). Hylids were most abundant, comprising 51.6% of recorded species, which aligns with previous general observations within the Atlantic Forest Ecoregion (Duellman 1988; Moura et al. 2012; Guimarães et al. 2020; Lima et al. 2021; Mendonça et al. 2022).

The anuran fauna of Ventania EPA represents 5.9% of the 529 species known in the Atlantic Forest Ecoregion (Haddad et al. 2013) and 18.7% of the 166 species recorded in the state of Rio de Janeiro (Rocha et al. 2004). Twenty of the 31 species found (64.5%) were endemic to this ecoregion, underscoring the EPA's role as a vital refuge for these taxa (Haddad et al. 2013).

Of the species recorded, *Dendropsophus pseudomeridianus* was the most abundant and *Proceratophrys boiei* was the least abundant, with only two individuals found in forest remnants. The difference in abundance was significant ($p = 0.0006$), highlighting the very different habitat requirements of the two species. Although *D. pseudomeridianus* thrives in anthropogenically altered landscapes, *P. boiei* relies on intact forests with streams or slow-flowing aquatic habitats, which are increasingly threatened by deforestation and habitat degradation (Cruz and Carvalho-e-Silva 2004; Borges-Najosa and Skuk 2010).

Endemic species are particularly significant for conservation, as their restricted distributions make them more vulnerable to habitat loss and climate change (Cahill et al. 2013; Shaffer 1981). The discovery of 20 endemic species within the Ventania EPA highlights its ecological importance but also its susceptibility to ongoing threats. Targeted conservation actions, including habitat restoration and the creation of ecological corridors, are crucial to maintaining these populations. Moreover, the identification of endemic species contributes to the recognition of Key Biodiversity Areas (KBAs), aiding in prioritizing conservation efforts (IUCN 2022).

We were unable to identify two species of *Crossodactylus* due to the sympatric occurrence of similar species (e.g., *C. grandis*, *C. boulengeri*, *C. werneri*, and *C. gaudichaudii*) in the region (Pimenta et al. 2014; Frost 2024). More conclusive taxonomic studies involving morphological and molecular evidence are required. These species are distinguished based on differences in advertisement calls and molecular data (Pimenta et al. 2014; Vittorazzi et al. 2021). Unfortunately, we were unable to record calls nor were molecular analyses performed on the two specimens.

We observed a predominance of generalist species (e.g., *Boana semilineata*, *Dendropsophus minutus*, *D. pseudomeridianus*, *Physalaemus cuvieri*, *Rhinella ornata*, and *Scinax fusco-*

Figure 3. Anurans species encountered in the Ventania EPA, Miracema, Rio de Janeiro, Brazil: *Rhinela ornata* (A), *Haddadus binotatus* (B), *Thoropa miliaris* (C), *Boana albomarginata* (D), *B. albopunctata* (E), *B. crepitans* (F), B. faber (G), *B. pardalis* (H), *B. semilineata* (I), *Dendropsophus bipunctatus* (J), *D. branneri* (K), *D. elegans* (L). Photographs by Lucas Custodio Lomba da Rocha.

Figure 3 (cont.). Anurans species found in the Ventania EPA, Municipality of Miracema, State of Rio de Janeiro, Brazil: *Dendropsophus minutus* (M), *D. pseudomeridianus* (N), *Ololygon flavoguttata* (O), *Scinax crospedospilus* (P), *S. eurydice* (Q), *S. fuscovarius* (R), *Leptodactylus fuscus* (S), *L. latrans* (T), *L. mystacinus* (U). Photographs by Lucas Custodio Lomba da Rocha.

varius) in areas with considerable anthropogenic disturbance (Fig. 1, sites 1, 3, 4), which is consistent with observations of generalists in such areas (Feio et al. 1998; Santana et al. 2010; Pereira et al. 2016; Frost 2024) and has been attributed to the presence of ample prey, shelter, and microhabitats suitable for reproduction (Feio and Ferreira 2005; Haddad and Prado 2005; Abrunhosa et al. 2006; Lucas and Fortes 2008; Almeida-Gomes et al. 2010). The generalist species *Rhinela ornata* exhibited considerable ecological plasticity by occurring in both open and forested situations, where we encountered them vocalizing in aquatic habitats and seeking refuge in litter.

In contrast, species such as *Phasmahyla lisbella*, *Proceratophrys boiei*, and *Phyllomedusa burmeisteri*, as well as the two unidentified species of *Crossodactylus*, which inhabit rocky streams in primary and secondary forests and do not occur in open areas, were found exclusively in forested habitats, highlighting both the ecological diversity of the Ventania EPA and its role as a refuge for sensitive species within a fragmented landscape (Gomides and Sousa 2012).

Comparisons with other studies conducted in the Atlantic Forest, particularly in nearby locations in the States of Rio de Janeiro and Minas Gerais (Table 3), reveal notable species richness. While the total number of species recorded

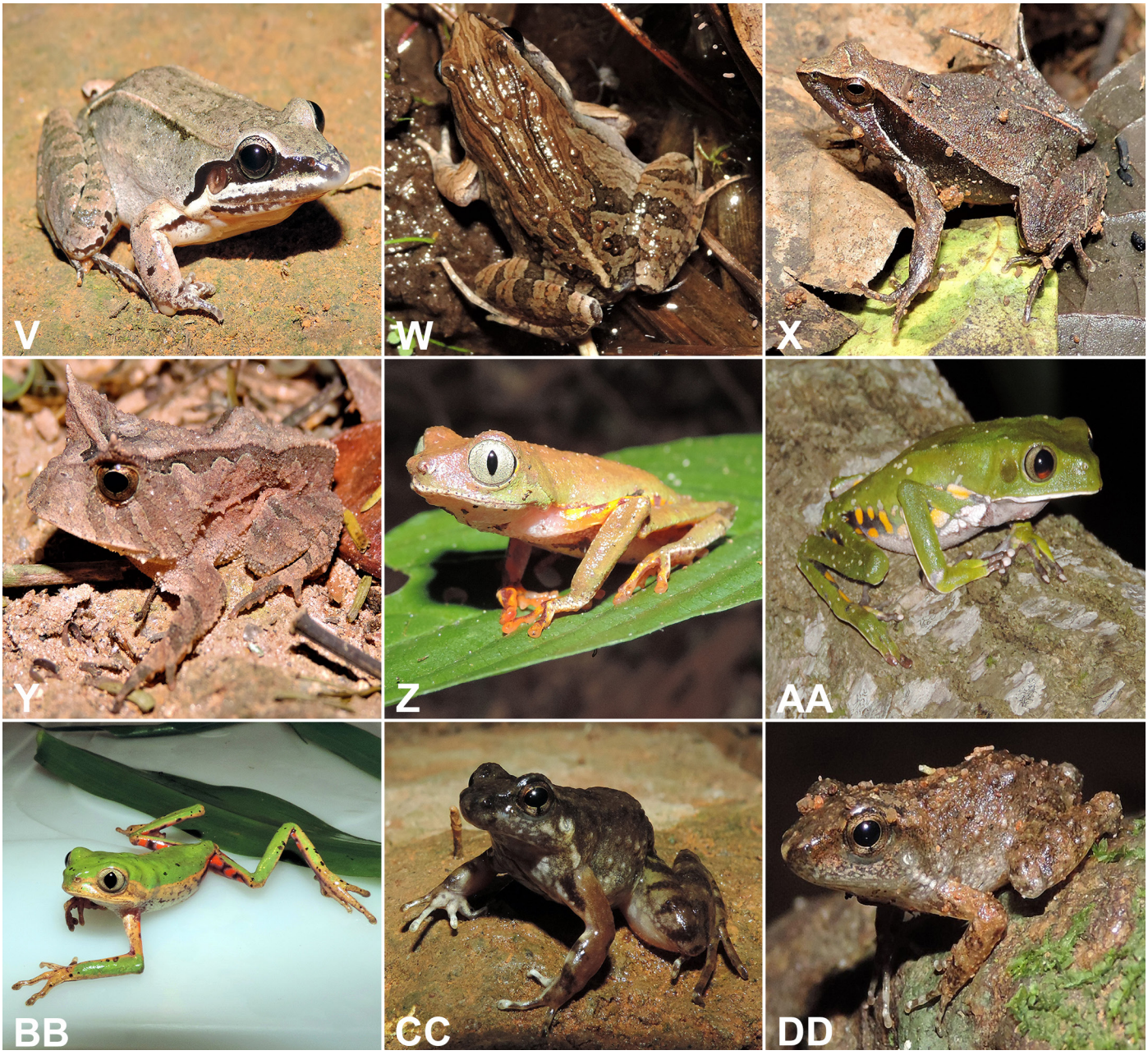

Figure 3 (cont.). Anurans species found in the Ventania EPA, Municipality of Miracema, State of Rio de Janeiro, Brazil: *Leptodactylus spixii* (V), *Physalaemus cuvieri* (W), *P. feioi* (X), *Proceratophrys boiei* (Y), *Phasmahyla lisbella* (Z), *Phyllomedusa burmeisteri* (AA), *P. rohdei* (BB), *Crossodactylus* sp. 1 (CC), *Crossodactylus* sp. 2 (DD). Photographs by Lucas Custodio Lomba da Rocha.

is relatively high, studies like that of Vrcibradic et al. (2011) found similar levels of richness in just one month of sampling, suggesting that sampling effort and methods used can strongly influence perceived richness.

We captured three species in pitfall traps, two of them (*Haddadus binotatus* and *Proceratophrys boiei*) exclusive to this method. We detected 19 species using Audio Strip Transects, although none were recorded exclusively by this method. Visual Encounter Surveys yielded the highest number of species (29), with nine (*Thoropa miliaris*, *Ololygon flavoguttata*, *Scinax eurydice*, *Leptodactylus latrans*, *L. mystacinus*, *L. spixi*, *Phyllomedusa burmeisteri*, and the two species of *Crossodactylus*) detected exclusively by this method. These results indicate that Visual Encounter Surveys were the most productive method; however, without the use of pitfall traps, some terrestrial species would not have been detected, demonstrating that each method complements the others and enhances overall sampling efficiency.

Beyond richness, the composition of species is crucial. The coexistence of generalists and specialists in the Ventania EPA underscores its ecological heterogeneity and conservation value. While generalist species like *Boana semilineata*,

Table 2. Anurans species recorded in the Ventania EPA, Miracema, Rio de Janeiro, Brazil. Species endemic to the Atlantic Forest are marked with an asterisk (*).

Taxon	Sampling Sites
Bufonidae	
Rhinela ornata (Spix 1824)*	1,3,4,5,7
Craugastoridae	
Haddadus binotatus (Spix 1824)*	2,5,6,7
Cycloramphidae	
Thoropa miliaris (SPIX Spix)*	2,4,5,6,7
Hylidae	
Boana albomarginata (Spix 1824)*	1,4,7
Boana albopunctata (Spix 1824)	1,3,4,7
Boana crepitans (Wied-Neuwied 1824)	1,7
Boana faber (Wied-Neuwied 1821)*	2,3,4
Boana pardalis (Spix 1824)*	3,4
Boana semilineata (Spix 1824)*	1,2,3,4,5,7
Dendropsophus bipunctatus (Spix 1824)*	1,3,4,7
Dendropsophus branneri (Cochran 1948)	1,3,4,7
Dendropsophus elegans (Wied-Neuwied 1824)*	1,3,4,7
Dedropsophus minutus (Peters 1872)	1,3,4,7
Dendropsophus pseudomeridianus (Cruz, Caramaschi, and Dias 2000)*	1,3,4
Ololygon flavoguttata (Lutz and Lutz 1939)*	2
Scinax alter (B. Lutz 1973)*	1,3,4
Scinax crospedospilus (A. Lutz 1925)*	3,4,7
Scinax eurydice (Bokermann 1968)*	3,4
Scinax fuscovarius (A. Lutz 1925)	1,2,3,4,7
Hylodidae	
Crossodactylus sp. 1*	7
Crossodactylus sp. 2*	7
Leptodactylidae	
Leptodactylus fuscus (Schneider 1799)	1,3,4,7
Leptodactylus latrans (Steffen 1815)	1,3,4,7
Leptodactilus mystacinus (Burmeister 1861)	6
Leptodactilus spixii Heyer 1983	1
Physalaemus cuvieri Fitzinger 1826	4
Physalaemus feioi Cassini, Cruz, and Caramaschi 2010	5
Odontophrynidae	
Proceratophrys boiei (Wied-Neuwied 1824)*	2
Phyllomedusidae	
Phasmahyla lisbella Pereira, Rocha, Folly, Silva, and Santana 2018*	2
Phyllomedusa burmeisteri Boulenger 1882*	4
Pithecopus rohdei (Mertens 1926)*	2,5

Dendropsophus minutus, and *Rhinella ornata* dominated in disturbed environments, the presence of habitat-specialist and forest-dependent taxa, such as *Phasmahyla lisbella*, *Proceratophrys boiei*, and *Crossodactylus* spp., indicated the ecological uniqueness of the Ventania EPA. The predominance of hylids also is consistent with data from other studies throughout the Atlantic Forest (Prado and Pombal 2005; Almeida-Gomes et al. 2010; Brassaloti et al. 2010; Siqueira et al. 2011a, 2011b; Wachlevski et al. 2014; Pereira et al. 2016).

Among the recorded species, only *Phasmahyla lisbella* is listed as Endangered (EN) on the IUCN Red List (IUCN SSC Amphibian Specialist Group and Instituto Boitatá de Etnobiologia e Conservação da Fauna 2023). Its type locality is a forest fragment surrounded by banana plantations within the Ventania EPA. In 2018, during the dry months, the stream in this area dried up and a fire ignited to maintain agricultural fields spread uncontrollably, resulting in the complete destruction of the forest remnant. Subsequent surveys at the type locality, including the stream, failed to detect any sightings of *P. lisbella*, raising concerns about the potential local extinction of this species in its original habitat (D.J. Santana, pers. comm.), highlighting the urgency of implementing conservation measures to prevent further losses.

The accumulation curve showed no tendency to asymptote and the estimated Ventania EPA richness was 34 species (Fig. 4). The Bootstrap index uses data from all species collected to estimate total richness. Although the estimated wealth suggests that at least three more species could occur in the region, sampling seemed adequate. Even so, noting that accumulation curves rarely stabilize, especially in tropical environments, is important (Santos 2004). As we did not sample during the day, additional species could be found, especially some in the genera *Hylodes* and *Pseudopaludicola*.

Additionally, some species found in the Municipality of Miracema are important biogeographic records. We recorded four species (*Boana albomarginata*, *Dendropsophus bipunctatus*, *D. branneri*, and *D. pseudomeridianus*) typical of coastal areas. All are distributed from the coastal regions of the state of Rio de Janeiro through the Paraíba River Valley to the Muriaé River region, reflecting a distribution pattern associated with river basins (e.g., Feio and Caramaschi 1995, 2002; Feio et al. 1998; Santana et al. 2010; Araújo et al. 2013; Pereira et al. 2016). These species tend to favor depressions and river valleys at low elevations, allowing incursions into inland areas, as seen in the Rio Doce Basin (Feio et al. 1998, 1999), Jequitinhonha (Feio and Caramaschi 1995, 2002), and Paraíba do Sul (Feio and Ferreira 2005). These records, similar to those in the Muriaé River Valley (Santana et al. 2010; Pereira et al. 2016; Rosado et al. 2019), suggest a biogeographical connection between coastal areas and the interior of this region. In addition, we encountered *Pithecopus rohdei*, which is one member of a species complex (Faivovich et al. 2010), and the area is in

Table 3. Number of anuran species recorded in various areas of the Atlantic Forest in Rio de Janeiro and Minas Gerais, Brazil. Time indicates the sampling period for that area. Protected areas are marked with an asterisk (*).

Locality	Number of species	Time	Reference
Miracema*	31	1.5 years	Present work
Barão do Monte Alto	29	2 years	Pereira et al. 2016
Guapimirim*	40	~27 years	Silva-Soares et al. 2010
Estação Ecológica Estadual do Paraiso*	25	~1 month	Vrcibradic et al. 2011
Cambuci*	20	~1 month	Almeida-Gomes et al. 2010
Parque Estadual do Desengano*	13	~1 month	Siqueira et al. 2011a
Parque Estadual dos Três Picos*	35	9 months	Siqueira et al. 2011b
Parque Estadual do Cunhambebe*	28	10 months	Ouvernay et al. 2012
Serra do Brigadeiro*	58	~27 years	Moura et al. 2012
Parque Natural da Taquara*	50	~2 years	Salles et al. 2009
Parque Natural Municipal de Grumarí*	22	~4 months	Telles et al. 2012
Reserva Rio das Pedras*	41	~9 years	Carvalho-e-Silva et al. 2008

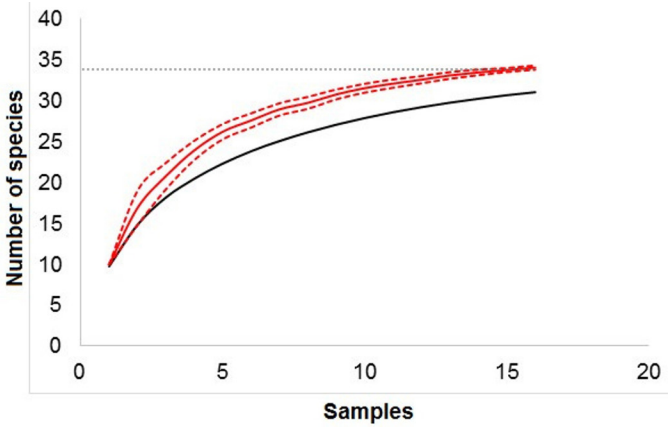

Figure 4. Accumulation curve for anurans sampled in the Ventania EPA, Municipality of Miracema, State of Rio de Janeiro, Brazil. Black line represents the accumulation curves, red line represents species estimates based on Bootstrap, red dashed lines represent its interval of confidence, and the black dashed line indicates the number of species estimated in the analysis (34 species).

a hybridization zone between *Rhinella crucifer* and *R. ornata* (Thomé et al. 2012), further studies of which might reveal additional taxonomic complexities.

During our surveys in the Ventania EPA, we identified multiple threats to local biodiversity. Activities such as illegal deforestation, uncontrolled burning, damming of streams, cattle grazing in forest fragments, improper waste disposal, and expansion of monocultures dramatically reduce habitat availability, affecting in particular habitat specialists like the endangered *Phasmahyla lisbella*, which depends on contiguous, healthy forests. The ongoing fragmentation and degradation of habitats exacerbates these threats, isolating populations, compromising reproduction, and increasing vulnerability to diseases and environmental stress (Haddad et al. 2008; Verdade et al. 2010).

In light of these concerns, continued research in the northwestern region of Rio de Janeiro, particularly in the Ventania EPA and the municipality of Miracema, is an urgent need. Faunal inventories are crucial for assessing the conservation status of anuran species and providing guidance for the development of effective management and conservation strategies. Habitat restoration, the establishment of ecological corridors, and the promotion of sustainable agricultural practices are obvious priorities.

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