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Amphibians of northwestern Buenos Aires province, Argentina: checklist, range extensions and comments on conservation

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Abstract: Northwestern Buenos Aires province is located within the Pampas region of Argentina. Agricultural practices have extensively modified native grasslands in a relatively short period, leaving no intact pristine areas. Based on data collected between 2006 and 2015 at 153 ponds in agricultural landscapes, we compiled an annotated list of the amphibians of northwestern Buenos Aires province. We report 13 extant species of amphibians, which represent 43% of the known amphibian diversity in Buenos Aires province, and extend the ranges of three species. Three species found only in herpetological collections extend the list to 16 species. Our study is the first long-term survey of amphibians conducted in this part of Argentina and provides valuable information for future conservation actions across agricultural landscapes.

Key words: Pampean region; Inland Pampas; agricultural landscapes; long-term survey

INTRODUCTION

Northwestern Buenos Aires province lies within the Inland Pampa, one of the ecological units of the Pampas (León et al. 1984). The Pampas region, with an area of ca. 540,000 km^2, occupies the center of Argentina and includes Buenos Aires, La Pampa, Córdoba, San Luis, Santa Fe and Entre Ríos provinces (Soriano et al. 1992). The continuing replacement of natural grassland by agro-ecosystems has created a complex mixture of agricultural landscapes dominated by pastures and croplands, leaving no intact pristine areas (León et al. 1984). The last remaining patches of semi-natural habitats are small, isolated, and frequently found in unproductive or marginal places such as roadsides, railway lines, or around lakes (León and Burkart 1998; Bilenca and Miñarro 2004). Studies have shown that agricultural practices cause loss of biodiversity and ecosystem services, as well as negative impacts on the quality of soil and the general quality of wetlands (Medan et al. 2011; Caride et al. 2012; Brandolin and Blendinger 2016). The homogenization of landscapes and contamination produced by pesticides and fertilizers are the main threats affecting biodiversity in the Pampas region (Herrera et al. 2013).

Inland Pampa is characterized by sandy soils with scattered ground elevations and grass-covered dunes. There are no natural streams or rivers in the region and the only wetlands are temporary ponds and permanent, mostly saline lakes (Soriano et al. 1992). The fauna of Inland Pampa is poorly known compared to other parts of the Buenos Aires province; this is true not only for amphibians, but also for other groups such as birds and mammals (Narosky and Di Giacomo 1993; Teta et al. 2010; González-Fischer et al. 2012; Abba et al. 2015). Available information on the amphibian communities of northwestern Buenos Aires province is scarce and decades old (Barrio 1964, 1965; Gallardo 1965, 1970, 1974), all of it before major agriculturization began in the 1990s (Bilenca et al. 2009). Recently, detailed information on one species (Agostini and Roesler 2011) and a taxonomic list of all amphibians from Buenos Aires province have been published (Williams 1991). Excluding these two publications, most of the information for Buenos Aires province comes from comprehensive amphibian inventories conducted in protected areas (Pereyra and Haene 2003; Williams and Kacoliris 2009; Agostini et al. 2012) and other sites in the northeastern and eastern areas of the province (Kacoliris et al. 2006; Agostini 2012), at least 450 km away from our study area.

Amphibians are highly sensitive to any habitat modification and represent the prime example of the modern biodiversity crisis because they are one of the most threatened and rapidly declining vertebrate groups (Pounds et al. 2006). Habitat loss and pesticide contamination, both related to agricultural practices, are mentioned as some of the major threats causing amphibian decline worldwide (Blaustein et al. 2011). Studies focusing on biodiversity are essential to our understanding of both the current effects of agricultural activities and possible future impacts (Weyland et al. 2014).

Here, we present a comprehensive list of the amphibians of northwestern Buenos Aires province, based on both field data and scientific collections. We also report range extensions for three species.

MATERIALS AND METHODS

Study site

The study area is located within the Inland Pampa and includes 12 departments of northwestern Buenos Aires province: Carlos Tejedor, Daireaux, Florentino Ameghino, General Villegas, Guaminí, Lincoln, Pehuajó,

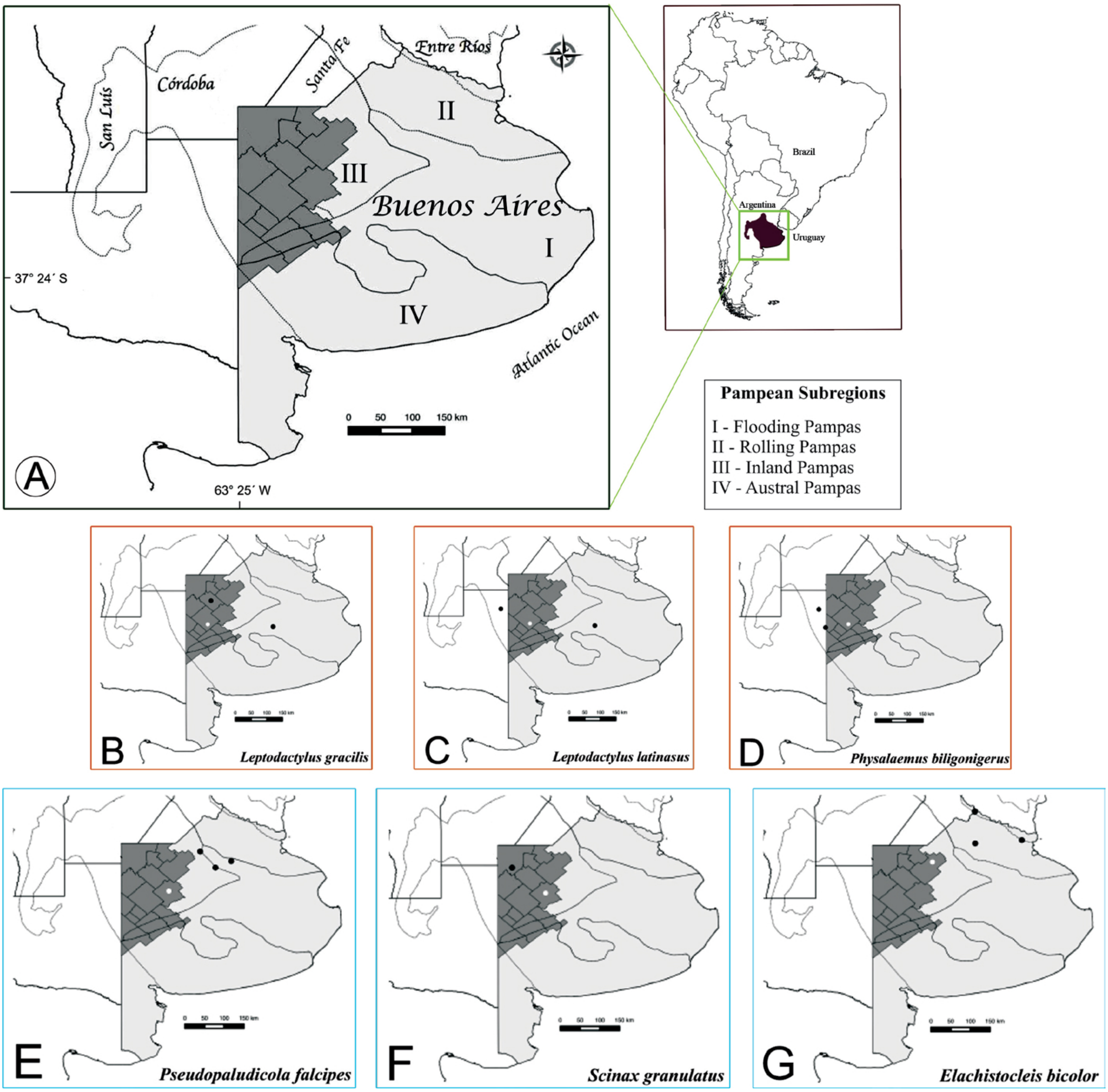

Figure 1. Maps of the study area, localities of species distribution limits in the study area and range extensions. **A.** Study area. Darker grey area represents the departments (=Carlos Tejedor, Daireaux, Florentino Ameghino, General Villegas, Guaminí, Lincoln, Pehuajó, Pellegrini, Rivadavia, Salliqueló, Trenque Lauquen and Tres Lomas) in Buenos Aires province (light grey area) where the amphibian surveys were conducted between 2006 and 2015. **B–D**: Maps showing the species with distribution limits in the study area (orange border). **E–G**: Maps showing distributional extensions (blue border). New localities records are shown with black circles and nearest previous records are shown with grey circles.

Pellegrini, Rivadavia, Salliqueló, Trenque Lauquen, and Tres Lomas (Figure 1A). The climate is sub-humid, with an annual rainfall gradient ranging from 900 to 600 mm, decreasing east to west. Mean annual temperature is ca. 16°C with a slight decrease in the same direction, and a large disparity between summer and winter temperatures, ranging from ca. 24°C to 8°C, respectively (Bilenca and Miñarro 2004). The soil has a sandy composition that facilitates excessive drainage, although during extremely wet periods floods are frequent (Viglizzo and Frank 2006).

Data collection

We monitored amphibians from 2006 to 2015. In each year, we surveyed at least three times per season in both spring (October to December) and summer (December to February), totaling 173 survey days per year. Because natural grasslands in the area are extremely scarce, we sampled 153 ponds in with agricultural areas (61 pastures, 82 soybean fields, and 10 rural roadways). Sampling was restricted to breeding sites, at which we employed auditory and visual methods for species detection (Heyer et al. 1994) during the night (between approximately 2200 hrs and 0200 hrs). We conducted surveys primarily during favorable wet periods, specifically before heavy rainfall that is coincident with high amphibian activity (Canavero and Arim 2009). To complement these auditory and visual surveys, we also sampled anuran tadpoles in 67 ponds using a quantitative method, utilizing 20 cm diameter hand nets with 1-mm mesh and 1-m passing rates for tadpole capture (Heyer et al. 1994).

Adult specimens were identified using general literature (Cei 1980) and specific descriptions for *Scinax granulatus* and *S. nasicus* (Kwet 2001). To identify reproductive calls, we used both Straneck (1993) and our own recordings. For larvae identification, we followed Kehr and Williams (1990) and Kehr and Duré (1995). We also relied on expert verification through the Herpetological Collection of the La Plata Museum (La Plata, Argentina). Specimens were deposited at the Herpetological Collection of the La Plata Museum, with collection authorized by Dirección de Flora y Fauna, Ministerio de Asuntos Agrarios Provincia de Buenos Aires (Expedient number 225500-11319/10).

To assess the efficiency and completeness of our inventory within the study area, we fit two asymptotic species accumulation models to our species accumulation data, the linear dependence model and the Clench model (Soberon and Llorente 1993). The species accumulation curve was obtained by taking the number of survey nights as sampling effort and the number of species recorded. To eliminate the influence of the order in which nights were added to the total, sample order was randomized 100 times using EstimateS software V 9.1.0 (Colwell 2000). For both models, we used a non-linear regression (Sigma Stat; Jandel Corporation 1995).

To complement our species list, we visited the three major Argentine herpetological collections, at Museo Argentino de Ciencias Naturales (MACN), La Plata Museum (MLP), and Centro Nacional Patagónico (CENPAT). We followed the systematic arrangement proposed by Frost (2015); species listed as sp., gr. (group of species), cf. (*confer*) or aff. (*affinis*) were excluded.

RESULTS

The amphibian list of northwestern Buenos Aires province includes 16 species (Table 1), 13 species was confirmed by 10 years of amphibian surveys (Figure 2) and three from herpetological collections.

Class Amphibia
Subclass Lissamphibia
Order Anura
Family Bufonidae

Rhinella arenarum (Hensel, 1867), Common Toad (Figure 2A)

Rhinella arenarum was commonly found in modified areas, mostly suburban places and around human settlements. We also recorded reproductive chorus and tadpoles in artificial ponds and roadside ditches during October and November.

Table 1. Species account. S: species found in our surveys. HC: species from herpetological collections.

Species	Voucher	S	HC
Bufonidae			
Rhinella arenarum (Hensel, 1867)	MLP A5745	X	X
Rhinella fernandezae (Gallardo, 1957)		X	X
Ceratophrydae			
Ceratoprhys ornata (Bell, 1843)		X	X
Hylidae			
Hypsiboas pulchellus (Duméril & Bibron, 1841)	MLP A5159; A5744; A5746	X	X
Scinax granulatus (Peters, 1871)		X	X
Scinax nasicus (Cope, 1862)	MLP A5781	X	
Pseudis minuta Günter, 1858			X
Leptodactylidae			
Leptodactylus gracilis (Duméril & Bibron, 1840)	MLP A5782	X	X
Leptodactylus latinasus Jiménez de la Espada, 1875	MLP A5157	X	X
Leptodactylus latrans (Steffen, 1815)	MLP A5158	X	X
Leptodactylus mystacinus (Burmeister, 1861)			X
Physalaemus biligonigerus (Cope, 1861)	MLP A5161	X	X
Physalaemus fernandezae (Müller, 1926)			X
Pseudopaludicola falcipes (Hensel, 1867)	MLP A5754- 55	X	
Microhylidae			
Elachistocleis bicolor (Guérin-Méneville, 1838)	MLP A5783- 84	X	
Odontophrynidae			
Odontophrynus americanus (Duméril & Bibron, 1841)	MLP A5160	X	X

Figure 2. Species of amphibians recorded in field surveys. **A**) *Rhinella arenarum*; **B**) *Rhinella fernandezae*; **C**) *Ceratophrys ornata*; **D**) *Odontophrynus americanus*; **E**) *Leptodactylus gracilis*; **F**) *Leptodactylus latinasus*; **G**) *Leptodactylus latrans*; **H**) *Physalaemus biligonigerus*; **I**) *Pseudopaludicola falcipes*; **J**) *Hypsiboas pulchellus*; **K**) *Scinax granulatus*; **L**) *Scinax nasicus*; **M**) *Elachistocleis bicolor*.

Rhinella fernandezae (Gallardo, 1957), Bella Vista Toad (Figure 2B)

Rhinella fernandezae was abundant in croplands and pastures. We recorded adults and tadpoles at every survey throughout the study area.

Family Ceratophrydae

Ceratoprhys ornata (Bell, 1843), Horned Frog (Figure 2C)

Ceratoprhys ornata was recorded in flooded grasslands and shallow artificial wetlands. We recorded tadpoles in a pond from General Villegas department during February.

Family Hylidae

Hypsiboas pulchellus (Duméril and Bibron, 1841), Common Treefrog (Figure 2J)

Hypsiboas pulchellus was recorded frequently (most of sampling events). Adults and tadpoles were found in semi-permanent ponds, artificial wetlands and roadside ditches, always when deep water (more than 1 meter) and emergent vegetation was present.

Scinax granulatus (Peters, 1871), unnamed treefrog (Figure 2K)

Scinax granulatus was found only at implanted forest patches. We recorded two males vocalizing in a pine patch in a rural area of Pehuajó department (vocalizations were recorded, but no specimens collected) and also several individuals were located at vegetated ponds of General Villegas, Trenque Lauquen, Lincoln and Tres Lomas departments.

Scinax nasicus (Cope, 1862), Lesser Snouted Treefrog (Figure 2L)

Scinax nasicus was uncommon within the study area. We only recorded tadpoles once (November 2015) in a pond located within a soybean field in Lincoln department.

Pseudis minuta Günter, 1858 Lesser Swimming Frog

Pseudis minuta was only mentioned based on specimens held at herpetological collections. This species was not recorded during our surveys.

Family Leptodactylidae

Leptodactylus gracilis (Duméril and Bibron, 1840), Dumeril's Striped Frog (Figure 2E)

Leptodactylus gracilis was an abundant species during our studies. We recorded adults, juveniles, reproductive chorus and tadpoles throughout the study area from October to February. This species mostly occurs in croplands and it reproduces principally in shallow ponds related to crops.

Leptodactylus latinasus Jiménez de la Espada, 1875, Oven Frog (Figure 2F)

Leptodactylus gracilis was an abundant species during our studies. We recorded adults, juveniles, reproductive chorus and tadpoles throughout the study area from October to February. This species mostly occurs in croplands and it reproduce mainly in shallow ponds related to crops.

Leptodactylus latrans (Steffen, 1815), Criolla Frog (Figure 2G)

Leptodactylus latrans was a common species during our studies. We recorded adults, juveniles, reproductive chorus and tadpoles in all the sampled departments of the study area. Although it often occurs in ponds in pastures, we also recorded reproductive chorus in artificial roadside ditches.

Leptodactylus mystacinus (Burmeister, 1861), Moustached Frog

Leptodactylus mystacinus was only represented in the study area by specimens held at herpetological collections. We did not record this species during our surveys.

Physalaemus fernandezae (Müller, 1926), Whistling Dwarf Frog

Leptodactylus fernandezae was only present by specimens held at herpetological collections. We did not record this species during our surveys.

Physalaemus biligonigerus (Cope, 1861), Weeping Frog (Figure 2H)

Physalaemus biligonigerus was the most common species in our study area occurring in sites whit different degrees of agricultural modification. We found reproductive chorus in all types of wetlands (temporal, permanents, natural and artificial ponds), flooded areas and deep canals from October to February.

Pseudopaludicola falcipes (Hensel, 1867), Hensel's Swamp Frog (Figure 2I)

Pseudopaludicola falcipes was an uncommon species (two males detected) during our studies, and it was found only in a shallow pond in a pasture plot in Pehuajó department.

Family Microhylidae

Elachistocleis bicolor (Guérin-Méneville, 1838), Two-colored Oval Frog (Figure 2I)

Elachistocleis bicolor was found in small ponds located in pastures and croplands. We recorded two juveniles and one female in Lincoln department during February 2015.

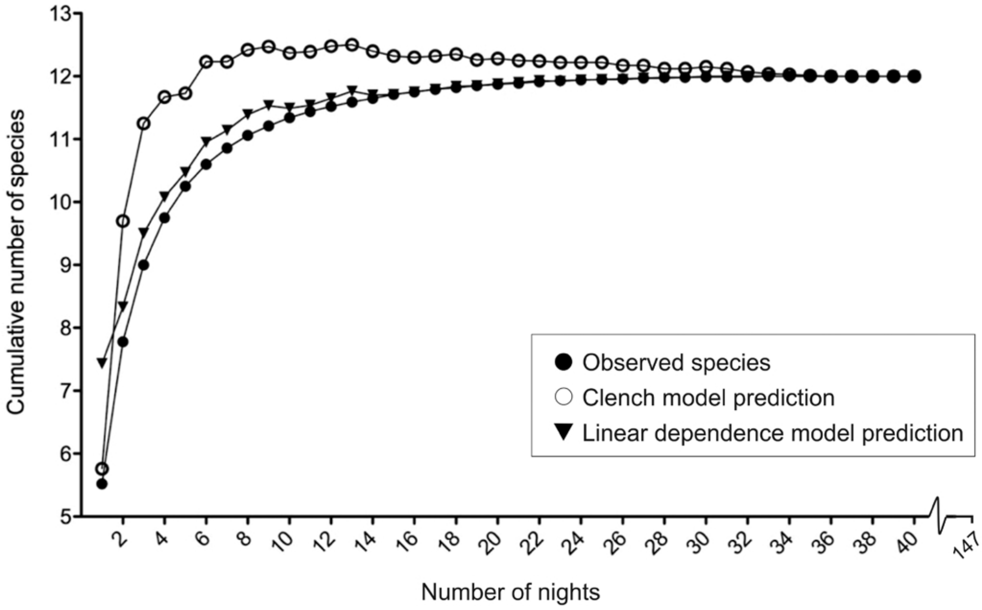

Figure 3. Original and predicted amphibian species accumulation curves (Clench model and Linear dependece model). Results only for visual and acoustic adult surveys (between 2006 and 2015) conducted in the northwestern Buenos Aires province.

Family Odontophrynidae

Odontophrynus americanus (Duméril and Bibron, 1841), Common Lesser Escuerzo (Figure 2D)

Odontophrynus americanus was a common species that inhabits crops and pastures. We recorded tadpoles and reproductive chorus in small ponds, flooded areas and artificial wetlands next to the roads within October to February.

Species accumulation curves and fitted models reached an asymptote (Figure 3). Both species accumulation models, the linear dependence model (a=4.68; b= 0.41; r^2= 0.92) and the Clench model (a=7.58; b= 0.59; r^2= 0.99), fit the data very well. According to the lower limits of both models, our amphibian inventory reached a satisfactory level of completeness. At the level of effort invested (nights surveyed), the linear dependence model and the Clench model showed 98.3% and 100% completeness respectively. Because we did not find adults of *Scinax nasicus*, this species was not included in our models and the total number of species observed was 12 (Figure 3).

We found three species (*L. gracilis*, *L. latinasus and P. biligonigerus*) that reach their southern limit of their ranges within our study area (Figure 1B–D); the southernmost locality at which we found them was near 30 de Agosto (36°19′57.9″ S, 062°36′58.8″ W). We also found range extensions for three species (Figure 1E–G). We recorded *P. falcipes* in Pehuajó department (36°17′05.5″ S, 062°32′51.7″ W) and *S. granulatus* males in a nearby area of Pehuajó department (35°01′03.8″ S, 061°56′24.9″ W). Additionally, we recorded *E. bicolor* in Lincoln department (34°53′23.53″ S, 061°33′32.2″ W).

DISCUSSION

The species detected during our fieldwork represent 43% of the amphibian species previously recorded in Buenos Aires province (Vaira et al. 2012). This low species richness in the Inland Pampa has already been mentioned for other groups of vertebrates (Pardiñas et al. 2010; González Fischer et al. 2012). It has been suggested that low species richness stems from the natural retraction of the Brazilian fauna during the Neopliocene (Ringuelet 1961). Another possible explanation is that low richness is caused by human activities (mostly agriculture) which have modified the natural grasslands for 400 years (Rapoport 1996). Regarding amphibians, several authors have noted that low species richness is undoubtedly a consequence of the climatic gradient between the Subtropical Domain and the Pampasic Domain, characterized by the decreasing levels of humidity and precipitation from east to west (Gallardo 1974; Cei 1980; Ringuelet 1981). Additionally, it is likely that the region's long dry periods, as well as the complete absence of natural streams and rivers, may make difficult the dispersion of frogs and toads. The lack of natural watercourses tends to be an obstacle for organisms with low dispersion capacity (Vitt and Caldwell 2014). However, we cannot dismiss the fact that the agricultural practices taking place in the Inland Pampas (causing habitat loss and aquatic contamination) may have influenced amphibian diversity in recent years. Several studies have reported that agricultural impacts can lead to the impoverishment of biological communities, reflected by the loss of the most sensitive species (Peltzer et al. 2006; Agostini et al. 2013; Moreira et al. 2016). In this regard, it is important to highlight that species richness is higher in eastern Buenos

Aires province at the same latitude as our study area (Williams 1991; Agostini 2012), even though this area is currently the most affected by agricultural activities in Argentina (Viglizzo and Frank 2006). However, annual precipitation there usually exceeds 1,000 mm (Soriano 1992) and favors a higher amphibian species richness compared to the Inland Pampa. We argue that the climatic, hydrological, and edaphic characteristics of the Inland Pampa could be the most important factors for explaining its low amphibian diversity. Nonetheless, studies involving biodiversity attributes besides species composition are necessary to determine agricultural impacts on amphibians in the Inland Pampa.

Species distribution limits in the study area

Several species reach the southern limits of their distribution within our study area in concordance with the maximum annual rainfall isohyets (600 and 700 mm) (Gallardo 1974; Cei 1980). This decrease in precipitation may determine the southern limits of *L. gracilis* and *L. latinasus,* because both species are typical of flooded grasslands (Maneyro and Carrerira 2012). We recorded *L. gracilis* and *L. latinasus* in the 30 de Agosto area (Trenque Lauquen department); the closest known records for *L. gracilis* are in Carlos Tejedor (MACN HE 25249) and Tapalqué (MACN HE 2646). For *L. latinasus*, there are museum specimens from Tapalqué (MACN HE 28088), and also from Maracó, La Pampa province (MACN HE 31676-31680). Thus, 30 de Agosto becomes the southwesternmost distributional limit of these species, while Tapalqué is the southernmost limit (Figure 1B–C).

The distributional situation of *P. biligonigerus* is complex and not easily summarized. The southernmost museum record is Henderson, in Buenos Aires province (MACN HE 35465-35472). In La Pampa province, this species has been collected as far south as General Pico (MACN HE 7098-7099), Catriló (CNP.A 1454), and it has been mentioned even further south in General Acha (Barrio 1965). We found it in 30 de Agosto, which represents its southernmost distributional limit (Figure 1D). We found high numbers of reproductive choruses of *P. biligonigerus* in the 30 de Agosto area, which suggests that the southernmost limit is probably even further south.

Range extensions

The reproductive choruses of *P. falcipes* recorded in Guanaco (Pehuajó department) represent a range extension of approximately 153 km westward from the previous known records (Figure 1E) in Buenos Aires province in Bragado (MACN 9190, 9247, 9257), Chivilcoy (MACN 9240), and Junín departments (MACN 25752). Gallardo (1974) recorded this species from 9 de Julio department, and although there are no voucher specimens to support this, we believe that its presence there is likely.

The presence of *S. granulatus* in the Inland Pampa has been confirmed recently (Agostini and Roesler 2011) and we occasionally recorded it during strong summer rains around General Villegas city. Furthermore, we recorded calling males in a rural area of Pehuajó department. This find represents a range extension of 140 km south from General Villegas, the nearest previous locality (Figure 1F). The infrequency of detection in the area may suggest recent colonization by this species, as already suggested (Agostini and Roesler 2011).

With regards to *E. bicolor* found in Lincoln department, the closest known localities are Salto (MLP A3965-GA36), Otamendi (MACN 39188), and San Nicolás (MACN 5535-36). This represents a distributional range extension of 135 km westward (Figure 1G).

Species present in herpetological collections but not recorded during surveys

There are museum specimens of *L. mystacinus* collected within the study area (MACN He25250, 30 de Agosto Town). Furthermore, there are specimens collected in General Pico (MACN 18316, 19273, 19440-41, 20995) and Santa Rosa (MACN 1268, 1319), both in La Pampa province. Additionally, there are specimens of *P. fernandezae* (MACN 29035-40) and *P. minuta* (MACN 20989-91) from 9 de Julio and Lincoln departments, respectively. Both *L. mystacinus* and *P. minuta* have broad distributional ranges and occur in varied habitats associated with flooded grasslands, even in agricultural lands (Cei 1980; Peltzer et al. 2006; Agostini et al. 2012; Maneyro and Carreira 2012). Both, *P. minuta* and *P. fernandezae,* occur in areas influenced by the Río de La Plata River and the Flooding Pampa in eastern Buenos Aires province (Barrio 1965; Cei 1980; Williams 1991), where maximum annual rainfall exceeds 1,000 mm and the grasslands are frequently flooded (Soriano et al. 1992). It is likely that the climatic and hydrological characteristics of the Inland Pampa restrict the occurrence of both species. After 10 years surveying the area without new records of these species, we suspect that the old records may represent vagrant individuals associated with exceptional flooding (Viglizzo and Frank 2006; Kuppel et al. 2015), and that these species may have not have maintained permanent populations in the area.

Conservation

We recorded *Ceratophrys ornata* several times during wetter periods. We detected individuals in all life cycle stages, including adult individuals dispersing, vocalizing, and in amplexus as well as tadpoles and juveniles. The frequency of detection in the area was noticeably higher than at other sites in Buenos Aires province, even compared to localities with high amphibian diversity (Pereyra and Haene 2003; Kacoliris et al. 2006; Williams and Kacoliris 2009; Agostini et al. 2012). In addition to the population from northwestern Buenos Aires province, we recently found several populations inhabiting psammophil grasslands near the Buenos Aires sea coast (M.G. Agostini, unpublished data). This may suggest a preference for grasslands on sandy soils, as reported in Uruguay (Maneyro and Carreira 2012). *Ceratophrys ornata* is considered to be nationally Vulnerable (Vaira et al. 2012) and globally Near Threatened (Kwet et al. 2004). Habitat loss and pesticide contamination caused by agricultural practices are the main factors driving population declines (Vaira et al. 2012). Because all of our records were made in ponds in agricultural lands, we suggest that more accurate studies are necessary to assess the contribution of agricultural practices to population declines.

Recently, the construction of drainage channels that draw water away from low-lying areas and conversion of these areas into cropland has become new and important threats to the local fauna. This practice is causing the loss of large wetlands, as well as negatively changing natural watersheds (Viglizzo and Frank 2006; Kuppel et al. 2015). The lack of wetland connectivity can cause isolation among populations, which tends to increase endogamy processes, and may lead to possible local extinctions of species (Storfer et al. 2014). However, these drainage channels and other artificial wetlands hold water even during dry periods when natural temporary ponds dried out. Artificial wetlands in the Inland Pampa could act as reservoirs for a high diversity of amphibians (Knutson et al. 2004; Casas et al. 2011) as mentioned for other arid regions where natural wetlands are scare or have been modified through agriculture (Dahl 2011). Further studies are needed to determine if the artificial wetlands are suitable habitats for amphibian populations.

Since the 1980s, agriculture has become the main economic activity of Buenos Aires province (Bilenca et al. 2009). The switch from extensive cattle pastures to intensive agriculture has generated a major change in habitat complexity, with the complete loss of the last patches of natural environment, mostly remnants of the Pampean grasslands. It is now almost impossible to assess the real effects of these environmental changes on amphibian populations, mostly because of the absolute lack of baseline data such as species' abundances, seasonality, and reproductive behavior. We believe our study will fill an important gap in the knowledge of amphibians of Buenos Aires province and will serve as the basis for further studies that are needed to understand how amphibians respond to habitat loss and fragmentation by the reduction of wetlands, and to contamination by pesticides. This information is essential for conservation planning and management.

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