



Amphibians of Serra das Torres Natural Monument: a reservoir of biodiversity in the Atlantic Forest of southeastern Brazil

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Abstract: The Brazilian Atlantic Forest holds a major part of the country's amphibian species richness and high rates of endemism. In this study, we conducted surveys using the Rapid Assessment (RA) method to sample the amphibian fauna of the Serra das Torres Natural Monument (MONAST), an Atlantic Forest remnant in southeastern Brazil. We sampled actively with a 6-10-person team to collect standard samples from 09:00 to 12:00 hours for the daytime period, and from 18:00 to 22:00 hours for the crepuscular/nighttime period, with a total of approximately 1,320 hours of sampling effort. We supplemented these data with 720 hours of passive sampling, using pitfall traps with drift fences (30 bucket-days). We recorded 54 amphibian species (two gymnophionans and 52 anurans), and the species richness estimated by the *Bootstrap* method indicates that a slightly larger number of species (n = 60) may occur in the study area. The most speciose family was Hylidae (n = 21), followed by Brachycephalidae (n = 8). Overall, 25% of the species (n = 13) were recorded only once (singletons) and 15% (n = 8) only twice (doubletons). Most amphibians recorded in this study (71%, n = 37 species) were restricted to the Atlantic Forest biome, two species (Euparkerella robusta and Luetkenotyphlus fredi) are endemic to the Espírito Santo state, and one of them, the leaf litter species, is endemic to the MONAST. Euparkerella robusta is currently listed as Vulnerable by the IUCN and is classified as Critically Endangered in the Espírito Santo State red list, while L. fredi has yet to be evaluated due to its recent description. Thoropa cf. lutzi is currently listed as Endangered (EN) by both the IUCN and in the State list. Nine species are listed as Data Deficient (DD) and populations of 13 species are considered to be declining by the IUCN. We extend the geographical distribution of two anuran species (Hylodes babax and Phasmahyla lisbella) and fill an important gap in the distribution of Siphonops hardyi. Amphibians associated with the forest floor represented 42% of the species richness from MONAST, and 43% of these species inhabit the leaf litter exclusively. Our study revealed that Serra das Torres preserves a considerable diversity of Atlantic Forest amphibians, which reinforces the need for the conservation of this forest remnant.

Keywords: Distribution extension, Filling gaps; Gymnophiona; Inventory; Leaf litter frogs; Rapid Assessment Method.

Anfíbios do Monumento Natural Serra das Torres: um reservatório da biodiversidade da Mata Atlântica no sudeste do Brasil

Resumo: A Mata Atlântica brasileira guarda importante porção da riqueza de anfibios e altas taxas de endemismos. Neste estudo, nós realizamos pesquisas usando o Método de Avaliação Rápida (RA) com o objetivo de inventariar a fauna de anfibios de um remanescente da Mata Atlântica no sudeste do Brasil, o Monumento Natural Serra das Torres (MONAST). Amostramos ativamente com uma equipe de 6 a 10 pessoas para coletar amostras padronizadas entre 09:00 e 12:00 horas durante o período diurno e entre 18:00 e 22:00 durante o período crepuscular/noturno, totalizando aproximadamente 1320

horas de esforço amostral. Complementamos estes dados com 720 horas de amostragem passiva usando armadilhas de queda com cercas-guia (30 dias de balde). Registramos 54 espécies de anfibios (dois gimnofionos e 52 anuros) e a riqueza de espécies estimada pelo Bootstrap indicou um número relativamente maior de espécies (n = 60). A família mais especiosa foi Hylidae (n = 21), seguida por Brachycephalidae (n = 8). No geral, 25% das espécies (n = 13) foram registradas apenas uma vez - (singletons) e 15% (n = 8) apenas duas vezes (doubletons). A maioria dos anfibios registrados neste estudo (71%, n = 37 espécies) esteve restrita ao bioma Mata Atlântica, duas espécies (Euparkerella robusta e Luetkenotyphlus fredi) são endêmicas do estado do Espírito Santo, sendo uma delas, a espécie de serapilheira E. robusta, endêmica do MONAST. Euparkerella robusta está atualmente listada como Vulnerável pela IUCN e classificada como Criticamente Ameaçada na lista vermelha do estado do Espírito Santo, enquanto L. fredi ainda não foi avaliada devido a sua descrição ser muito recente. Thoropa cf. lutzi está atualmente listada como Ameaçada (EN) pela IUCN e na lista estadual. Nove espécies estão listadas como Deficiente de Dados (DD) e as populações de 13 espécies são consideradas em declínio pela IUCN. Estendemos a distribuição geográfica de duas espécies de anuros (Hylodes babax e Phasmahyla lisbella) e preenchemos uma importante lacuna na distribuição de Siphonops hardyi. Os anfibios associados ao chão da floresta representaram 42% das espécies do MONAST e 43% destas espécies habitavam exclusivamente a serapilheira. Nosso estudo revelou que a Serra das Torres preserva diversidade considerável de anfibios da Mata Atlântica, o que reforça a necessidade de conservação desse remanescente florestal.

Palavras-chave: Anfibios do folhiço; Distribuição geográfica; Gymnophiona; Inventário; Método de Avaliação Rápida.

Introduction

Brazilian amphibians are among the world's most diverse, with approximately 1136 recognized species (Segalla et al. 2019). The Atlantic Forest is home of an important portion of this richness (around 600 species) and most species are found in ombrophilous forest vegetation, which has high rates of endemism, in just over half (approximately 52%) of the species (Rossa-Feres et al. 2017). The heterogeneity of Atlantic Forest habitats is probably one of the factors that have led to this high amphibian diversity (Carnaval et al. 2009, Bastazini et al. 2007, Oliveira et al. 2017), once it provides several favorable microhabitats for anuran development that contribute, for example, to species specificity and endemism (Sá 2013). Although considerable knowledge exists on the amphibian diversity of the Atlantic Forest, it is assumed that many species have yet to be discovered (Pimm et al. 2010) and new species are described every year (e.g., Ferreira et al. 2015, Baêta et al. 2016, Maciel et al. 2019). In addition, the amphibian fauna of many forest remnants is still under-sampled or completely unknown (Silvano & Segalla 2005). The state of Espírito Santo, in southeastern Brazil, is located entirely within the Atlantic Forest biome (IBGE 2004), although the state's forest cover has been reduced to only 483,087 hectares (SOS Mata Atlântica/INPE 2017/2018), even though 85% of its 46,000 km² area was originally covered with forest (Atlas dos Ecossistemas do Espírito Santo 2008). Several forest remnants in the Espírito Santo state are still poorly sampled, mainly within the ombrophilous forest vegetation, which implies a persistent knowledge bias regarding the composition of the amphibian communities of many areas (Almeida et al. 2011).

In this study, we surveyed a major forest remnant of the Atlantic Forest biome in southeastern Brazil, in southern Espírito Santo state. We evaluated parameters of species richness and abundance, spatial distribution, and habitat use by the amphibians of the Serra das Torres Natural Monument. We also add range extensions and fill gaps for three amphibian species.

Material and Methods

1. Study site

We collected samples at the Serra das Torres Natural Monument (Monumento Natural Serra das Torres – MONAST: -21.0209°,

-41.2378°, WGS84 datum), which is located in the southern portion of the Espírito Santo state, in the municipalities of Atílio Vivacqua, Mimoso do Sul, and Muqui in southeastern Brazil (Figure 1). The MONAST encompasses the largest complex of forest remnants in southern Espírito Santo, with approximately 10,450 hectares of Atlantic Forest. The habitats of this remnant include mountains that rise to more than 1,000 m asl at their highest point, and are covered by seasonal forest, semi-deciduous forest, dense rainforest, and dense submontane rainforest (Magnago et al. 2008). Several rocky outcrops (*inselbergs*) covered with tank bromeliads are found in the MONAST, many of which are relatively prominent, with steep slopes (Figure 2). The mean annual temperature of the study area is approximately 24.5°C and the mean annual rainfall is around 1290 mm (Oliveira et al. 2013). The area surrounding the MONAST is occupied by private properties with extensive areas of farmland, primarily coffee and banana plantations.

2. Amphibian survey

We used the Rapid Assessment (RA) method to assess the species richness and abundance of amphibians during three months of the rainy season of 2018. The RA is an effective sampling method that permits the collection of reliable and replicable data over a short period of time (Patrick et al. 2014). We did not include acoustic records in the assessment of the species richness and abundance data.

We surveyed the MONAST over 10 consecutive days in each of the three municipalities in which the protected area is located, with a total of 30 sampling days: Atílio Vivacqua (January 2018), Mimoso do Sul (February 2018), and Muqui (March 2018). The survey was conducted by a team of six to 10 people, at 19 different sample sites (Figure 1). We conducted time-limited active searches (Crump & Scott Jr. 1994), from 09:00 to 12:00 in daytime period, and from 18:00 to 22:00 in crepuscular/nighttime period, with a total sampling effort of approximately 1320 hours (considering variation in team members and sampling hours per day). We conducted the active searches in preserved fragments of forest that were located as far as possible from areas of anthropogenic impact at altitudes from ca. 600 m to 1000 m asl. We installed all transects at least one kilometer from the nearest trail to avoid pseudo-replication, and to sample the largest possible area of forest.

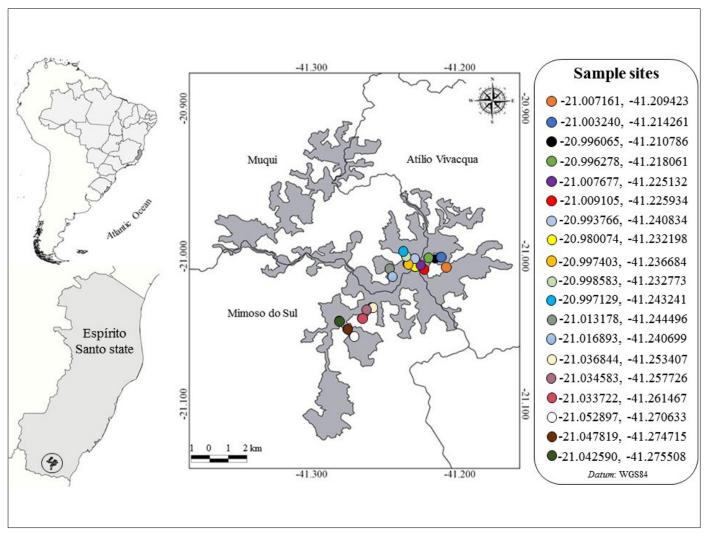


Figure 1. Points sampled in the Serra das Torres Natural Monument (MONAST) in the Brazilian state of Espírito Santo (black dots), southeastern Brazil. The enlarged figure to the right shows the limits of the MONAST in relation to the three municipalities in which it is located (Atílio Vivacqua, Mimoso do Sul, and Muqui).

We also used four systems of pitfall traps with drift fences (Corn 1994), each consisting of 40 buckets (20 L each), arranged in a line within each study area. We placed the pitfall traps in the best-preserved habitats at different forest fragments and altitudes and, as far as possible from each other. We installed the traps three days prior to the sampling period, with all the buckets remaining open until the 10th consecutive day of sampling. We removed the buckets and fences from each forest at the end of the sampling period, and we then moved them to the next sampling area. The overall sampling effort of the pitfall traps was approximately 720 hours.

We collected data on the microhabitats used by the amphibians encountered during the active search (e.g., leaf litter, streams, trees, ponds, and bromeliads). We also recorded the height above ground (cm) which each individual was encountered in the microhabitat. In the case of species associated with streams, we also measured the distance (cm) between the individual and the stream, together with the depth (cm), width (cm), and temperature (°C) of the stream at the point closest to the location of the sampled individual.

Voucher specimens were collected under the authorization of SISBIO/RAN No 57085-6 and the Espírito Santo State Environment

Institute (IEMA), license number 033-2017. The specimens were deposited in the following Brazilian collections (Table 1): Museu Nacional (MNRJ) in Rio de Janeiro state, Museu Paraense Emílio Goeldi (MPEG), Pará state and, Museu de Biologia Professor Mello Leitão (MBML), Espírito Santo state.

3. Data analysis

We compiled a species accumulation curve based on the cumulative number of species (*S*) recorded during the RA, as a function of sampling effort (n_days). We estimated species richness by the Bootstrap method, for which we considered the diversity index best suited to our data (Magurran 2004). Species recorded opportunistically during fieldwork were included in the richness counts but were not used to plot the accumulation curve or to quantify abundance. We analyzed the abundance data using Whittaker plots (Whittaker 1960), which rank the log abundance of each species (Krebs 1999). We assessed the vertical distribution of the species in the habitat using bloxplots. We analyzed the Whittaker plots in PAST 2.17 (Hammer et al. 2011). The species accumulation and rarefaction curves were plotted in EstimateS 8.0 (Colwell 2005).



Figure 2. Sampling areas in the Serra das Torres Natural Monument (MONAST), Espírito Santo state, southeastern Brazil. (A) Forests in the municipality of Atílio Vivácqua (Photograph: Pedro Fatorelli); (B) Rocky outcrop in the municipality of Muqui (Photograph: Pedro Fatorelli); (C) Bromeliad Aechmea flavorosea on a rocky outcrop in the municipality of Atílio Vivácqua (Photographs: Marcelo Simonelli); (D) Forest on the municipal limits of Atílio Vivácqua and Muqui (Photograph: Jane C. F. Oliveira); (E) Stream in the municipality of Atílio Vivácqua (Photograph: Pedro Fatorelli), (F) Forests in the municipality of Atílio Vivácqua (Photograph: Jane C. F. Oliveira).

Results

We recorded 495 individuals belonging to 54 amphibian species distributed in two orders (Gymnophiona = two species; Anura = 52 species) (Table 1; Figures 3, 4 and 5). The cumulative species curves did not reach the asymptote (Figure 6), and species richness estimated by the Bootstrap procedure (S = 61 species) was higher than that recorded in the active searches (S = 54).

We extended the known geographical distribution of two anuran species, *Hylodes babax* and *Phasmahyla lisbella* (Figure 7). *Hylodes babax* was previously known from its type locality (Caparaó National Park) Serra do Brigadeiro State Park, and Uaimií State Forest, in the southern Espinhaço mountain range (Pirani et al. 2010), all in the state of Minas Gerais, and from Santa Teresa municipality, in Espírito Santo state (Table 2, Figure 7). The record of *H. babax* in the MONAST extends the known geographic range of this species by approximately 86 km from the nearest locality, in the Caparaó National Park. *Phasmahyla lisbella* was recently described from Ventania Environmental Protection Area, in the Miracema municipality, Rio de Janeiro state (Pereira et al. 2018), and here we extend its known geographic range in Brazil by approximately 110 km to the north. We also filled a distributional gap in the known range of *Siphonops hardyi*, for which no records are

available from northern Rio de Janeiro or southern Espírito Santo states (Table 2, Figure 7).

Hylidae was the anuran family with the highest species richness (n = 21), followed by the Brachycephalidae (n = 9), whereas Odontophrynidae was represented by only a single species. The caecilian species were relatively rare, with *Luetkenotyphlus fredi* being represented by only three individuals, and *Siphonops hardyi* by a single specimen (Table 1). *Haddadus binotatus* was the most abundant species (n = 117 individuals; 26% of total sample), followed by *Thoropa miliaris* (n = 67; 15%), and *Hylodes lateristrigatus* (n = 35; 8%). Overall, 40% of the species were recorded only once (13 species) or twice (8 species). The best-fitting Whittaker abundance model for the MONAST amphibian community was the logarithmic series model (Figure 8).

Most amphibians recorded in the MONAST (72%; n = 38 species) are endemic to the Atlantic Forest biome. Two of them, *Luetkenotyphlus fredi* and *Euparkerella robusta*, are endemic to Espírito Santo state, in which, the frog *E. robusta* is endemic to the MONAST and listed as Critically Endangered (CR) in the Espírito Santo state red list (Ferreira et al. *in press*) and Vulnerable (VU) by the IUCN (2020). The gymnophionan *L. fredi* was described during this study, based on specimens collected by us from this study site, and this species is endemic to the Espírito Santo state. Furthermore, the frog *Thoropa* cf. *lutzi* is listed as Endangered (EN) in the Espírito Santo

Table 1. Amphibian species recorded in the Serra das Torres Natural Monument (MONAST) in Espírito Santo state, southeastern Brazil, showing abundance (number of records), voucher number in herpetological collections, endemism (End/AF = endemic to the Atlantic Forest; End/ES = endemic to Espírito Santo state), and conservation status of each species based in global (The IUCN Red List, IUCN 2020), national (Ministério do Meio Ambiente, ICMBio 2018) and Espírito Santo state lists (Lista Vermelha do Espírito Santo - ES, Ferreira et al. in press). * = secondary data (Oliveira et al. 2013). Conservation status: CR = Critically Endangered; DD = Data Deficient; EN = Endangered; LC = Least Concern; VU = Vulnerable. MBML = Museu de Biologia Mello Leitão; MPEG = Museu Paraense Emílio Goeldi; MNRJ = Museu Nacional, Rio de Janeiro.

					Cons	ervation sta	itus	_
	Voucher	Abundance (%)	End/ AF	End/ ES	Global	National	ES	Population trend (IUCN)
ORDEM								
GYMNOPHIONA								
Family Siphonopidae								
Luetkenotyphlus fredi Maciel, Castro, Sturaro, Silva, Ferreira, Santos, Risse- Quaioto, Barboza, Oliveira, Sampaio & Schneider, 2019	MBML 11620	3 (0.6)	X	x	_	-	_	-
Siphonops hardyi Boulenger, 1888	MPEG 41563	1 (0.2)	X	_	LC	LC	DD	Stable
ANURA								
Family Bufonidae								
Rhinella crucifer (Wied-Neuwied, 1821)	MNRJ 93680-1	18 (3.6)	x	_	LC	LC	LC	Decreasing
Rhinella granulosa (Spix, 1824)	MNRJ 93672	7 (1.4)	-	-	LC	LC	LC	Stable
Family Leptodactylidae								
Leptodactylus fuscus (Schneider, 1799)	MNRJ 93768	7 (1.4)	_	_	LC	LC	LC	Stable
Leptodactylus latrans (Steffen, 1815)	MNRJ 93760	2 (0.4)	_	_	LC	LC	LC	Stable
Leptodactylus mystacinus (Burmeister, 1861)	MNRJ 93769	1 (0.2)	_	_	LC	LC	LC	Stable
Family Leptodactylidae (Leiuperinae)								
Adenomera bokermanni (Heyer, 1973)*	MNRJ 66710	_	X	_	LC	LC	LC	Stable
Physalaemus crombiei Heyer & Wolf, 1989	MNRJ 93802-6	1 (0.2)	X	-	LC	LC	LC	Decreasing
Physalaemus cuvieri Fitzinger, 1826	MNRJ 93801-2	3 (0.6)	-	-	LC	LC	LC	Stable
Family Brachycephalidae								
Ischnocnema abdita Canedo & Pimenta, 2010	MNRJ 93775-8	16 (3.2)	X	_	-	LC	LC	-
Ischnocnema cf. izecksohni (Caramaschi & Kisteumacher, 1989)	MNRJ 93697	13 (2.6)	_	_	-	_	-	-
$\it Ischnocnema verrucosa$ (Reinhardt & Lütken, 1862)	MNRJ 93689	7 (1.4)	X	-	DD	LC	LC	Unknown
Brachycephalus didactylus (Izecksohn, 1971)	MNRJ 58974	1 (0.2)	X	_	LC	LC	DD	Decreasing
Ischnocnema bolbodactyla (Lutz, 1925)	MNRJ 66698	17 (3.4)	X	_	LC	LC	LC	Decreasing
Ischnocnema parva (Girard, 1853)	MNRJ 66699	3 (0.6)	X	_	LC	LC	LC	Decreasing
Ischnocnema sp.	MNRJ 93717	1 (0.2)	_	_	-	_	_	_
Ischnocnema cf. guentheri (Steindachner, 1864)	MNRJ 93701	3 (0.6)	_	_	_	_	_	_

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Family Hylidae								
Aplastodiscus arildae (Cruz & Peixoto, 1987)	MNRJ 93780	2 (0.4)	X	_	LC	LC	LC	Stable
Boana sp.	MNRJ 93715	4 (0.8)	_	_	_	_	_	_
Boana aff. polytaenia (Cope, 1870)	MNRJ 93682	2 (0.4)	X	_	_	_	_	_
Boana albomarginata (Spix, 1824)	MNRJ 93710	3 (0.6)	X	_	LC	LC	LC	Stable
Boana albopunctata (Spix 1824)	MNRJ 93779							
Boana crepitans (Wied-Neuwied, 1824)	MNRJ 93696	1 (0.2)						
Boana faber (Wied-Neuwied, 1821)	MNRJ 93674-8	17 (3.4)	_	_	LC	LC	LC	Stable
Boana pardalis (Spix, 1824)	MNRJ93762	3 (0.6)	X	_	LC	LC	LC	Stable
Bokermannohyla caramaschii (Napoli, 2005)	MNRJ93699, 93702, 93703	3 (0.6)	x	_	LC	LC	LC	Unknown
Dendropsophus bipunctatus (Spix 1824)	MNRJ93761	13 (2.6)	X	_	LC	LC	LC	Stable
Dendropsophus elegans (Wied-Neuwied, 1824)	MNRJ93711	7 (1.4)	X	_	LC	LC	LC	Stable
Dendropsophus minutus (Peters, 1872)	MNRJ93673	10 (2.0)	_	_	LC	LC	LC	Stable
Dendropsophus sp.	MNRJ93685-6, 93708, 93814	5 (1.0)	_	_	-	-	_	_
Fritziana ohausi (Wandolleck, 1907)	MNRJ93687	1 (0.2)	X	_	LC	LC	LC	Stable
Ololygon argyreornata (Miranda-Ribeiro, 1926)	MNRJ93690-5	13 (2.6)	X	_	LC	LC	LC	Stable
Scinax cf. belloni (Faivovich, Gasparini & Haddad, 2010)		- (-)						_
Ololygon gr. perpusilla	MNRJ 93791-2	1	_	_	_	_	_	_
Ololygon cf. tripui (Lourenço, Nascimento & Pires, 2010)	MNRJ 93818	5 (1.0)	x	-	_	_	DD	-
<i>Phasmahyla lisbella</i> Pereira, Rocha, Folly, da Silva & Santana, 2018	MNRJ 58975	4 (0.8)	x	_	_	_	_	-
Phyllodytes luteolus (Wied-Neuwied, 1821)	MNRJ 93698	2 (0.4)	X	_	LC	LC	LC	Decreasing
Phyllomedusa burmeisteri Boulenger, 1882	MNRJ 93759	8 (1.6)	X	_	LC	LC	LC	Stable
Scinax gr. ruber	MNRJ 93763	1 (0.2)	_	_	LC	LC	LC	Stable
Scinax cf. x-signatus (Spix, 1824)	MNRJ 93707	1 (0.2)	X	_	_	_	_	Stable
Trachycephalus mesophaeus (Hensel, 1867)	MNRJ 93679	1 (0.2)	X	-	LC	LC	LC	Decreasing
Family Craugastoridae (Craugastorinae)								
Haddadus binotatus (Spix, 1824)	MNRJ 93770-1	117 (22.9)	X	-	LC	LC	LC	Stable
Euparkerella robusta Izecksohn, 1988	MNRJ 60996-4	2 (0.4)	x	X	VU	DD	CR	Decreasing
Family Cycloramphidae								
Crossodactylus gaudichaudii Duméril & Bibron, 1841	MNRJ 93688	23 (4.6)	x	_	LC	LC	LC	Decreasing
Thoropa cf. lutzi Cochran, 1938	MNRJ 93829- 30	3 (0.6)	x	-	EN	DD	EN	Decreasing
Thoropa miliaris (Spix, 1824)	MNRJ 93788-9	67 (13.5)	X	-	LC	LC	LC	Stable
Zachaenus parvulus (Girard, 1853)	MNRJ 93764	2 (0.4)	X	_	LC	LC	DD	Decreasing

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Family Hylodidae								
Hylodes babax Heyer, 1982	MNRJ 92566	6 (1.2)	x	_	_	_	DD	_
Hylodes lateristrigatus (Baumann, 1912)	MNRJ 93112	35 (7.0)	x	_	LC	LC	LC	Decreasing
Family Odontophrynidae								
Proceratophrys boiei (Wied-Neuwied, 1824)	MNRJ 93819	1 (0.2)	X	_	LC	LC	LC	Stable
Family Microhylidae (Gastrophryninae)								
Chiasmocleis ef. lacrimae Peloso, Sturaro, Forlani, Gaucher, Motta & Wheeler, 2014	MNRJ 93717	2 (0.4)	-	-	_	-	-	_
Chiasmocleis schubarti Bokermann, 1952	MNRJ 93709	1 (0.2)	_	_	_	_	_	_
Myersiella microps (Duméril & Bibron, 1841)	MNRJ 93852	1 (0.2)	X		LC	LC	LC	Stable

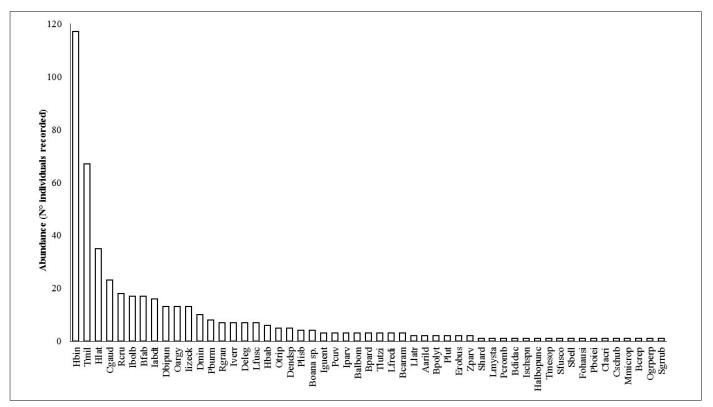


Figure 3. Species richness and abundance of amphibians recorded in the Serra das Torres Natural Monument (MONAST), Espírito Santo state, southeastern Brazil. Hbin = Haddadus binotatus; Tmil = Thoropa miliaris; Hlat = Hylodes lateristrigatus; Cgaud = Crossodactylus gaudichaudii; Rcru = Rhinella crucifer; Ibolb = Ischnocnema bolbodactyla; Bfab = Boana faber; Iabdt = Ischnocnema abdita; Dbipun = Dendropsophus bipunctatus; Oargy = Ololygon argyreornata; Iizeck = Ischnocnema cf. izecksohni; Dmin = Dendropsophus minutus; Pburm = Phyllomedusa burmeisteri; Rgran = Rhinella granulosa; Iverr = Ischnocnema verrucosa; Deleg = Dendropsophus elegans; Lfusc = Leptodactylus fuscus; Hbab = Hylodes babax; Otrip = Ololygon cf. tripui; Dendsp = Dendropsophus sp; Plisb = Phasmahyla lisbella; Boana sp. = Boana sp; Iguent = Ilschnocnema cf. guentheri; Pcuv = Physalaemus cuvieri; Iparv = Ischnocnema parva; Balbom = Boana albomarginata; Bpard = Boana pardalis; Tlutzi = Thoropa cf. lutzi; Lfredi = Luetkenotyphlus fredi; Bcaram = Bokermannohyla caramaschii; Llatr = Leptodactylus latrans; Aarild = Aplastodiscus arildae; Bpolyt = Boana aff. polytaenia; Plut = Phyllodytes luteolus; Erobus = Euparkerella robusta; Zparv = Zachaenus parvulus; Shard = Siphonops hardyi; Lmysta = Leptodactylus mystacinus; Pcromb = Physalaemus crombiei; Bdidac = Brachycephalus didactylus; Ischspn = Ischnocnema sp.; Halbopunc = Hypsiboas albopunctatus; Tmesop = Trachycephalus mesophaeus; Sfusco = Scinax cf. x-signatus; Sbell = Scinax cf. belloni; Fohausi = Fritziana ohausi; Pboiei = Proceratophrys boiei; Clacri = Chiasmocleis cf. lacrimae; Cschub = Chiasmocleis schubarti; Mmicrop = Myersiella microps; Bcrep = Boana crepitans; Ogrperp = Ololygon gr. perpusilla; Sgrrub = Scinax gr. ruber.

state red list (Ferreira et al. *in press*) and by the IUCN (2021). Nine anuran species are classified as Data Deficient, DD (IUCN 2021), and the populations of 12 species are considered to be declining (IUCN 2020, Table 1).

In the MONAST, the amphibians were found predominantly in five types of microhabitat: leaf litter, bromeliads, trees, ponds, and streams. The microhabitat occupied most frequently was the leaf litter of the forest floor, followed by ponds, streams, trees, and bromeliads (Figure 9). We recorded



Figure 4. Amphibian species recorded in the Serra das Torres Natural Monument (MONAST), Espírito Santo state, southeastern Brazil. (A) Phasmahyla lisbella; (B) Euparkerella robusta; (C) Ololygon argyreornata; (D) Ololygon cf. belloni; (E) Chiasmocleis schubarti; (F) Ischnocnema abdita; (G) Zachaenus parvulus; (H) Ischnocnema verrucosa. Photographs: Jane C. F. Oliveira.

25 species perched in vegetation at heights up to three meters above the ground, while 22 species (43%) were observed exclusively in the leaf litter (Figure 10). The stream-dwelling species were recorded on the ground and in the vegetation at heights up to 1.2 m. The streams at which the amphibians were encountered had a mean width of 96.2 cm, mean depth 10.58 cm, and mean temperature of 20.5°C (Table 2).

Discussion

Species richness and abundance. The Atlantic Forest is one of the world's most threatened biodiversity hotspots (Myers et al. 2000, Mittermeier et al. 2005), and the remaining forests probably contain more than 600 amphibian species (Rossa-Feres et al. 2017). In the present study, we recorded an important sample of this diversity, which represents approximately 9% of the amphibian species found in the Atlantic Forest.

The Serra das Torres Natural Monument (MONAST) has relatively higher amphibian species richness and abundance in comparison with the nearest remnants of ombrophilous forest that have been surveyed in the



Figure 5. Amphibian species recorded in the Serra das Torres Natural Monument (MONAST), Espírito Santo state, southeastern Brazil. (I) Ololygon cf. tripui; (J) Luetkenotyphlus fredi; (K) Siphonops hardyi; (L) Hylodes lateristrigatus; (M) Phyllomedusa burmeisteri; (N) Aplastodiscus arildae; (O) Thoropa cf. lutzi; (P) Crossodactylus gaudichaudii. Photographs: Jane C. F. Oliveira.

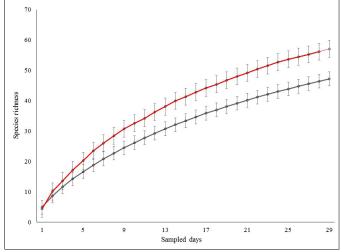


Figure 6. Accumulation curve (black line) and the species estimates (red line) based on Bootstrap estimator, both with 95% confidence intervals, for amphibians recorded at the Serra das Torres Natural Monument (MONAST) in Espírito Santo state, southeastern Brazil.

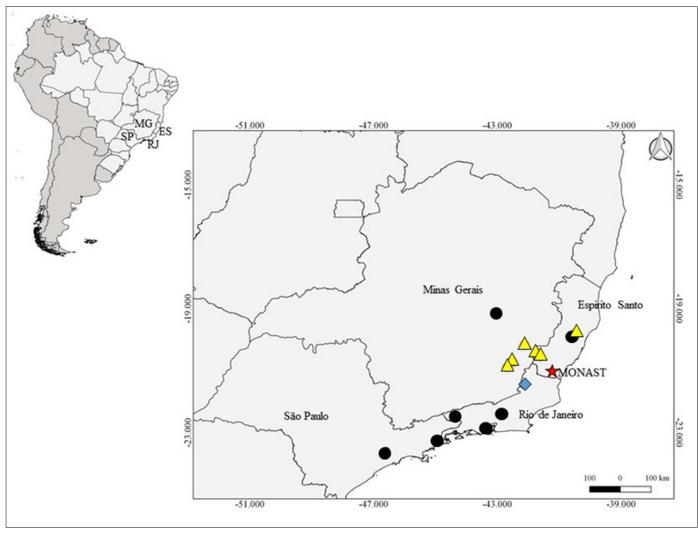


Figure 7. Geographic distribution of amphibian species recorded in the Serra das Torres Natural Monument (MONAST: red star), Espírito Santo state, southeastern Brazil: black dot, nearest previously known locality of *Siphonops hardyi*; yellow triangles, the nearest previously known locality of *Hylodes babax*; blue diamond, the nearest previously known locality of *Phasmahyla lisbella*.

Atlantic Forest, as Duas Bocas Biological Reserve, Forno Grande State Park, São Roque Canaã, Mata das Flores State Park, and Marechal Floriano, all in Espírito Santo state, and the Desengano State Park, in the Rio de Janeiro state (Figure 11). These differences are even more accentuated in many cases if the variation in sampling is taken into consideration, given that our study was based on only 30 days of survey. The 43 anuran species (and no gymnophionans) known to occur in the Forno Grande State Park were recorded over six years of sampling (Montesinos et al. 2012), while only 13 anuran species were recorded during a 10-day winter survey in the Desengano State Park, which is the nearest reserve to the MONAST, and has twice the area of forest (Siqueira et al. 2011). Only the Guapiaçu Ecological Reserve is known to have a higher species richness (n = 73 species) than the MONAST, although this total was recorded over a sampling period of more than 10 years (Almeida-Gomes et al. 2014). In addition, it is also important to note that the cumulative species curves for the MONAST did not reach the asymptote, with estimated (n = 60) exceeding observed species richness (n = 54) by more than 10%. The total number of amphibian species currently known to occur in the Brazilian state of Espírito Santo is 133 (Almeida et al. 2011), of which 39% are found in the MONAST, which reinforces the importance of this remnant for conservation and as a reservoir of genetic

diversity. Our findings also reinforce the efficacy of the Rapid Assessment method for the evaluation of species richness from other forest remnants in the region, which have yet to be surveyed systematically.

The richest anuran family recorded in the MONAST was Hylidae, which is consistent with the composition of amphibian communities in other Atlantic Forest remnants in the region, such as the Mata das Flores State Park (Pereira-Ribeiro et al. 2019), the Pedra Azul-Forno Grande Corridor (Montesinos et al. 2012), and the Desengano State Park (Siqueira et al. 2011). The Hylidae is the second most speciose anuran family, worldwide (see Frost 2020), and is the richest family in the ombrophilous formations of the Atlantic Forest, in which about 470 hylid species are currently known to occur (Rossa-Feres et al. 2017). The second richest family at MONAST was the Brachycephalidae, direct-developing leaf litter frogs that inhabit the forest floor. These species were similarly abundant in previous surveys of the MONAST (Oliveira et al. 2013) and their abundance is usually associated with the leaf litter depth (Van Sluys 2007, Oliveira et al. 2013). A deeper leaf litter layer may also maintain a higher level of humidity on the forest floor, which may influence the occurrence of the litter-dwelling species in the MONAST (Oliveira et al. 2013). Within the forest, the presence

Table 2. Previous and present records of amphibian species whose geographical distribution were increased or filled in Serra das Torres Natural Monument (MONAST), Espírito Santo state, southeastern Brazil. Geographical coordinates are in decimal degrees, WGS84 datum. MPGE = Museu Paraense Emílio Goeldi, Pará, Brazil; MNRJ = Museu Nacional, Rio de Janeiro, Brazil. Source data: CRIA/speciesLink: http://www.splink.org.br/ (last access on 13/03/2021).

Taxon	Previous record: locality, municipality and/or Brazilian state, coordinates, source data	Record in the present study: municipality, coordinates
Hylodes babax (MNRJ 92566)	Caparaó State Park (type locality), Minas Gerais/Espírito Santo, -20.5414 -41.6672, CRIA/SpeciesLink	Atílio Vivacqua, -21.0097 -41.2249
	Uaimií State Forest, -20.4833 -43.9500, Ouro Preto, Minas Gerais, Pirani et al. (2010)	
	Santa Teresa, Espírito Santo, -19.9167 -40.6, Cria/SpeciesLink	
	Simonésia, Minas Gerais, -20.1239 - 42.0014, Cria/SpeciesLink	
	Araponga, Minas Gerais, -20.6667 -42.5208, Cria/SpeciesLink	
	Ervalia, Minas Gerais, -20.8400 -42.5208, Cria/Specieslink	
Phasmahyla lisbella (MNRJ 58975)	Ventania Environmental Protection Area, Rio de Janeiro (type locality), -21.3353 -42.2042, Pereira et al. (2018)	Atílio Vivacqua, -21.0097 -41.2249
Siphonops hardyi (MPEG41563)	Ipiranga, São Paulo, -23.590612 -46.605462, Maciel et al. (2009)	Mimoso do sul, -21.0107 -41.2476
	Cunha, São Paulo, -23.075969 -44.956067, Maciel et al. (2009)	
	Porto Real, Rio de Janeiro (type locality), -22.422361 -44.301557, Maciel et al. (2009)	
	Pedra Branca State Park, Rio de Janeiro, -22.932691 -43.472243, Maciel et al. (2009)	
	Tijuca National Park, Rio de Janeiro, -22.964565 -43.268453, Maciel et al. (2009)	
	Serra dos Órgãos, Rio de Janeiro, -22.401149, -42.828546, Maciel et al. (2009)	
	Augusto Ruschi Biological Reserve, Espírito Santo, -19.910980 -40.549299, Maciel et al. (2009)	
	Ferros, Minas Gerais, -19.248330 -42.999445, Maciel et al. (2009)	

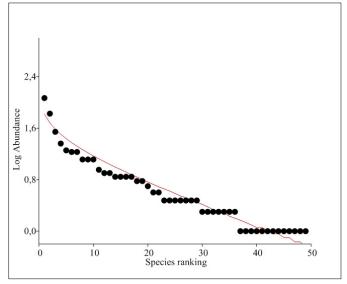


Figure 8. Whittaker abundance, plotted on a log₂ scale, and the log-series adjusted abundance of the amphibian community of the Serra das Torres Natural Monument (MONAST), Espírito Santo state, southeastern Brazil.

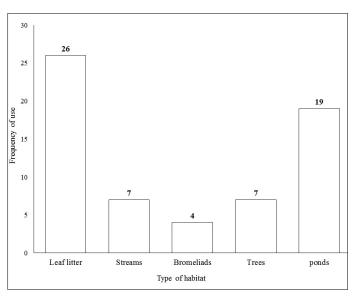


Figure 9. Frequency of the microhabitats used by the amphibian species recorded in the Serra das Torres Natural Monument (MONAST), Espírito Santo state, southeastern Brazil.

Table 3. Mean depth, width, and temperature of water from the streams sampled in the present study, and the distance between the sites at which specimens were recorded and the streams.

Stre	am depth (cm)	Stre	am width (cm)		
Mean	Max.	Min	Mean	Max.	Min	
10.58	60.0	1.0	96.2	260.0	2.0	

Water	temperatui	e (°C)			oetween specimen stream (cm)		
Mean	Max.	Min	Mean	Max.	Min		
20.5	25.0	19.0	50.0	1000.0	0		

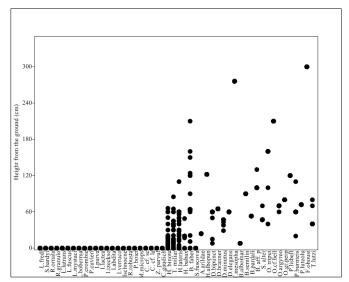


Figure 10. Vertical distribution, according to microhabitat use, of the amphibian species recorded in Serra das Torres Natural Monument (MONAST), Espírito Santo state, southeastern Brazil.

of deep leaf litter is generally the result of a greater local canopy cover, and the maintenance of the forest remnants in the MONAST clear of human impact will be essential for the conservation of their natural habitats and, in turn, the species they contain.

The abundance of anurans recorded in the present study followed a logarithmic model of distribution, which is typical of a community in which most species are rare and found within a limited area (Magurran 2004). Indeed, we recorded only three relatively high abundant species: the leaf litter inhabitant *Haddadus binotatus* (n = 117 individuals, 25.3% of the total abundance), and two stream-dwelling frogs, Thoropa miliaris (n = 67 or 14.5%) and Hylodes lateristrigatus (n = 35, 7.5%). Haddadus binotatus is a direct-developing leaf litter frog which makes this species independent of water bodies on the ground for its reproduction (Canedo & Rickli 2006, Nogueira-Costa & Carvalho-e-Silva 2010), but it is often associated with deep leaf litter and humid substrates (Oliveira et al. 2013). Thoropa miliaris inhabits rocky habitats in forest environments, presents semi-terrestrial tadpoles that live in thin, slow-flowing films of water on rocky surfaces bordering rivulets (Rocha et al. 2002), and it is not frequently abundant (e.g., 2.9% of anuran abundance in Ilha Grande State Park, Rio de Janeiro state, Rocha et al. 2011; 1.2% of anuran abundance in Duas Bocas Biological Reserve, Espírito Santo

state, Linause et al. 2020). Although there is an extensive database on the stream-dwelling species of the Atlantic Forest, the variation in the abundance of these species, and the environmental factors that determine this variation, are still poorly understood. *Hylodes lateristrigatus* occurs in the states of Espírito Santo and Rio de Janeiro, and the presence of this species in the MONAST fills a major gap in its known distribution (Vrcibradic et al. 2014). Species of the genus *Hylodes* are associated with rheophilic habitats in the Atlantic Forest (e.g., Pombal et al. 2002, Canedo & Pombal 2007), and they may be considered indicators of habitat quality (e.g., Weygoldt 1989).

Most species in a community tend to be rare (Bracken & Low 2012) and have unique traits that contribute to the long-term stability of the ecosystem (Mouillot et al. 2013, Jain et al. 2014). Some characteristics of rare species, such as their reduced abundance, limited geographic ranges, and greater susceptibility to environmental impacts, makes them more vulnerable to the risk of local extinction (Wilsey & Polley 2004, Purvis et al. 2000). This is typical for most of the rare species in the MONAST. The two gymnophionan species (Luetkenotyphlus fredi and Siphonops hardyi), for example, have restricted ranges and their biology is poorly known (e.g., Maciel et al. 2009, 2019). Luetkenotyphlus fredi was recorded and described during the present study and is currently known only from the MONAST and one other small forest in southern Espírito Santo state, the Mata do Ouvidor, in the municipality of Itapemirim (Maciel et al. 2019). Only four individuals of this species have been captured up to now (one from Mata do Ouvidor and three from the MONAST) and all these individuals were recorded in preserved habitats.

In the present study, the least abundant anuran species were associated with the leaf litter, including *Brachycephalus didactylus*, *Chiasmocleis* cf. *schubarti*, and *Myersiella microps*. The flea-toad *Brachycephalus didactylus* is known to be abundant at the present study site (see Oliveira et al. 2013), although it is probably restricted to a small portion of the MONAST, and we recorded this species at just one of the 18 sample sites. This miniaturized leaf litter frog (SVL = 11.0 mm) is one of the world's smallest tetrapods (Estrada & Hedges 1996, Lehr & Catenazzi 2009). The MONAST is the only forest in Espírito Santo state known to have a population of *B. didactylus* (Oliveira et al. 2012), with all the other known localities of the species being found in Rio de Janeiro state (Almeida-Santos et al. 2011).

Geographic distribution and gaps. We extend the known geographic distribution of two anuran species in the present study. One of these species, *Phasmahyla lisbella*, was described in early 2018, and our record is its northernmost known locality, which represents a range extension of 110 km to the northeast of the nearest previous record, in the municipality of Miracema (21°20'S, 42°12'W, WGS84 datum), in the state of Rio de Janeiro (Pereira et al. 2018). In 2019, we published the first record of this species in the MONAST (Oliveira et al. 2009), although we identified the specimen as *P. guttata* because *P. lisbella* was not available at that time. Given this, we are hereby correcting this account and presenting the first record of *P. lisbella* for the Espírito Santo state.

The second species, *Hylodes babax*, was previously known from four localities: (i) type locality, Caparaó National Park, on the division between Minas Gerais and Espírito Santo states, (ii) Serra do Brigadeiro State Park, in the Mantiqueira mountain range; (iii) Uaimií State Forest, in the southern Espinhaço range (Pirani et al. 2010); and (iv) in the municipality of Santa Teresa (Ferreira et al. *in press*). *Hylodes babax* is

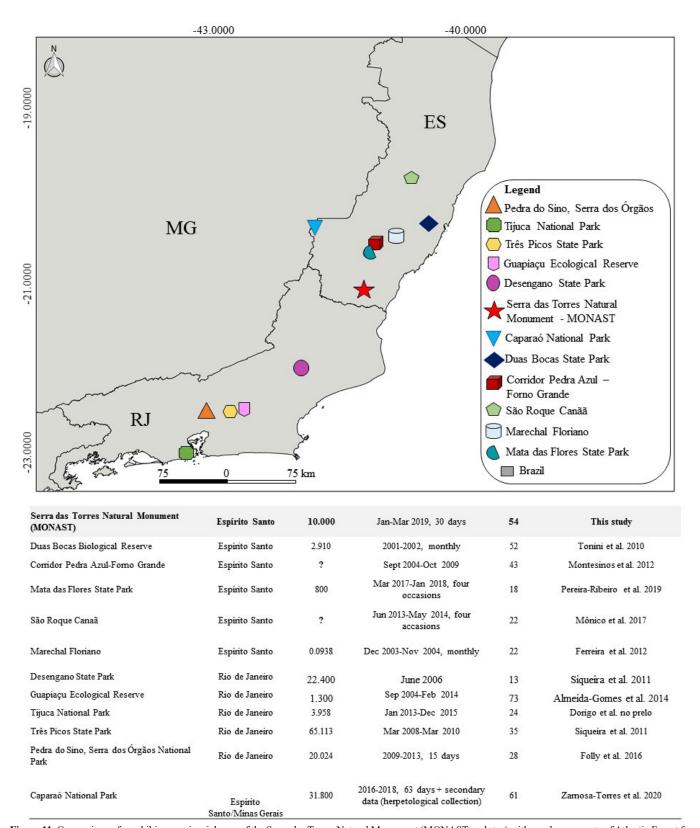


Figure 11. Comparison of amphibian species richness of the Serra das Torres Natural Monument (MONAST; red star) with nearby remnants of Atlantic Forest from the Espírito Santo and Rio de Janeiro states in southeastern Brazil. ES = Espírito Santo state; RJ = Rio de Janeiro state; MG = Minas Gerais state.

considered to be Data Deficient by the IUCN (2020) and in the Espírito Santo state (Ferreira et al., *in press*) mainly due to the limited data on its range (Rocha et al. 2004a). Here, we extend its known geographical

range approximately 86 km from the nearest locality, in the Caparaó National Park, representing the southernmost known record of this species in Brazil. *Hylodes babax* occurs in the MONAST together with

H. lateristrigatus, in Atílio Vivacqua and Muqui municipalities, as well as in Santa Teresa municipality, all in the Espírito Santo state (Heyer & Cocroft 1986).

Siphonops hardyi has a highly disjunct distribution, with gaps in its known occurrence in southeastern Brazil, in several disconnected remnants (Maciel et al. 2009). This species has been recorded in Ipiranga municipality and Serra da Mantiqueira, both in São Paulo state, Porto Real municipality (type locality), Pedra Branca State Park, Tijuca National Park, and the Serra dos Órgãos (regional designation for Serra do Mar range), all in Rio de Janeiro state, and in the municipality of Ferros, in Minas Gerais state. In the Espírito Santo state S. hardyi is known only from Santa Teresa and Domingos Martins municipalities (Maciel et al. 2009, 2019), and therefore our record from the MONAST fills a major gap in the known geographical distribution of this caecilian species.

Endemism, threats, and our contributions. The amphibians recorded in the MONAST are important representatives of the biological diversity of the Atlantic Forest. Most species recorded by us (71%) are endemic to this forest remnant, and among them two species are endemic to the Espírito Santo state: Euparkerella robusta and Luetkenotyphlus fredi. The only gymnophionan species known to be endemic to the Espírito Santo state is L. fredi (Maciel et al. 2019), although there is no data on the ecology of this species. Species of the genus Euparkerella are small leaf litter terrestrial frogs with globular bodies (Izecksohn 1988). Up to now, E. robusta was known to be endemic to the municipality of Mimoso do Sul, where the first individuals were recorded and described (Izecksohn 1988), while we have now recorded for the Espírito Santo state a second population, which is protected within the MONAST in the municipality of Atílio Vivacqua (Oliveira et al. 2013; present study). As there are no records of E. robusta outside the MONAST, this conservation unit may be responsible for the protection of this species.

Several threatened species were recorded in the MONAST. Euparkerella robusta is listed as Critically Endangered (CR) in the Espírito Santo state (Ferreira et al. in press) and as Vulnerable (VU) by the IUCN (2020). Thoropa cf. lutzi is classified as Endangered (EN) in the IUCN red list and in the state red list. Thoropa cf. lutzi has been recorded previously in the states of Rio de Janeiro, Minas Gerais, and Espírito Santo, although no populations have been confirmed in Rio de Janeiro state for more than 30 years (ICMBio 2018), and the MONAST probably protects the last remaining population of this species in the Espírito Santo state. Nine are listed as Data Deficient (DD): Brachycephalus didactylus, Euparkerella robusta, Hylodes babax, Ischnocnema cf. izecksohni, , Ischnocnema lactea, Ischnocnema verrucosa, Siphonops hardyi, Thoropa cf. lutzi, and Zachaenus parvulus (IUCN 2020). Two of these species (B. didactylus and Z. parvulus) were recorded in the Espírito Santo state for the first time in the MONAST (Oliveira et al. 2012) and this is the only known locality for these species in the state. The large number of species evaluated as endemic, threatened, data deficient, and unknown conservation status (Table 1) reinforces the need for the conservation of this forest remnant.

Spatial distribution of species and associated habitats. The leaf litter was the habitat most used by the amphibians recorded in the MONAST, and five families (42% of the species) are found exclusively in this component of the forest, followed by pond environments. Leaf litter-dwelling amphibians are an important component of the forest anuran community and a wide variety of biotic and abiotic factors are associated with their species richness, density, and abundance, as altitude

(Siqueira et al. 2014), structure and composition of the litter layer (Van Sluys et al. 2007), and humidity and depth of leaf litter (e.g., Giaretta et al. 1997, Oliveira et al. 2013). Our study reinforces previous findings in the MONAST (Oliveira et al. 2013), which indicate the importance of this forest remnant for the conservation of the region's leaf litter fauna. Data on the structure of leaf litter frog communities from the Espírito Santo state are available only from the MONAST (Oliveira et al. 2013) and Duas Bocas Biological Reserve (Vagmaker et al. 2020) and there is a considerable gap on the knowledge of these communities in the Atlantic Forest further north along the eastern coast of Brazil.

Six species recorded in the MONAST are streams inhabitants. *Hylodes* lateristrigatus and H. babax were recorded in the same streams, and the sharing of both spatial and acoustic niches by these species is an interesting phenomenon, which should be investigated further. The stream-dwelling species used the habitat vertically from zero to 1.25 m, which probably reflects their morphological characteristics. Although we surveyed all habitats extensively, most species were recorded in small rivulets, relatively small and shallow bodies of water. The presence of H. babax and H. lateristrigatus (the latter, in most sampled streams) indicates good water quality and well-preserved environments (e.g., Motta-Tavares et al. 2019). These species require clean water and are sensitive to anthropogenic impacts (Weygoldt 1989, Hatano et al. 2002, Motta-Tavares et al. 2019), and although additional studies are recommended for species of the genus Hylodes, it is known that amphibians that breed in ephemeral and often isolated bodies of water (e.g., headwater streams) are especially vulnerable to changes in temperature (Rome et al. 1992). Once again, we emphasize the need to isolate the MONAST forest from human disturbance because even minor alterations of these aquatic environments may impact the temperature of the water and its quality, leading to local extinction of these frogs (e.g., Weygoldt 1989, Blaustein et al. 2010).

Two other microhabitats used most frequently by amphibians are trees and tank bromeliads. The MONAST has a high diversity of bromeliads, with several rocky outcrops (inselbergs) covered by *Alcantarea extensa* (Magnago et al. 2008), which may store a large volume of water and that is usually inhabited by several anuran species in the Brazilian Atlantic Forest (e.g., Rocha et al. 2004b, Pontes et al. 2013). The low number of anuran species recorded by us in these microhabitats in the MONAST is possible due to their relative inaccessibility (high height of trees and steepness of inselbergs).

Recommendations for the conservation of amphibians in the MONAST. The MONAST is one of the largest Atlantic Forest conservation units in the Brazilian state of Espírito Santo, and one of the state's last remaining refuges of substantial ombrophilous forest cover. The combination of the large area of the MONAST with the quality of its forests contributes to its herpetological diversity, including its reptiles (Oliveira et al. 2020). We recommend the following measures to guarantee the conservation of these species in the MONAST: (1) the implementation of reforestation programs in areas of unused pasture to guarantee the connectivity of the forest fragments, a measure already recommended by Magnago et al. (2008); (2) the protection of rocky outcrops, including the limitation of rock climbing, due to the abundance of tank bromeliads that may contain many animal and plant species, including frogs; (3) the expansion of the programs that already involve the local residents in the vicinity of the MONAST, for the protection of local springs, including the "Aguas da Comunidade" project, which is currently restricted to a small area of the MONAST; (4) the implementation of programs for the recovery of the riparian forests throughout the entire area of the MONAST; (5) the implementation of long-term education programs for the residents of the area surrounding the MONAST to limit the use of pesticides, and encourage the sustainable use of natural resources; and (6) the implementation of sustainable tourism practices, including the prohibition of new trails, the access of visitors to the forest interior, and the presence of motor vehicles within the areas of forest. In other words, tourism should be limited to the existing areas of access.

The Serra das Torres Natural Monument has a significant amphibian fauna and constitutes an important reservoir of the amphibian diversity of the Brazilian state of Espírito Santo and of the Atlantic Forest biome. This conservation unit includes several endangered amphibian species and one endemic anuran (*Euparkerella robusta*), and currently, it represents the only reserve in the Espírito Santo that has populations of *B. didactylus*, *E. robusta*, *P. lisbella*, and *Z. parvulus*, which reinforces the importance for its conservation in coming years.

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Author contributions

Jane C. F. Oliveira: Substantial contribution to the conception and design of the study; contribution to the acquisition of the data; contribution to the analysis and interpretation of the data; contribution to the writing of the manuscript; contribution to the critical review of the manuscript, appending intellectual content.

Rafael dos Santos, Mateus Leite Lopes-Silva, Bárbara Risse-Quaioto, Cátia Moura Militão, and Flávia A. L. Belmoch: contribution to data collection.

Lorena P. Vasconcelos Barros and Pedro Fatorelli: Contribution to data collection; contribution to the critical review of the manuscript.

José P. Pombal Jr: Contribution to the critical review of the manuscript; contribution to identification of the species.

Carlos Frederico Duarte Rocha: Substantial contribution to the conception and design of the study; contribution to the acquisition of the data; contribution to the analysis and interpretation of the data; contribution to the writing of the manuscript; contribution to the critical review of the manuscript, appending intellectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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