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Amphibians of grasslands in the state of Paraná, southern Brazil (*Campos Sulinos*)

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Abstract. Amphibian conservation depends on knowledge about species composition and distribution. This emphasizes the need for inventories, especially in poorly sampled areas. This is the case of Southern grasslands (*Campos Sulinos*) associated with *Araucaria* forest in the state of Paraná, southern Brazil. We sampled amphibians in 105 environments from 2004 to 2013 using transect sampling, active search and surveys at breeding sites. We found 61 anuran species and two caecilians. This is the first comprehensive list of amphibian species inhabiting grasslands in Paraná. This landscape deserves high conservation priority before these natural grasslands vanish.

Key words. Anura, regional pool diversity, geographic distribution, inventory.

Introduction

Amphibians have a wide variety of life histories strategies, playing important roles in aquatic and terrestrial food webs (Wells, 2007). They are particularly sensitive to environmental changes, due to their morphological and physiological traits, such as a highly permeable skin and complex life cycles (Duellman and Trueb, 1986). As a result, habitat loss, fragmentation, accompanied by microclimatic changes in temperature and humidity, are the main threats to local populations (Cushman, 2006; Becker et al., 2007).

Recent research involving Brazilian amphibians has focused on species richness patterns and spatial distributions in poorly sampled regions (e.g., Conte and Rossa-Feres, 2006; Bastazini et al., 2007; Canelas and Bertoluci, 2007; Menin et al., 2008). Nonetheless, many gaps remain to understand the geographic distribution of amphibian species, such as in the grasslands (*Campos Sulinos*) of southern Brazil (reviewed in Santos et al., 2014).

Campos Sulinos are the oldest vegetation formation in southern Brazil formed during the Pleistocene (Behling and Pillar, 2007). Over time, warmer and wetter climates have favored the introgression of patches of forests scattered among grasslands. Together, these grasslands and scattered forests form a natural mosaic landscape (Maack, 2012).

The grasslands of the South Brazilian Plateau, which are associated with *Araucaria* forest (Pillar and Vélez, 2010), occur in the states of Paraná, Santa Catarina, and Rio Grande do Sul. This phytophysiognomy is characterized by low hills, well-distributed rainfall

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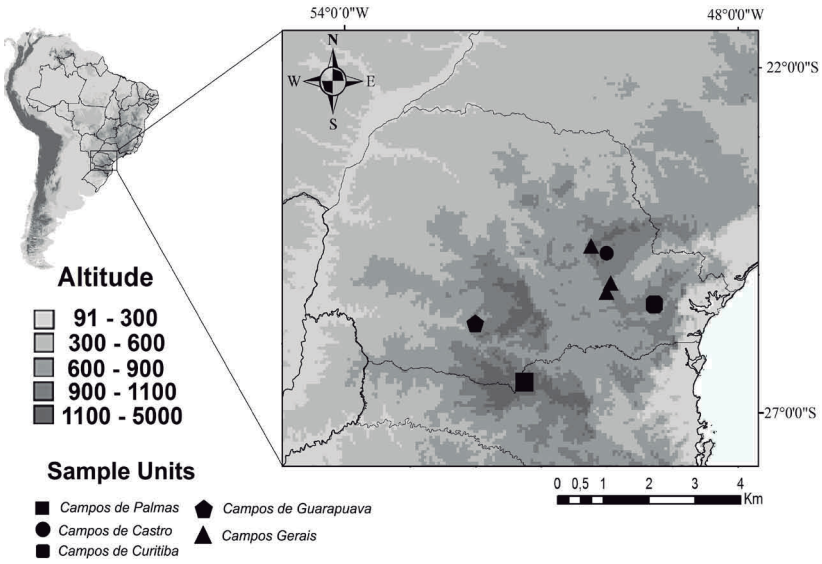

Figure 1. Map of South America showing the state of Paraná and sampling locations.

throughout the year, average annual temperatures varying between 16 and 22 ºC (Nimer, 1990), with eventual frosts and snow in the winter. Grasslands associated with *Araucaria* forest originally comprise approximately 30,000 km^2 in the state of Paraná (Maack, 2012), and most of it is being conversed to agriculture (including forestry with exotic species) and pasture (MMA, 2000).

Information about amphibian species distribution in the grasslands of the state of Paraná (southern Brazil) is scarce. This gap poses difficulties in understanding potential areas of endemism, conservation and taxonomic status of populations, and the human impact on their habitats (Crivellari et al., 2012). Here, we start to fill this knowledge gap by providing a list of amphibians inhabiting grasslands in Paraná, southern Brazil.

Material and Methods

Sampling

We sampled 105 environments, totalizing three rivers, nine permanent and 24 temporary pools, nine marshes, 13 forest transects, 18 streams and 29 ponds using three sampling methods: transection sampling (Jaeger, 1994), survey at breeding site (Scott Jr. and Woodward, 1994), and active search (*sensu* Conte and Rossa-Feres, 2007).

We conducted 10 visits between July 2005 and April 2006 and sampled 15 sites in municipal parks in the city of Curitiba. We sampled 13 sites between September 2010 and March 2011 in seven visits in and near Caxambu State Park (PEC); in *Campos Gerai*s, 12 visits and 52 environments between January 2013 and December 2013 in Vila Velha State Park (VVSP; 16 sites), Guartelá State Park (GSP; 15 sites), Campos Gerais National Park (CNP; 21 sites); nine visits in two periods: November 2001 to January 2002 and January, February, September, October, November and December 2006 and 11 sites in and near Santa Clara State Park (SCSP); 12 visits and 14 sites in Palmas Wildlife Refuge (PWR) between January 2013 and December 2013 (Table 1, Figure 1).

Voucher specimens are housed in the herpetological collections of the Capão da Imbuia Natural History Museum (MHNCI) in Curitiba, Paraná, and in the Department of Zoology and Botany (DZSJRP), State University of São Paulo, São José do Rio Preto, São Paulo, Brazil.

Habitat use

We considered generalists (G) in habitat use those species found in forests, forest edges, and open areas; as forest specialist species (F) those only found in the interior of forest; as open area species (O) those found only in open areas; as forest edge species (FE) those found only in forest and forest edges; and as open edge species (OE) those found in open areas and occasionally in forest edges. Use of habitat was recorded for calling males or amplectant pairs. Species which were recorded a single individual were not assessed for their habitat use.

Table 1. Sampling sites in the grasslands associated with *Araucaria* forest in the state of Paraná, Southern Brazil. National Park (NP), State Park (SP), Wildlife Refuge (WR), City Park (CP). *Municipal (City) Parks in Curitiba: Bosque João Paulo II (4.6 ha), Bosque Municipal do Alemão (3.8 ha), Barigui City Park (140 ha), G. Iberê de Mattos City Park (15.20), Iguaçu City Park (177.8 ha), Franchete Rischibieter Botanical Garden (22.49 ha).

City	Protected Areas	Geographical Coordinates	Altitude
Ponta Grossa, Castro, Carambeí	NP dos Campos Gerais	-49.9500 -25.0500	980
Ponta Grossa	SP de Vila Velha	-50.0000 -25.2333	950
Tibagi	SP do Guartelá	-50.2333 -22.5667	1125
Palmas	WR dos Campos de Palmas	-51.6167 -26.5167	1144
Curitiba	CP	-49.2500 -25.4167	920
Castro	SP do Caxambú	-50.0000 -24.6667	1025
Candói, Pinhão, Foz do Jordão	SP Santa Clara	-51.9833 -25.6000	890

Results and Discussion

We recorded 63 amphibian species, being 61 anurans and 2 caecilians (Table 2, Figs. 2-9). Morphological and acoustic parameters indicate that 6 taxa may represent new species (Table 2). Hylidae (N = 30, 48%) was the most species-rich family, followed by Leptodactylidae (14, 22%), Bufonidae (7, 11%), Odontoprhynidae (3, 5%), Microhylidae (2, 3%), Caeciliidae (2, 3%), Brachycephalidae, Ranidae, Centrolenidae, Craugastoridae, and Hylodidae, with one species each. We found 21 species were generalist in habitat use, 20 species occurred only in open areas, and 19 species were associated to forest or forest edges (Table 2).

The species found represent 44% of richness known from the state of Paraná (Conte et al., 2010) and 6% of Brazil (Segalla et al., 2012). The highest species richness observed for Campos Sulinos of Atlantic Forest domain are mainly due to the location of these grasslands scattered among forest formations present in the Southern Plateau, beyond the ecotone influences of adjacency with other phytophysionomies, likely campos of Pampa on south, and Cerrado savanna formations on north (*sensu* Overbeck et al., 2007). Probably, due these intermixing vegetation matrix and the variety of water body types herein allows different patterns of habitat use (Conte and Rossa-Feres, 2007), enabling the similar species richness among habitat (21 species of generalist, 19 species of forest and forest edges, 20 species in open areas) as a reflect of these pattern.

Of those species that not yet been formally described or species taxonomically difficult which did not have clear distribution patterns (Table 2). Clearly, both Linnean (taxonomic uncertainty) and Wallacean (distribution uncertainty) shortfalls (Bini et al., 2006) are still a challenge in Brazil and hinder conservation efforts. The lack of taxonomic studies and the recognition of many taxa under a single nominal species may underestimate the diversity of amphibians in Brazil and misinterpretation of ecological patterns and geographical distribution (Conte et al., 2010). This effect is even more worrying considering that the geographic distribution is one of the main criteria used to define the threat status of a species (IUCN, 2012). Thus, the distribution of many species can be over or underestimated. Additionally, many species are classified as "Data Defficient" in regional or national red lists, reflecting the initial stage of knowledge about anurofauna at large part of Brazil (e.g. Garey et al., 2014; Guerra and Bastos, 2014).

Only 19% of the species (N = 12) were found using more than one survey method. A total of 42 species (67%) was found by searching in breeding sites, while only five (8%) species were found by active search, and four (6%) by transect method (Table 2). Clearly more species are found via by searching in breeding sites (Armstrong and Conte, 2010: 84%, rain forest; Bernarde et al., 2013: 66%, Amazon; Santos et al., 2009: 100%, semideciduous forest; Conte and Rossa-Feres, 2007: 84%, *Araucaria* forest; Conte et al., 2013: 80%, *Cerrado*). Nonetheless, for a more complete record, other methods must also be used (Conte and Rossa-Feres, 2007; Silva, 2010), especially for species that reproduce away from typical bodies of water (e.g., *Adenomera nana* and *Ischnocnema henselii*) or bromeliads (e.g., *Melanophryniscus alipioi* and *M. vilavelhensis*), as well as cryptic and fossorial species (*Microcaecilia* sp. and *Siphonops paulensis*).

While many species are widely distributed (Frost, 2014), more than 70% occur in less than 14% (N = 15) of the sampled environments (Figure 10), with several found in two or fewer (e.g., *Melanophryniscus alipioi*, *Phyllomedusa rustica, Hypsiboas pulchellus, Trachycephalus dibernardoi, Haddadus binotatus,* and *Pseudis cardosoi*; Table 2, Figure 10).

It is known amphibians exhibited habitat restrictions with respect to breeding sites (Cardoso et al., 1989; Haddad and Prado, 2005), and dispersal ability limited (Gibbs, 1998; Smith and Green, 2005). These peculiarities of amphibians emphasize the importance of protected areas, especially because loss of habitat is the main conservation problem for amphibians in Brazil

Table 2. Amphibian species recorded in grasslands associated with *Araucaria* forest in the state of Paraná, southern Brazil. Curitiba City Parks (CCP); Caxambu State Park (CSP), Vila Velha State Park (VVSP), Guartelá State Park (GSP), Campos Gerais National Park (CGNP), Santa Clara State Park (SCSP), Palmas Grasslands Wildlife Refuge (PWR). Distribution pattern: Ample (A), Atlantic Forest (AF), *Araucaria* forest (RF), new species (NEW), taxonomic difficulties (TAX). Habitat: Generalist, Forest (F), Open (O), Forest Edge (FE), Open Edge (OE). Type of survey: T – transect, A – active search, B – breeding sites.

	Distribution	Habitat	Sampling method	CCP	CSP	VVSP	GSP	CGNP	SCSP	PWR
ORDER ANURA										
Family Brachycephalidae										
Ischnocnema henselii (Peters,1872)	AF	F	T		1	1	1	1	1	1
Family Bufonidae										
Rhinella abei (Baldissera, Caramaschi & Haddad, 2004)	AF	FE	A, B,T	1	1	1	1	1		
R. henseli (A. Lutz,1934)	RF	FE	B						1	
R. icterica (Spix,1824)	A	OE	A, B,T	1				1	1	1
Melanophryniscus sp.1 (gr. *tumifrons*)	NEW	A	A, B			1				
Melanophryniscus sp.2 (gr. *tumifrons*)	NEW	F	B							1
M. vilavelhensis Steinbach-Padilha, 2008	RF	A	B			1	1			
M. alipioi Langone, Segalla, Bornschein & de Sá, 2008	AF	F	T					1		
Family Centrolenidae										
Vitreorana uranoscopa (Müller, 1924)	AF	F	B		1			1	1	
Family Craugastoridae										
Haddadus binotatus (Spix, 1824)	A	F	A, B					1		
Family Odontoprhynidae										
Odontophrynus americanus (Duméril & Bibron, 1841)	A	FE	A, B, T		1	1	1	1	1	1
Proceratophrys brauni Kwet & Faivovich, 2001	RF	FE	B			1	1		1	1
P. boiei (Wied-Neuwied, 1825)	AF	F	A, B, T	1	1			1		
Family Hylodidae										
Crossodactylus cf. *caramaschii*	TAX	F	B		1		1	1		
Family Hylidae										
Aplastodiscus albosignatus (A. Lutz & B. Lutz, 1938)	AF	FE	B, T	1	1	1	1	1		
A. perviridis A. Lutz in B. Lutz, 1950	A	FE	B, T	1	1	1	1	1	1	1
Bokermannohyla circumdata (Cope, 1871)	AF	F	B				1	1		
Dendropsophus microps (Peters, 1872)	AF	FE	B	1	1	1		1		
D. minutus (Peters, 1872)	A	FE	B	1	1	1	1	1	1	1
D. sanborni (Schmidt, 1944)	A	FE	B		1	1		1		1
Hypsiboas albopunctatus (Spix, 1824)	A	OE	B		1	1	1	1	1	
H. bischoffi (Boulenger, 1887)	AF	FE	B	1	1	1	1	1		1
Hypsiboas cf. *stellae*	TAX	OE	B				1		1	1
H. caingua (Carrizo, 1991 "1990")	A	A	B						1	
H. faber (Wied-Neuwied, 1821)	A	FE	A, B, T	1	1	1	1	1	1	1
H. leptolineatus (P.Braun & C. Braun, 1977)	RF	OE	A, B, T						1	1
H. prasinus (Burmeister, 1856)	AF	FE	B	1	1		1	1	1	1
H. pulchelus (Duméril & Bibron, 1841)	A	A	B							1
Phyllomedusa distincta A. Lutz in B. Lutz, 1950	AF	FE	B					1		

Table 2. Continued.

	Distribution	Habitat	Sampling method	CCP	CSP	VVSP	GSP	CGNP	SCSP	PWR
Phyllomedusa tetraploidea Pombal & Haddad, 1992	AF	FE	B		1	1	1		1	
*Phyllomedusa rustic*a Bruschi, Lucas, Garcia & Recco-Pimentel	RF	A	B							1
Pseudis cardosoi Kwet, 2000	RF	A	B							1
Scinax aromothyella Faivovich, 2005	A	FE	B		1	1			1	1
Scinax cf. *catharinae*	TAX	F	B					1	1	
S. fuscovarius (A. Lutz, 1925)	A	FE	A, B, T	1	1	1	1	1	1	
S. granulatus (Peters,1871)	A	FE	A, B, T							1
S. perereca Pombal Jr., Haddad & Kasahara, 1995	AF	FE	B	1	1	1	1	1	1	
S. rizibilis (Bokermann, 1964)	AF	FE	B	1	1	1		1		
Scinax sp. (gr. *ruber*)	NEW	A	B			1	1	1		
S. squalirostris (A. Lutz, 1925)	A	OE	B		1	1	1	1	1	1
S. uruguayus (Schimidt, 1944)	A	A	B				1	1		1
Sphaenorhynchus caramaschii (Toledo, Garcia, Lingnau & Haddad, 2007)	AF	FE	B			1		1		
S. surdus (Cochran,1953)	RF	FE	B	1				1		1
Trachycephalus dibernardoi Kwet & Solé, 2008	RF	F	B					1		
Family Leptodactylidae										
Adenomera aff. *marmorata*	NEW	F	T					1		
Adenomera nana (Müller, 1922)	AF	F	T	1						
Leptodactylus fuscus (Schneider, 1799)	A	A	B				1			
L. gracilis (Duméril & Bibron, 1841)	A	A	B			1	1	1		
L. cf. *latrans*	TAX	FE	B	1	1	1	1	1	1	1
L. labyrinthicus (Spix,1824)	A	A	B				1			
L. mystacinus (Burmeister, 1861)	A	OE	B						1	
L. notoaktites Heyer,1978	AF	FE	B	1				1		
L. plaumanni Ahl, 1936	A	OE	B						1	1
Physalaemus cuvieri Fitzinger, 1826	A	FE	A, B, T	1	1	1	1	1	1	1
Physalaemus aff. *gracilis*	NEW	FE	A, B, T		1	1	1	1	1	1
P. lateristriga (Steindachner, 1864)	AF	FE	B	1				1		
P. nanus (Boulenger,1888)	AF	FE	B					1		1
Pleurodema cf. *bibroni*	TAX	?	A, B							1
Family Microhylidae										
Chiasmocleis leucosticta (Boulenger, 1888)	AF	FE	B				1	1		1
Elachistocleis bicolor (Guérin-Méneville, 1838)	A	A	B	1					1	1
Family Ranidae										
Lithobates catesbeianus (Shaw, 1802)	EXO	OE	B	1						
ORDER GYMNOPHIONA										
Family Caeciliidae										
Microcaecilia sp.	NEW	?	A, B						1	
Siphonops paulensis Boettger, 1892	A	?	A, B				1			
Total				21	24	26	29	39	27	29

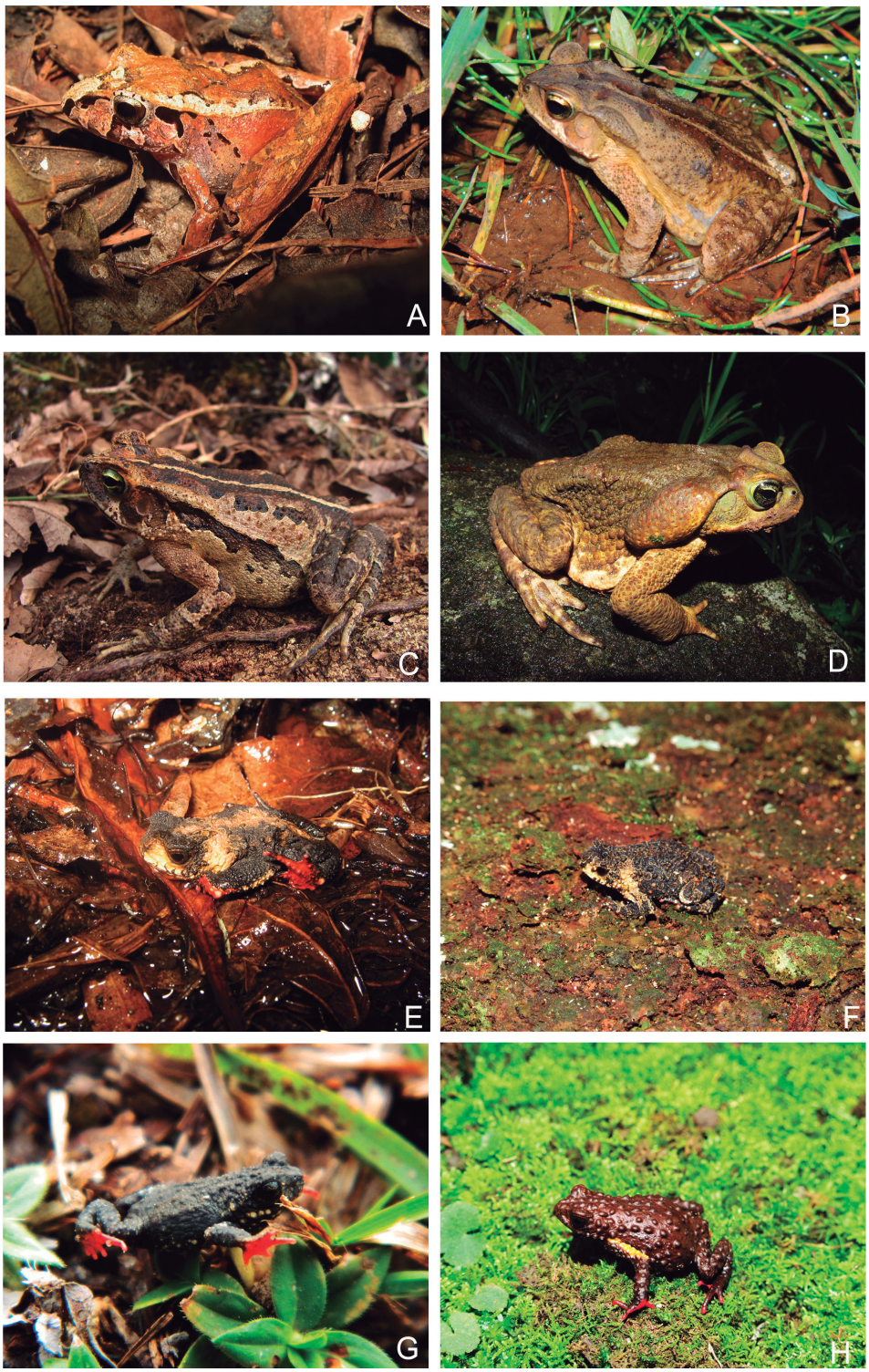

Figure 2. Amphibian species found in grasslands associated with Atlantic Forest in southern Brazil. A. *Ischnocnema henseli*; B. *Rhinella abei*; C. *Rhinella henseli*; D. *Rhinella icterica*; E. *Melanophryniscus* sp.1 (gr. *tumifrons*); F. *Melanophryniscus* sp.2 (gr. *tumifrons*); G. *Melanophryniscus vilavelhensis*; H. *Melanophryniscus alipioi.*

Figure 3. Amphibian species found in grasslands associated with Atlantic Forest in southern Brazil. A. *Vitreorana uranoscopa*; B. *Haddadus binotatus*; C. *Odontophrynus americanus*; D. *Proceratophrys brauni*; E. *Proceratophrys boiei*; F. *Crossodactylus* cf. *caramaschii*; G. *Aplastodiscus albosignatus*; H. *Aplastodiscus perviridis*.

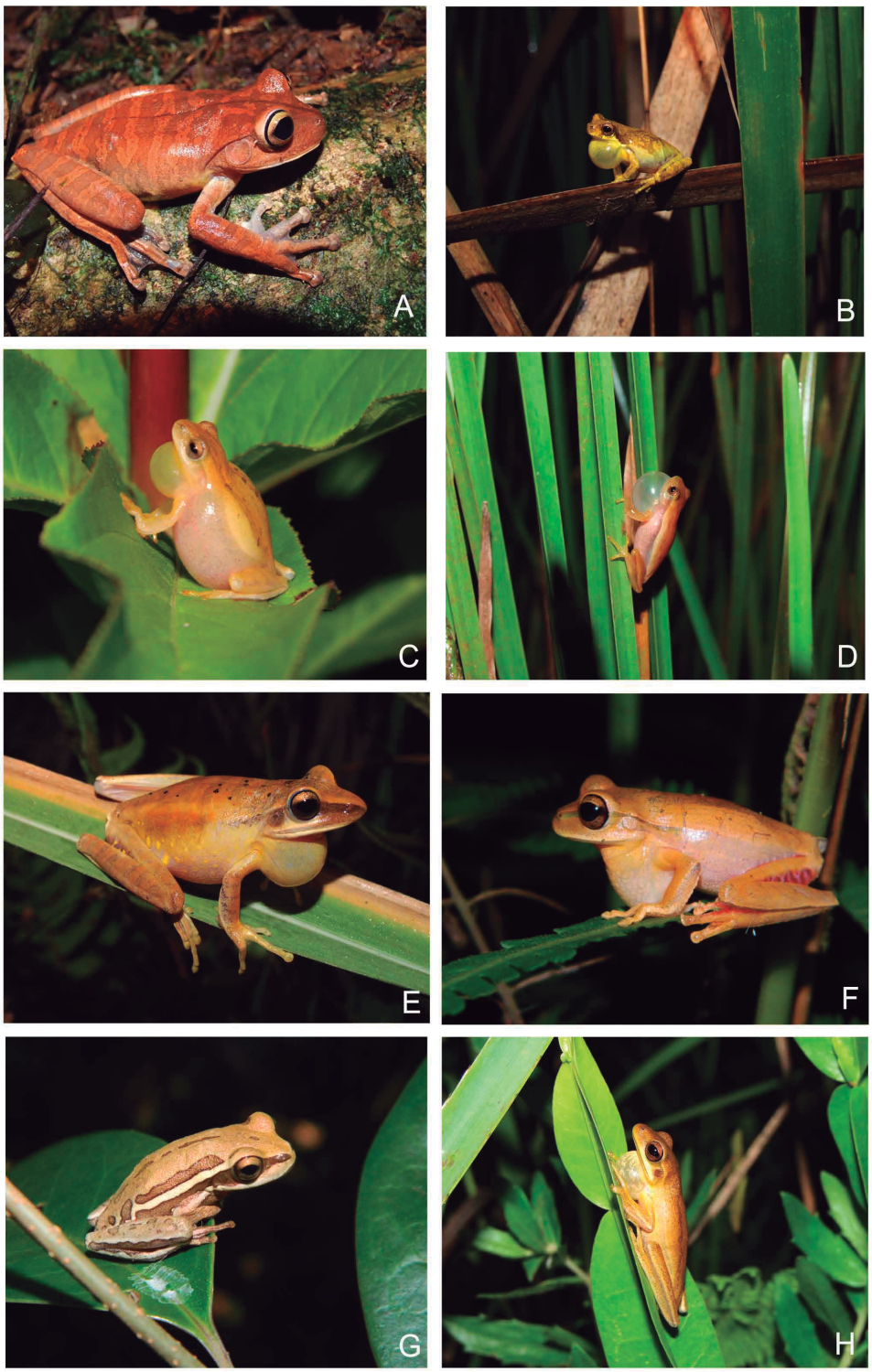

Figure 4. Amphibian species found in grasslands associated with Atlantic Forest in southern Brazil. A. *Bokermannohyla circumdata*; B. *Dendropsophus microps*; C. *Dendropsophus minutus*; D. *Dendropsophus sanborni*; E. *Hypsiboas albopunctatus*; F. *Hypsiboas bischoffi*; G. *Hypsiboas* cf. *stellae*; H. *Hypsiboas caingua.*

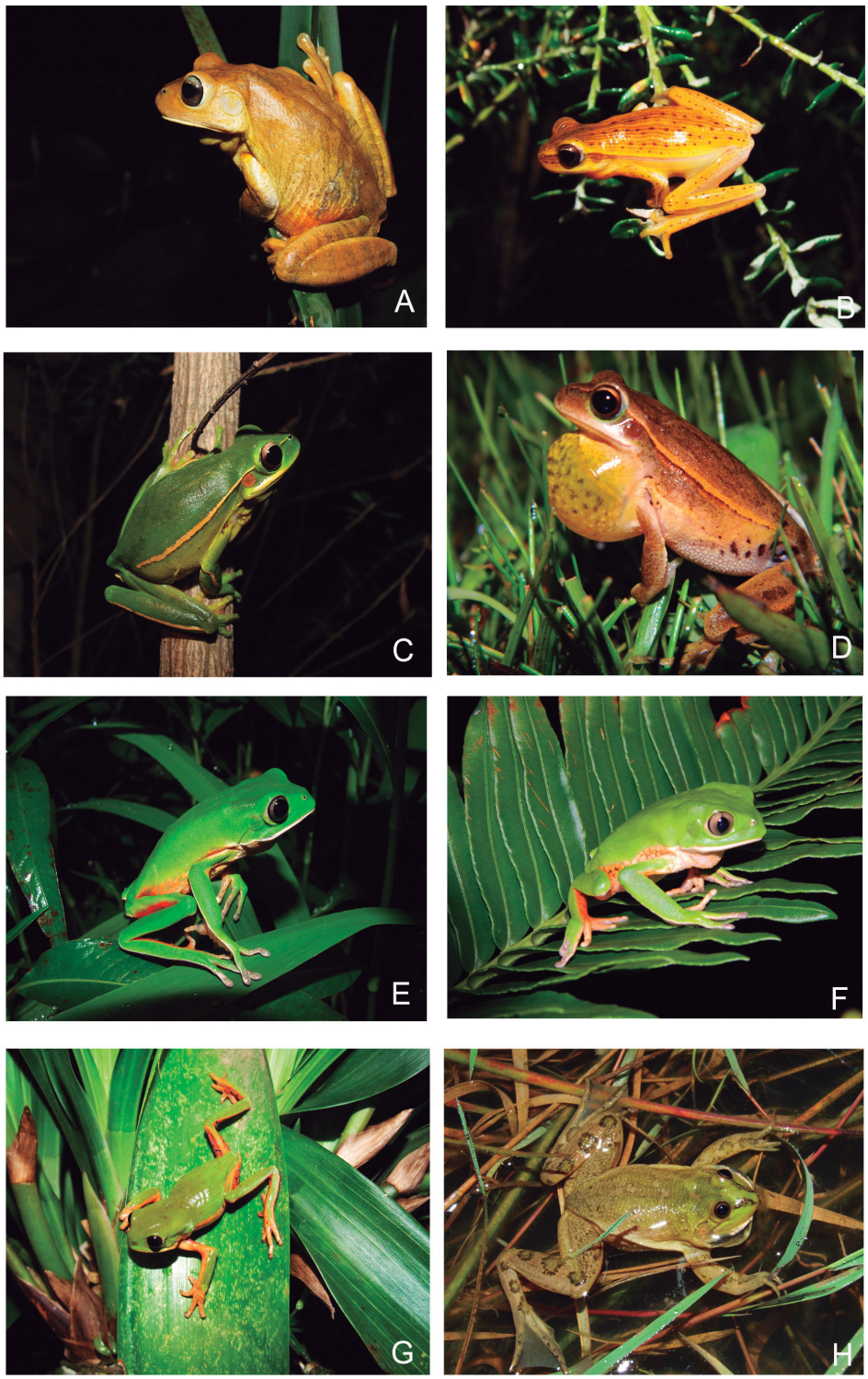

Figure 5. Amphibian species found in grasslands associated with Atlantic Forest in southern Brazil. A. *Hypsiboas faber*; B. *Hypsiboas leptolineatus*; C. *Hypsiboas prasinus*; D. *Hypsiboas pulchellus*; E. *Phyllomedusa distincta*; F. *Phyllomedusa tetraploidea*; G. *Phyllomedusa rustica* ; H. *Pseudis cardosoi.*

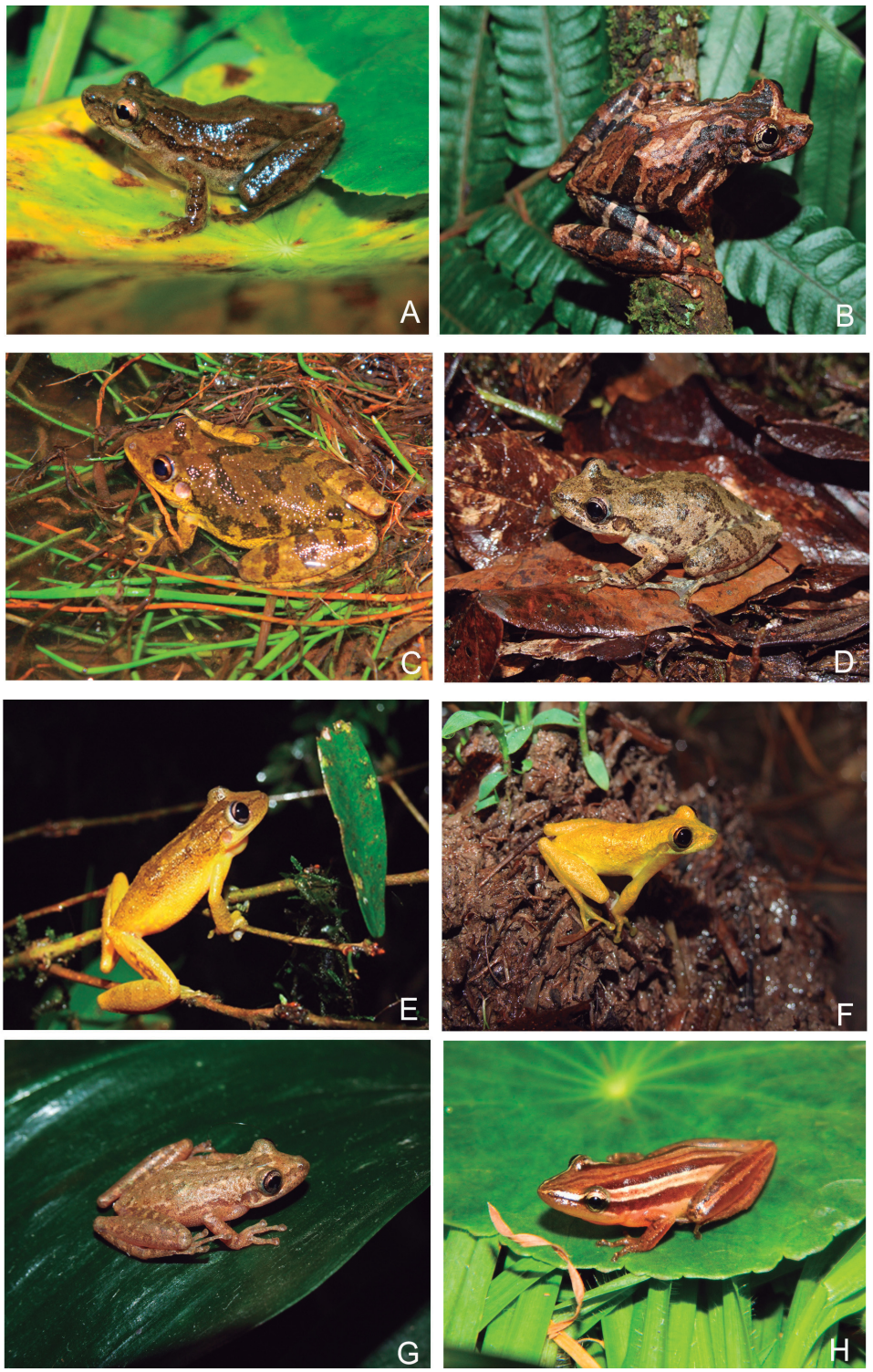

Figure 6. Amphibian species found in grasslands associated with Atlantic Forest in southern Brazil. A. *Scinax aromothyella*; B. *Scinax* sp. (aff. *catharinae*); C. *Scinax fuscovarius*; D. *Scinax granulatus*; E. *Scinax perereca*; F. *Scinax rizibilis*; G. *Scinax* sp. (gr. *ruber*); H. *Scinax squalirostris*.

Figure 7. Amphibian species found in grasslands associated with Atlantic Forest in southern Brazil: A. *Scinax uruguayus*; B. *Sphaenorhynchus caramaschii*; C. *Sphaenorhynchus surdus*; D. *Trachycephalus dibernardoi*; E. *Adenomera nana*; F. *Adenomera* sp. (aff. *marmorata*); G. *Leptodactylus fuscus*; H. *Leptodactylus notoaktites.*

Figure 8. Amphibian species found in grasslands associated with Atlantic Forest in southern Brazil: A. *Leptodactylus* cf. *latrans*; B. *Leptodactylus labyrinthicus*; C. *Leptodactylus mystacinus*; D. *Leptodactylus gracilis*; E. *Leptodactylus plaumanni*; F. *Physalaemus cuvieri*; G. *Physalaemus* sp. (aff. *gracilis*); H. *Physalaemus lateristriga.*

Figure 9. Amphibian species found in grasslands associated with Atlantic Forest in southern Brazil: A. *Physalaemus nanus*; B. *Chiasmocleis leucosticta*; C. *Elachistocleis bicolor*; D. *Lithobates catesbeianus*; E. *Microcaecilia* sp.; F. *Siphonops paulensis*. Photos by Conte,C.E.

(Silvano and Segalla 2005). A variety of human activities, such as pasture and agriculture, as well as for pine and *Eucalyptus* plantations (Overbeck et al., 2007; Machado et al. 2012) also, generate fragmentation and habitat split (*sensu* Becker et al., 2007). Because grasslands are favored for agriculture and pasture, they are especially fragile in terms of conservation, yet also receive little attention (Overbeck et al., 2007). Thus, protection is urgent, especially for relicts of Atlantic Forest mixed with grasslands, as conducted by the National Action Plan for the Conservation of Amphibians and Reptiles in Southern Brazil (BRASIL 2012, Portaria Nº 25).

We also report the expansion of distribution of two species, being the first records outside their type localities. *Melanophryniscus vilavelhensis* was previously restricted to the Vila Velha State Park (Table 1, Figure 1), in central-east Paraná (Steinbach-Padilha, 2008). However, we found this species in the Guartelá State Park (Table 1, Figure 1), which extended its distributions by 80 km towards the northwest. Considering the scale

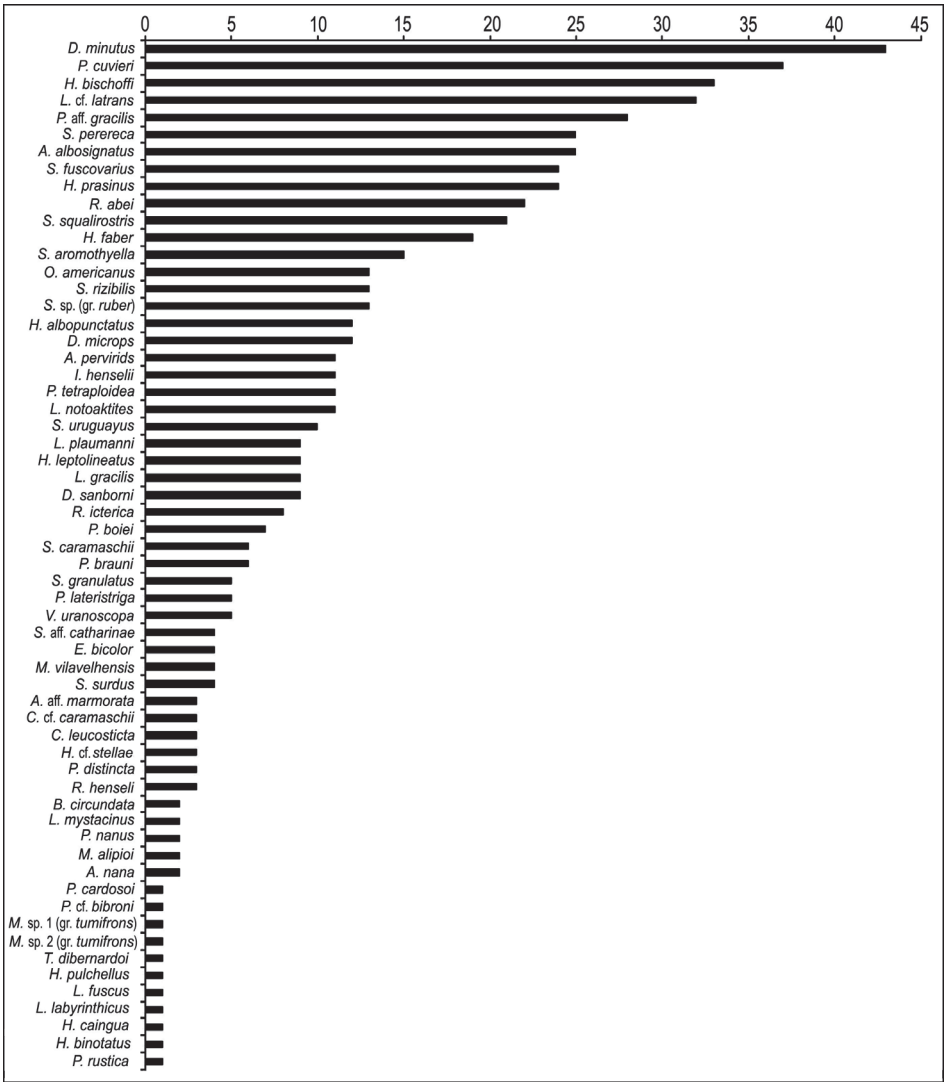

Figure 10. Frequency of occurrence of species.

of our sampling effort, we suggest that the distribution of this species might be limited to hydrophilic grasslands with phytotelmata (e.g., bromeliads of the genera *Eriocaulon* and *Eryngiun*). *M. vilavelhensis* calls only at night (Steinbach-Padilha, 2008), in contrast to other *Melanoprhryniscus* species that call during daylight and reproduce in temporary bodies of water (Maneyro et al., 2008). *Melanoprhyniscus alipioi*, was found in the Campos Gerais National Park, central-eastern Paraná (Table 1, Figure 1), about 110 km away from the Serra do Capivari to which it was restricted previously (Langone et al., 2008). We found *M. alipioi* calling in a tank bromeliad (*Aechmea* sp.) on the ground. In contrast to previous studies (Langone et al., 2008), males were calling at night from flowering bracts, where egg laying also occurs, within a forest fragment about 500 m from the edge.

Additionally, we made the third record of *L. catesbeianus* in this phytophysiognomy (Conte and Rossa-Feres, 2006; Leivas et al., 2012), and the first in a protected area in the state of Paraná. There is no study determining the impact of exotics species, but several studies indicate that the bullfrog preys on or competes with native species and may be a threat (Boelter and

Cechin, 2007; Giovanelli et al., 2007; Both et al., 2011; Boelter et al., 2012; Leivas et al., 2012). At the same time, the bullfrog may be a carrier of the fungus, *Batrachochytrium dendrobatidis*, which is causing amphibian population declines worldwide (Lips et al., 2005), and has been found in the southern Brazilian states of Paraná (Vieira et al., 2012), Santa Catarina and Rio Grande do Sul (Toledo et al., 2006).

This study provided the most comprehensive list of amphibians in grasslands of the state of Paraná, southern Brazil. Our results added key information for the conservation of amphibians in this region and point to the role of the mosaic of grasslands and Atlantic Forest promoting habitat heterogeneity (Conte & Rossa-Feres, 2007) and allowing species coexistence at the regional scale.

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