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Composition and diversity of anurans in the largest conservation unit in Pampa biome, Brazil

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Abstract: Grassy biomes occupy about 20% of the earth's surface, and are characteristic of northern Australia, Africa and South America, being biodiversity in these environments poorly understood. The Área de Proteção Ambiental (APA) do Ibirapuitã includes areas with the best-preserved grassland areas in the Pampa biome in Rio Grande do Sul state, Brazil. This study aimed to determine anuran species richness, abundance, constancy of occurrence, and reproductive modes, and to compare the taxonomic composition in the APA with other localities within the grassland areas of the southernmost of South America. We collected frogs from September to November 2012 and in November 2013 by sampling of reproductive sites. We also examined specimens deposited in herpetological collections. In total, 32 frog species were identified from the combination of larval and adult sampling and analysis of specimens deposited in scientific collections. The registered anurofauna is typical of grassland areas, with at least 10% restricted to the subtropical region of South America and at least two species considered endangered in state and global scales. The most abundant species was *Pseudopaludicola falcipes* and *Hypsiboas pulchellus* was the species most frequently found among sites. Five reproductive modes were recorded, and the most common mode consists of development of exotrophic tadpole and deposition in to lentic bodies of water (57.5%). Cluster analysis of 16 communities representing grassland locations presented five groups with more than 50% similarity, whose structure was influenced by geographic distance but can be partially interpreted by regional peculiarities (e.g. height and phytophysiognomies). Our results consist of initial knowledge base on the anurofauna of APA do Ibirapuitã, supporting recommendations for future conservation actions to APA and also for the grassland biomes, which are increasingly threatened by human activities.
Keywords: *anuran communities, native grasslands, tadpoles, reproductive modes, similarity.*

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Resumo: Biomas campestres ocupam cerca de 20% de toda a superfície terrestre, e são característicos do norte da Austrália, África e América do Sul, sendo a biodiversidade nesses ambientes pobremente conhecida. A Área de Proteção Ambiental (APA) do Ibirapuitã possui as áreas de campo mais bem preservadas do bioma Pampa no Rio Grande do Sul, Brasil. Este estudo objetivou determinar a riqueza de espécies de anuros, abundância, constância de ocorrência, modos reprodutivos e comparar a composição taxonômica da APA com outras localidades inseridas em áreas campestres do extremo sul da América do Sul. Nós coletamos os anuros de setembro a novembro de 2012 e novembro de 2013, utilizando o método de busca em sítios de reprodução. Nós também examinamos espécimes em coleções herpetológicas. No total, registramos 32 espécies de anuros pela combinação da amostragem de larvas, adultos e análise de espécimes depositados em coleções científicas. A anurofauna registrada é típica de áreas campestres, com pelo menos 10% das espécies restritas à região subtropical da América do Sul e pelo menos duas espécies consideradas ameaçadas em escala estadual e global. *Pseudopaludicola falcipes* foi a espécie mais abundante e *Hypasiboas pulchellus* a mais frequentemente encontrada nos sítios amostrados. Cinco modos reprodutivos foram registrados, sendo o modo mais comum a deposição e desenvolvimento de larvas exotróficas em corpos d'água lênticos (57,5%). A análise de similaridade entre as 16 comunidades representando localidades campestres, apresentou cinco grupos com mais de 50% de similaridade, cuja estrutura foi influenciada pela distância geográfica, mas pode ser parcialmente interpretada por peculiaridades regionais (e.g. altitude e fitofisionomias). Nossos resultados representam uma base inicial de dados sobre a anurofauna da APA do Ibirapuitã, bem como incluem recomendações para futuras ações de conservação dos biomas campestres, que são cada vez mais ameaçados pelas atividades humanas.
Palavras-chave: *comunidades de anuros, campos nativos, girinos, modos reprodutivos, similaridade.*

Introduction

Grassy biomes are ancient on earth, and have persisted in some landscapes for tens of thousands of years (Bond & Parr 2010). Biota associated with these biomes has been unevenly studied, and include, for example, the unique flora and fauna of the Cerrado, one of the world's biodiversity hotspots (MMA 2007). Technically, grassy biomes are described as having open vegetation that is subject to periodic droughts, with ground cover dominated by grasses and other similar species, typically occurring in areas with at least 10-15 trees per hectare (Risser 1997). Grassy biomes occupy around 20% of the earth's surface, and are more prominent in northern Australia, South America and Africa, and in most parts of the world the associated biodiversity is still poorly known (see Bond & Parr 2010). Despite a paucity of attention given to these biomes, they are known to harbor diverse and distinct fauna (Bond & Parr 2010). For example, the Brazilian Cerrado has high diversity and endemism of a number of animal groups, including birds, small mammals, herpetofauna and insects (Vitt & Caldwell 1993, Da Silva 1997, Lacher & Alho 2001, Da Mata et al. 2008, Vasconcelos et al. 2008, Morais et al. 2012).

In the most southern parts of Brazil, grassland physiognomies are referred to as Southern Grasslands, and are present in two Brazilian biomes: the Atlantic Forest biome (which includes areas of grasslands in the south Brazilian plateau, forming mosaics with forests in the northern half of Rio Grande do Sul and neighboring states); and the Pampa biome (approximately corresponding to the southern half of Rio Grande do Sul state) (IBGE 2004, Overbeck et al. 2007). In Brazil, the Pampa biome exists only in Rio Grande do Sul, where it occupies about 60% of the state territory and 2% of the Brazilian territory (Suertegaray & Silva 2009). Brazilian, Argentinian and Uruguayan Pampa combined have a total biome area of approximately 700,000 km^2 (Suertegaray & Silva 2009).

The Pampa biome has its own unique fauna and flora, and contains great diversity (Pillar et al. 2009). In terms of fauna, it is a rich ecosystem with numerous endemic species, including some frogs of the genus *Melanophryniscus*, whose center of diversity is understood to be open formations in the subtropical/temperate zone of South America (Pramuk et al. 2008). However, there is a lack of data available for accurate estimates of the richness of most vertebrate groups, including amphibians, in grassy ecosystems (Bencke 2009). Basic research on amphibians in the Pampa biome, including studies to construct species lists, acquisition of natural history information, and community studies are still scarce and recent (Santos et al. 2008, Both et al. 2011, Maragno et al. 2013). These approaches can provide valuable information on patterns of local diversity (richness, abundance and evenness), as well as on the spatial and temporal distribution of species (Duellman & Trueb 1994).

The southern Brazilian grasslands are in general of private domain, are typically used for pasture, and are under constant threat of conversion for other land uses (Pillar & Vélez 2010). Thus, the Pampa has suffered great loss of habitat and biodiversity due to the accelerated expansion of agriculture (especially soybeans), exacerbated by the conversion of large grassland areas into monoculture plantations (MMA 2007, Gautreau & Vélez 2011), and by introduction of invasive species often spread by irresponsible agricultural practices (Ferreira et al. 2012). Further, conservation of grassy ecosystems is of fundamental importance for biodiversity, because these areas maintain assemblages of characteristic biota and protect various floral and faunal organisms within them (known or unknown), and preserve ecosystem processes (Pillar et al. 2006).

Only about 3% of the Pampa biome is legally protected into 15 Conservation Units (CUs) in Brazil and most of them still was not truly implemented (Vélez et al. 2009). Compared to other Brazilian terrestrial biomes, the Pampa has a high percentage of agricultural land use with one of the lowest percentages of legally protected area (Ferreira et al. 2012). Moreover, forests are more represented in CUs than are grasslands, and is lacking adequate technical of management to ensure the conservation of grassland's biodiversity against forest invasion (Pillar & Vélez 2010).

Another worrying aspect for Pampa biome conservation is the lack of basic knowledge of biodiversity in the established protected areas. Determination of how many species are present in a given habitat, as well as species abundance and how they interact are important issues for ecology and conservation (Dood Jr. 2010). Thus, considering the historical and cultural problems in conservation of the Pampa biome, the objectives of this study were to characterize the frog assemblage in a conservation unit in Brazilian Pampa, located in the Planalto da Campanha, Rio Grande do Sul state, regarding species richness and reproductive modes, taxonomic composition, abundance and constancy of occurrence, as well as to compare the anuran assemblage of this CU with other frog assemblages in grasslands of the southernmost of South America.

Material and Methods

The present work was developed in the Área de Proteção Ambiental (APA) do Ibirapuitã (30° 51' 57.41" S; 55° 38' 59.63" W north extreme and 29° 57' 20.52" S; 55° 40' 16.80" W south extreme, Figure 1). The APA has approximately 318,000 ha of extension in to Pampa biome and consists almost exclusively of rural private property (Vélez et al. 2009) distributed in the municipalities of Alegrete, Quaraí, Rosário do Sul and Santana do Livramento, all in the state of Rio Grande do Sul (RS). This type of conservation unit fits the IUCN Category VI (protected with sustainable use of natural resources area), and aims to conserve ecosystems and habitats in a manner harmonious with associated cultural values and traditional systems of natural resource management (Dudley 2008). Thus, the main objective of this type of protection area is non-industrial use of natural resources that are compatible with nature conservation.

The APA do Ibirapuitã houses part of the Ibirapuitã River basin, and is located on the western border of the state. The southern boundary of the APA coincides with the Brazil-Uruguay international boundary, with no geographic or other physical barrier to separate the Uruguayan territory (Brasil 1992). The APA is situated between mosaics of the central depression of the state (including butte hills and soft grassy hills of the plain) and the Planalto da Campanha (grassy hill surface plateau) (Zaions 1989), with altitude varying from 30 to 400 meters (Hasenack et al. 2010).

The climate is classified as TEUM (temperate sub-humid) and STEUM (sub-humid temperate) (Maluf 2000), with annual

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rainfall of about 1,500 mm, with the lowest rainfall in August and the highest in October (MMA/IBAMA 1999). The average annual temperature is 18.6°C, with a minimum recorded temperature of -4.1°C and maximum of 40.4°C (MMA/IBAMA 1999). The predominant vegetation type is characterized as 'grasslands on shallow soil' sensu Hasenack et al. (2010) and constitute the best preserved grasslands of Rio Grande do Sul, developing on rocky soils (derived from basalt and sandstone), with low moisture retention and water deficit in summer. Additional forest inclusions occur along the banks of rivers and streams (Boldrini 2009, Boldrini & Longhi-Wagner 2011).

Samples were collected in September, October and November 2012 and November 2013, period that comprises the seasons most favorable to amphibian activity in austral region due to longer photoperiod (Both et al. 2008). Additionally, complementary sporadic collections were performed in July 2013. 40 ponds and 24 streams were all sampled once, totaling 64 water bodies distributed within the APA and surrounding region. The number of sites sampled in studies of frog communities varied according to the distribution and natural history of frog species studied, and with respect to the research questions of interest and other factors (Dorcas et al. 2010). The sampling of a large number of water bodies (over 50, as recommended by Dorcas et al. 2010) in a short period of time was opted in order to cover the greatest diversity of habitats possible and to minimize the effect of drought, which typically drastically limits water body availability from late spring to early summer (pers. obs.).

Sampling of adult frogs was performed by sampling sites of reproduction (Scott Jr. & Woodward 1994). During frog breeding season, lentic (natural ponds and weirs to cattle) and lotic waterbodies (streamlets) were sampled. Pond areas varied from 43.26 to 15740.32 m^2 (554.83 ± 2,933.54) and stream lengths were sections of 100 m. Pond depths varied from 7.1 to 96.67 cm (21.67 ± 20.9), and stream depths varied from 9.67 to 50.71cm (24 ± 10.13). Distance among waterbodies varied from 0.02 to 124.37 km (39.5 ± 25.7) for ponds and from 2.98 to 105.11 km (35.4 ± 22) for streamlets. The search for frogs was conducted during twilight and at night, along the banks of water bodies. Sampling effort was proportional to environmental size and complexity (Scott Jr. &Woodward 1994). During sampling all visualized individuals were registered, and estimations were made for the abundance of calling males for each species (e.g., Goottsberger & Gruber 2004, Vasconcelos & Rossa-Feres 2005, Santos et al. 2007).

Additionally, tadpoles were collected during the day using a long-handled dip net and 3 mm^2 metal mesh. Sampling consisted of performing a full scan of the water body one time only, along the edge of the pools. Thus, the sampling effort for different bodies of water was roughly proportional to their size (Heyer 1976, Santos et al. 2007, Both et al. 2009).

To complement the species list, we consulted four main scientific collections of the Rio Grande do Sul state in search of amphibian records for the municipalities that make up the APA do Ibirapuitã: the Scientific Collection of the Department of Zoology, Department of Biology, Federal University of Santa Maria (ZUFSM); the Amphibian Collection of the Museum of Science and Technology, Catholic University of Rio Grande do Sul (MCP); the Amphibian Collection of the Department of Herpetology, Department of Zoology, Federal University of Rio Grande do Sul (UFRGS); and the Collection of Amphibians at the Museum of Natural Sciences of the Zoobotanical Foundation of Rio Grande do Sul (MCN). Voucher specimens were deposited in the Scientific Collection of the Zoology section in the Department of Biology, Federal University of Santa Maria (ZUFSM) (license SISBIO/ICMBio # 33975).

Sampling sufficiency was assessed by constructing a species accumulation curve from 500 randomizations, and by using five qualitative richness estimators (Bootstrap, Chao II, ICE, Jackknife I and II) (Gotelli & Colwel 2001). The curve was constructed based on records of collected tadpoles and on audio/visual recordings of adults in the study area. To analyze the relative abundance of adults, we built a Whittaker plot in which the relative abundance of each species is presented as percentage of the number of males with vocalization activity of males in the total of water bodies (sensu Krebs 1999, Magurran 2013). Reproduction modes were classified according to Haddad & Prado (2005). The frequency of species occurrence in water bodies (expressed as a percentage) was determined by the presence of adults and/or tadpoles, and was analyzed by the constancy of occurrence index (c) (Dajoz 1983) for ponds and streams separately, such as to all water bodies together (i.e. pooled).

The composition of the APA do Ibirapuitã anurofauna was compared with the composition recorded in other areas characterized as native grassland vegetation in the southernmost of South America (see Figure 1 and Table 2). The comparison between anuran communities from different areas can be affected by unequal sampling effort, size of the sampling area, characteristics and conservation status of the localities, and differences in taxonomic concepts used by researchers (Santos et al. 2009, Iop et al. 2011). Therefore, some precautions were taken to minimize potential limitations in the comparison: work was included in the analyses that had sampling effort for at least a year or reproductive season (temporal and/or spatial), species listed as sp, gr. (group of species), cf. (*confer*) or aff. (*affinis*) were excluded, and the exotic species *Lithobates catesbeianus* was not considered in the analysis.

The similarity between the communities of species was calculated using the 'coefficient of graphical resemblance' (CGR) (Duellman 1990), where GSC = 2NS/ NA + NB (NS = number of species in both areas, NA = number of species in area A, NB = number of species in area B). This index ranges from 0 (maximum dissimilarity) to 1 (maximum similarity). The similarity matrix is represented by cluster analysis (UPGMA) and the statistical significance of all possible groups was determined by the similarity profile test (SIMPROF) (Clarke & Gorley 2006). The SIMPROF is based on a series of permutation tests as null hypothesis and the lack of structuring of the samples. Thus, the test calculates an expected profile similarity permuted 1,000 times from input variables in the subset of samples, which produces a null average profile (i.e., unstructured group) that is statistically compared (999 times) with the real profile similarity by absolute distances (Phi) (Clarke & Gorley 2006).

To check the influence of geographic distance (measured in km) on the similarity matrix for frog species composition among the locations studied, a Mantel test was conducted (Manly 2000). This test correlates matrices using the Z statistic, where Z depends on the number and size of the matrix elements to be compared. Therefore, normalization was carried out in order to transform the Z coefficient (r) to a value ranging from

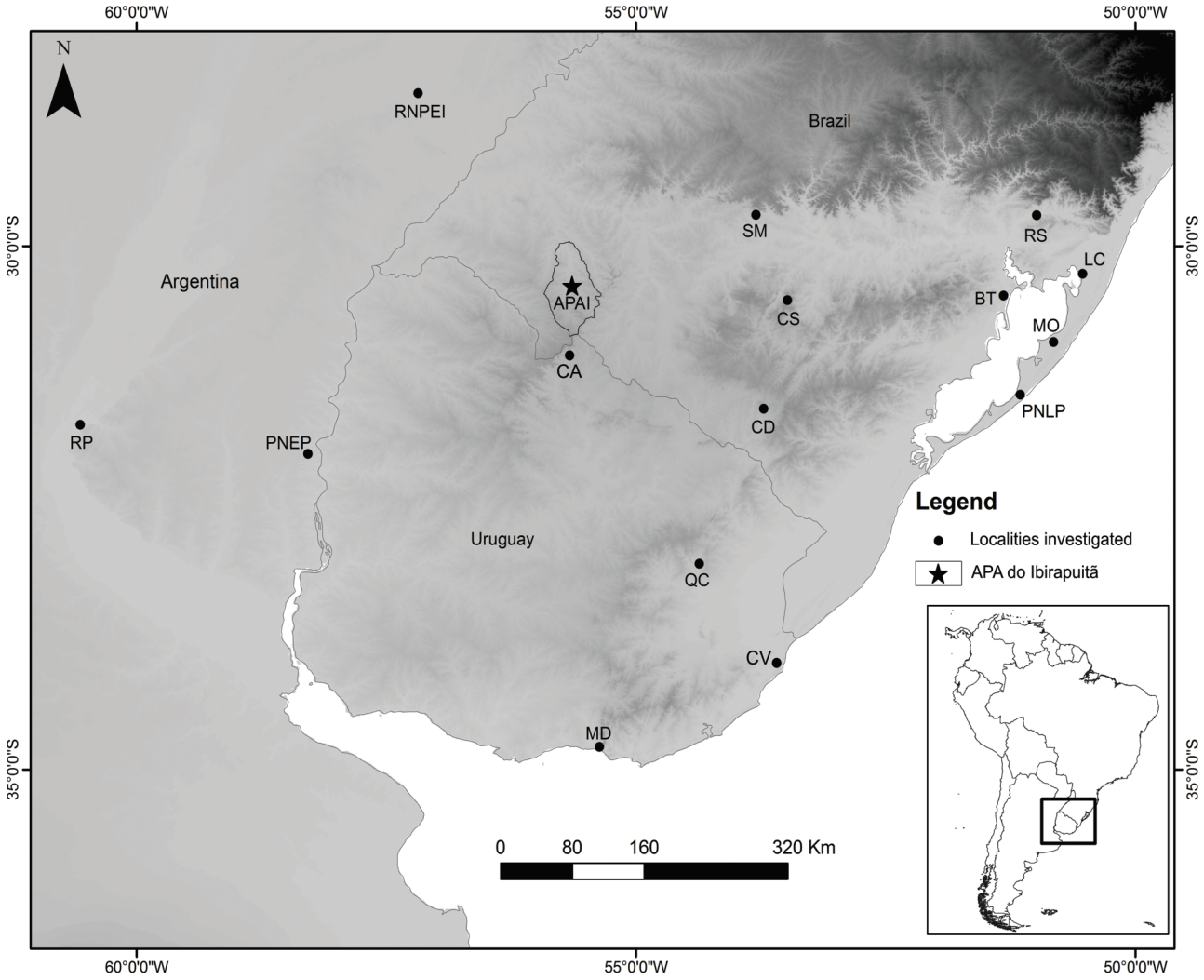

Figure 1. Location of the Área de Proteção Ambiental do Ibirapuitã (APAI) in Rio Grande do Sul, Brazil, and 15 locations as compared to the composition of frog species in the similarity analysis (Abbreviations: Table 2). Map: Carolina Pietczak.

+1 to -1, and statistical significance was determined using 5,000 Monte Carlo permutations (Smouse et al. 1986).

Results

The study resulted in 32 anuran species being recorded, belonging to six families: Alsodidade (1 species), Bufonidae (6 species), Hylidae (11 species), Leptodactylidae (12 species), Microhylidae (1 species) and Odontophrynidae (1 species) (Table 1). A total of 26 species were recorded during field activities (Figures 2 and 3) and six other species (*Dendropsophus nanus*, *Hypsiboas albopunctatus*, *Leptodactylus fuscus*, *Melanophryniscus sanmartini*, *Rhinella dorbignyi* and *R. fernandezae*), were recorded during the review of scientific collections. The species accumulation curve is still on the rise and the estimators showed a richness of 27.38 (± 0; Bootstrap), 32.38 (± 8.06; Chao II), 32.35 (± 0.01; ICE), 30.91 (± 2.31; Jackknife I) and 35.77 (± 0; Jackknife II) (Figure 4a).

Five reproductive modes were recorded among the 32 species of frogs in the APA and surroundings. Mode 1, with eggs and exotrophic tadpoles in lentic bodies of water, was the most common (n = 18, 57.5%). The second most recorded was mode 11, with eggs in a nest of floating foam in lentic bodies of water (n = 7, 21.2%). The third most used reproductive mode was 30, which consists of a foam nest with eggs and early larval stages in nests built underground (n = 4, 12.1%), followed by mode 2 which consists of eggs and exotrophic tadpoles in lotic water bodies (n = 2; 6.1%). Finally, the least common observation was mode 24, which consists of arboreal eggs that hatch exotrophic tadpoles that fall into lentic bodies of water (n = 1, 3.1%, Table I).

In total the most abundant species for APA and surroundings was *Pseudopaludicola falcipes* (29.3%, n = 130), followed by *Physalaemus biligonigerus* (12.6%, n = 56) and *Hypsiboas pulchellus* (9.53%, n = 42) (Figure 4b). *H. pulchellus* was the species most frequently registered in ponds (68%) and streams (75%) as well as in total water bodies (70%). *Odontophrynus americanus* was the second most commonly occurring species among the total number of sampled water bodies (47%). In streams, *Limnomedusa macroglossa* was the second most frequent species (63%) and occurred only in those environments, followed by *O. americanus* (58%). In ponds, the second most common species was *P. biligonigerus* (58%), followed by *P. falcipes* (50%) (Table 1).

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Table 1. Species of amphibians recorded in the Área de Proteção Ambiental do Ibirapuitã and surrounding areas, Rio Grande do Sul, Brazil. RM = reproductive mode, FR = form of registration (a = adult, t = tadpole, sc = scientific collection, * = recorded in complementary sampling), CO = constancy of occurrence (%), P = pond, S = stream, T = Total (ponds and streams).

Família/Espécie	RM	FR	CO		
			P	S	T
Alsodidae					
Limnomedusa macroglossa (Duméril & Bibron, 1841)	2	a, t	0	63	24
Bufonidae					
Melanophryniscus atroluteus (Miranda-Ribeiro, 1920)	1	a, t	3	8	5
Melanophryniscus devincenzii Klappenbach, 1968	2	a, t	0	4	2
Melanophryniscus sanmartini Klappenbach, 1968	1	sc	-	-	-
Rhinella dorbignyi (Duméril & Bibron, 1841)	1	sc	-	-	-
Rhinella fernadezae (Gallardo, 1957)	1	sc	-	-	-
Rhinella schneideri (Werner, 1894)	1	a, t	10	0	6
Hylidae					
Dendropsophus minutus (Peters, 1872)	1	a, t	3	0	2
Dendropsophus nanus (Boulenger, 1889)	1	sc	-	-	-
Dendropsophus sanborni (Schmidt, 1944)	1	a, t	5	0	3
Hypsiboas albopunctatus (Spix, 1824)	1	sc	-	-	-
Hypsiboas pulchellus (Duméril & Bibron, 1841)	1	a, t	68	75	70
Phyllomedusa iheringii Boulenger, 1885	24	a	0	4	2
Pseudis minuta Günther, 1858	1	a, t	43	4	28
Scinax fuscovarius (Lutz, 1925)	1	a	10	4	8
Scinax granulatus (Peters, 1871)	1	a, t	35	8	25
Scinax squalirostris (Lutz, 1925)	1	a, t	25	13	20
Scinax uruguayus (Schmidt, 1944)	1	a, t	13	4	9
Leptodactylidae					
Leptodactylus chaquensis Cei, 1950	11	a	-	-	-
Leptodactylus fuscus (Schneider, 1799)	30	sc	-	-	-
Leptodactylus gracilis (Duméril & Bibron, 1840)	30	a, t	23	28	25
Leptodactylus latinasus Jiménez de la Espada, 1875	30	a, t	40	17	31
Leptodactylus latrans (Steffen, 1815)	11	a, t	18	0	11
Leptodactylus mystacinus (Burmeister, 1861)	30	a, t	18	42	27
Physalaemus biligonigerus (Cope, 1861)	11	a, t	58	4	38
Physalaemus cuvieri Fitzinger, 1826	11	a	3	0	2
Physalaemus gracilis (Boulenger, 1883)	11	t	3	0	2
Physalaemus henselii (Peters, 1872)	11	a,t, *	-	-	-
Physalaemus riograndensis Milstead, 1960	11	t	20	13	17
Pseudopaludicola falcipes (Hensel, 1867)	1	a, t	50	33	44
Microhilidae					
Elachistocleis bicolor (Guérin-Méneville, 1838)	1	a, t	43	13	31
Odontophrynidae					
Odontophrynus americanus (Duméril & Bibron, 1841)	1	a, t	40	58	47

Cluster analysis between grassland localities showed the formation of five groups with more than 50% similarity in frog species composition: Group 1 comprised localities of flat terrain and low elevation (30 m) (Hasenack et al. 2010); Group 2 consisted of localities from relatively mild to strongly ridged (mountainous areas) with altitudes between 30-500 m (Evia & Gudynas 2000, Hasenack et al. 2010); Group 3 consists of localities in the Coastal Plain of Rio Grande do Sul (Zaions 1989); Group 4 consists just one locality in a coastal plain of Uruguay; Group 5 consists of localities lowlands of de chaquean region in Argentina (Morrone 2010) (Figure 5). The Mantel test showed that anuran communities geographically closest are also more similar in composition ($r = 0.64$, $p < 0.01$).

Discussion

Species richness for the APA do Ibirapuitã and surroundings is high, representing about 40% of amphibians known to exist in Brazilian Southern Grasslands (Santos et al. 2014) and approximately 55% of the total known species for the Uruguayan Savanna ecoregion (Garcia et al. 2007). Despite the species accumulation curve not showing a trend towards stabilization, the richness estimators showed a maximum value very close to the total obtained after incorporate records in scientific collections. At least four other species of frogs (*Leptodactylus furnarius, Melanophryniscus langonei*, *Rhinella achavali* and *Scinax nasicus*), have confirmed occurrence in relatively nearby locations near the study area (e.g., Maneyro & Carreira 2012), which, if present in

Table 2. Communities of amphibians in native open grasslands compared with the records obtained in the Área de Proteção Ambiental do Ibirapuitã, Rio Grande do Sul, Brazil.

Localities	References	Abbreviation
Área de Proteção Ambiental do Ibirapuitã	Present study	APAI
Butiazais de Tapes, Rio Grande do Sul, Brazil	Borges-Martins et al. 2007	BT
Caçapava do Sul, Rio Grande do Sul, Brazil	Both et al. 2011	CS
Campo del Abasto, Rivera, Uruguay	Maneyro 2008	CA
Candiota, Rio Grande do Sul, Brazil	Di-Bernardo et al. 2004	CD
Cerro Verde, Rocha, Uruguay	Bardier & Maneyro 2015	CV
Lagoa do Casamento, Rio Grande do Sul, Brazil	Borges-Martins et al. 2007	LC
Maldonado, Uruguay	Canavero et al. 2008	MD
Mostardas, Rio Grande do Sul, Brazil	Machado & Maltchik 2010	MO
Parque Nacional da Lagoa do Peixe, Rio Grande do Sul, Brazil	Machado et al. 2012, Moreira et al. 2010	PNLP
Parque Nacional El Palmar, Entre Ríos, Argentina	Gangenova et al. 2012	PNEP
Quebrada de los Cuervos, Departamento de 33, Uruguay	Prigioni et al. 2011	QC
Reserva Natural Provincial Esteros del Iberá, Corrientes, Argentina	Ingaramo et al. 2011	RNPEI
Rio dos Sinos, Rio Grande do Sul, Brazil	Moreira et al. 2008	RS
Rio Paraná, Entre Ríos, Argentina	Peltzer & Lajmanovich 2004	RP
Santa Maria, Rio Grande do Sul, Brazil	Santos et al. 2008	SM

future studies would indicate a total richness of 36 frog species in the APA do Ibirapuitã.

The characteristics of the APA do Ibirapuitã landscape are reflected by the low diversity of reproductive modes, and by the predominance of generalized modes and/or those that favor resistance to desiccation (modes 1 and 11, 78.7%). Reproductive mode diversity tends to be higher in forested habitats (most representative of terrestrial reproductive modes), mainly due to high temperatures, high rainfall and high atmospheric humidity, which in combination prevent egg desiccation (Prado et al. 2005). Indeed, the hypothesis that sites with high humidity levels support higher diversity of reproductive modes than drier sites has been reforced in studies in geographic scale of Brazilian biomes and inside the Atlantic Forest biome (Vasconcelos et al. 2010, Da Silva et al. 2012, respectively). Due to both summer drought associated with grassy biome and the physical homogeneity of open habitats, more general reproductive modes were expected to predominate in our study area (Duellman & Trueb 1994, Prado et al. 2005, Santos et al. 2008). Likewise, many intermediate modes, such as foam nests, are found in dry areas or seasonal environments with high temperatures and flutuating water levels (Crump 2015). These environmental features also are present in the study area, what can explain the high contribution of species with foam nests (modes 11 and 30). In this context, we highlight that future studies are needed to better understand and access patterns of reproductive modes inside the geographical scale of southern grassland ecosystems.

The composition of anurofauna recorded in this study is similar to other frog communities sampled in Pampa (e.g., Di-Bernardo et al. 2004, Santos et al. 2008, Both et al. 2011, Prigioni et al. 2011), with greatest representation from the families Hylidae and Leptodactylidae. A similar pattern has been reported in other Brazilian biomes, including Cerrado (Kopp et al. 2010), Atlantic Forest (Bastiani & Lucas 2013, Garey et al. 2014), Amazon (Bernarde 2007), Pantanal (Prado et al. 2005) and Caatinga (Vieira et al. 2007), locally reflecting historical patterns of global diversification of Hylidae, and Neotropical diversification of Leptodactylidae, respectively (Frost 2014).

Most species that make up the frog community in the APA do Ibirapuitã are characteristic of open areas (natural and/or anthropic) of Brazil, and those of neighboring countries (sensu Santos et al. 2014). Among the species recorded, *Melanophryniscus devincenzii* and *M. sanmartini* are threatened with global extinction, and are currently in the endangered category (EN) (IUCN 2014) due to restricted geographic distribution and habitat fragmentation. *M. sanmartini* is still considered threatened at the state level, the category Near Threatened (NT) (Rio Grande do Sul 2014).

The pattern of distribution of species abundance in the study area can be interpreted as indicative of strong species dominance, as the most abundant species in the Whittaker diagram represented more than twice of the relative abundance of the second species. This heterogeneity in the distribution of abundance in the study area may be a consequense of environmental characteristics at regional level (Tews et al. 2004, Werner et al. 2009, Magurran 2013), including shallow and stony soils with low moisture retention and drought in summer; these and other factors have lead researchers to indicate the site as a stressful environment (Boldrini 2009, Boldrini & Longhi-Wagner 2011). Indeed, these environmental conditions cause desiccation of most ponds and streams in the hottest period of the year, restricting the supply of breeding sites, and a pattern of strong species dominance is expected under such environmental stress (Odum 1988). This pattern of distribution of species abundance related to the environmental characteristics also occurs in other groups like mammals (Gheler-Costa et al. 2012), birds (Enríquez-Lenis et al. 2006) and reptiles (Cruz-Elizalde & Ramírez-Bautista 2012).

Most of the species recorded in the study area are considered generalists with respect to habitat use (62.5% occurred both in ponds and in streams). But at least two species, including *Limnomedusa macroglossa* and *Melanophryniscus devincenzii*, are typical of streams. *L. macroglossa* exhibits high habitat affinity, found exclusively in rocky areas associated with rivers and streams with clear water (Di-Bernardo et al. 2004, Kwet et al. 2010, Maneyro & Carreira 2012). The three most abundant species (*Pseudopaludicola falcipes, Physalaemus biligonigerus* and

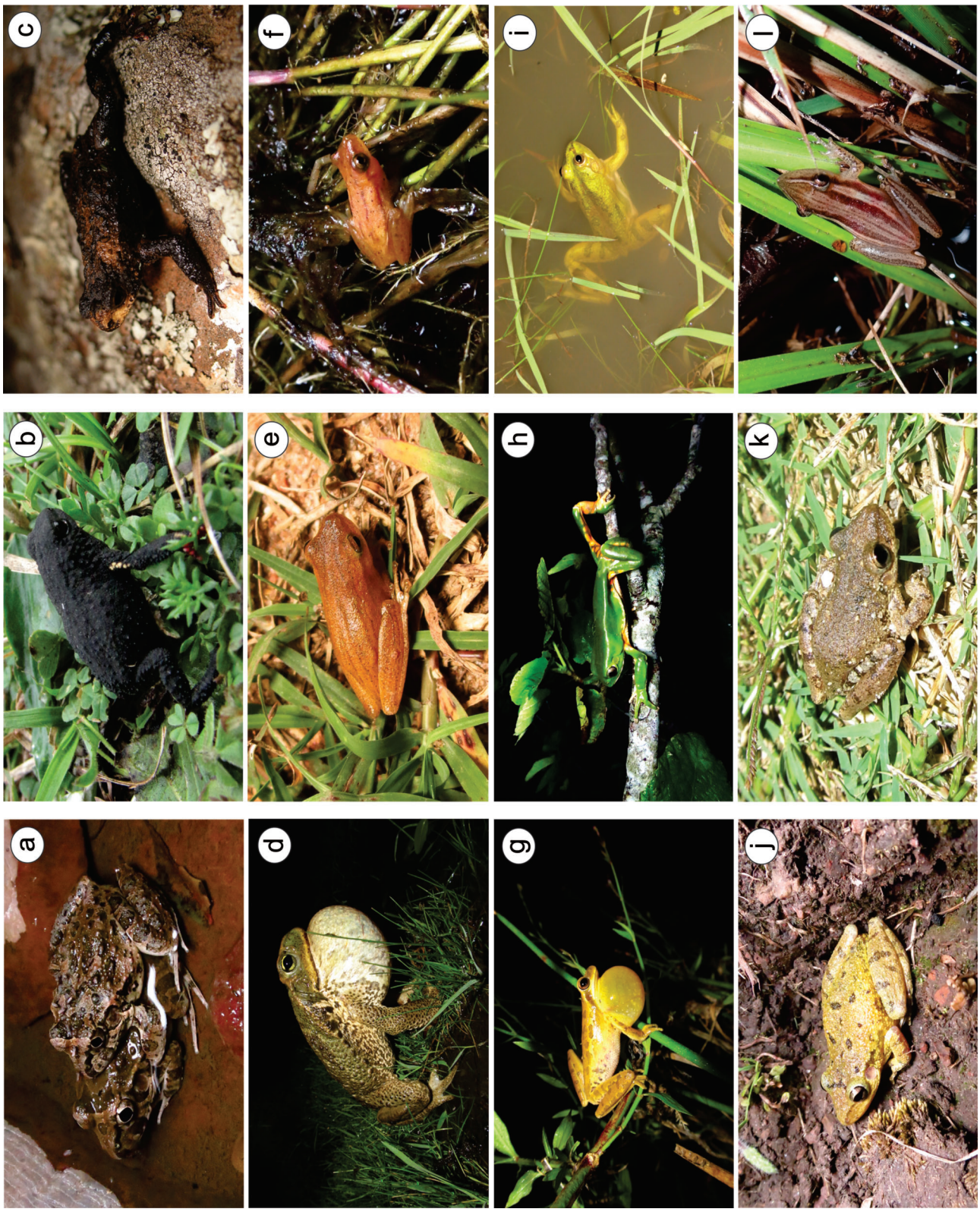

Figure 2. Amphibians species found in the Área de Proteção Ambiental do Ibirapuitã, Rio Grande do Sul, Brazil. a = *Limnomedusa macroglossa*, b = *Melanophryniscus atroluteus*, c = *M. devincenzii*, d = *Rhinella schneideri*, e = *Dendropsophus minutus*, f = *D. sanborni*, g = *Hypsiboas pulchellus*, h = *Phyllomedusa iheringii*, i = *Pseudis minuta*, j = *Scinax fuscovarius*, k = *S. granulatus*, l = *S. squalirostris*. Photos: Ana Maria R. Bolzan (a, e, f, h, i, j,), Suélen S. Alves (l), Tiago G. dos Santos (b, c), Vitor F. Oliveira (d, g, k).

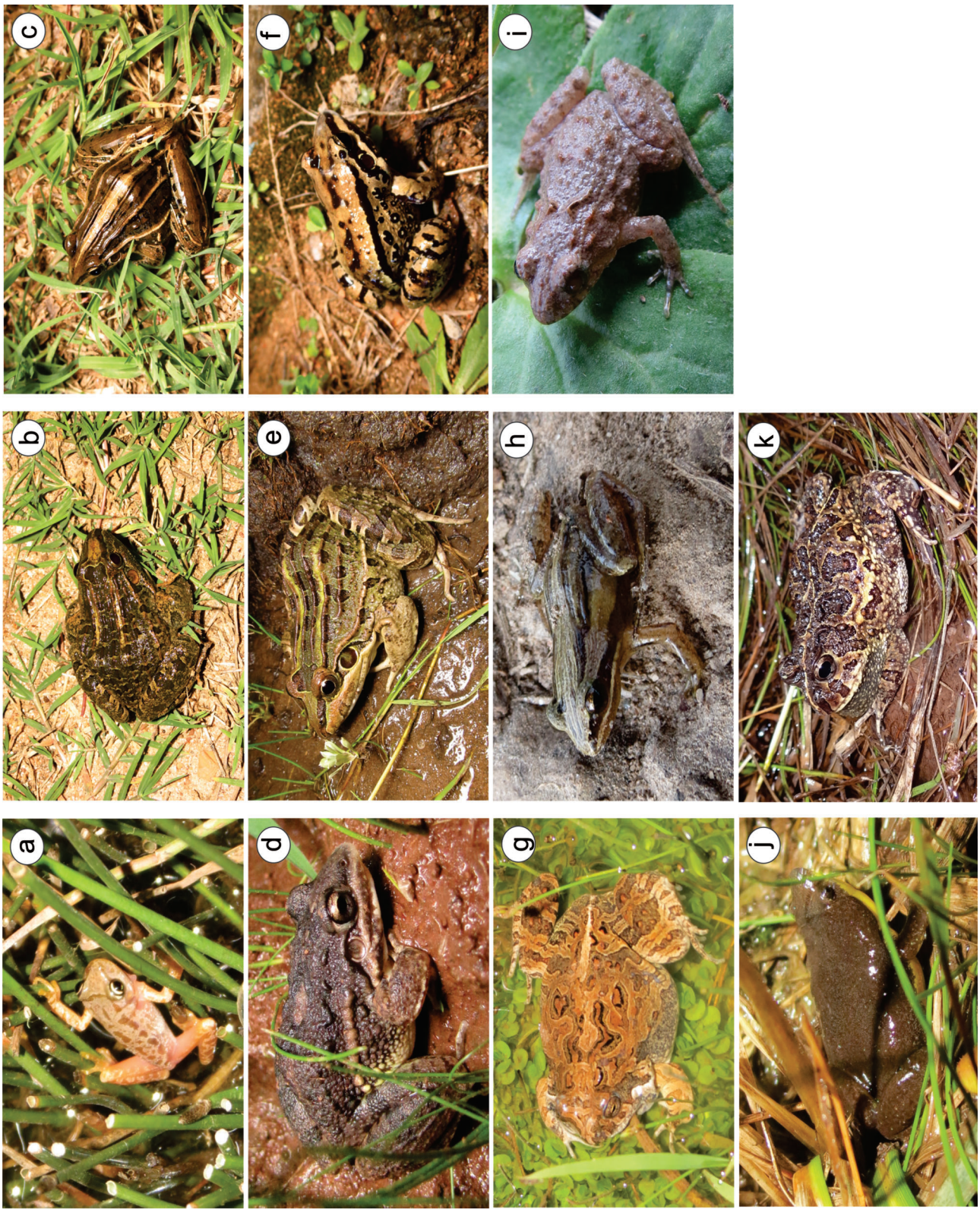

Figure 3. Amphibians species found in the Área de Proteção Ambiental do Ibirapuitã, Rio Grande do Sul, Brazi. a = *Scinax uruguayus*, b = *Leptodactylus chaquensis*, c = *L. gracilis*, d = *L. latinasus*, e = *L. latrans*, f = *L. mystacinus*, g = *Physalaemus biligonigerus*, h = *P. henselii*, i = *Pseudopaludicola falcipes*, j = *Elachistocleis bicolor*, k = *Odontophrynus americanus*. Photos: Ana Maria R. Bolzan (a, b, c, f, g, h, i, j), Suélen S. Alves (k), Tiago G. dos Santos (d), Vitor F. Oliveira (e).

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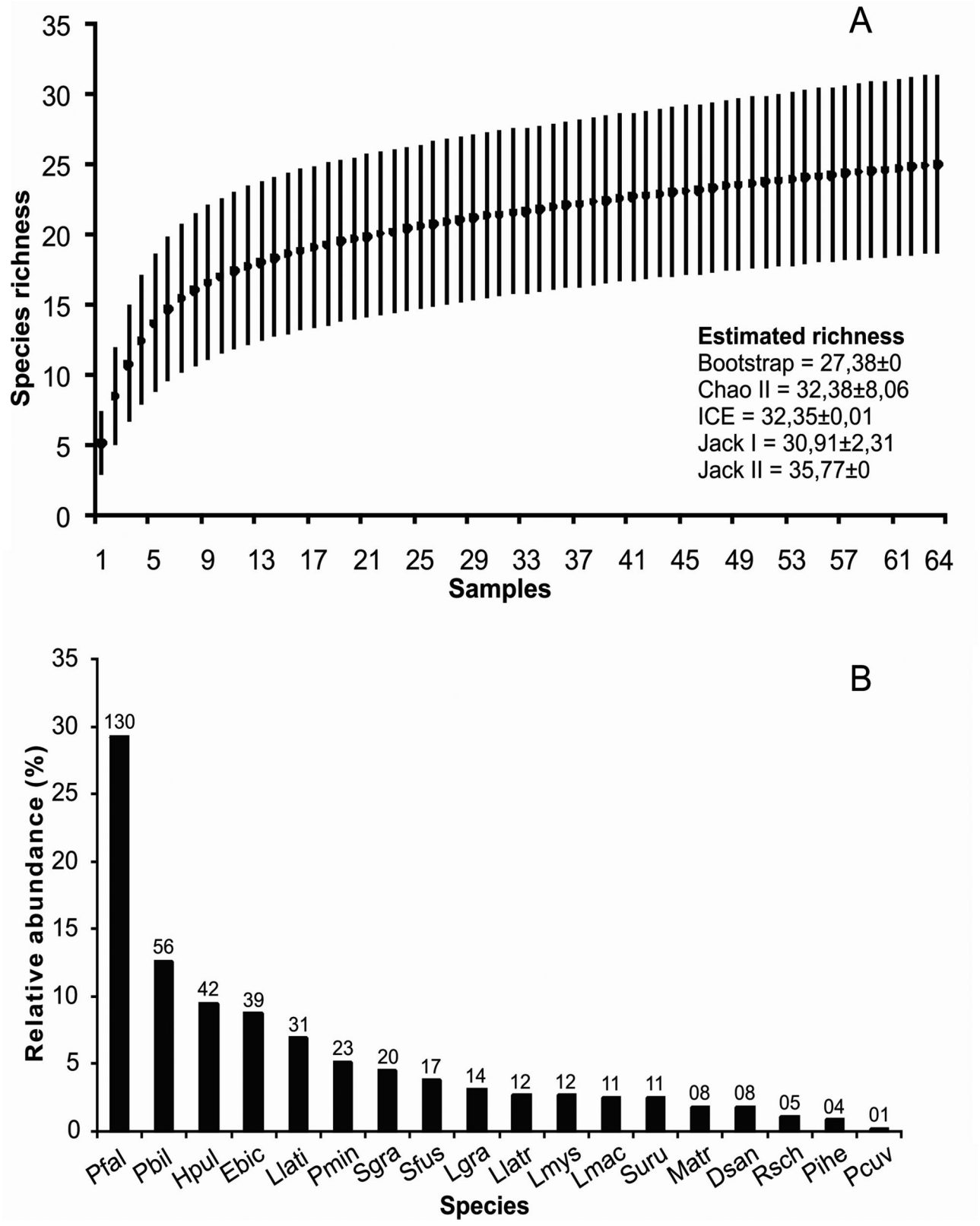

Figure 4. Frog species accumulation curve (a). The line represents the average curve generated from 500 randomizations and the vertical bars represent 95% confidence intervals. Whittaker plot (b), bars represents the relative abundance (%) and top numbers the total abundance of species in calling activity registered in the Área de Proteção Ambiental do Ibirapuitã, Rio Grande do Sul, Brazil. Dsan = *Dendropsophus sanborni*, Ebic = *Elachistocleis bicolor*, Hpul = *Hypsiboas pulchellus*, Llati = *Leptodactylus latinasus*, Llatr = *L. latrans*, Lmys = *L. mystacinus*, Lmac = *Limnomedusa macroglossa*, Matr = *Melanophryniscus atroluteus*, Pihe = *Phyllomedusa iheringii*, Pbil = *Physalaemus biligonigerus*, Pcuv = *P. cuvieri*, Pmin = *Pseudis minuta*, Pfal = *Pseudopaludicola falcipes*, Rsch = *Rhinella schneideri*, Sgra = *Scinax granulatus*, Suru = *S. uruguayus*.

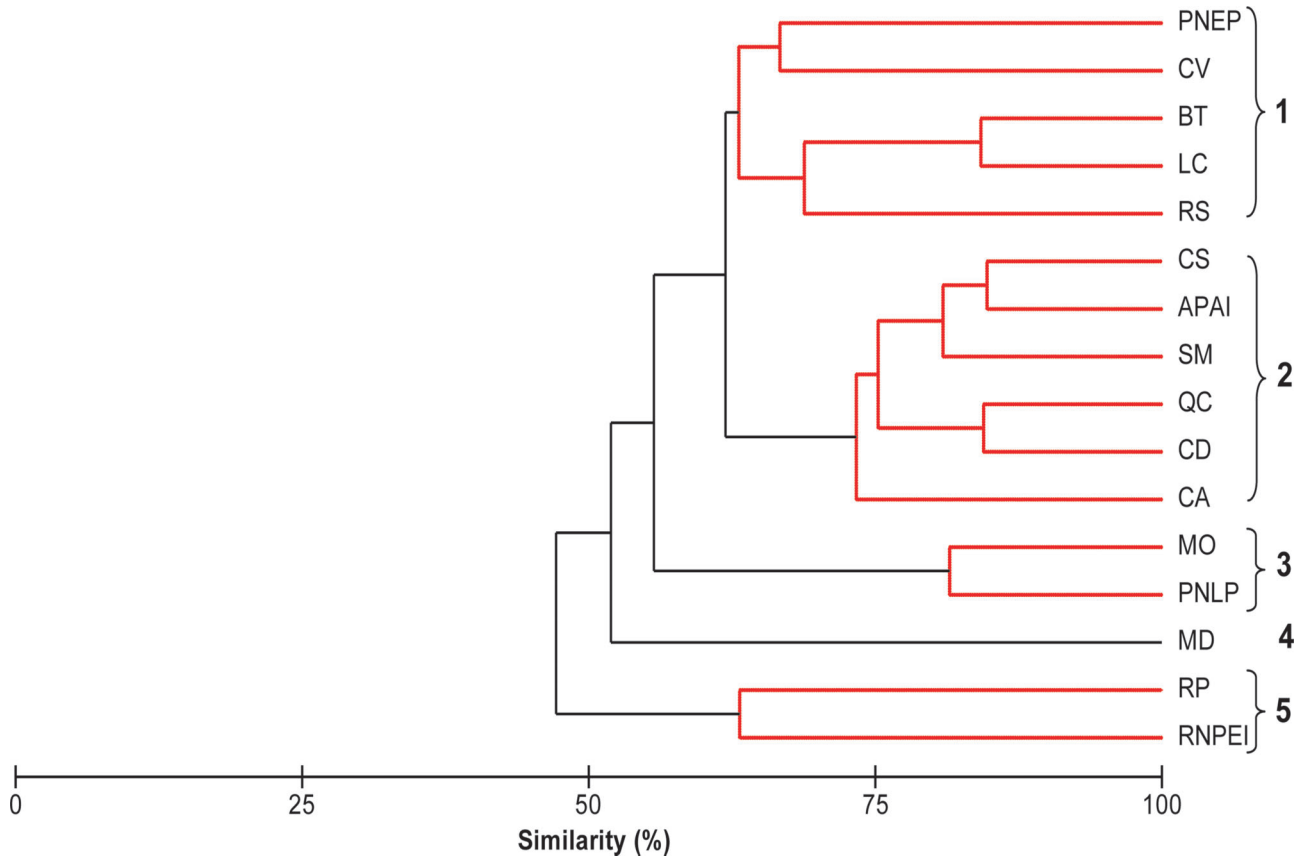

Figure 5. Similarity between anuran communities in grassland areas of the southernmost of South America, using the Coefficient of Geographical Resemblance (CGR). Groups: 1 = locations composed of flat terrain up to 30m above sea level; 2 = composed of localities with ridged and/or smooth ground in Brazil and Uruguay; 3 = consists of localities belonging to the coastal region of Rio Grande do Sul; 4 = consists of Maldonado, Uruguay localities; 5 = consists of Argentinian localities with Chaquenha influence (Abbreviations: see Table 2). The black lines represent statistically significant groups ($p < 0.05$), while the red lines indicate statistical absence ($p > 0.05$) in the subgroup structure.

Hypsiboas pulchellus) were also occurred more frequently among the total sampled bodies of water, and are very common species in Uruguayan Savannah ecoregion grasslands (Santos et al. 2008, Machado & Maltchik 2010, Maragno et al. 2013).

The results of the similarity analysis were heavily influenced by geographical proximity between locations, since the geographically closest locations (such as most locations in flat terrain of Rio Grande do Sul in Group 1, and those in center-southern Rio Grande do Sul and center Uruguay in Group 2) were also more similar in anurofauna. On the other hand, locations geographically apart (such as those in lowlands of de chaquean region of Argentina in Group 5, and the cost plain of Uruguay in Group 4) presented low similarity in anurofauna with other locations. Similar effects of geographical proximity were previously reported on analyses of taxonomic composition in anuran assemblages (see Bertoluci et al. 2007; Santos et al. 2009; Iop et al. 2011) and these spatial structures can be related to neutral process of dispersion (Leibold et al. 2004), as wells as to spatially-structured environment (Legendre & Legendre 2012). Our study was not specifically delineated to assess the origins of these spatial structures, nor can exclude other possible effects like anthropogenic pressure, but is expected that neutral processes are dominant in open areas (Prado & Rossa-Feres 2014).

Anurofauna of Group 1 is primarily under Atlantic and/or lagoon influences, including species such as *Hypsiboas faber* and *Trachycephalus mesophaeus* present in some locations, and two exclusive species (*Argenteohyla siemersi* and *Melanophryniscus montevidensis*) in one location of north coastal Uruguay. The former two species utilize forest areas (primary or secondary) and forest edges for reproduction (Armstrog & Conte 2010, Kwet et al. 2010, Maffei et al. 2011), while the second couple reproduces in coastal environments (Maneyro & Carreira 2012).

The Group 2 is associated to a landscape in mosaic composed by grasslands in hills with patches of forests on the slopes of hills and rocky outcrops, and generally narrow valleys with streams with riparian vegetation (Evia & Gudynas 2000). At least three species give consistency to this group: *Limnomedusa macroglossa*, *Phyllomedusa iheringii* and *Scinax uruguayus*. *L. macroglossa* shows high habitat affinity and its distribution is always associated with rocky (Gudynas & Gehrau 1981, Achaval & Olmos 2007, Maneyro & Carreira 2012). *P. iheringii* is associated with riparian forest and shrub vegetation, and has a preference for lotic water bodies (Maneyro & Carreira 2012). *S. uruguayus* is a typical hill species, and uses environments with grass and shrub vegetation (Maneyro & Langone 2001, Di-Bernardo et al. 2004, Prigioni et al. 2011).

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Group 3 is related to assemblages with low species richness in two coastal plain locations of Rio Grande do Sul state that feature flat terrain and low altitude (0 to 30m) (Hasenack et al. 2010). These locations resemble by most wide distributed anuran species and just one species (*Odontophrynus maisuma*) considered an integral part of the coastal faunal association (Maneyro & Carreira 2012).

Group 4 represent a location in south cost plain of Uruguay (Maldonado) characterized by a low richness of anurans (Canavero et al. 2008). All species recorded in this location are generalists and occurred in the other groups. Finally, most species in Group 5 are related to the eastern portion of the Brazilian states of Mato Grosso, Mato Grosso do Sul, Paraná, Santa Catarina, Rio Grande do Sul, and in neighboring countries (Maneyro & Carreira 2012, Frost 2014, IUCN 2014). Seven species give consistency to this group: *Hypsiboas raniceps, Leptodactylus podicipinus, Lysapsus limellum, Phyllomedusa azurea, P. hypocondrialis, Physalaemus albonotatus* and *Pseudis platensis* (Peltzer & Lajmanovich 2004, Ingaramo et al. 2012).

Our work shows that native grasslands of Pampa biome are important for maintenance of anuran diversity, since the anurofauna in the APA do Ibirapuitã shows high species richness, typical of natural open areas, and includes species endemic to subtropical grasslands of South America. The APA lies within a region with the best-preserved grasslands of the Pampa biome in Brazil (Boldrini 2009, Boldrini & Longhi-Wagner 2011), with high priority for amphibian conservation (MMA 2007). This includes the presence of globally endangered species (e.g., *Melanophryniscus devincenzii*, *M. sanmartini*) and species with high habitat affinity (e.g., *Limnomedusa macroglossa*).

The results of our work are the first knowledge base for anurofauna in the APA do Ibirapuitã, paving a path for future conservation actions for CUs, and stimulating future studies in this area. We highlighted that the lack of effective legislative restrictions on land use in this type of environmental protection area (Brazilian Federal Law n°. 9985/2000, art. 14 and 15) is a serious problem for the conservation of regional biodiversity, since this stimulate the increase of monoculture practices and power production responsible by fragmentation and habitat loss. While traditional extensive livestock is considered a sustainable activity promoting conservation of freshwater environments used as breeding sites by anurans at the grassland ecosystems (Moreira et al. 2015), like the APA do Ibirapuitã, fast changes in the productive matrix (mainly soybean cultivation and expansion of wind energy parks) represents a challenge for biodiversity conservation in this region that needs urgent attention.

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