Composition of anuran species in the Parque Natural Municipal Augusto Ruschi in Paraíba Valley, São Paulo State, Brazil

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Abstract. In this article, we characterize species richness, reproductive modes, constancy of occurrence, and taxonomic distinctness of the anuran community of the Parque Natural Municipal Augusto Ruschi (PNMAR) in Paraíba valley, southeastern Brazil. We sampled three distinct points in the area between August 2015 and July 2016. Three methodologies were used for anuran sampling (i) visual search, (ii) auditory search, and (iii) search at breeding site. We recorded 32 species of anurans distributed across nine families, belonging to 19 genera. Across all the sampled environments, a greater number of rare species were found, followed by occasional and frequent species. The forest area presented greater species richness and a greater taxonomic distinctness in relation to the open area and the forest edge. The PNMAR still retains a large part of its original vegetation cover, which provides different microclimatic conditions across its area, likely providing suitable habitats for species restricted to forest environments. We reinforce the need to sample localities in the southern portion of the Serra da Mantiqueira in the Paraíba Valley region.

Keywords. Amphibia, Anura, frogs, Atlantic Forest, biodiversity

Introduction

The Atlantic Forest domain is considered one of the world's biodiversity hotspots, recognized for its enormous species richness and a high rate of endemism, mainly of anurans (Myers et al., 2000; Haddad et al., 2013). For the state of São Paulo (southeastern Brazil) are known 27% of all species described for the country, however this is one of the states most impacted by anthropogenic action (Rossa-Feres et al., 2008). The number of species in the State is outdated, as in

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recent years new species have been described and new distribution records have added more species to this list (e.g., Moroti et al., 2017; Forlani et al., 2017; Malagoli et al., 2017).

The Paraíba Valley is largely located in the state of São Paulo but comprises a small portion of the state of Rio de Janeiro. This region is home to important remnants of the Atlantic Forest, located between two mountain formations, the Serra da Mantiqueira and the Serra do Mar. The region has undergone an intense urbanization process since the 1950s, whereby most of its forest cover was affected (Silva et al., 2017). Despite this, forest environments on steeper terrain were maintained and are partially protected through conservation units (Silva, 2015). The Paraíba Valley region has a high diversity of flora and fauna, especially in protected areas (Silva, 2015). The protection of these areas is of vital importance for the conservation of the local anurofauna, considering the descriptions and the extension of the distribution of anurans in the region (e.g., Cassini et al., 2010; Moroti et al., 2016; Forlani et al., 2017).

In the São Paulo stretch of the Paraíba Valley 74 species of frogs are known (Martins and Gomes, 2007; Cassini et al., 2010; Moroti et al., 2016; Forlani et al.,

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Figure 1. Map showing location of Parque Natural Municipal Augusto Ruschi, São Paulo State, municipality of São José dos Campos, Brazil. We inventoried three areas in PNMAR, in which (a) open area, (b) forest edge, and (c) forest area.

2017; Moroti et al., 2017). However, the vast majority of herpetofauna inventories in the Paraíba Valley region are concentrated in the portion that includes the Serra do Mar (e.g. Hartmann et al., 2009; Garey et al., 2014; Silva et al., 2017). Thus, there is a knowledge gap of species in the southern portion of the Mantiqueira.

In this context, we aimed to characterize the community of anurans, including species richness, reproductive modes, constancy of occurrence and taxonomic distinctness of the anurans of the Parque Natural Municipal Augusto Ruschi, in the Paraíba Valley, South of the Serra da Mantiqueira, southeastern Brazil.

Materials and Methods

Study area.—The sampled area is within the Parque Natural Municipal Augusto Ruschi (PNMAR), an Integral Protection Conservation Unit located in the region of the Paraíba Valley, municipality of São José dos Campos, São Paulo State, Brazil (Fig. 1). The natural landscape is highly fragmented, with only 10.17% of its area covered by fragments of Atlantic Forest (Morelli et al., 2003). The PNMAR is located on elevated terrain with altitudes varying between 640 and 1,040m (23.0722°S, 45.9322°W; datum WGS 84).

The park has an area of 2,467,436.75 m² (Silva, 1989), which added to the areas surrounding the PNMAR, constitutes a large portion of forest within the municipality (Dela Rosa, 2015). The PNMAR forest consists of Montana Semideciduous Seasonal

Forest, belonging to the Atlantic Forest domain, of which 62.87% is in the advanced stage of regeneration, 31.55% in the middle stage and 1.75% in the initial stage of succession, totaling 95.97% of the area of the park with forest cover (Dela Rosa, 2015).

The climate of the region is classified as Aw (tropical rainy with dry winter; Alvares et al., 2013b). The maximum temperature varies between 23.3° and 30.2°C and the minimum temperature varies between 11.7°C and 19.8°C (Alvares et al., 2013a). The months from June to August are the driest, with rain values lower than 50mm, while January is the wettest month, with 210.7mm (Alvares et al., 2013b).

Data collection.—The fieldwork began in May 2015 with visits for recognition and establishment of the study area. For this, we made three visits, one visit per month (May, June, and July 2015). Data collection was carried from August 2015 to July 2016, totalling 12 months. Five monthly visits were carried out, distributed throughout each month between 16:00h and 00:00h, totalling ca. 480 hours of sampling.

Three areas were inventoried in each sample: (i) open area (OA), which is located at the entrance of the park, consisting of one trail (1.0km) with the elevation range varying from 620m to 650m. This trail is characterized mainly by herbaceous and grassy vegetation, with a stream that crosses the forest area (Fig. 1a), (ii) forest edge (FE), consists of two trails (1.0km and 1.2km) with elevation ranges varying from 620m to 650m. This area is characterized as being a transitional area, with mainly herbaceous vegetation and medium-sized woody trees. In this area there is a dam across one of the streams which pass through the forest area, forming a lake with herbaceous marginal vegetation (Fig. 1b); and (iii) forest area (FA), consisting of two trails (1.5km and 1.2km) of medium to advanced succession stages with elevation ranges varying from 620m to 860m. Each trail has two independent streams where native Atlantic Forest preserved vegetation is present (Fig. 1c).

Three methodologies were used for anuran sampling (Heyer et al., 1994): (i) visual search, which consisted of searching for amphibians in trunks, rocks, bromeliads and other refuges, (ii) auditory search, which occurred on two trails already existing within the study area, and (iii) search in a breeding site, whereby the trails were slowly traversed in order to sample anurans that vocalize in temporary puddles, among leaf litter and in forest streams or distant from bodies of water.

The vouchers specimens were manually captured, euthanized in 5% lidocaine, fixed in 10% formalin and

preserved in 70% alcohol (Pisani, 1973). Tissue samples were not taken. The voucher specimens were deposited in the scientific collections of Universidade Estadual de Campinas (ZUEC) and Universidade Federal do Mato Grosso do Sul (UFMS) (Appendix 1).

Data analysis.--We evaluated the efficiency of the sample effort throughout the year, each day considered as a sample and the species richness plotted in relation to the number of individuals visualized. In addition, the observed rarefaction curve was extrapolated to obtain the expected species richness, generated from 1000 randomizations. To estimate the richness of species, we used the first-order Jackknife estimators and their respective confidence intervals calculated using the program Estimates v.9.10. The occurrence of the registered species was evaluated by the use of the constancy index of occurrence (Dajoz, 1978), classified as: abundant (present in > 50% of samples), common (present in 25 to 50% of samples) or rare (present in < 25% of samples). Reproductive modes of recorded frogs were classified according to the literature (sensu Haddad and Prado, 2005; Haddad et al., 2013). To verify the taxonomic distinctness between the sampled environments (open area, forest edge, and forest area), the averages of taxonomic distinctness (Δ +), and the variation in taxonomic distinctness (Λ +) (Clarke and Warwick, 1998; 2001) were calculated with PRIMER software version 7.0 (PRIMER-E Ltd.).

Results

We recorded 32 species of anurans distributed across nine families, belonging to 19 genera (Fig. 2-5). Through the accumulation of species, a real richness of 32 species of anurans and an estimated richness of $32 \pm$ 0.9 species (amplitude = 32 to 33 species) was obtained for PNMAR (Fig. 6). The PNMAR anuran community is composed mostly of species of the family Hylidae (n = 15; 45.5%), followed by the families Leptodactylidae (n = 5; 18.2%), Brachycephalidae (n = 3, 9.1%), Bufonidae (n = 2; 6.25%), Microhylidae (n = 2; 6.25%), and Phyllomedusidae (n = 2; 6.25%). The other families (Craugastoridae, Hylodidae and Odontophrynidae), presented only one species each. Of the 32 species listed, 24 are considered endemic to the Atlantic Forest domain (Table 1).

The number of rare species recorded was higher and represented 45% of the community (n = 15), followed by occasional species (n = 12; 36%) and frequent species (n = 6; 18%; Table 1). Ten different reproductive



Figure 2. Anuran species recorded in Parque Natural Municipal Augusto Ruschi, São Paulo State, municipality of São José dos Campos. (a) *Brachycephalus ephippium*, (b) *Ischnocnema* sp. (aff. *parva*), (c) *Ischnocnema* sp. (aff. *guentheri*), (d) *Rhinella ornata*, (e) *R. icterica*, (f) *Haddadus binotatus*, (g) *Aplastodiscus arildae*, and (h) *A. leucopygius*. Photographs by Rafael Menegucci (b) and Matheus Moroti (a, c, d, e, f, g,h).



Figure 3. Anuran species recorded in Parque Natural Municipal Augusto Ruschi, São Paulo State, municipality of São José dos Campos. (a) *Boana faber*, (b) *B. pardalis*, (c) *B. prasina*, (d) *B. semilineata*, (e) *Bokermannohyla circumdata*, (f) *B. luctuosa*, (g) *Dendropsophus elegans*, and (h) *D. sanborni*. Photographs by Karoline Ceron (h) and Matheus Moroti (a-g).

Figure 4. Anuran species recorded in Parque Natural Municipal Augusto Ruschi, São Paulo State, municipality of São José dos Campos. (a) *Ololygon obtriangulata*, (b) *Scinax cardosoi*, (c) *S. crospedospilus*, (d) *S. fuscovarius*, (e) *S. hayii*, (f) *Hylodes* sp., (g) *Adenomera marmorata*, and (h) *Leptodactylus fuscus*. Photographs by Matheus Moroti.

Figure 5. Anuran species recorded in Parque Natural Municipal Augusto Ruschi, São Paulo State, municipality of São José dos Campos. (a) *Leptodactylus labyrinthicus*, (b) *L. mystacinus*, (c) *Physalaemus olfersii*, (d) *Chiasmocleis mantiqueira*, (e) *Elachistocleis cesarii*, (f) *Proceratophrys boiei*, (g) *Phyllomedusa burmeisteri*, and (h) *Pithecopus rohdei*. Photographs by Matheus Moroti.

Figure 6. Species accumulation curve (solid black line), richness index (first order Jackknife; solid red line) and its respective confidence intervals (dashed red lines) for anurans sampled during field surveys in the Parque Natural Municipal Augusto Ruschi, São Paulo State, municipality of São José dos Campos.

modes were observed for the sampled community, with reproductive mode 1 (exotrophic tadpoles in standing water) being the most common, present in 12 of 32 recorded species (37.5%). The forest area presented a greater number of reproductive modes (n = 7), followed by the forest edge (n = 6) and by the open area (n = 5).

The forest area presented a greater species richness (n = 16), followed by the open area (n = 13), and forest edge (n = 12). Thirteen species were recorded only in the forest environment, seven species only in the open area, and four species only at the forest edge. *Boana faber* was the only species found in all sampled environments. Among the forest environment species, only *Ololygon obtriangulata* and *Physalaemus olfersii* were found in other habitats, both at the forest edge (Table 1).

The analysis of taxonomic distinctness (Δ +) for the sampled environments was similar, mainly between the open area and the forest edge (Fig. 7a). The forest area presented greater species richness and taxonomic distinctness in relation to the open area and the forest edge. All areas were distributed within the confidence limits (95%) of the analysis. In the analysis of taxonomic distinctness variation (Λ +), the open area was outside the upper confidence interval. The forest area, although presenting a greater species richness, presented a smaller variation of the taxonomic distinctness in relation to the other areas (Fig. 7b).

Discussion

The richness of anuran species registered in PNMAR represents about 45% of the species registered for the Paraíba Valley (Martins and Gomes, 2007), 13.5 % of the State of São Paulo (Rossa-Feres et al., 2011), and about 6% of the species recorded for the Atlantic Forest (Haddad et al., 2013). The species Chiasmocleis mantiqueira and Scinax cardosoi were recently registered for PNMAR (Moroti et al., 2016; 2017) adding two species to the list of Martins and Gomes (2007). The prevalence of Hylidae and Leptodactylidae families in relation to the other anuran families in the area of study is a common result in other locations (e.g. Garey et al., 2014; Neves et al., 2017). The richness accumulation curve stabilized, showing that sampling was sufficient to record most of the PNMAR species, and that new sampling efforts are unlikely to add further species to this list.

According to the Brazilian Official Threatened Fauna Species List (MMA, 2014) and the list of Endangered Fauna in the State of São Paulo (Garcia et al., 2009), none of the recorded species are threatened. However,

Figure 7. The 95% probability funnels (dotted and solid line) for (a) taxonomic distinctness (Delta +), and (b) variation in taxonomic distinctness (Lambda +), for anurans recorded on three areas in the Parque Natural Municipal Augusto Ruschi (August 2015 to July 2016). FA = forest area, OA = Open area, and FE = Forest edge.

Table 1. Anurans recorded in Parque Natural Municipal Augusto Ruschi, São Paulo State, Brazil, between August 2015 and July 2016. RM = Reproductive mode (*sensu* Haddad and Prado, 2005 and Haddad et al., 2013), FO = Frequency of occurrence, CI = constancy index (Dajoz, 1983), A (abundant species), C (Common species), and R (rare species). *Species not endemic to the Atlantic Forest.

Taxon	Open area	Forest edge	Forest area	RM	FO (%)	CI
Family Brachycephalidae						
Brachycephalus ephippium (Spix, 1824)			x	23	52	А
Ischnocnema sp. (aff. parva)			x	23	18	R
Ischnocnema sp. (aff. guentheri)			x	23	38	С
Family Bufonidae						
Rhinella icterica (Spix, 1824)*	х			1	16	R
Rhinella ornata (Spix, 1824)	x	x		1,2	36	С
Family Craugastoridae						
Haddadus binotatus (Spix, 1824)			x	23	54	А
Family Hylidae						
Aplastodiscus arildae (Cruz and Peixoto, 1987)			x	5	38	С
Aplastodiscus leucopygius (Cruz and Peixoto, 1985)			x	5	68	А
Boana faber (Wied-Neuwied, 1821)	х	х	x	4	56	А
Boana pardalis (Spix, 1824)	х			2,4	64	А
Boana prasina (Burmeister, 1856)	х	x		1	40	С
Boana semilineata (Spix, 1824)		x		1	20	R
Bokermannohyla circumdata (Cope, 1871)			x	4	18	R
Bokermannohyla luctuosa (Pombal and Haddad, 1993)			x	4	56	А
Dendropsophus elegans (Wied-Neuwied, 1824)	х	x		1	38	С
Dendropsophus sanborni (Schmidt, 1944)*	х	x		1	14	R
Ololygon obtriangulata (Lutz, 1973)		x	x	2	28	С
Scinax cardosoi (Carvalho-e-Silva and Peixoto, 1991)			x	1	18	R
Scinax crospedospilus (Lutz, 1925)	х	x		1	36	С
Scinax fuscovarius (Lutz, 1925)*	х			1	12	R
Scinax hayii (Barbour, 1909)			x	1	22	R
Family Hylodidae						
Hylodes sp. (aff. sazimai)			x	3	42	С
Family Leptodactylidae						
Adenomera marmorata Steindachner, 1867		x		32	12	R
Leptodactylus fuscus (Schneider, 1799)*	х			30	22	R
Leptodactylus labyrinthicus (Spix, 1824)*	х			11	12	R
Leptodactylus mystacinus (Burmeister, 1861)*	х			30	12	R
Physalaemus olfersii (Lichtenstein e Martens, 1856)		x	x	11	38	С
Family Microhylidae						
Chiasmocleis mantiqueira Cruz, Feio, and Cassini, 2007			x	1	14	R
Elachistocleis cesarii (Miranda-Ribeiro, 1920)*	x			1	16	R
Family Odontophrynidae						
Proceratophrys boiei (Wied-Neuwied, 1824)			х	2	26	С
Family Phyllomedusidae						
Phyllomedusa burmeisteri Boulenger, 1882		х		24	38	С
Pithecopus rohdei (Mertens, 1926)		х		24	26	С
Richness	13	12	16	10		

the species *Chiasmocleis mantiqueira* is listed as Data Deficient, with little information on the extent of occurrence and ecological requirements of the species (Angulo, 2008). Verdade et al. (2010) reinforce the importance of preserving anuran species classified as Data Deficient, especially in the southeastern region of Brazil, since these areas suffer constant anthropic pressures. Another important factor is that ca. 78% of the inventoried species are endemic to the Atlantic Forest domain, corroborating the high rate of endemism of anurans found in this domain (Haddad et al., 2013).

The index of constancy of occurrence showed that a greater number of rare species were found (Table 1). This number can be considered inflated because the species *Leptodactylus labyrinthicus*, *L. mystacinus*, and *Scinax fuscovarius* were recorded only in one sample (singletons). Likewise, the high number of rare species may be related to species with explosive-breeding strategies or that the methodologies used were inadequate. For example, *Scinax cardosoi* and *S. hayii* were observed in the area showing breeding activity for only few days during the rainy season (M.T. Moroti, pers. obs.). The leaf-litter species (e.g. *Adenomera marmorata* and *Ischnocnema* spp.), were more difficult to capture with the methodology used, even when using auditory search techniques.

The species Leptodactylus fuscus, L. labyrinthicus, Rhinella icterica, and Scinax fuscovarius were recorded only in the open area of the PNMAR and according to our sampling method were considered rare (<25%). However, these species are widely distributed in Brazil, mainly in impacted areas, originally inhabiting areas of open scrubland (Brasileiro et al., 2005). These same species are found in abundance in pasture areas around PNMAR (M.T. Moroti, pers. obs.). According to Haddad (1998), the most general species expand their geographical distribution mainly in places where the natural landscape changes and where there is extensive deforestation, can reach high levels of dominance (Giaretta et al., 1999). This result reinforces the importance of preserving the natural landscape of PNMAR, since these species, although abundant in the surroundings, are not found in abundance within the conservation unit, which still offers support capacity for species restricted to forest fragments (e.g. Brachycephalus ephippium, Hylodes sp. (aff. sazimai), Ischnocnema spp., and Proceratophrys boiei).

From the 27 reproductive modes recorded for the Atlantic Forest (Haddad et al., 2013), 10 reproductive modes were recorded for the PNMAR community. More reproductive modes were found within the forest

area than in comparison to other habitats. This result is probably related to limitations in reproductive modes, restricting the use of specific habitats (Bertoluci and Rodrigues, 2002; Moraes et al., 2007). For example, the species of the genus *Aplastodiscus*, which use underground burrows excavated by males along stream banks (Mode 5; *sensu* Haddad and Prado, 2005) and the family Brachycephalidae (Mode 23; *sensu* Haddad and Prado, 2005), which utilizes direct development in leaf litter and were only found within the forest area.

The presence of Boana faber in all environments sampled can be explained by the ecological plasticity of the species, which inhabits forest areas, edges of the forest and open areas (Höbel, 2008; Luza et al., 2015). Furthermore, the exclusiveness of some species of this study to certain environments (e.g. Hylodes sp. (aff. sazimai) and Proceratophrys boiei), was due to the specialist nature of these species, which inhabit only forest environments containing freshwater streams (Zina et al., 2007). The greatest number of species being attributed to the forest environment is due to this environment being more heterogeneous and presenting a greater number of microhabitats that the open area and forest edge (Morellato and Haddad, 2000). Similarly, Pfeifer et al. (2017) demonstrated that forest environments present a greater richness of amphibians, reptiles and mammals compared to the forest edge, as found here for anurans.

In the analysis of variation of the taxonomic distinctness, the open area falls above the upper limit of the Λ + funnel, indicating a higher than expected variation in distinctness of species pairs. A possible implication is that some genera or families are represented only by a single species whilst others are relatively species-rich. This effect would then be approximately neutral on the average statistic Δ +, but would elevate the variance statistic Λ + (Clarke and Warwick, 2001). The lower variation of the taxonomic distinctness of the forest area in relation to other areas is related to the uniformity of the taxonomic tree of this community, since the Λ + is simply the variance of these pairwise path-lengths and reflects the unevenness of the taxonomic tree (Clarke and Warwick, 2001).

The highest average of the taxonomic distinctness of the forest area is related to the greater number of families and genera that compose this community, which entails greater taxonomic distances between the pairs of species analyzed. In the same way, the affinity of the average taxonomic distinctness between the open area and forest edge is due to the similarity in the composition of these communities, in which species of the families Hylidae and Leptodactylidae predominated and which entails similar taxonomic distances. Many species that breed in open areas may take shelter in forest fragments or forest edge areas (Silva and Rossa-Feres, 2007). These areas can be used by anurans in three non-exclusive ways: (i) as refuge areas during the dry season, (ii) as day shelters during the breeding season, and (iii) as a foraging area (Silva and Rossa-Feres, 2007). This may result in a similar species composition between the environments (Lucas and Fortes, 2008).

Although the PNMAR is a remnant and small fragment of forest, our study uncovered a representative sample of the anurofauna of the Paraíba Valley and the state of São Paulo. In addition, the PNMAR still has a large part of its original vegetation cover, creating different microclimatic conditions and providing support capacity for species restricted to forest areas. Thus, we reinforce the need to sample localities in the southern portion of the Serra da Mantiqueira in the Paraíba Valley region. The region contains endemic species to the Atlantic Forest that only use preserved forest fragments, and it may harbour still non-described species or provide new records of species distributions, especially the rare ones.

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Appendix 1. Vouchers of anuran species collected in the Parque Natural Municipal Augusto Ruschi in Paraíba Valley, São Paulo State, Brazil. Individuals were deposited in the Museu de Zoologia "Adão José Cardoso", Universidade Estadual de Campinas, Campinas, São Paulo State, Brazil (ZUEC) and Coleção Zoológica de Referência, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil (ZUFMS). The advertisement call of *Ischnocnema* sp. (aff. *parva*) was deposited in the Fonoteca Neotropical Jacques Vielliard, Universidade Estadual de Campinas, Campinas, São Paulo State, Brazil (FNJV).

Family Brachycephalidae: Brachycephalus ephippium (ZUEC-AMP 23296); Ischnocnema sp. (aff. guentheri) (ZUEC-AMP 23320-22); and Ischnocnema sp. (aff. parva) (ZUEC-AMP 23303-04; FNJV-AMP 34244). Family Bufonidae: Rhinella icterica (ZUEC-AMP 23312); Rhinella ornata (ZUEC-AMP 23309). Family Craugastoridae: Haddadus binotatus (ZUEC-AMP 23310-11). Family Hylidae: Aplastodiscus arildae (ZUEC-AMP 23398); Aplastodiscus leucopygius (ZUEC-AMP 23299); Bokermannohyla circumdata (ZUEC-AMP 23325); Bokermannohyla luctuosa (ZUEC-AMP 23318-19); Dendropsophus elegans (ZUEC-AMP 23300); Dendropsophus sanborni (ZUEC-AMP 23301); Boana faber (ZUEC-AMP 23306); Boana pardalis (ZUEC-AMP 23302, 23336); Boana prasina (ZUEC-AMP23297); Boana semilineata (ZUEC-AMP 23316); Scinax cardosoi (ZUEC-AMP 23323); Scinax crospedospilus (ZUEC-AMP 23314); Scinax fuscovarius (ZUFMS-AMP 06457); Scinax havii (ZUEC-AMP 23326); Ololygon obtriangulata (ZUEC-AMP 23331-34). Family Hylodidae: Hylodes sp. (aff. sazimai) (ZUEC-AMP 23324). Family Leptodactylidae: Adenomera marmorata (ZUEC-AMP 23305, 23330); Leptodactylus fuscus (ZUEC-AMP 23315); Leptodactylus labyrinthicus (ZUEC-AMP 23328); Leptodactylus mystacinus (ZUEC-AMP 23327); Physalaemus olfersii (ZUEC-AMP 23317). Family Microhylidae: Chiasmocleis mantiqueira (ZUFMS-AMP 03738); Elachistocleis cesarii (ZUEC-AMP23329). Family Odontophrynidae: Proceratophrys boiei (ZUEC 23307). Family Phyllomedusidae: Phyllomedusa burmeisteri (ZUEC-AMP 23308); Pithecopus rohdei (ZUEC-AMP 23313).