

Original Article

# Anurans from the upper basins of the Rio Grande and Rio Preto: eastern portion of the Serra da Mantiqueira Environmental Protection Area, state of Minas Gerais, Brazil

Anfíbios anuros das bacias hidrográficas do alto rio Grande e alto rio Preto: porção leste da Área de Proteção Ambiental da Serra da Mantiqueira, estado de Minas Gerais, Brasil

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## Abstract

The Neotropical zone stands out for having the greatest richness of amphibians in the world. About 625 species of anurans are known on Atlantic Forest. The southeastern mountainous regions of Brazil called Serra do Mar and Serra da Mantiqueira, are very important areas for the diversity of anurans in the country because they shelter remaining strata of the Atlantic Forest that have several endemic species. The present study aims to expand the knowledge about the Serra da Mantiqueira anurans, specifically from the Rio Preto and Rio Grande upper basins. The study area comprised 10 sample centers. We realized 38 campaigns, lasting two days each. During this period, we used active search as visual and auditory cues. The passive search was comprised of pitfall traps. Bioacoustic analyses were used to determine the cryptic species. The sufficiency of the sampling effort was estimated through the accumulation curve using Bootstrap and Jackknife-1 as estimators. During the study 55 species were found, 40 of them in the basin of the Rio Preto, and 44 in the basin of the Rio Grande. Twenty-nine species were found in both areas. The Hylidae family was predominant, followed by Leptodactylidae. In addition to species of the Atlantic Forest, the place houses typical components of the Cerrado biome and altitudinal swamps with unexpected sympatries. This inventory expands the area of occurrence of *Scinax duartei*, a species considered threatened. Although adjacent to the oldest national park in Brazil, the composition of anuran species of this area was unknown until then.

**Keywords:** amphibian, Atlantic Forest, diversity, Itatiaia National Park, inventory.

## Resumo

A região Neotropical se destaca por possuir a maior riqueza de anfíbios do mundo. Cerca de 625 espécies de anuros são conhecidas na Mata Atlântica. As regiões montanhosas do sudeste do Brasil, denominadas Serra do Mar e Serra da Mantiqueira, são áreas muito importantes para diversidade de anuros no país, pois abrigam estratos remanescentes da Mata Atlântica que possuem diversas espécies endêmicas. O presente estudo visa ampliar o conhecimento sobre os anuros da Serra da Mantiqueira, especificamente das altas bacias dos rios Preto e Grande. A área de estudo compreendeu 10 centros amostrais. Realizamos 38 campanhas, com duração de dois dias cada. Nesse período, utilizamos a busca ativa como pistas visuais e auditivas. A busca passiva foi composta por armadilhas de queda. Análises bioacústicas foram usadas para determinar as espécies crípticas. A suficiência do esforço amostral foi estimada através da curva de acumulação usando Bootstrap e Jackknife-1 como estimadores. Durante o estudo foram encontradas 55 espécies, sendo 40 delas na bacia do Rio Preto e 44 na bacia do Rio Grande. Vinte e nove espécies foram encontradas em ambas as bacias. A família Hylidae foi predominante, seguida por Leptodactylidae. Além de espécies da Mata Atlântica, o local abriga componentes típicos do bioma Cerrado e brejos de altitude com simpatrias inesperadas. Este inventário amplia a área de ocorrência de *Scinax duartei*, espécie considerada ameaçada. Embora adjacente ao parque nacional mais antigo do Brasil, a composição de espécies de anuros dessa área era desconhecida até então.

**Palavras-chave:** anfíbio, Mata Atlântica, diversidade, Parque Nacional do Itatiaia, inventário.

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Received: January 15, 2022 – Accepted: April 29, 2022



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## 1. Introduction

The Atlantic Forest biome is one of the five world conservation hotspots (Mittermeier et al., 2011). Although this biome has a high level of fragmentation (Pereira et al., 2016), the heterogeneity on climatic conditions, relief, and types of vegetation throughout the geographical distribution of the Atlantic Forest support a great diversity of species and phylogenetic groups (Duellman, 1999; Martins and Haddad, 2010), with a high degree of endemisms (Forlani et al., 2010; Mittermeier et al., 2011; Silva et al., 2018). Despite its importance, the knowledge about the diversity of the herpetofauna in the Atlantic Forest is still incipient (Van Sluys et al., 2009; Forlani et al., 2010; Ribeiro et al., 2015; Silva et al., 2017). This deficiency is even more pronounced when we consider altitudes above 1,000 meters (Rossa-Feres et al., 2008; Martins and Haddad, 2010; Ribeiro et al., 2015), where the development of tolerance to unique climatic conditions can lead to a high level of endemism (Cruz and Feio, 2007; Ribeiro et al., 2015; Silva et al., 2018). In the Serra da Mantiqueira, the animals and plants have different origins, from elements of the coastal plains at the base of the mountain range to the Andean relicts of the altitude fields (Gouvêa, 1985; Pineschi, 2012).

The Atlantic Forest houses about 625 species of amphibians (Rossa-Feres et al., 2017), of which 472 are endemic to this biome (Haddad et al., 2013). It represents approximately 56% of the Brazilian richness of known species of this group (Segalla et al., 2021). Punctual data indicate that the Serra da Mantiqueira complex is an extremely important region for the anurans conservation (Neves et al., 2017, 2018; Silva et al., 2018). There are 234 species registered, of which 88 are endemic (Cruz and Feio, 2007; Silva et al., 2018), of these, about 15 species are recorded for the Itatiaia massif in its portion of state of Rio de Janeiro (Van Sluys et al., 2009). The total richness estimated for the Itatiaia National Park is, at least, 64 species (Gouvêa, 1985). Despite this, the microregion of the Itatiaia plateau, located in the oriental portion of the Serra da Mantiqueira where are the basins of the upper Rio Preto and upper Rio Grande, does not present studies related to its anurans.

The study area is inserted in the meridional portion of Serra da Mantiqueira (Cruz and Feio, 2007), a transition area between the Atlantic Forest biome and Cerrado enclaves (Gatto et al., 1983; IBGE, 2012). An area indicated as of very high importance for the biodiversity preservation (Drummond et al., 2005). Adding to this complexity, the existence of altitude swamps, and abandoned meanders of the Rio Grande makes peculiar the biotic characteristics of these localities, making extremely relevant the implementation of a methodical study of its faunistic composition.

Species inventories are important tools in conservation measures (Bastos et al., 2003; Segura et al., 2012; Haddad et al., 2013; Silva et al., 2018). They help to improve the knowledge about the conservation status of species (Heyer et al., 1994; Becker et al., 2013; Bruscagin et al., 2014) as well as the knowledge about their biogeographic patterns (Cruz and Feio, 2007; Segura et al., 2012; Neves et al., 2018). In this context, and as recommended by Drummond et al. (2005, 2009), the present study seeks

to increase the knowledge on the anurans of the Serra da Mantiqueira that presents only punctual studies in view of the complexity of these mountain ranges.

## 2. Material and Methods

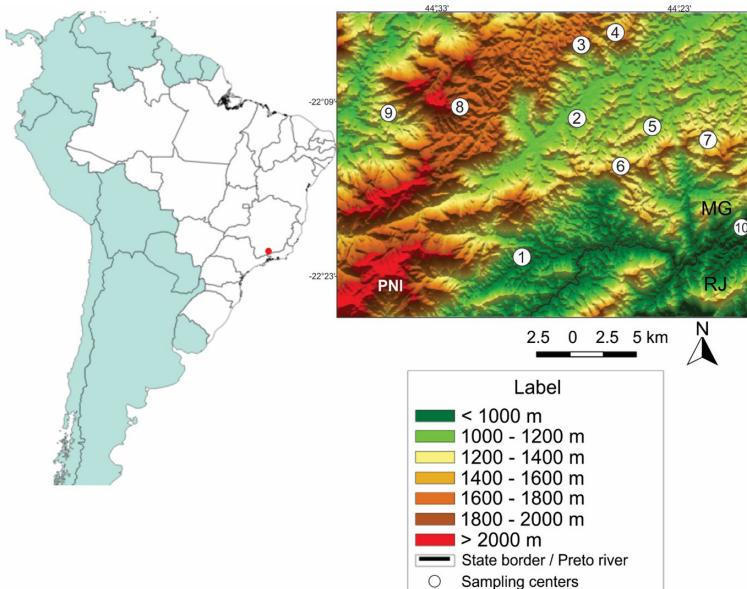
### 2.1. Study area

The inventory of the anurans was carried out in the municipalities of Alagoa ( $22^{\circ}11'S$ ;  $44^{\circ}36'W$ ), Bocaina de Minas ( $22^{\circ}09'S$ ;  $44^{\circ}23'W$ ), and Passa Vinte ( $22^{\circ}10'S$ ;  $44^{\circ}16'W$ ), south/southwest mesoregion of the state of Minas Gerais, comprising part of the Serra da Mantiqueira Environmental Protection Area (APA da Serra da Mantiqueira – ICMBio, 2018) (See Figure 1). The municipality of Alagoa comprises de basin of Rio Aiuruoca (subbasin of Rio Grande), Passa Vinte is inserted in the Rio Preto basin and, Bocaina de Minas is inserted in both basins (Grande and Preto).

The meridional Serra da Mantiqueira presents altitudes varying from just over 900 m, north of the Rio Preto, to 2,790 m at the Pico do Itatiaia, on the Itatiaia plateau. The regional climate is sub-humid to humid, with an average annual rainfall of 1,500 mm, reaching up to 2,300 mm in the Itatiaia massif, where the springs of the Rio Grande and Rio Preto are located, with a maximum in January and minimum in July (Gatto et al., 1983). Under these conditions, the Montane Ombrophilous Dense Forests, Upper Montane Forests, and areas of ecological tension with Mixed Ombrophilous Forest, Cerrado enclaves, and altitudinal fields developed. However, a great part of these vegetal conformations has been replaced by pastures, secondary vegetation, and reforestation with eucalyptus (Gatto et al., 1983; Ururahy et al., 1983), a scenario that persists nowadays.

The study area comprises forest formations, rupestrian fields and Cerrado vegetation enclaves (Open Tree Savanna according to Ururahy et al., 1983). The relief varies from 900 to 2,149 meters of altitude. The climate is subtropical highland with cold and dry winters and hot and humid summers – Cwb of Köpen (Alvares et al., 2014; CBH, 2018). Its average temperature varies between 18 and 19 °C, and the average annual rainfall varies between 1,200 and 1,500 mm (CBH, 2018).

The study area comprised 10 sample centers (1 to 8 in the municipality of Bocaina de Minas; 9 in the municipality of Alagoa; 10 in the municipality of Passa Vinte) and their surrounding areas: (1) RPPN Fazenda Boa Vista, situated in the district of Mirantão, region adjacent to the Itatiaia National Park, which comprises the basin of the upper Rio Preto; (2) floodplains of the Rio Grande along its course within the municipality of Bocaina de Minas; (3) RPPN Ave Lavrinha, in the Serra da Aparecida; (4) Arunachala farm, head of the Carrascal stream, located in the Serra da Aparecida; (5) lotic and lentic ecosystems along highway MG 81, and vicinal roads of the municipality of Bocaina de Minas; (6) Serra do Palmital, and Bagres locality; (7) Serra da Bocaina in the central region of the municipality; (8) Serra Verde, near Mitra do Bispo, which represents the higher mountain of the municipality; (9) municipality of Alagoa, comprising the Rio Aiuruoca basin; (10) municipality of Passa Vinte, Rio Preto basin (See Figure 2).



**Figure 1.** Sampling area in the Serra da Mantiqueira, south portion of Minas Gerais, Brazil: (1) RPPN Fazenda Boa Vista; (2) floodplains of the Rio Grande; (3) RPPN Ave Lavrinha; (4) Arunachala farm; (5) lotic and lentic water bodies; (6) Serra do Palmital, and Bagres locality; (7) Serra da Bocaina; (8) Serra da Aparecida/RPPN Mitra do Bispo; (9) Municipality of Alagoa; (10) Municipality of Passa Vinte. MG - State of Minas Gerais; RJ - State of Rio de Janeiro; PNI - Parque Nacional do Itatiaia (Itatiaia National Park).

## 2.2. Sample design

Thirty-eight field samplings were carried out from 2015 to 2021. Twenty-two in the rainy, and 16 in the dry season. The campaigns lasted at least two days each. The anurans were sampled through (i) active search, consisting of the visual and auditory search in which one or more people actively search for anurans in its habitats (Lima and Pederassi, 2015); (ii) pitfall traps (Cechin and Martins, 2000) composed of five buckets of 60 liters distant five meters each other. Two lines of pitfall traps were installed in the RPPN Fazenda Boa Vista, being one at 1,000, and another at 1,200 meters above sea level (m.a.s.l.); one line in the RPPN Ave Lavrinha, at 1,400 m.a.s.l.; one line in the Arunachala farm, at 1,300 m.a.s.l., parallel to the head of the stream Carrascal, and one line in Serra Verde at 1600 m.a.s.l. between an altitudinal swamp and a cloud forest. The traps were opened at the beginning of each campaign and closed at the end. The minimum was two days, and the maximum was seven days open, totaling more than 2,000 hours of sampling effort per bucket; (iii) bioacoustic record, performed with digital recorder Tascam DR-05 coupled to a super-cardioid microphone Rode Ntg-2 shotgun condenser, and (iv) accidental encounters of the anurans, for example, during displacement by roads (Bernarde, 2004).

## 2.3. Taxonomic characterization and deposition of the specimens

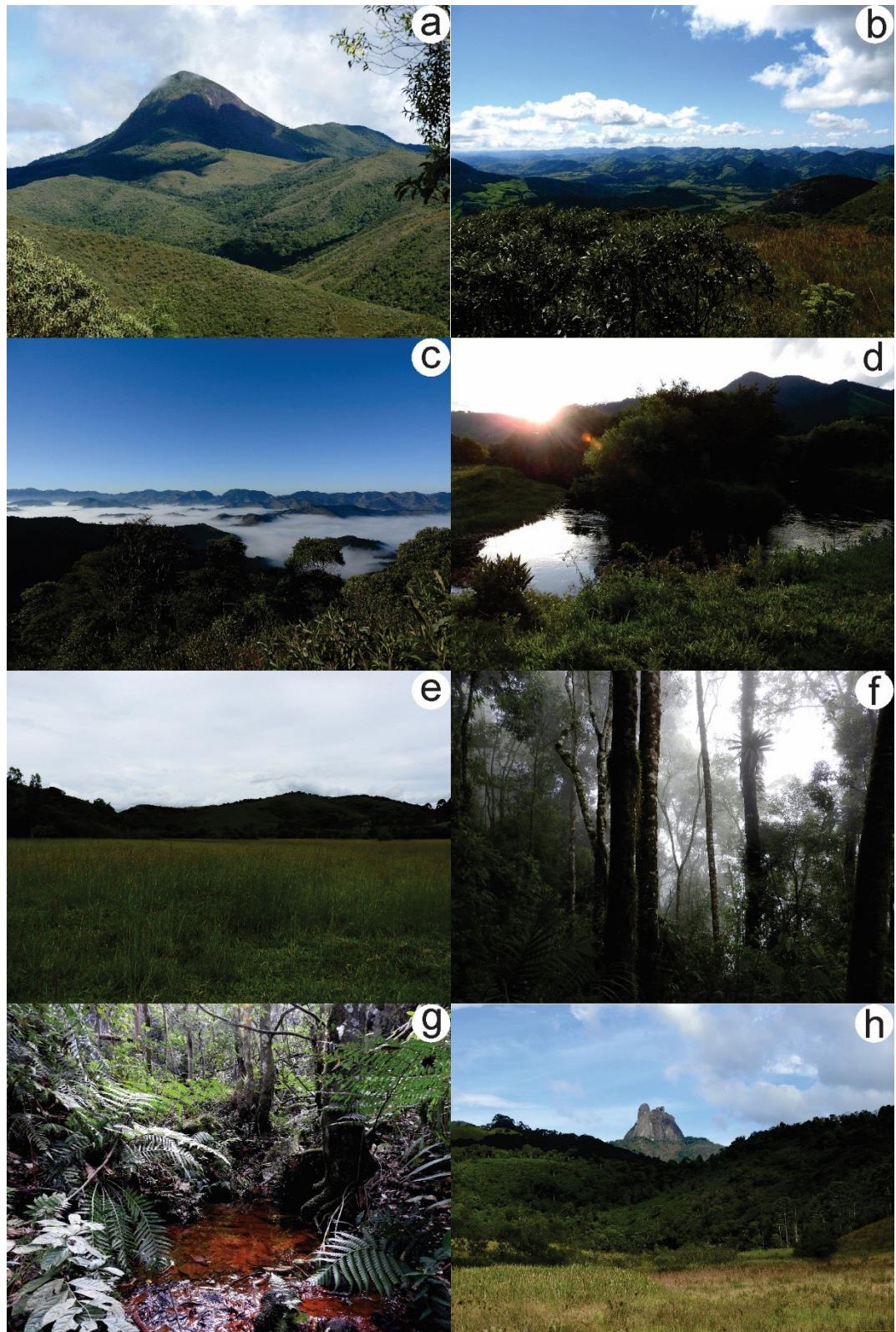
The collected specimens were taxonomically characterized according to their external morphology and bioacoustic parameters. The taxonomic nomenclature follows Frost (2021). The bioacoustic analysis was performed in Raven Pro 1.6.1 software (CornellLab, 2019)

using the parameters call duration, notes per call, pulses per note, and dominant frequency as proposed by Köhler et al. (2017). The bioacoustic was useful to compare related species like *Scinax* sp.1 and sp.2, and to determine the identity of *Leptodactylus jolyi*.

Specimens collected were fixed in 10% formaldehyde and preserved in 70% ethanol. Tissues of the specimens were preserved in absolute ethanol. The specimens were collected with permission from SISBIO/IBAMA # 50,094, and were deposited in the herpetological collection of the Museu Nacional, Rio de Janeiro (MNRJ) and Coleção de História Natural da Universidade Federal do Piauí (CHNUFPI).

## 2.4. Ecological analysis

To verify the sufficiency of the sampling effort we constructed the species accumulation curve and calculated the estimated richness using the Bootstrap and the first-order Jackknife estimators (Gotelli and Colwell, 2001) through EstimateS® 9.0 software (Colwell, 2013) considering 1,000 randomizations (Colwell and Coddington, 1994). The Bootstrap estimator was selected because it differs from the others since it considers the data of all the species collected to estimate the total richness, not being restricted to the rare species (Santos, 2006). The first-order Jackknife estimator was selected as a non-parametric method that provides a more accurate estimate of the community and produces confidence intervals based on rare species (Krebs, 1999). The first-order Jackknife is considered more accurate and less biased when compared to other methods of extrapolation (Palmer, 1990).



**Figure 2.** Environmental characteristics of the sampling areas. (a) Peak of Mitra do Bispo in Serra da Aparecida; (b) basin of the Rio Grande from Serra da Aparecida; (c) Basin of the Rio Grande from RPPN Ave Lavrinha; (d) Rio Grande; (e) characteristic swamp fields of the Rio Grande basin; (f) cloudy forest in RPPN Ave Lavrinha; (g) semi-lotic water body in the forests of Arunachala farm; (h) characteristic geomorphology in the basin of the Rio Preto.

The similarity between the river basins was determined through the Sorensen index ( $S_s$ ). This index is more robust because it considers the simultaneous occurrence without considering the double absence of species (Valentin, 2000).

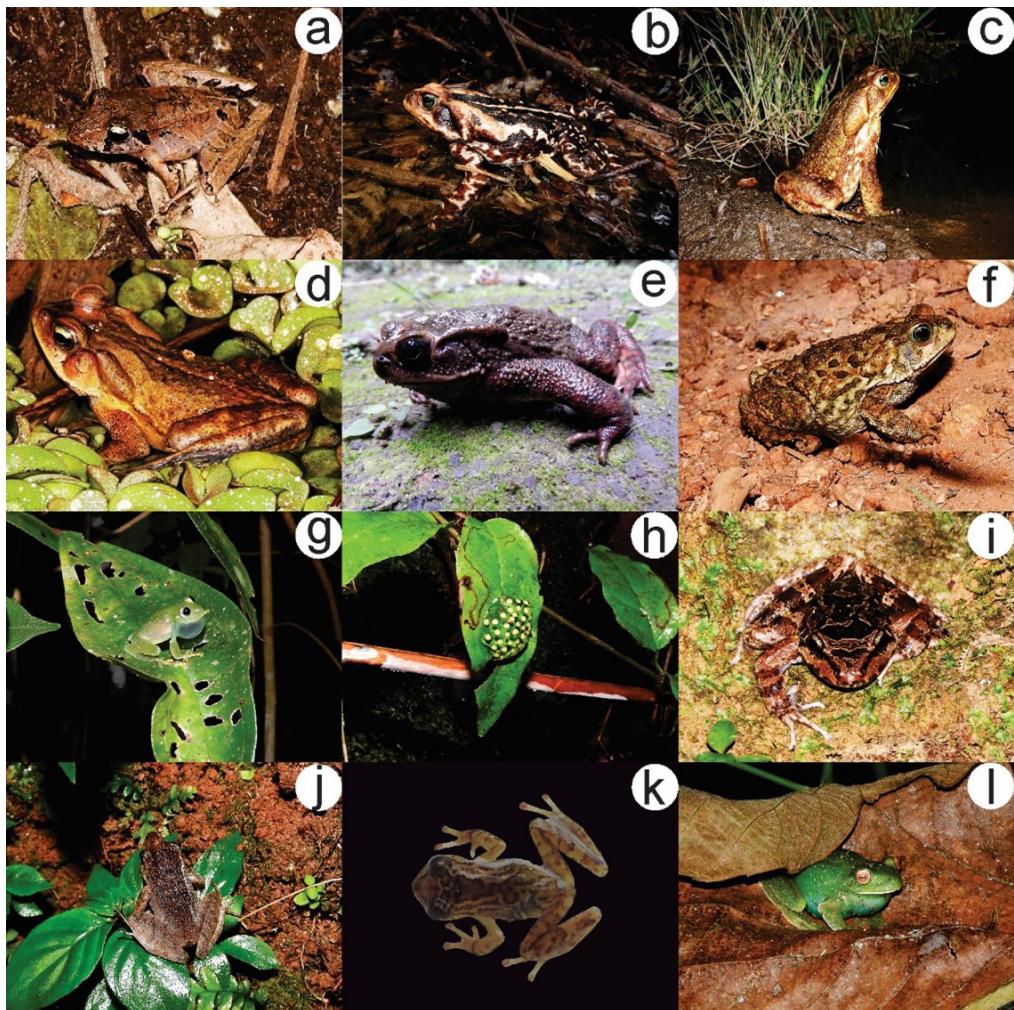
For access the conservation status of the species recorded, we used the official Brazilian list of endangered species (Brasil, 2014), the list of endangered species in the state of Minas Gerais (Drummond et al., 2008), the red list of the International Union for the Conservation of Nature (IUCN, 2021), and, due to the Rio Preto basin being shared also by the state of Rio de Janeiro, we have checked the list of endangered species of this state as well (Instituto LIFE, 2000).

### 3. Results

Fifty-five species of anurans were recorded during the study. Of which, 40 were found in the Rio Preto basin and 44 in the Rio Grande basin, with 29 common

species among the two basins. Meaning that 15 species were exclusive from Rio Grande basin and 11 from Rio Preto basin (See Table 1). This richness is distributed in 20 genera and 11 families: Brachycephalidae (two species), Bufonidae (three species), Centrolenidae (two species), Cycloramphidae (one species), Craugastoridae (one species), Hemiphractidae (one species) (See Figure 3); Hylidae (28 species) (See Figures 3, 4 and 5); Hylodidae (one species) (See Figure 5); Leptodactylidae (11 species) (See Figure 5 and 6); Microhylidae (two species); and Odontophrynidae (four species) (See Figure 6).

The estimated richness accumulation curves (See Figure 7) showed values between  $60.0 \pm 0.1$  and  $66.0 \pm 3.1$ , respectively for Bootstrap and Jackknife-1. The curve generated by the Bootstrap showed values closer to the observed species richness ( $S_{obs} = 55$  spp.). Considering only the performance of Jackknife-1 (estimator of richness that presented the highest value), the 55-species recorded represented 83% of the estimated theoretical richness,



**Figure 3.** Sampled anuran species in the basins of the Rio Grande and Rio Preto in Bocaina de Minas, MG. (a) *Ischnocnema* aff. *guentheri*; (b) female of *Rhinella icterica*; (c) male of *R. icterica*; (d) *R. ornata*; (e) *R. rubescens*; (f) *R. rubescens*; (g) *Vitreorana uranoscopa*; (h) spawning of *V. uranoscopa*; (i) *Thoropa miliaris*; (j) *Haddadus binotatus*; (k) *Fritziana goeldii*; (l) *Aplastodiscus leucopygius*.

**Table 1.** List of species recorded by river basin and sample localities.

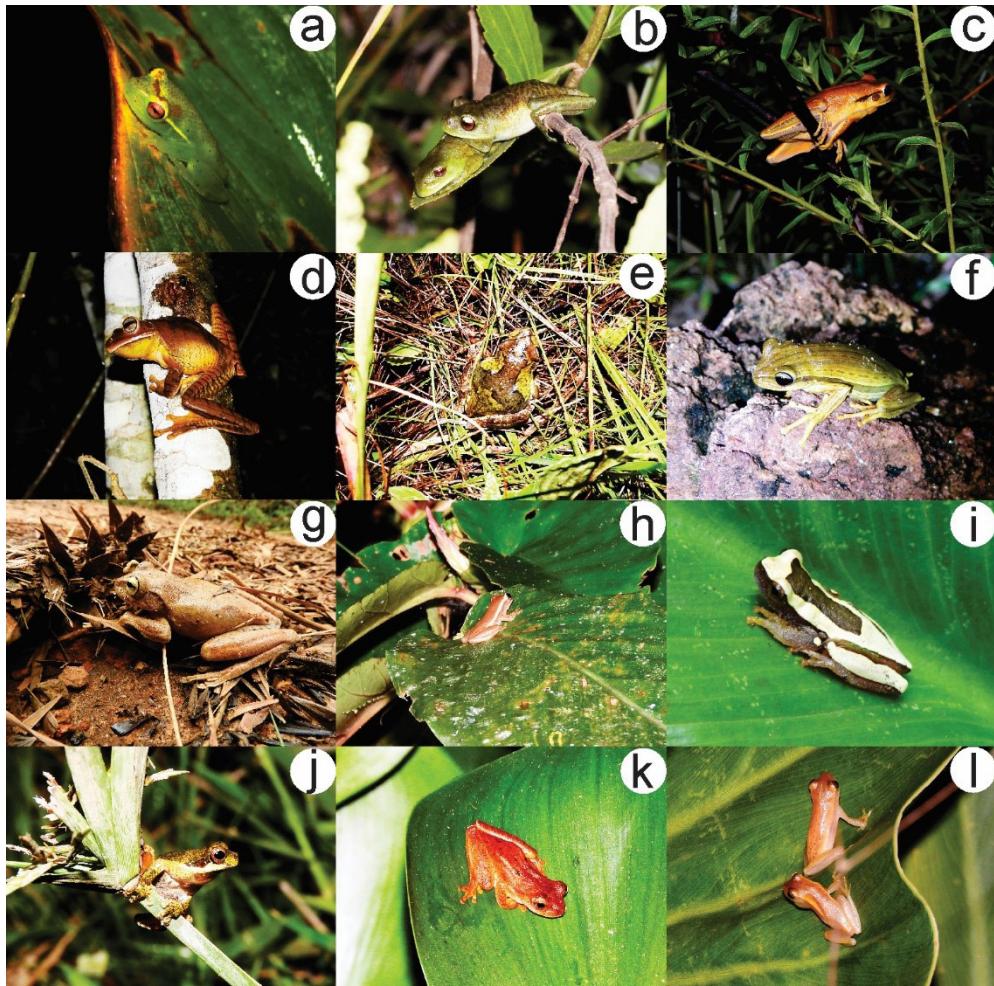
TAXON	OBS.	POINTS BY RIVER BASIN	
		RIO GRANDE	RIO PRETO
<b>Brachycephalidae</b>			
<i>Ischnocnema aff. guentheri</i> (Lineage CS4 Gehara et al., 2013)	CR	2, 3, 4, 8, 9	1, 6, 7, 10
<i>Ischnocnema parva</i> (Girard, 1853)	CR	3, 4	1
<b>Bufoidae</b>			
<i>Rhinella icterica</i> (Spix, 1824)	CR	2, 3, 4, 8, 9	1, 6, 7, 10
<i>Rhinella ornata</i> (Spix, 1824)	CR		1, 10
<i>Rhinella rubescens</i> (Lutz, 1925)	C	4, 5	
<b>Centrolenidae</b>			
<i>Vitreorana eurygnatha</i> (Lutz, 1925)	R	3	
<i>Vitreorana uranoscopa</i> (Müller, 1924)	CR	3, 4, 8, 9	1
<b>Cycloramphidae</b>			
<i>Thoropha miliaris</i> (Spix, 1824)	C		1
<b>Craugastoridae</b>			
<i>Haddadus binotatus</i> (Spix, 1824)	C		1
<b>Hemiphractidae</b>			
<i>Fritziana goeldii</i> (Boulenger, 1895)	C		1
<b>Hylidae</b>			
<i>Aplastodiscus arildae</i> (Cruz and Peixoto, 1987)	CR	3, 4, 8, 9	1
<i>Aplastodiscus leucopygius</i> (Cruz and Peixoto, 1985)	CR	3, 4, 8, 9	1
<i>Aplastodiscus pereirae</i> Lutz, 1950	CR	2, 5	
<i>Boana albopunctata</i> (Spix, 1824)	CR	2, 3, 4, 5, 9	1, 6, 7, 10
<i>Boana faber</i> (Wied-Neuwied, 1821)	CR	2, 4, 5, 8, 9	1, 6, 7, 10
<i>Boana pardalis</i> (Spix, 1824)	CR	2, 4, 9	1, 10
<i>Boana polytaenia</i> (Cope, 1870)	CR	2, 3, 4, 5, 8, 9	1, 6, 7, 10
<i>Boana prasina</i> (Burmeister, 1856)	R	8	
<i>Bokermannohyla luctuosa</i> (Pombal and Haddad, 1993)	C	8	1
<i>Dendropsophus decipiens</i> (Lutz, 1925)	CR	2	10
<i>Dendropsophus elegans</i> (Wied-Neuwied, 1824)	R		1, 10
<i>Dendropsophus microps</i> (Peters, 1872)	C	2	10
<i>Dendropsophus minutus</i> (Peters, 1872)	CR	2, 5, 9	1, 6, 7, 10
<i>Dendropsophus seniculus</i> (Cope 1868)	L	3	
<i>Dendropsophus sanborni</i> (Schmidt, 1944)	CR	2, 5	1, 10
<i>Scinax crospedopilus</i> (Lutz, 1925)	CR	2, 3, 4, 5, 8, 9	1, 6, 7, 10
<i>Scinax duartei</i> (Lutz, 1951)	CR	4	1
<i>Scinax dolloi</i> (Werner, 1903)	C		1
<i>Scinax fuscomarginatus</i> (Lutz, 1925)	CR	2	
<i>Scinax hayii</i> (Barbour, 1909)	CR	2, 4, 9	1, 10
<i>Scinax hiaralis</i> (Haddad and Pombal, 1987)	CR		1
<i>Scinax rogerioi</i> Pugliese, Baêta and Pombal, 2009	R	2	
<i>Scinax similis</i> (Cochran, 1952)	R	2	1
<i>Scinax squalirostris</i> (Lutz, 1925)	CR	2	
<i>Scinax x-signatus</i> (Spix, 1824)	C	2, 5	1, 10
<i>Scinax sp.1</i>	R	3	
<i>Scinax sp.2</i>	R		1
<b>Hydrididae</b>			
<i>Hylodes phylloides</i> Heyer and Crocroft, 1986	CR		1, 10
<b>Leptodactylidae</b>			
<i>Adenomera bokermanni</i> (Heyer, 1973)	R	9	
<i>Adenomera marmorata</i> Steindachner, 1867	R	8, 9	10
<i>Leptodactylus furnarius</i> Sazima e Bokermann, 1978	CR	2, 4, 5, 8, 9	1, 6, 7, 10
<i>Leptodactylus fuscus</i> (Schneider, 1799)	CR	2, 4, 5, 9	1, 6, 7, 10
<i>Leptodactylus labyrinthicus</i> (Spix, 1824)	C	2	
<i>Leptodactylus latrans</i> (Steffen, 1815)	CR	2, 4, 9	1, 6, 10
<i>Leptodactylus jolyi</i> Sazima and Bokermann, 1978	CR	2, 4, 5, 8, 9	1, 6, 7, 10
<i>Physalaemus cuvieri</i> Fitzinger, 1826	CR	2, 4, 5, 8, 9	1, 6, 7, 10
<i>Physalaemus jordanensis</i> Bokermann, 1967	C	8, 9	
<i>Physalaemus olfersii</i> (Lichtenstein and Martens, 1856)	CR		10
<i>Pseudopaludicolamurundu</i> Toledo, Siqueira, Duarte, Veiga-Menoncello, Recco-Pimentel and Haddad, 2010	CR	2	

Label: C = collected; R = Recorded; L = Listed.

**Table 1.** Continued...

TAXON	OBS.	POINTS BY RIVER BASIN	
		RIO GRANDE	RIO PRETO
<b>Microhylidae</b>			
<i>Elachistocleis cesarii</i> (Miranda-Ribeiro, 1920)	CR	2	
<i>Myersiella microps</i> (Duméril and Bibron, 1841)	CR	3, 8	1
<b>Odontophryniidae</b>			
<i>Odontophrynus americanus</i> (Duméril and Bibron, 1841)	CR	2, 4, 8, 9	
<i>Proceratophrys boiei</i> (Wied-Neuwied, 1824)	CR	3, 4, 8, 9	1, 10
<i>Proceratophrys mantiqueira</i> Mângia, Santana, Cruz and Feio, 2014	C		1
<i>Proceratophrys melanopogon</i> (Miranda-Ribeiro, 1926)	CR	3, 4, 8	1, 7, 10

Label: C = collected; R = Recorded; L = Listed.



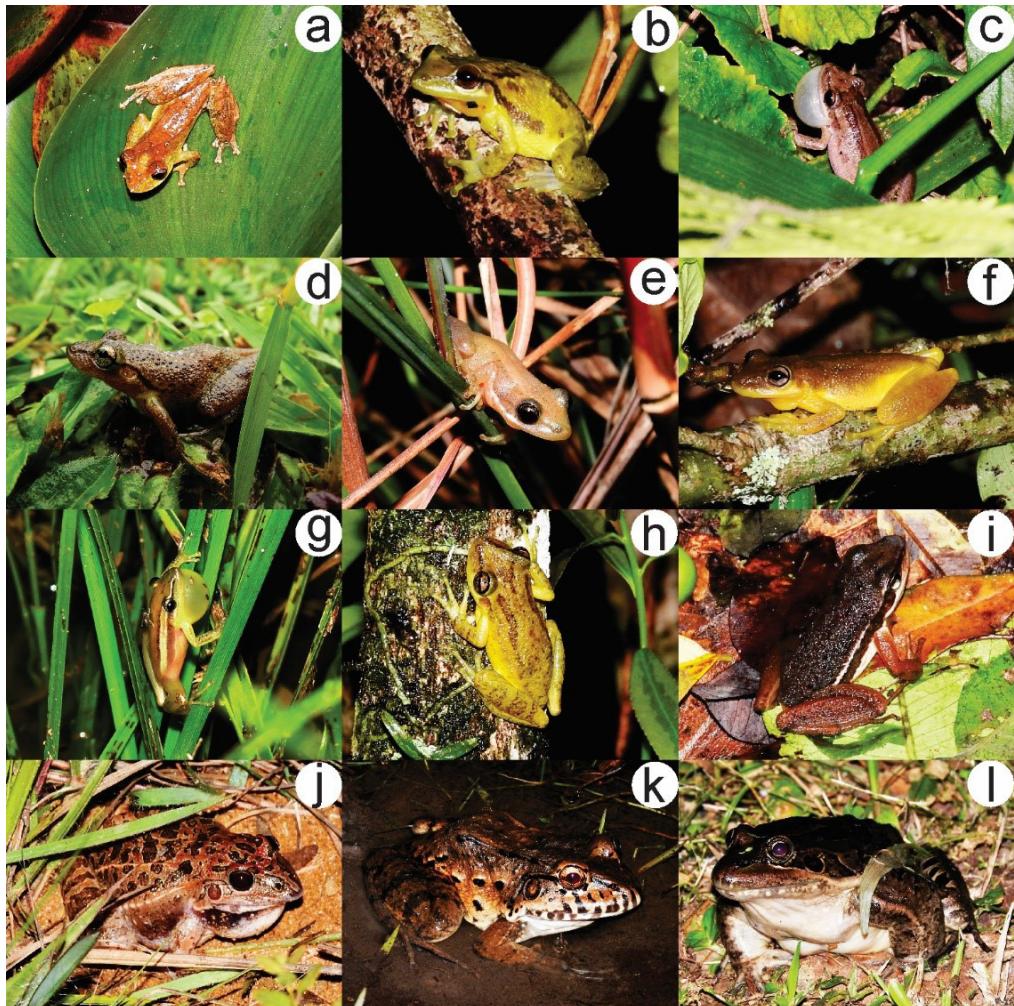
**Figure 4.** Sampled anuran species in the basins of the Rio Grande and Rio Preto in Bocaina de Minas, MG. (a) *Aplastodiscus arildae*; (b) *A. p. viridis*; (c) *Boana albopunctata*; (d) *B. faber*; (e) *B. pardalis*; (f) *B. polytaenia*; (g) *Bokermannohyla luctuosa*; (h) *Dendropsophus decipiens*; (i) *D. elegans*; (j) *D. microps*; (k) *D. minutus*; (l) *D. sanborni*.

demonstrating that the sampling effort is close to its sufficiency.

The species similarity found between the river basins was approximately 70% ( $Ss \approx 0,70$ ). The areas differ mainly due to the altitudinal gradient (See Figure 1). While, along the Rio Preto basin, we found primarily Ombrophilous

Forests and degraded pastures; along the Rio Grande basin, besides these habitats, we found altitudinal swamps, rupestrian fields, and proximity with Cerrado enclaves.

We highlight the occurrence of *Rhinella rubescens*, *Scinax duartei*, *S. hiemalis*, *S. rogerioi*, *Pseudopaludicola murundu*, and *Physalaemus jordanensis*. These species



**Figure 5.** Sampled anuran species in the basins of the Rio Grande and Rio Preto in Bocaina de Minas, MG. (a) *Scinax hiemalis*; (b) *S. crospedospilus*; (c) *S. duartei*; (d) *S. dolloi*; (e) *S. fuscomarginatus*; (f) *S. hayii*; (g) *S. squalirostris*; (h) *S. x-signatus*; (i) *Hylodes phyllodes*; (j) *Leptodactylus fuscus*; (k) *L. labyrinthicus*; (l) *L. latrans*.

were not expected for the locality. The sympatry between the related species *Scinax duartei* and *S. rogerioi* was also unexpected. *Scinax rogerioi* is a rare species in the locality being registered only by its unequivocal bioacoustic. Furthermore, the species *Boana prasina*, *Dendropsophus seniculus*, *Fritziana goeldii*, *Physalaemus jordanensis*, *P. olfersii*, *Pseudopaludicola murundi*, *Scinax rogerioi*, *Thoropa miliaris*, and *Vitreorana eurygnatha* had punctual occurrences, being found only in specific localities in the study area.

*Scinax* sp.1 and *Scinax* sp.2 were not identified at the species level since they represent only two individuals recorded only by their respective vocalizations that differ from each other.

#### 4. Discussion

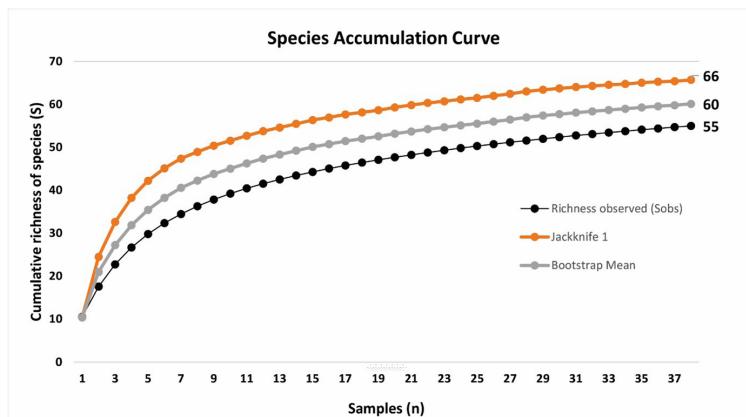
The observed richness represents 19% of the genera, 55% of the families of Brazilian anurans (Segalla et al., 2021),

and 24% of the amphibian species estimated for the Serra da Mantiqueira (Silva et al., 2018). It is equivalent to the richness observed in Serra Negra da Mantiqueira, in the Zona da Mata of Minas Gerais, by Neves et al. (2017), but with a partially distinct specific composition. The richness observed in the present study also approximates the estimated richness for Itatiaia National Park by Gouvêa (1985), which is 64 species, very close to the 66 species estimated by Jackknife-1 for the area of the present study. Hylidae was the most numerous family, followed by Leptodactylidae, a result also found by Neves et al. (2017), and a common pattern for the Neotropical region according to Heyer et al. (1990).

The similarity between the basins ( $S_s=0,70$ ) could be explained by the punctual occurrence of some species. These were found only in specific localities within the basins. Such specificity could be related to the altitudinal difference associated with habitat heterogeneity. For example, *Fritziana fissilis* was reported only in



**Figure 6.** Sampled anuran species in the basins of the Rio Grande and Rio Preto in Bocaina de Minas, MG. (a) *Leptodactylus jolyi*; (b) *Physalaemus cuvieri*; (c) *P. olfersii*; (d) *Pseudopaludicola murundu*; (e) *Elachistocleis cesarii*; (f) *Myersiella microps*; (g) *Odontophryne americanus*; (h) *Proceratophrys boiei*; (i) *P. melanopogon*.



**Figure 7.** Species accumulation curve during the 38 samples.

bromeliads of the Ombrophilous Forests of RPPN Boa Vista. *Rhinella rubescens* and *Scinax rogerioi* are species related to the Cerrado biome and were found only in the Rio Grande area, probably due to the occurrence of Cerrado enclaves in this basin. *Physalaemus jordanensis* was found only in the altitudinal swamps of Serra Verde (Rio Grande Basin). This study increases the distribution area of these last three species. Thus, in addition to species commonly

found in the Atlantic Forest biome, the study area harbors components of the Cerrado biome and altitudinal swamps. The co-occurrence of the sibling species *Scinax duartei* and *S. rogerioi* was a not expected sympatry. The region presents, as expected, many Atlantic Forest species, but also species known to occur in Cerrado and altitude fields, demonstrating unexpected sympatries as observed between *Scinax duartei* and *S. rogerioi*.

**Table 2.** Comparison between the bioacoustic parameters of *Leptodactylus jolyi* and *L. sertanejo*.

Species	SVL	Call duration (sec)	Notes/call	Pulses/note	Dominant Frequency (kHz)	Reference
<i>Leptodactylus jolyi</i>	45	0.03-0.04	1	1-3	1.40-2.40	Giaretta and Costa (2007)
<i>Leptodactylus sertanejo</i>	51-54	0.02-0.03	1	1-2	2.00-2.40	Giaretta and Costa (2007)
<i>Leptodactylus cf. jolyi</i>	51-53	0.02-0.04	1	1	2.15-2.33	Neves et al. (2017)
<i>Leptodactylus jolyi</i>	46-52	0.02-0.05	1	1	1.97-2.25	Present study

SVL = snout-vent length; sec = seconds.

Only one species, *Scinax duartei*, is considered Vulnerable according to the conservation criterion of Brasil (2014). Four species, *Dendropsophus microps*, *D. sanborni*, *Scinax duartei*, and *Myersiella microps*, appear as "Deficient data" in the red list of the state Minas Gerais (Drummond et al., 2008). No species recorded is threatened according to IUCN (2021) or Instituto LIFE (2000) criterions. However, *Ischnocnema parva*, *Vitreorana eurygnatha*, *V. uranoscopa*, *Bokermannohyla luctuosa*, *Hylodes phyllodes*, and *Proceratophrys melanopogon* present populations in decline, while the conservation status of the populations of *Rhinella ornata* and *Leptodactylus jolyi* is unknown (IUCN, 2021).

*Scinax duartei* occupies open areas at altitudes above 1,000 meters. This species had as its only confirmed locality until then, its type locality on the plateau of the Itatiaia National Park, in two very close water bodies (Haddad et al., 2016). In the present study, *S. duartei* was found in both, the Rio Preto basin (sheltered in the bromeliad *Quesnelia arvensis*), and in the Rio Grande basin, respectively, about 20 and 40 kilometers from its type locality (Brejo da Lapa, Itatiaia National Park, at 2,160 m.a.s.l.), and approximately 1,000 meters below the altitude of the type locality. *Scinax duartei*, considered threatened, may be distributed further along the Rio Grande basin which indicates the need to go further in studies on this watershed and possibly a re-evaluation of its conservation status.

*Leptodactylus jolyi* is widely dispersed throughout the Rio Grande and Rio Preto basins, along the studied area. Although for the region, the occurrence of *L. sertanejo* Giaretta and Costa, 2007 is plausible due to the Cerrado enclaves, the original description does not precisely delimit *L. sertanejo* from *L. jolyi* and Neves et al. (2017) question the taxonomic validity of *L. sertanejo*. According to Giaretta and Costa (2007), *L. sertanejo* differs from *L. jolyi* mainly due to its larger size, robustness, and bioacoustic characters. However, Table 2 shows the overlapping of the bioacoustic characters between the two species, while the larger size of *L. sertanejo* could represent only an adaptation to the habitat, Cerrado for *L. sertanejo* and Atlantic Forest for *L. jolyi* (Neves et al., 2017). For this reason, we consider only the occurrence of *L. jolyi* in the study area.

The anuran community of this highland region is composed by endemic, rare, and also species widely dispersed

throughout the Brazilian biomes. The species have complex distributions in these mountains and only long-term studies can provide a better knowledge of their peculiarities. This complexity is the result of the evolution of the environment itself, but also the evolutionary and biogeographical history of each species. The complexity of the Mantiqueira ridges and the disjointed distributions of the populations of anurans did not allow this survey to be absolute; however, the present study represents a pioneer, and important approximation of the local anurans richness. Despite representing an area adjacent to the oldest national park in Brazil, its species composition was unknown until now. This complex species composition is under threat due to irregular human occupation throughout the area, with deforestation, altitudinal swamps embankment, and fire to open pastures.

### Acknowledgements

We are grateful to the ICMBIO and IBAMA for authorizing the research in the Área de Proteção Ambiental (APA) da Serra da Mantiqueira (no. 50094), the Pró-Fundação Mantiqueira (president Lino Matheus de Sá Pereira and his wife, Nívea) for authorizing the research at the RPPN Fazenda Boa Vista and logistic support, the managers of RPPN Ave Lavrinha (especially the president Mr. Vicente Paulo da Costa, and the executive director Nieta Lindenberg do Monte), Letícia de Abreu, Ricardo Alves (UNEMAT), and farmers and residents in the region for authorizing the research in their properties. UC acknowledges the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for support.

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