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Anurans Associated with Streams and Riparian Zones in a Brazilian Atlantic Forest Remnant: Diversity, Endemism and Conservation

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Abstract.—**The Atlantic Forest biome contains > 50% of the anuran species described in Brazil and is considered a vital biodiversity hotspot; however, negative anthropogenic actions have been increasing over time, promoting wide-scale deforestation of this biome. Deforestation can impact the maintenance of anuran populations associated with streams and riparian zones because most of these species have high habitat specificity and are thus more susceptible to local extinction. We studied aspects of the anuran community (i.e., composition, richness, abundance, spatio-temporal distribution, diversity of reproductive modes, and endemism) associated with streams and riparian zones in a protected area of the Atlantic Forest (Duas Bocas Biological Reserve - DBBR) in the state of Espírito Santo, southeastern Brazil. We sampled from February 2018 to March 2019 using the active search method with visual and auditory sampling in 22 transects distributed across streams in three areas of the reserve. We recorded a total richness of 22 anuran species and 10 reproductive modes in streams and riparian zones with highest abundance of Kautsky's Snouted Treefrog, *Ololygon kautskyi*, and Gaudichaud's Frog, *Crossodactylus* aff. *gaudichaudii*. Anuran communities differed significantly between the three studied areas and between the dry and rainy seasons. About 80% of the recorded species are endemic to the Atlantic Forest biome and one is endemic to the state of Espírito Santo. Our study shows the importance of DBBR as a remnant of the Atlantic Forest and as a reservoir for rare and endangered species, and it is thus a priority area for anuran conservation.**

Key Words.—amphibians; community ecology; frogs; rainforest; richness

Introduction

Among Brazilian biomes, the Atlantic Forest contains the highest richness of anuran species: at least 625 species are estimated to occur in this biome, representing over 50% of the anuran species described in Brazil (Rossa-Feres et al. 2017). Of the anuran species in the Atlantic Forest, about 80% are endemic (Rocha et al. 2004; Rossa-Feres et al. 2017). Reproductive mode diversity is also high among Atlantic Forest anurans with 27 of the 39 known reproductive modes represented, making this a priority biome for amphibian conservation (Haddad and Prado 2005; Condez et al. 2009; Rossa-Feres et al. 2017). The wide variety of environments in the Atlantic Forest provides many favorable microhabitats for anuran development that contribute to species specificity, and thus endemism (Sá 2013). Unfortunately, negative anthropogenic actions have led to wide-scale deforestation of this biome (Colombo and Joly 2010). The maintenance of habitat conditions that allow a particular reproductive mode to be locally performed is one of the primary factors determining the persistence of amphibian species in altered Atlantic Forest environments (e.g., fragments; Almeida-Gomes and Rocha 2015).

Anurans associated with streams and riparian zones of the Atlantic Forest are closely tied to their environments throughout their life cycle, making them vulnerable to loss of riparian forest, pollution, and damming, which results in changes in water flow, depth, and pH (Kupferberg et al. 2012; Almeida-Gomes et al. 2014). In this context, some species may be eliminated or suffer population declines after habitat disturbance due to their high microhabitat specificity, with only a few species persisting in the altered landscape matrix (Almeida-Gomes et al. 2014, 2016a,b). In addition, studies suggest that anurans associated with streams and riparian zones are more susceptible to infection by the fungus *Batrachochytrium dendrobatidis*, which causes chytridiomycosis, than species in other habitats (e.g., Lips 1998; Hero et al. 2005) due to favorable abiotic conditions for the reproduction of the pathogen (Berger et al. 2004; Piotrowski et al. 2004).

Ecological and taxonomic studies are useful for the conservation of biodiversity, as they provide the basic knowledge necessary for planning actions to protect

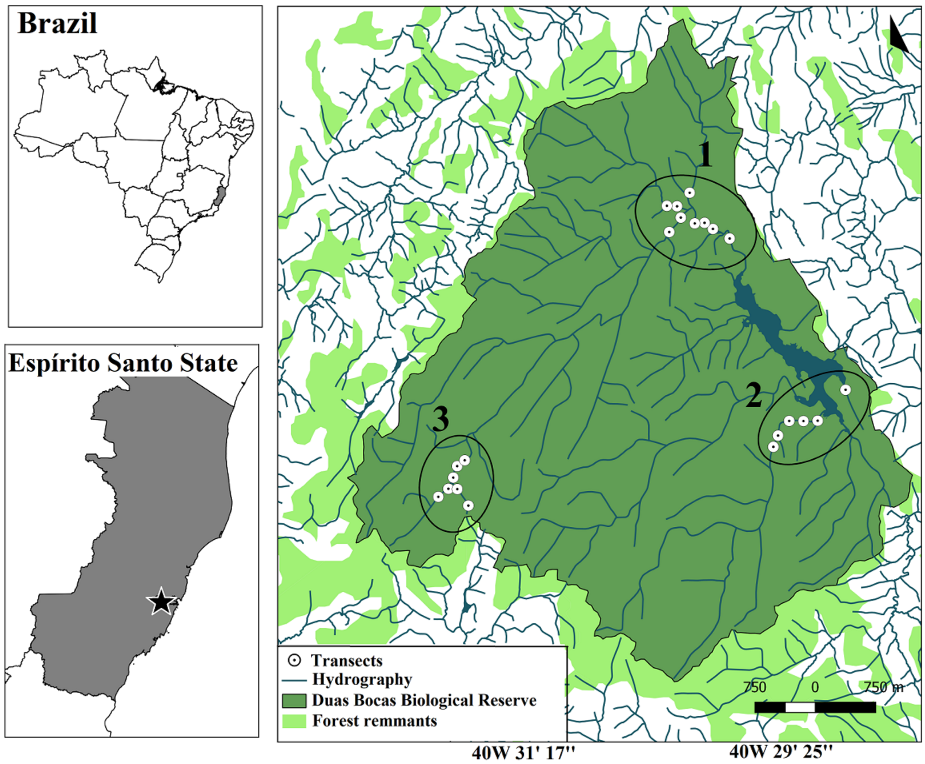

Figure 1. Location of sampling transects along streams in Duas Bocas Biological Reserve, Cariacica, Espírito Santo, Brazil. Areas of study are: 1 = Represa Velha, 2 = Panelas, and 3 = Alto Alegre.

species (Mace 2004; Sutherland et al. 2013; Doi and Takahara 2016). These studies allow for better categorization of conservation status (Doi and Takahara 2016), especially of Atlantic Forest endemic species on the Global and Brazilian Endangered Species List (Almeida-Gomes et al. 2014). They also contribute to the discovery of new species, as the presence of Data Deficient (DD) species may highlight places with high potential for the discovery of new species (Trindade-Filho et al. 2012; Almeida-Gomes et al. 2014).

The Duas Bocas Biological Reserve (DBBR), in the municipality of Cariacica, is an important Atlantic Forest remnant in the state of Espírito Santo (Tonini et al. 2010) both because of its local biodiversity and because it includes much of the protective forests of the Duas Bocas River Basin, an important water source. Many species of anurans, including numerous endemic species, occur in riparian environments along local rivers and streams; however, studies on anurans in the area are limited to a general species list (Tonini et al. 2010) and a study of the distribution of anurans in permanent ponds (Prado and Pombal Jr. 2005). Thus, our current knowledge of DBBR does not allow determination of which species are associated with streams or their potential conservation status and degree of endemism. Here, we characterize the amphibian community associated with streams and riparian zones of DBBR, Espírito Santo, Brazil, evaluating the richness, composition, abundance, spatio-temporal distribution, and diversity of reproductive modes, in addition to identifying endemic and threatened species.

Material and Methods

Study site.—Duas Bocas Biological Reserve (DBBR) is located in the municipality of Cariacica, in the state of Espírito Santo, southeastern Brazil (20º14’04’’S to 20º18’30’’S; 40º28’01’’W to 40º32’07’’W; Fig. 1). DBBR constitutes about 40% of the Duas Bocas River Basin area (Bastos 2015). Within DBBR, the main springs of the river basin are found: the Sertão Velho, Panelas, Naiá-Assú, and Pau Amarelo streams (López 2016; Santos 2016). The Pau Amarelo stream, which represents the main channel, together with Panelas and Naia-Assú form the Duas Bocas River, which supplies the DBBR dammed reservoir (López 2016; Santos 2016).

The DBBR has a total area of 2,910 ha, with elevation varying between 200 and 738 m above sea level, and comprises about 80% native forest with the remaining 20% distributed among secondary forest, dam, streams and riparian zones (Tonini et al. 2010; José et al. 2016). It has a humid tropical climate with monthly average temperatures ranging from 19° C in winter to 25.5° C in summer, average annual rainfall of approximately 1,500 mm, and relative air humidity above 70% (Prado and Pombal 2005; Tonini et al. 2010; José et al. 2016). Its vegetation type is dense ombrophilous forest (Novelli 2010).

With 51 anuran species recorded for the area and 38% of the species known for the state, DBBR plays an important role in biodiversity conservation in Atlantic Forest remnants in the state of Espírito Santo (Tonini et al. 2010; Almeida et al. 2011). Furthermore, DBBR is integral to one of the priority ecological corridors for state conservation and of fundamental importance for the water supply to the population of Cariacica municipality (Boni et al. 2009; Instituto Estadual de Meio Ambiente e Recursos Hídricos do Espírito Santo [IEMA]. 2018. Reserva Biológica Duas Bocas. IEMA, Brazil. Available from https://iema.es.gov.br/REBIO_Duas_Bocas. [Accessed 12 August 2018]).

We conducted our study in three areas of DBBR: (1) Represa Velha (hereafter Area 1), which has an elevation between 202 m and 213 m above sea level. This area is composed of secondary forest, where banana (*Musa* spp.), coffee (*Coffea* spp.), and pasture crops were introduced, in addition to a predominance of Jackfruit (*Artocarpus heterophyllus*), intercalated with other native plant species (Boni et al. 2009; Novelli 2010). During the study period, the mean air temperature of this area was 23.8° C (range, 21.4°–26.8° C) and the relative humidity averaged 85.8% (range, 69% –94.5%). (2) Panelas (hereafter Area 2), which has an elevation between 204 m and 270 m above sea level and is composed of a forest with a high density of large trees and little evidence of disturbance. During the study period, the mean air temperature in this area was 24.6° C (range, 21.9°–29.8° C) and the relative humidity averaged 78.8% (range, 58.1%–92.3%). (3) Alto Alegre (hereafter Area 3), which has an elevation between 300 m and 738 m above sea level and is composed of forest with a high density of large trees, abundant leaf litter covering the soil, and little evidence of disturbance (Boni et al. 2009; Novelli 2010). During the study period, the mean air temperature in this area was 21.9 °C (range, 19.6°–24.4° C) and the relative humidity averaged 83.3% (range, 68.5%–95%).

Data collection.—We sampled from February 2018 to March 2019, with monthly campaigns of 2-4 d, using the active search method with visual and auditory sampling (Crump and Scott 1994). We sampled during the day (0800–1700) and night (1800–2300) to increase the possibility of recording a higher variety of anuran species with different activity periods (Rocha et al. 2015; Pereira-Ribeiro et al. 2019). We distributed 22 sampling transects across different streams of the reserve, each 50 m long, with a distance of at least 50 m between them (Fig. 1). Nine transects were in Area 1, six were in Area 2, and seven were in Area 3 (Fig. 1). We sampled 16 transects four times, twice in the daytime and twice at night, and six transects (located in Area 3) three times, twice in the daytime and once at night. The difference in sampling was due to logistical difficulties accessing the six latter transects. We sampled each transect twice in the dry season and twice in the rainy season, except for the six transects mentioned above, which we sampled once in the dry season and twice in the rainy season. In each sampling of a transect, we searched for anurans for 30 to 50 min with a minimum of two observers looking for individuals on the soil, on rocks and within rock crevices, under vegetation, leaf litter, tree roots, and water, on tree trunks, and on the stream banks. We included all individuals found (visually or by their calling activity) within 2-m wide zone on each side of the stream bank. For each individual found, we recorded the species, the date and time, and the microhabitat in which it was initially observed.

We identified anuran species in the field with the help of Atlantic Forest anuran-specific field guides (Gasparini 2012; Haddad et al. 2013) and we follow the taxonomy of Frost (2019). When possible, we collected an individual of each anuran species for specific identification and later deposited it in the herpetological collection of the Museu Nacional, in Rio de Janeiro (MNRJ), as a voucher specimen. We euthanized collected anurans with 1.8% Lidocaine ointment and fixed them in 10% formaldehyde. After fixation, we preserved individuals in 70% alcohol.

Data analysis.—Using the species recorded as a function of sampling effort, we made a rarefaction curve using the program EstimateS 9.2 (http://viceroy.eeb.uconn.edu/estimates/) to evaluate the extent to which our sampling reached the predicted richness for DBBR. We used the Bootstrap estimator with 1,000 randomizations because the community was relatively stable, with few rare species present (Magurran 2004). We used the Shannon-Wiener index (*H'*) to quantify species diversity in each of the three study areas in DBBR (Krebs 1999), according to the following formula:

$$H' = -\sum_{i=1}^{S} P_i \ln P_i$$

Where: H' = the Shannon diversity index; S = the number of species encountered; $\sum$ = sum from species 1 to species S; P_i = the relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individual in the community: n_i/N; n_i = the number of individual in species I; N = the total number of all individuals (Shannon 1948).

Additionally, we performed a One-way ANOVA to test if study areas differed in anuran species richness.

We tested the normality and homoscedasticity and both met the assumptions of the analysis. To quantify β diversity between study areas, we used the betadisper function in the R software vegan package version 2.0–4 (Oksanen et al. 2019). This analysis defines β diversity as the average distance-to-centroid, measured as the average distance using the Jaccard dissimilarity index (or compositional dissimilarity) from a plot to the centroid of the group of all plots within an area. Finally, we performed a Wilcoxon signed rank test to determine if anuran abundance differed between the dry and rainy seasons.

To determine which species are endemic to the Atlantic Forest, we used the Atlantic Forest amphibian guide (Haddad et al. 2013) and other literature about anurans from this biome (Rossa-Feres et al. 2017). To ascertain the conservation status of each species, we used the global International Union for Conservation of Nature (IUCN) Red List (IUCN 2019), the List of Endangered Brazilian Fauna (Instituto Chico Mendes de Conservação da Biodiversidade [ICMBIO] 2018) and the list of endangered species of Espírito Santo (Gasparini et al. 2007). Reproductive modes are according to Haddad and Prado (2005).

Results

We recorded 248 individuals of 22 anuran species belonging to nine families (Table 1, Fig. 2). Nighttime surveys were more productive (171 individuals of 18 species) than daytime surveys (77 individuals of 10 species). The family Hylidae had the highest species richness (13 species, 59.1%), followed by Hylodidae (two, 9.1%), and Brachycephalidae, Bufonidae, Craugastoridae, Cycloramphidae, Leptodactylidae, Odontophrynidae, and Phyllomedusidae (each with one, 4.54%). The rarefaction curve predicted a richness of approximately 26 species for the DBBR stream and riparian zone areas (Bootstrap Estimator = 25.89 ± [SD] 2.33) and the species accumulation curve showed a tendency towards stabilization (Fig. 3).

The most abundant species was Kautsky's Snouted Treefrog (*Ololygon kautskyi*, 93 individuals, 37.5%), followed by Gaudichaud's Frog (*Crossodactylus* aff. *gaudichaudii*) and Lesser Foam Frog (*Leptodactylus latrans*; Table 1). The species with the lowest abundances were Cruz's Treefrog (*Aplastodiscus cavicola*), Weygoldt's Treefrog (*Aplastodiscus* cf. *weygoldti*), White-edged Treefrog (*Boana albomarginata*), Espirito Santo Robber Frog (*Ischocnema oea*), Rio Mutum Snouted Treefrog (*Ololygon argyeornata*), Burmeister's Frog (*Phyllomedusa burmeisteri*), *Scinax* sp. 1, and *Scinax* sp. 2 (one individual each, 1.1%). Anuran species diversity varied between the three sampled areas (Table 1), with Area 1 having the highest richness and diversity (16 species, H' = 1.88), followed by Area 2 (14 species, H' = 1.67), and Area 3 (10 species, H' = 1.50). Species richness significantly differed between the three sampled areas ($F_{2,19}$ = 5.36, P = 0.014). In relation to β diversity, Area 1 showed a 67% similarity in species composition with Area 2 and 30% similarity with Area 3. The similarity of Area 2 and Area 3 was 26%. The abundance of anurans varied significantly between the dry and rainy seasons, with higher values in the rainy season (W = 181.5, df = 22, P = 0.023; Table 1).

Of the 22 species we found, 18 are endemic to the Atlantic Forest biome (81.8%) and one of them (*Ololygon kautskyi*) is endemic to Espírito Santo. In terms of conservation status, three species are categorized as Near Threatened by the IUCN Red List (*Ischnocnema oea, Aplastodiscus cavicola, A. weygoldti*) and one is classified as Data Deficient (*O. kautskyi*). Considering the list of endangered species of Espírito Santo (Gasparini et al. 2007), one species is classified as Endangered (Mottled Leaf Frog, *Phasmahyla exilis*) and two species are classified as Data Deficient (*I. oea* and *O. kautskyi*; Table 1). It is noteworthy that none of the species we documented is classified as Threatened in the List of Endangered Brazilian Fauna (ICMBIO 2018).

We classified the 22 species recorded in streams and riparian zones in DBBR into 10 reproductive modes (Table 1). Most species (8, 36.4%) have reproductive mode 1, in which the deposition of eggs and the development of tadpoles occur in lentic environments. Five species have a reproductive mode associated with streams: Santo Smooth Horned Frog (*Proceratophrys schirchi*) and *O. kautskyi* have reproductive mode 2, in which the deposition of eggs and the development of tadpoles occur in lotic environments; Baumann's Tree Toad (*Hylodes lateristrigatus*) and *Crossodactylus* aff. *gaudichaudii* have reproductive mode 3, with eggs and early larval stages in constructed subaquatic chambers and exotrophic tadpoles in streams; and *P. exilis* lays eggs in vegetation and the tadpoles develop in lotic environments (mode 25).

Discussion

We found that DBBR has a richness of at least 22 anuran species associated with streams and riparian zones. The rarefaction curve showed a tendency towards stabilization, with a predicted richness of 26 species, indicating that our sampling effort was satisfactory (84.6%). Using other survey methods, such as pitfall traps, may yield the additional species predicted to occur. Other studies on stream- and riparian zone-associated anuran assemblages reported similar richness to what we found in DBBR. For example, Almeida-Gomes et al. (2015) analyzed the anuran community along two Atlantic Forest rivers in southeastern Brazil

Table 1. List of anuran species recorded in streams and riparian zones of the Duas Bocas Biological Reserve, Cariacica, Espírito Santo, Brazil, including abundance in the dry and rainy seasons and reproductive mode (RM, Haddad and Prado 2005). Area refers to the sampling areas where species were recorded (Area 1 = Represa Velha, Area 2 = Panelas, Area 3 = Alto Alegre) while E = endemism for which AF indicates endemism to the Atlantic Forest. For Conservation Status, NT = Near Threatened, LC = Least Concern, EN = Endangered, DD = Data Deficient with sources indicated in parentheses (ES = List of endangered species of the State of Espírito Santo, IUCN = International Union for Conservation of Nature).

	Abundance					
Species	Dry	Rainy	RM	Area	E	Conservation Status
Brachycephalidae						
Espirito Santo Robber Frog, *Ischnocnemaoea*	1	0	23	2	AF	NT (IUCN), DD (ES)
Bufonidae						
Striped Toad, *Rhinella crucifer*	4	2	1 or 2	1, 3	AF	LC (IUCN)
Craugastoridae						
Clay Robber Frog, *Haddadus binotatus*	6	8	23	1, 2	AF	LC (IUCN)
Cycloramphidae						
Military River Frog, *Thoropa miliaris*	3	0	19	1, 2	AF	LC (IUCN)
Hylidae						
Cruz's Treefrog, *Aplastodiscus cavicola*	0	1	5	1	AF	NT (IUCN)
Weygoldt's Treefrog, *Aplastodiscus* cf. *weygoldti*	0	1	5	2	AF	NT (IUCN)
White-edged Treefrog, *Boana albomarginata*	0	1	1	1,2	AF	LC (IUCN)
Blacksmith Treefrog, *Boana faber*	0	6	1 or 4	1, 2	-	LC (IUCN)
Boana semilineata	1	3	1 or 2	1, 2, 3	AF	LC (IUCN)
Rio Mutum Snouted Treefrog, *Ololygon argyreornata*	0	1	1	1, 2	AF	LC (IUCN)
Kautsky's Snouted Treefrog, *Ololygon kautskyi*	39	54	2	1, 2, 3	AF	DD (IUCN, ES)
Rio Lime Treefrog, *Sphaenorhynchus planicola*	0	2	1	1, 2	AF	LC (IUCN)
Crubixa Snouted Treefrog, *Scinax alter*	0	14	1	1, 2, 3	AF	LC (IUCN)
Maracas Snouted Treefrog, *Scinax eurydice*	1	1	1	1, 2	AF	LC (IUCN)
Scinax sp. 1	0	1	-	1	-	-
Scinax sp. 2	1	0	-	1	-	-
Hylodidae						
Gaudichaud's Frog, *Crossodactylus* aff. *gaudichaudii*	26	43	3	1, 2, 3	AF	LC (IUCN)
Baumann's Tree Toad, *Hylodes lateristrigatus*	0	3	3	3	AF	LC (IUCN)
Leptodactylidae						
Lesser Foam Frog, *Leptodactylus latrans*	2	17	11	1, 2, 3	-	LC (IUCN)
Odontophrynidae						
Santo Smooth Horned Frog, *Proceratophrys schirchi*	1	2	2	3	AF	LC (IUCN)
Phyllomedusidae						
Mottled Leaf Frog, *Phasmahyla exilis*	2	0	25	3	AF	LC (IUCN), EN (ES)
Burmeister's Frog, *Phyllomedusa burmeisteri*	1	0	24	2	AF	LC (IUCN)
Total	88	160				

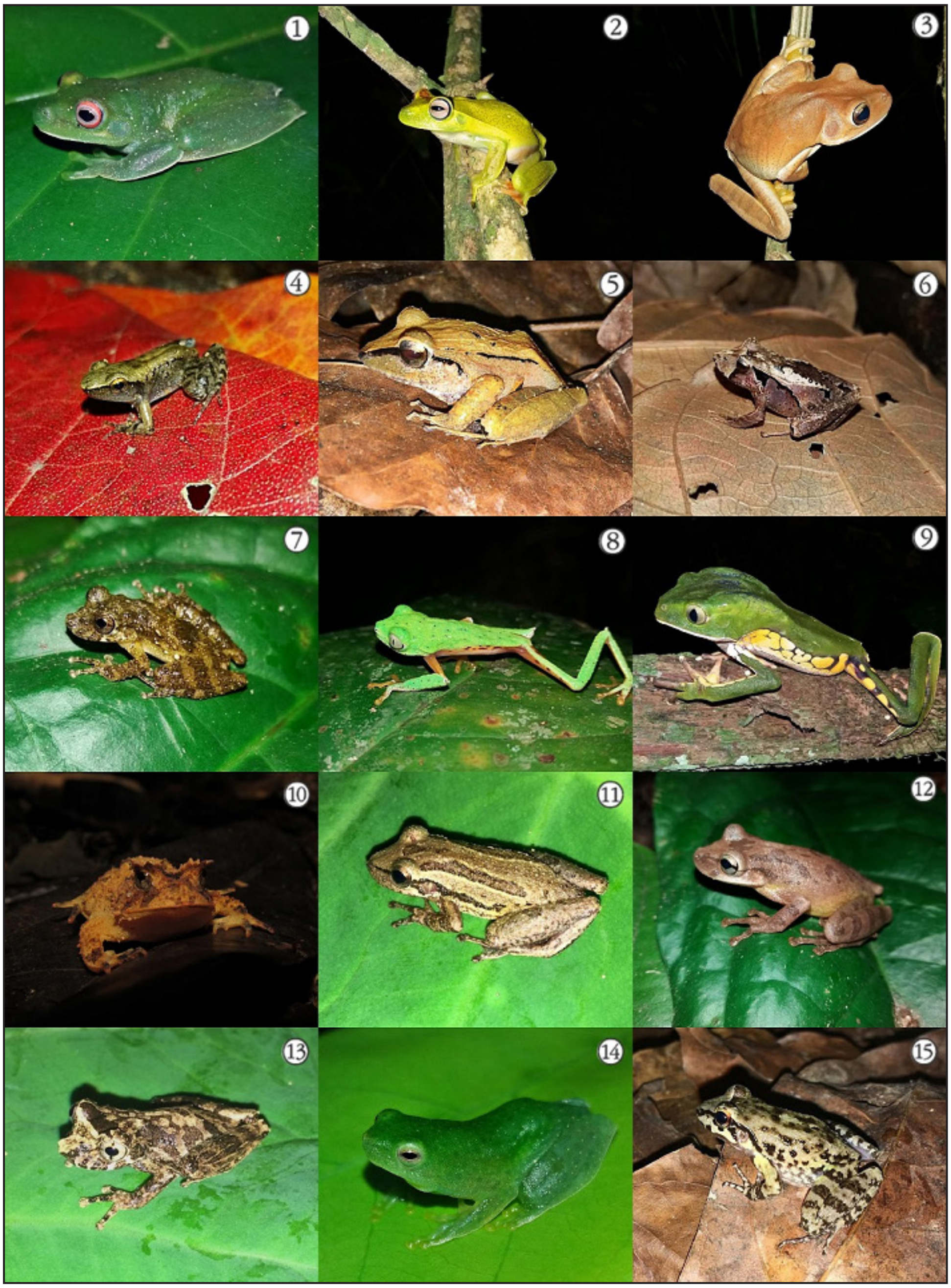

Figure 2. Some of the anuran species we recorded in streams and riparian zones of Duas Bocas Biological Reserve, Espírito Santo, Brazil. 1. Cruz's Treefrog (*Aplastodiscus cavicola*) 2. White-edged Treefrog (*Boana albomarginata*) 3. Blacksmith Treefrog (*Boana faber*) 4. Gaudichaud's Frog (*Crossodactylus* aff. *Gaudichaudii*) 5. Clay Robber Frog (*Haddadus binotatus*) 6. Espirito Santo Robber Frog (*Ischnocnema oea*) 7. Rio Mutum Snouted Treefrog (*Ololygon argyreornata*) 8. Mottled Leaf Frog (*Phasmahyla exilis*) 9. Burmeister's Frog (*Phyllomedusa burmeisteri*) 10. Santo Smooth Horned Frog (*Proceratophrys schichi*) 11. Crubixa Snouted Treefrog (*Scinax alter*) 12. Maracas Snouted Treefrog (*Scinax euridyce*) 13. Kautsky's Snouted Treefrog (*Ololygon kautskyi*) 14. Rio Lime Treefrog (*Sphaenorhynchus planicola*) 15. Military River Frog (*Thoropa miliaris*). (Photographed by Thais Meirelles Linause [1, 4-8, 11-15], Jonathan Silva Cozer [2, 3, 9] and Juliane Pereira-Ribeiro [10]).

and found a richness of 22 species. Other studies in Brazil, in different biomes, reported anuran richness for stream and riparian zones ranging from 14 to 28 species (e.g., Eterovick 2003; Rojas-Ahumada and Menin 2010; Tsuji-Nishikido and Menin 2011; Ribeiro et al. 2012).

Of the species we registered, more than 50% belonged to the family Hylidae. We expected this because Hylidae has high species richness, especially in tropical South America (Frost 2019). In fact, several studies on anuran communities in the Atlantic Forest (e.g., Silvano

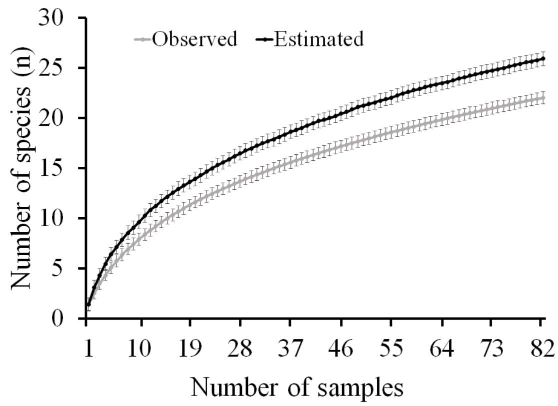

Figure 3. Rarefaction curve for number of amphibian species based on 82 samples of 22 50-m transects, sampled at Duas Bocas Biological Reserve, Cariacica, Espírito Santo, Brazil, from February 2018 to March 2019.

and Pimenta 2003; Camurugi et al. 2010; Mira-Mendes et al. 2018) found this family to be the most speciose. The most abundant species (*Ololygon kautskyi* and *Crossodactylus* aff. *gaudichaudii*) were generally those with an ecology strongly related to riparian vegetation and streams, respectively. Due to the recent expansion of agriculture and human settlements, *O. kautskyi* (Hylidae) has an increasingly fragmented distribution (ICMBIO. 2019. Anfíbios - *Scinax kautski*. Instituto Chico Mendes de Conservação da Biodiversidade, Brazil. Available from http://www.icmbio.gov.br/portal/faunabrasileira/estado-de-conservacao/7752-anfibios-scinax-kautskyi. [Accessed 5 May 2019]). *Ololygon kautskyi* is endemic to the Atlantic Forest biome of southeastern Brazil, and is known only from the state of Espírito Santo, with records in the municipalities of Domingos Martins, Santa Teresa, Aracruz, and Cariacica (Peixoto and Pimenta 2004; Tonini et al. 2010; Frost 2019; ICMBIO, *op. cit*.). According to the IUCN (2019), there is insufficient information regarding the ecology, population size and structure, and distribution of the species for this species, and thus the species is currently categorized as Data Deficient (DD); the status of the species is the same on the list of endangered species of Espírito Santo (Gasparini et al. 2007). *Crossodactylus* aff. *gaudichaudii* (Hylodidae) is also endemic to the Atlantic Forest of southeastern Brazil, occurring in the states of Rio de Janeiro, São Paulo, and Espírito Santo (Tonini et al. 2010; Frost 2019). This species has diurnal habits (Rocha et al. 2015) and is commonly associated with rocky streams within primary and secondary forest where females oviposit in cracks under rocks and stones (Weygoldt and Carvalho-e-Silva 1992; Almeida-Gomes et al. 2007; Frost 2019).

We found differences in species richness (α diversity) and β diversity of the anuran communities across the three sampled areas. Areas 1 and 2 had higher richness and diversity than Area 3. In addition, the composition of anuran species in Areas 1 and 2 were similar, but both differed from Area 3. These patterns may be related to elevation of areas. Areas 1 and 2 are at 200–270 m elevation whereas Area 3 is a higher elevation (300–738 m). Elevational gradients vary in biotic and abiotic factors that influence the structure of communities (Rahbek 1995). For example, with the increase in altitude, temperature decreases, ultraviolet radiation increases, and habitat complexity changes, among other factors (Siqueira and Rocha 2013). These factors, individually or in combination, can limit the distribution of species, depending on their biology (e.g., Halloy 1989; Navas 1996; Naniwadekar and Vasudevan 2007). In general, anuran α diversity tends to decline with increasing elevation (e.g., Fauth 1989; Phochayavanich et al. 2010; Villacampa et al. 2019), although some studies show different relationships (e.g., Naniwadekar and Vasudevan 2007). Area 3, however, despite having lower diversity, had the highest exclusivity of species when compared to the other areas (e.g., *Ischnocnema oea, Hylodes lateristrigatus, Proceratophrys schirchi*, and *Phasmahyla exilis*), and thus it makes an important contribution to the biodiversity and conservation value of DBBR as a whole. The lower species richness found in Area 3 may also be, in part, due to lower sampling effort in this area.

Species abundance varied significantly between the dry and rainy seasons, with most species being more abundant in the rainy season (October to March). In general, amphibians in tropical regions tend to be more abundant in months with higher accumulation of rain and with higher temperatures, as they are characteristics that favor the reproductive activity of most amphibian species in this region (Duellman and Trueb 1994). It is important to note, however, that the availability of water in the sampled environments remained stable during the entire study period, with no total drought of the streams (pers. obs.). Bastos et al. (2015) demonstrated that, despite having months with higher precipitation, the Duas Bocas drainage basin receives rain throughout the year, allowing streams to be supplied continuously, which can favor the occurrence of anuran species in the dry season.

The 10 reproductive modes among the 22 species recorded in our study represents 25.6% of the anuran reproductive modes recognized worldwide (Haddad and Prado 2005) and 37% of the reproductive modes recognized in the Atlantic Forest (Haddad and Prado 2005). Our results are similar to the 22 species and 11 reproductive modes found in the study by Almeida-Gomes et al. (2015) along two rivers in the Atlantic Forest. The high diversity of reproductive modes in Atlantic Forest anurans is attributed to the high availability of humid microhabitats and the efficient use of these environments by species (Haddad and Prado 2005). Additionally, the repetition of such microhabitats across the Atlantic Forest landscape may be directly related to the persistence of rare species (Brown and Brown 1992); however, much of the Atlantic Forest has been destroyed by intense exploitation of natural resources and deforestation, reducing the extent of this biome to only 28% of its original area and resulting in extensive fragmentation (Colombo and Joly 2010; Rezende et al. 2018). Deforestation of Atlantic Forest can result in a generalized impoverishment in terms of species richness and anuran reproductive modes, as these areas become drier and more seasonal, reducing the number of species by eliminating those that depend on humid microhabitats (Haddad and Prado 2005). Of the species we observed, most depend on water for reproduction during oviposition and/or in the development of tadpoles (except mode 23). In addition, *Hylodes lateristrigatus, Crossodactylus gaudichaudii, Phasmahyla exilis, Ololygon kautskyi*, and *Proceratophrys schirchi* have reproductive modes strictly related to streams. The term habitat split, caused by forest loss and fragmentation, tends to negatively affect anuran species with aquatic larvae, especially those that breed in rivers and/or streams, because the discontinuity between suitable aquatic and terrestrial habitats forces this group to migrate through unfavorable environments to reproduce (Becker et al. 2007). In this sense, the occurrence of species dependent on stream environments for breeding in DBBR reinforces the need for monitoring and conservation of these environments.

Approximately 80% of the anuran species recorded in our study are endemic to the Atlantic Forest biome and one of these is endemic to the state of Espírito Santo. Endemic species are important targets of global conservation efforts because they tend to have small ranges and population sizes and, given that they have few places for conservation intervention, they are more vulnerable to extinction (Loyola et al. 2007). The study by Loyola et al. (2007) highlighted the importance of endemic species as the most effective criterion for assessing the classification and conservation value of ecoregions, thus endemicity is indispensable for identifying priority conservation areas in Brazil. Based on this, we would expect that most endemic species would have some official conservation status, but only four of the 18 endemic species recorded in our study are considered Endangered. Moreover, the occurrence of Data Deficient species is an aggravating factor for species conservation, as conservation funds are limited and focus primarily on species identified with high risk of extinction (Stuart et al. 2004; Morais et al. 2012; Howard and Bickford 2014) while neglecting species that are little known (Brito 2010; Howard and Bickford 2014). Despite several Data Deficient species, the high number of endemic species in DBBR indicates that it is an important conservation fragment of Atlantic Forest.

Another important point to consider with respect to conservation of stream and riparian zone anurans is that their threat status may be underestimated. This is because criteria for assessing species encompass the total area of occurrence rather than the area of suitable habitat; when a species is broadly distributed, the determination is low risk of extinction. The distributions of many stream and riparian species are restricted to the channels of rivers and streams and their margins, and not the vast areas between. As a result, the actual occupied area is much smaller than the full spatial extent of geographical distribution (Almeida-Gomes et al. 2014). Thus, many of the exclusively stream and riparian anuran species that are currently categorized as Least Concern may actually be in some degree of threat.

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