REPTILES & AMPHIBIANS

Anurans in the Ecotone Between Atlantic Forest and Cerrado in the Campos das Vertentes, Southeastern Brazil

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Abstract.—Knowing the biodiversity of anurans in poorly studied regions, especially those in biomes declining in area as a result of human activities, such as the Campos das Vertentes in Minas Gerais, Brazil, is essential for developing and implementing management and conservation strategies for the biota and for monitoring environmental quality. Our objective was to inventory the anurans of forest remnants in an ecotonal area between Atlantic Forest and Cerrado in Barroso and Prados Municipalities, Minas Gerais, Brazil. From October 2020 to March 2021, four researchers sampled anurans during three nighttime hours over 24 days for a total of 168 person-hours. We recorded 26 anuran species (16 in the Family Hylidae) in 13 genera and seven families. Forest fragments, such as those sampled, are important for the protection of anurans but are disappearing rapidly, mostly due to conversion to agricultural activities. Reduction or even local extinctions of anuran populations render conservation actions urgent.

The order Anura (Amphibia) comprises about 7,700 species, of which 1,187 occur in Brazil (Segalla et al. 2021; Frost 2024). These vertebrates are particularly sensitive to changing ecosystems (Becker et al. 2007), climate change (Pounds et al. 2006; Luedtke et al. 2023), and environmental pollution (Goessens et al. 2022; Jacinto-Maldonado et al. 2023). Additionally, many species are habitat specialists, requiring heterogeneous and pristine habitats to survive (Trochet et al. 2016; Cândido et al. 2024). Consequently, they are frequently used as bioindicators of environmental quality (Toledo 2009). These organisms also play important trophic roles as both predators or prey, either in larval or adult stages, for a wide variety of animals (Brandão et al. 2020; M.S. Oliveira et al. 2023).

Anurans are declining globally (Luedtke et al. 2023), with diminishing population and extinctions directly attributable to human activities. For example, water pollution (Jacinto-Maldonado et al. 2023) by agricultural contaminants (Goessens et al. 2022), even in sublethal amounts, can be harmful and has been shown to alter the ability of immature individuals to detect predators (Polo-Cavia et al. 2016). However, loss and fragmentation of natural habitats (Becker and Zamudio 2011; Trochet et al. 2016; Luedtke et al. 2023), the spread of diseases (Becker and Zamudio 2011; Luedtke et al. 2023; Jacinto-Maldonado et al. 2023), and the introduction of exotic species (Falaschi et al. 2020) are the principal factors responsible for declines and increased risks of extinction for Brazilian anurans.

In southeastern Brazil, the state of Minas Gerais boasts a rich (Haddad et al. 2013; Rossa-Feres et al. 2017) and largely endemic anuran fauna (Lima et al. 2021), reflecting the various ecosystems in the state (Drummond et al. 2005). Among the phytogeographic domains present in Minas Gerais, Atlantic Forest harbors the greatestest amphibian diversity in Brazil (Segalla et al. 2021) and the Cerrado supports the third highest richness in the country (Toledo and Batista 2012).

Despite the existing alterations and reductions of natural areas, both Atlantic Forest and Cerrado biomes suffer from ongoing antropogenic threats (Fines and Curvo 2019; Branco et al. 2021). Nevertheless, information regarding the anuran fauna, especially in areas that are not part of Conservation Units (CUs), is lacking. However, many of these areas have a high potential to become CUs, and/or to become parts of mosaics of protected areas, such as the sites sampled in this

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study (G.C.S. Oliveira et al. 2021; Gouvêa et al. 2023). Conservation Units are areas with relevant natural characteristics, established by public authorities, the purposes of which include conservation of biota and natural resources and sustainable use and recovery of ecosystems (Salvio 2017), largely because they contain contiguous forest remnants or constitute transition regions between different domains. Understanding the anuran fauna, especially in these areas, can be useful for monitoring environmental quality as well as developing conservation strategies for these organisms, in itself a critical need due to the large number of species threatened with extinction in Brazil; 58 of which were listed by ICMBio (2022).

Methods

The study was conducted in the municipalities of Barroso (-21.18694 -43.97583) and Prados (-21.22583 -44.07972), in Campos das Vertentes, a mesic region in south-central Minas Gerais (Cruz et al. 2006) (Fig. 1). The Campos das Vertentes is in a transition area between Atlantic Forest and Cerrado, characterized by isolated forest remnants among agroecosystems, pastures, and monocultures (Morikawa et al. 1998; Menini-Neto et al. 2004; Sousa et al. 2010). According to Spatial Data from the State System of Environment and Water Resources of Minas Gerais (IDE-SISEMA 2024), the two municipalities are located in a zone of "extreme priority" (Prados) and a "special" zone (Barroso) for conservation, an area of "very high" priority for recovery. Additionally, they border the buffer zone of the Conservation Unit: Environmental Protection Area (APA) Serra de São José and Wildlife Refuge (REVIS) Libélula da Serra de São José, in the municipality of Tiradentes.

We conducted surveys in gallery forests called "Cachoeiras" in three non-continuous forest fragments, named Cachoeira da Lajinha (Fig. 2A–B; 32.25 hectares; -21.22591, -44.02888), Cachoeira do Padeiro (Fig. 1C–D; 26.43 hectares; -21.22388, -43.99166), and Mata do Baú (Fig. 1E–F; 38.4 hectares; -21.20194, -43.93027). The three sampled fragments have remnants of gallery forests, a phytophysiognomy of the Cerrado characterized by arboreal vegetation forming corridors along watercourses, and Semideciduous Seasonal Forest, a forest formation belonging to the Atlantic Forest domain in which approximately 50% of tree species lose their leaves during the dry season (Oliveira Filho 2006) and with lotic (streams) and lentic environments (swamps and ponds) (Fig. 1). They are subject to anthropogenic pressures such as fragmentation, wildfires, livestock farming, uncontrolled tourism, and eucalyptus cultivation (Gouvêa et al. 2023). We actively searched each fragment once during each sampling campaign (continuous days of field sampling in a given period) (Conte and Rossa-Feres, 2006) in October and December 2020 and January and March 2021, totaling 24 non-consecutive days. Four researchers conducted active searches from 1800 h to 2100 h each day (this period was defined by transport logistics in the area), totaling 288 hours over the four sampling events. Anurans were photographed in the field and some specimens were collected and anesthetized using a lethal dose of 60–100 mg/kg Thiopental administered intraperitoneally after applying a topical sedative (lidocaine) to the abdomen to avoid physiological stress or pain. Specimens were then preserved and deposited in the amphibian collection of the Museum of Biological Diversity (MDBio), Unicamp, Campinas, São

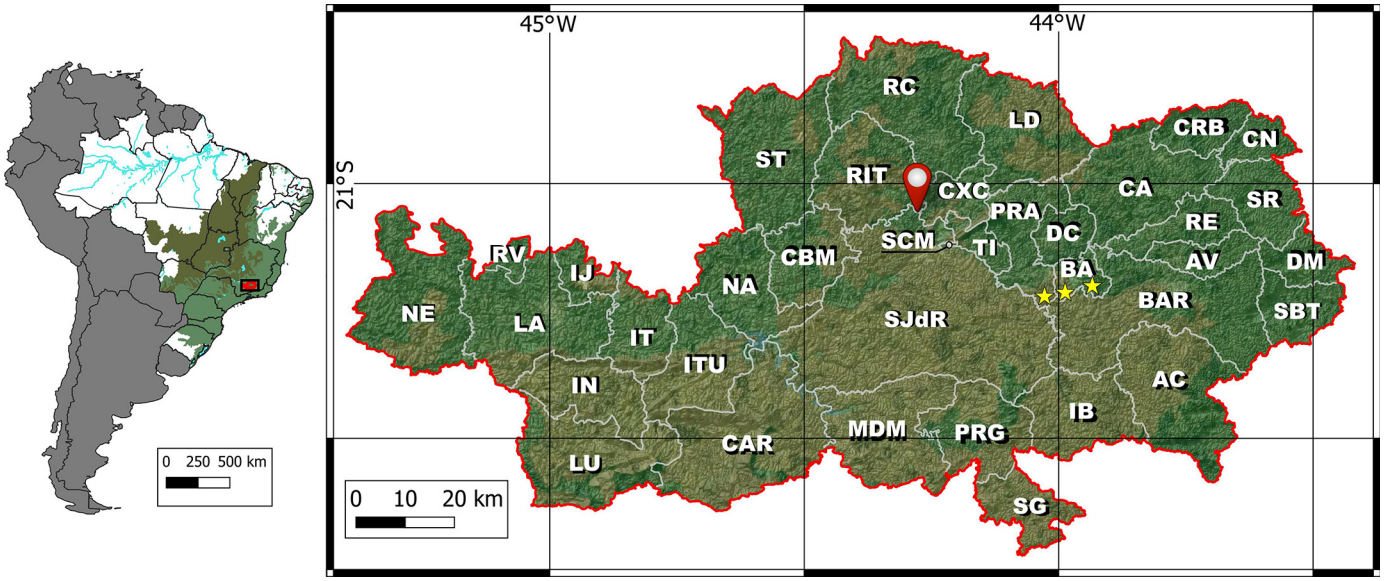

Figure 1. Ecotonal area between Atlantic Forest (deep green) and Cerrado (brown) in the municipalities comprising the Campos das Vertentes region, Minas Gerais, southeastern Brazil, noting those with information on the anuran fauna: Ritápolis National Forest in Ritápolis (RIT) (red marker) and the present study in Barroso (BA) and Prados (PRA) Municipalities (yellow stars).

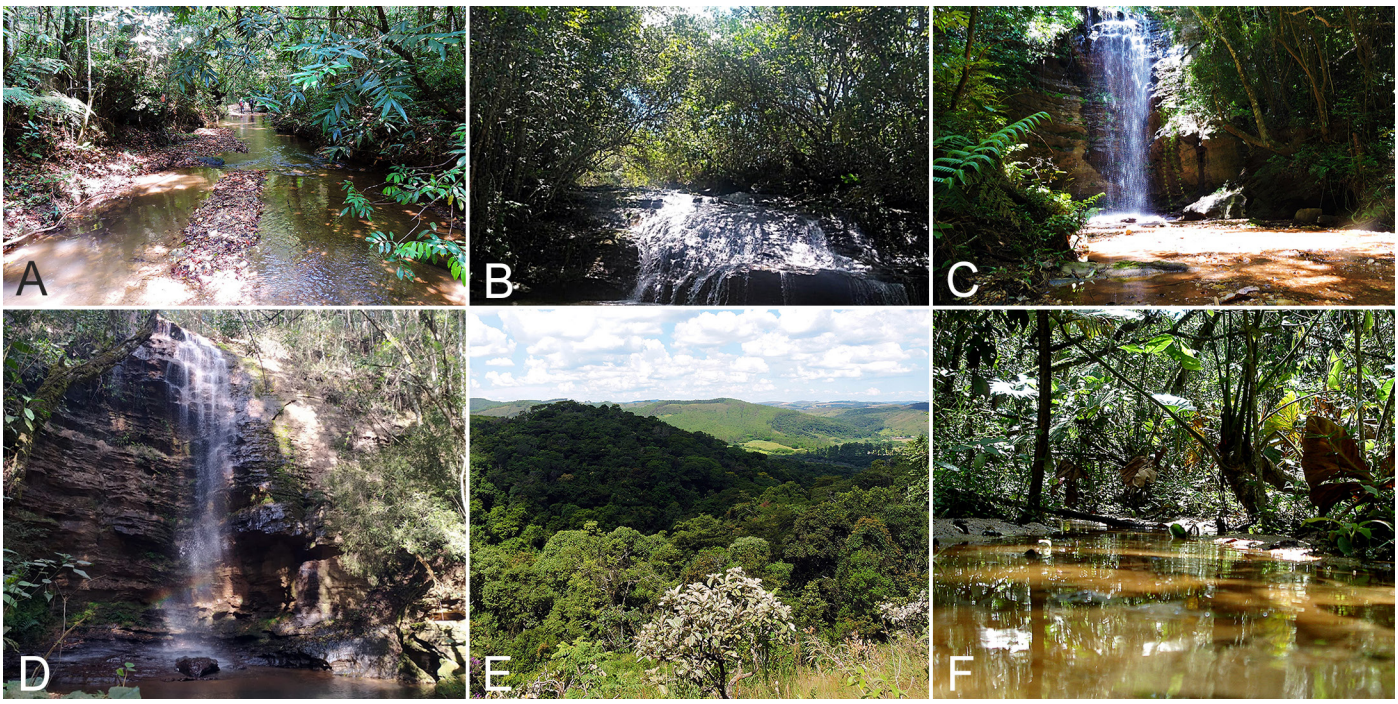

Figure 2. Forest fragments of the Cerrado and Atlantic Forest in the Campos das Vertentes region, Barroso and Prados Municipalities, Minas Gerais, Brazil: Lajinha Waterfall (A–B), Bakers Waterfall (C–D), and Baú Forest (E–F). Photographs by Marcos Magalhães.

Paulo. Species were identified by LFT with the assistance of specialists João Victor Andrade de Lacerda, Jose Perez Pombal Junior, and Pedro Paulo Goulart Taucci. Lacking DNA samples or recordings of vocalizations from collected frogs, we were unable to identify all of the specimens to species. Nevertheless, all were deposited in the museum and future examinations might provide additional insights.

A collection permit was granted by ICMBio (SISBio #76125-1) and all activities were regulated by the university's animal ethics committee (CEUA #402407052). Data regarding the conservation status of the species were obtained from IUCN (2023) and the Chico Mendes Institute for Biodiversity Conservation (ICMBio 2024). To assess regional sampling effort, we built a species-accumulation curve using observed abundance (numbers of individuals per species collected and accessioned in a biological collection or documented with photographs taken in the field) and richness, with a 95% confidence interval, and a Bootstrap 1 estimator, using EstimateS 9.1.0 Software (Cowell and Elsensohn 2014).

Results and Discussion

We documented a total of 109 anurans of 26 species, of which 84 were collected (Table 1; Fig. 3). Most (55% of all records) were in the family Hylidae, which has a wide geographic distribution (Duellman 1988) both in Atlantic Forest (Moura et al. 2012; Guimarães et al. 2020; Lima et al. 2021; Mendonça et al. 2022) and in Cerrado (Maffei et al. 2011; Teixeira et al. 2022). Due to their morphology and climbing habits, most hylids readily adapt to different microhabitats (Gondim et al. 2013), occupying all levels of various phytophysiognomies.

Most of the recorded anuran species are abundant in preserved natural habitats (Haddad et al. 2013). However, our records indicated exactly the opposite for the sampled region, with most species encountered in low numbers and in only one sampling campaign (Table 1), except for *Ololygon flavoguttata* (Lutz and Lutz 1939) (Hylidae) and *Vitreorana parvula* (Boulenger 1895) (Centrolenidae), with frequencies of 60% and 30%, respectively.

These two species have been reported to have been infrequently encountered in natural habitats (Haddad et al. 2013). However, in our samples, we found them during all sampling events in all campaigns (Table 1). *Ololygon flavoguttata* is considered a habitat specialist, usually found in primary and secondary forests and rocky fields in the Atlantic Forest domain in southeastern region of Brazil, but sensitive to open modified areas such as pastures and crops, as it requires more natural lotic environments for reproduction (IUCN SSC Amphibian Specialist Group & Instituto Boitatá de Etnobiologia e Conservação da Fauna 2023a). However, *O. flavoguttata* can tolerate forest formations with some anthropic disturbance (Lima et al. 2021). Therefore, the high abundance and sampling frequency recorded in this study suggests that the conservation of forest fragments can sustain populations of these frogs, and expanding efforts to restore surrounding areas could be an effective conservation strategy for the regional biota.

Table 1. Anuran species recorded in the ecotonal area between Atlantic Forest and Cerrado in the Campos das Vertentes region, Minas Gerais, southeastern Brazil, documented by photographs or specimens deposited at MDBio, Unicamp; month of sampling; threat of extinction according to the IUCN Red List (VU = Vulnerable, LC = Least Concern); and MDBio specimen numbers.

Species	Status	Oct	Dec	Jan	Mar	(N)	MDBio numbers
Brachycephalidae							
Ischnocnema cf. *juipoca* Sazima and Cardoso 1978	LC	1	1	0	0	2	25484, 25531
Bufonidae							
Rhinella rubescens (Lutz 1925)	LC	1	1	2	0	4	25480–11
Centrolenidae							
Vitreorana parvula (Boulenger 1895)	VU	2	4	4	0	10	25483, 25518, 25542, 25552, 25555
Craugastoridae							
Haddadus binotatus (Spix 1824)	LC	4	0	3	0	7	25492, 25499–500, 25503, 25527, 25548
Hylidae							
Aplastodiscus cf. *albosignatus* (Lutz and Lutz 1938)	LC	1	0	0	0	1	25541
Aplastodiscus cf. *arildae (*Cruz and Peixoto 1987*)*	LC	5	1	0	0	6	25515, 25517
Aplastodiscus cf. *leucopygius* (Cruz and Peixoto 1985)	LC	2	0	0	0	2	25563
Boana albopunctata (Cruz and Peixoto 1985)	LC	0	0	1	0	1	25510
Boana cf. *pardalis* (Spix 1824)	LC	1	0	2	0	3	25488, 25504, 25560
Boana cf. *crepitans* (Wied-Neuwied 1824)	LC	1	3	3	1	8	25488, 25501, 25504, 25525
Boana faber (Wied-Neuwied 1821)	LC	3	3	0	3	9	25482, 25486–7,
Ololygon cf. *cosenzai* Lacerda, Peixoto and Feio 2012	LC	4	1	2	0	7	25497–8, 25530–40
Ololygon luizotavioi Caramaschi and Kisteumacher 1989	LC (declining)	1	0	0	0	1	25556
Ololygon flavoguttata (Lutz and Lutz 1939)	LC	5	5	8	2	20	25485, 25493, 25507, 25534, 25543–6, 25553–4, 25557–8
Scinax fuscovarius (Lutz 1925)	LC	1	2	1	2	6	25559
Scinax sp. 1	—	1	0	0	0	1	25505
Scinax sp. 2	—	0	1	0	0	1	25516
Scinax sp. 3	—	1	0	0	0	1	25519
Hylidae sp. 1	—	1	0	0	0	1	25495
Hylidae sp. 2	—	1	0	0	0	1	25521
Leptodactylidae							
Leptodactylus latrans (Steffen 1815)	LC	2	0	0	0	2	25512
Adenomera sp. 1	—	1	0	0	0	1	25490
Adenomera sp. 2	—	0	0	0	1	1	25562
Physalaemus sp.	—	0	0	1	0	1	25511
Odontophrynidae							
Odontophrynus cultripes Reinhardt and Lütken 1861	LC	2	1	0	0	3	25508–9
Proceratophrys sp.	—	0	4	0	0	4	25494

The status of *Vitreorana parvula* was listed as Data Deficient (DD) until the most recent assessment, in which they were considered vulnerable (VU) (IUCN SSC Amphibian Specialist Group & Instituto Boitatá de Etnobiologia e Conservação da Fauna 2022). However, a recent study (Zucchetti and Castroviejo-Fisher 2024) indicated that the distribution of *V. parvula* in Brazil extends beyond the state of Santa Catarina and includes the states of Rio Grande do Sul, Paraná, São Paulo, Rio de Janeiro, Espírito Santo, Minas Gerais, and Bahia, differing from the range described in the IUCN Red List. This suggests that a future reassessment of the species likely will result in an adjustment of its conservation status.

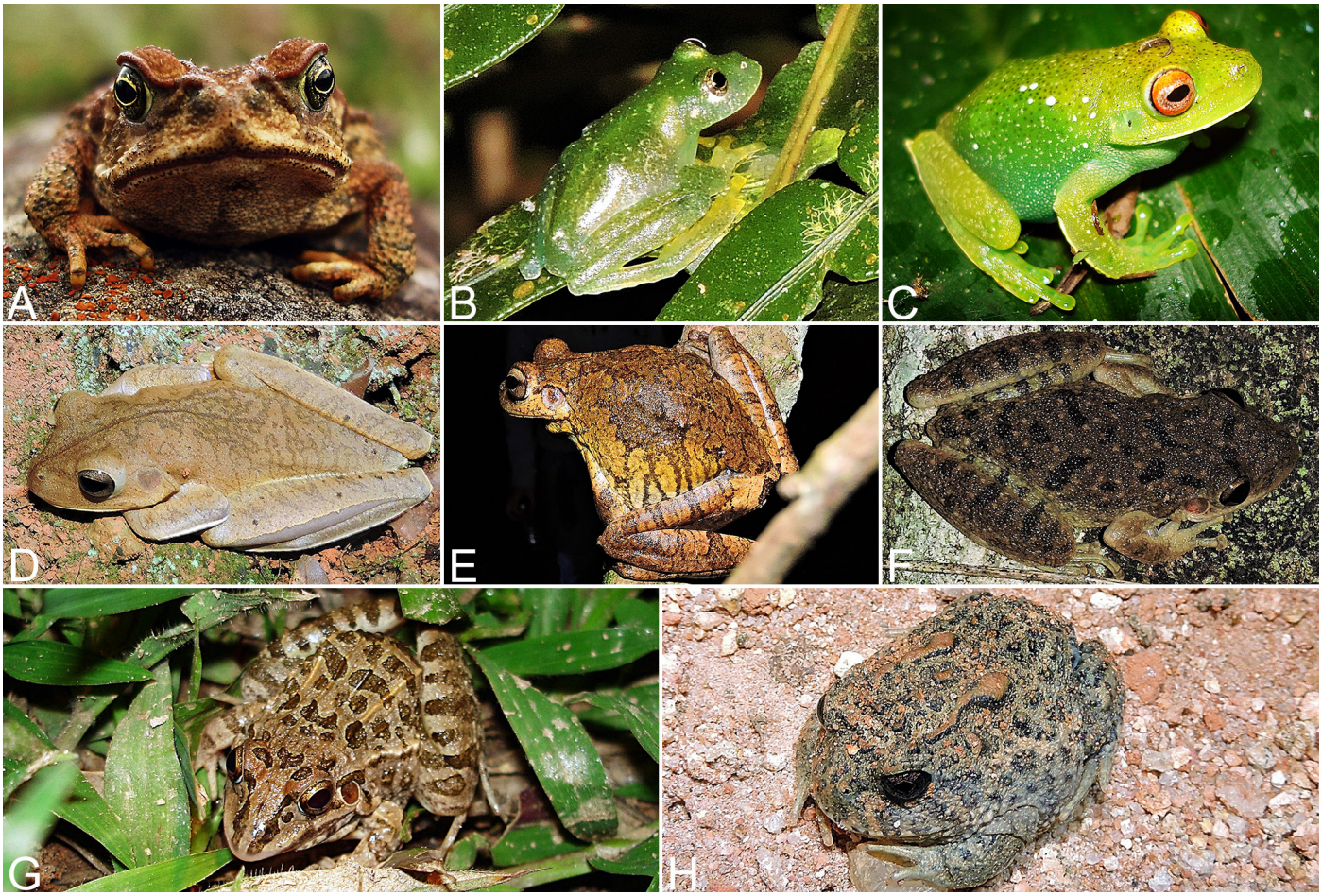

Figure 3. Anurans encountered in forest fragments in Barroso and Prados Municipalities in the Campo das Vertentes region, Minas Gerais, Brazil: *Rhinella rubescens* (A); *Vitreorana parvula* (B); *Aplastodiscus* cf. *leucopygius* (C); *Boana faber* (D); *Boana crepitans* (E); *Scinax fuscovarius* (F); *Leptodactylus latrans* (G); *Odontophrynus cultripes* (H). Photographs by Marcos Magalhães.

The main threats to amphibian populations, in order of importance, are effects of climate change, habitat fragmentation, and diseases (Luedtke et al. 2023), such as panzoic chytridiomycosis (Scheele et al. 2019). In addition to favoring generalist species, the destruction and/or conversion of natural habitats contribute to geographic isolation and limits gene flow between populations in forest fragments, resulting in population declines (Rivera-Ortíz et al. 2014; Ferrante et al. 2017), and could be responsible for the large number of poorly represented species in our samples. However, another contributing factor likely is the difficulty in sampling certain taxa, such as *Odontophrynus cultripes*, the semi-fossorial habits of which render encounters rare (Vaz-Silva et al. 2020). Regardless of which factors are most responsible for the relative rarity of some species in our samples, noting that the bootstrap 1 estimator (Fig. 4) suggested that regional richness could increase to 31 species is important.

Also worth noting is that *Aplastodiscus* cf. *albosignatus*, *Aplastodiscus* cf. *arildae*, *Aplastodiscus* cf. *leucopygius*, *Boana pardalis*, *Ololygon flavoguttata*, *Ololygon luizotavioi*, and *Vitreorana parvula* were not recorded during a seven-year herpetological survey in the Ritápolis National Forest (FLONA), located 104 km from our study area (Hudson 2020). This reinforces the importance of sampled forest fragments for the regional species pool in the Campos das Vertentes, as they can

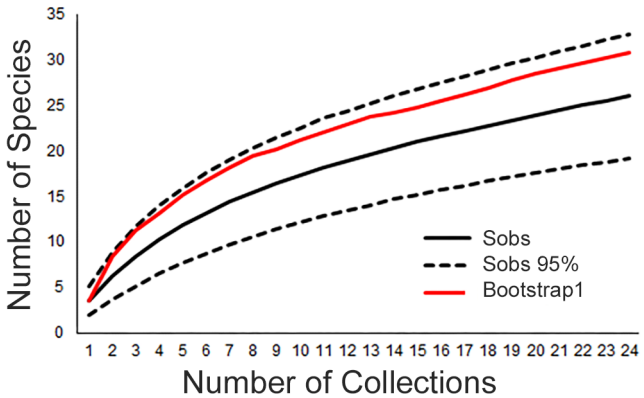

Figure 4. Accumulation curve of anuran species collected in forest fragments of Barroso and Prados Municipalities, Minas Gerais, Brazil, based on observed species richness with a 95% trustworthy interval and estimated species richness (Bootstrap 1).

serve as corridors for anurans and other vertebrates with limited dispersal capabilities. Additionally, the presence of species such as *Aplastodiscus* cf. *albosignatus*, *A.* cf. *arildae*, *A.* cf. *leucopygius*, *Boana* cf. *pardalis*, *Haddadus binotatus*, *Ololygon flavoguttata*, *O. luizotavioi*, and *V. parvula*, which are endemic to the Atlantic Forest (Rossa-Feres et al. 2017), as well as *Rhinella rubescens*, which is typical of open habitats such as Cerrado and Caatinga (Valdujo et al. 2011), illustrates the ecotonal nature of this region.

Another species deserving attention is *Ololygon luizotavioi,* this frog is endemic to the Atlantic Forest, occurring in the central, eastern, and Zona da Mata regions of Minas Gerais (Rossa-Feres et al. 2017). Although generally more abundant in natural habitat, populations are declining (IUCN SSC Amphibian Specialist Group & Instituto Boitatá de Etnobiologia e Conservação da Fauna 2023b), which might be the case in the study area, explaining the single record of one individual in one sampling event. Local expansion of livestock farming and eucalyptus cultivation has led to reductions of the studied fragments, as also was indicated by studies on dragonflies (Souza et al. 2013; Gouvêa et al. 2023) and social wasps (Souza and Prezoto 2005; Coelho et al. 2023) in the region.

On the other hand, the presence of generalist species such as *Boana faber* and *Boana albopunctata*, which occur in open areas or within or at the edges of forests (Santana et al. 2008), and have extensive ranges (IUCN SSC Amphibian Specialist Group 2023), could be indicative of an increase in anthropogenic pressure, represented mainly by pastures and monocultures, which form the vegetational matrix in which the forest fragments are embedded (Lima et al. 2021). This could affect the richness, abundance, and composition of amphibian communities (Ferrante et al. 2017). Another threat to the anurans of Campos das Vertentes is the possible contamination of water resources by metals and other substances from irregular mining and other industrial activities in the region (Zuliane et al. 2017; Hudson 2020; Amâncio et al. 2021). Although the species recorded during this study are not in imminent risk of extinction, some are under some degree of threat (e.g., ICMBio 2022).

This study contributes to the knowledge of the herpetofauna in an under-sampled region in Minas Gerais. Although the region's reptiles have been examined (Sousa et al. 2010; Rios et al. 2017), the anuran fauna of Campos das Vertentes was known previously only from Hudson's (2020) study in the Ritápolis National Forest (Fig. 1), during which he recorded 31 species, ten of which (*Boana* cf. *crepitans*, *B. albopunctata*, *B. faber*, *B.* cf. *pardalis*, *Haddadus binotatus*, *Ischnocnema* cf. *juipoca*, *Leptodactylus latrans*, *Odontophrynus cultripes*, *Rhinella rubescens*, and *Scinax fuscovarius*) were also recorded in the present study.

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