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Trabajo

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Description of a new species of the *Liolaemus elongatus* group (Squamata: Iguania) through integrative taxonomy

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ABSTRACT
Liolaemus is the genus with the highest number of lizard species described in South America up to date, with approximately 290. One of the groups within the genus, the *Liolaemus elongatus* group, is distributed in central-south Argentina and Chile. In this work, we describe a new species belonging to the *L. petrophilus* clade using an integrative approach that includes the analysis of morphological (lepidosis, morphometric, coloration and hemipenis characters) and molecular evidence (genetic distances and phylogeny). We provide information about the geological formation of the study area and the clade distribution, and present an extensive description of its natural history (including diet, habitat, behaviour, phytogeography, mode of life, and sympatric species). Additionally, we present a time divergence analysis of internal branches for all representatives of the *L. elongatus* group. This newly described species shows character states (morphological and molecular) that allow its clear distinction from the other members of the *L. elongatus* group, as well as from the remaining *Liolaemus* species. According to our results, the *L. elongatus* group originated during the Miocene, whereas the new species diverged from its sister taxon during the Pleistocene.

Key words: Divergence times; Lizards; Patagonia; South America; Phylogeny

RESUMEN
Liolaemus es el género con el mayor número de especies de lagartijas descritas en América del Sur hasta la fecha, con aproximadamente 290 especies. Uno de los grupos dentro del género, el grupo de *Liolaemus elongatus*, se distribuye en el centro-sur de Argentina y Chile. En este trabajo, describimos una nueva especie perteneciente al clado de *L. petrophilus* usando un enfoque integrador que incluye el análisis de evidencia morfológica (lepidosis, morfométrica, coloración y caracteres de hemipenes) y evidencia molecular (distancias genéticas y filogenia). Brindamos información sobre la formación geológica del área de estudio y la distribución del clado, y presentamos una descripción extensa de su historia natural (que incluye dieta, hábitat, comportamiento, fitogeografía, modo de vida y especies simpátricas). Además, presentamos un análisis de tiempos de divergencia de las ramas internas y de todos los representantes del grupo de *L. elongatus*. Esta especie recién descrita muestra estados de carácter (morfológicos y moleculares) que permiten su clara distinción de los otros miembros del grupo de *L. elongatus*, así como de las restantes especies de *Liolaemus*. Según nuestros resultados, el grupo de *L. elongatus* se originó durante el Mioceno, mientras que la nueva especie se separó de su taxón hermano durante el Pleistoceno.

Palabra claves: Tiempos de divergencia; Lagartos; Patagonia; Sudamérica; Filogenia

Introduction

The family Liolaemidae (Frost *et al.,* 2001) is the most species-rich family of lizards in South America. One of the genera within this family, *Liolaemus* (Wiegmann, 1834), is the second most diverse genus in the world, with ~290 small and medium-sized species described in South America (Abdala *et al.,*

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2021a; Arapa-Aquino *et al.,* 2021; Avila *et al.,* 2021; Bulacios Arroyo *et al.,* 2021; Quiroz *et al.,* 2021; Ruiz *et al.,* 2021; Troncoso-Palacios and Ramírez-Álvarez, 2021; Valladares-Faúndez *et al.,* 2021). *Liolaemus* has been the subject of numerous studies in various disciplines. The interest in this genus can be explained by its great morphological, functional and ecological diversity. The species that compose this taxon occur in different substrates (there are saxicolous, arboreal, and sand-dwelling species), have two distinct reproductive modes (viviparous and oviparous), and present a wide variety of diets (herbivorous, insectivorous and omnivorous) (Ramírez Pinilla, 1991; Schulte *et al.,* 2000; Martínez Oliver and Lobo, 2002; Espinoza *et al.,* 2004; Halloy *et al.,* 2013; Esquerré *et al.,* 2019). Regarding coloration patterns, in some species there is no sexual dichromatism, whereas in other this phenomenon is conspicuous; there are also varying levels of polymorphism (Abdala and Quinteros, 2014). Geographically, *Liolaemus* is distributed from Tierra del Fuego, in the southernmost point in South America, to the Peruvian Andes in the north. Its range covers regions in Argentina, Uruguay, Bolivia, Chile and Peru, with an altitudinal range from sea level to over 5000 m a.s.l. (Aparicio and Ocampo, 2010). Thus, this group has captured the attention of herpetologists for years, and is still the subject of many ongoing studies, since there are still many interesting questions that remain unanswered.

Regarding the phylogenetic relationships within the genus *Liolaemus*, Laurent (1983, 1985, 1995) and Etheridge (1995) split the *Liolaemus* genus into two main groups, subgenus *Liolaemus* (sensu stricto) (Laurent, 1983) or "Chileno group", and *Eulaemus* (Laurent, 1983) or "Argentino group", distributed mainly to the west and east of the Andes, respectively. This split was supported by many phylogenetic analyses (Schulte, 2000; Espinoza *et al.,* 2004; Pyron *et al.,* 2013; Olave *et al.,* 2014; Zheng and Wiens, 2016). Different phylogenetic hypotheses have been proposed for *Liolaemus* sensu stricto subgenus, and several subgroups have been recognized inside (Lobo *et al.,* 2010; Abdala and Quinteros, 2014; Morando *et al.*, 2020; Abdala *et al.*, 2021b, Esquerré *et al.*, 2022). One of them is the *L. elongatus* group, proposed by Cei (1974) as "*L. elongatus* complex" and later renamed as *L. elongatus* group (Cei, 1986), which is distributed in central-south Argentina and Chile, along the Andean and extra-Andean regions (Abdala *et al.*, 2010; Avila *et al.*, 2015; Troncoso-Palacios *et al.*, 2018).

Morando *et al.* (2003) performed a phylogeographic analysis of the relationships within *Liolaemus elongatus* group, including specimens of all recognized species at that time. Morando *et al.* (2003) and Avila *et al.* (2004) recognized three groups: the *L. elongatus*, the *L. kriegi*, and the *L. petrophilus* groups, and renamed the most inclusive group as the *L. elongatus-kriegi* complex. In addition, Morando *et al.* (2003) mentioned the existence of many candidate species within this complex. Subsequently, Lobo (2005) and Díaz Gómez and Lobo (2006) proposed a new clade within the *L. elongatus* group, named *L. capillitas* group, which included the species of the *L. petrophilus* group by Morando *et al.* (2003), distributed in north-western Argentina. Based on a consensus of previous studies (Etheridge, 1995; Schulte *et al.,* 2000; Lobo, 2001, 2005; Espinoza *et al.,* 2004), Lobo *et al.* (2010) defined the *L. kriegi* group (four species) and the *L. elongatus* group, which includes the *L. capillitas* clade (15 species), grouping the species in the *L. elongatus* and *L. petrophilus* groups by Morando *et al.* (2003). More recently, Avila *et al.* (2010) recovered four clades: the *L. elongatus*, *L. kriegi*, *L. petrophilus* (including the *L. capillitas* subclade), and *L. punmahuida* clades. The relationships among the clades are as follows: the *L. elongatus* clade is sister to the *L. kriegi* clade; in turn, the *L. petrophilus* group is sister to the group formed by the *L. elongatus* and *L. kriegi* clades. Finally, the *L. punmahuida* clade is sister to all of them. Abdala and Quinteros (2014) performed an update of the study of Lobo *et al.* (2010) and proposed the same groups but included all the new species described at that time in their phylogeny. Avila *et al.* (2015) recovered four clades (which are similar to the complexes of Morando *et al.,* 2003): the *L. elongatus*, *L. kriegi*, *L. petrophilus*, and *L. punmahuida* clades. The same groups (except for *L. punmahuida* clade) were recovered by Medina *et al.* (2015), who studied the *L. kriegi* complex in detail. Escobar-Huerta *et al.* (2015) and Troncoso-Palacios *et al.* (2016) recovered the same clades and relationships as Avila *et al.* (2012). Medina *et al.* (2017) conducted a phylogeographic study of the *L. elongatus* group based on mitochondrial and nuclear genes. They did not retrieve the clades proposed for the group so far, since the results contained multiple polytomies among the group's internal clades. Later, Medina *et al.* (2018) added more evidence, both mitochondrial and nuclear, and analysed the *L. elongatus* group including all the representatives described until then. In this case, the four clades proposed before were recovered (Avila *et al.,* 2012) and the relationships between them were in agreement with the results of Avila *et al.* (2015) and in part with the results of Medina *et al.* (2015), who reported that the *L. elongatus* clade is sister to the *L. petrophilus* clade, and the *L. kriegi* clade is sister to the group formed by the two mentioned groups. The most recent contributions to the *L. elongatus* group continue to disagree with respect to the internal relationships of their clades. Troncoso-Palacios *et al.* (2018) and Ruiz *et al.* (2020) recovered the same clades and relationships as those recovered by Avila *et al.* (2012), whereas Escobar-Huerta *et al.* (2015), Troncoso-Palacios *et al.* (2016) and Avila *et al.* (2021) recovered the relationships of Avila *et al.* (2015) and Medina *et al.* (2018). Finally, Esquerré *et al.* (2022) performed a DNA phylogenetic study and proposed that the *L. elongatus-kriegi* complex is formed by six groups: *L. austromendocinus* Cei, 1974, *L. capillitas* Hulse, 1979, *L. elongatus* Koslowsky, 1896, *L. kriegi* Müller and Hellmich, 1939, *L. petrophilus* Donoso-Barros and Cei, 1971 and *L. punmahuida* Avila *et al.* 2003, but with two different arrangements for mitochondrial and nuclear DNA data.

Since, higher taxonomy of these groups is still matter of debate; here we deal with the *Liolaemus elongatus* group in the wide sense (all proposed groups at date excluding the *L. kriegi* group). The distribution of the *L. elongatus* group ranges from the locality of Tafí del Valle in Tucumán province, northern Argentina, along the Andes in Chile and Argentina, to Chubut province in Argentina. The species of the *L. elongatus* group are characterized by their elongated body shape; they are usually saxicolous, viviparous and omnivorous (Cei, 1986; Quatrini *et al*., 2001; Robles and Halloy, 2011). Currently, the group is composed of 34 valid species, many of which have been described in the last 10 years (Abdala *et al*., 2010; Avila *et al*., 2010; Avila *et al*., 2012; Avila *et al*., 2015; Escobar-Huerta *et al*., 2015; Troncoso-Palacios *et al*., 2016; Ruiz *et al*., 2019; Troncoso-Palacios *et al*., 2019; Abdala *et al*., 2021a; Avila *et al*., 2021; Troncoso-Palacios and Ramírez-Álvarez, 2021).

In recent years, several authors analysed representatives of the *Liolaemus elongatus* group to estimate phylogenies (relationships among families, genera or species), divergence times or to study the evolution of certain biological features (Schulte, 2013; Medina *et al.,* 2014; Zheng and Wiens, 2016; Medina *et al.,* 2017; Esquerré *et al.,* 2019; Ruiz *et al.,* 2020; Esquerré *et al.,* 2022). Table 1 summarises the ages of the group and clades estimated by different studies. Very few systematic studies on *Liolaemus* incorporated morphological evidence (Lobo, 2001; Abdala, 2005; Lobo, 2005; Abdala, 2007; Lobo *et al.,* 2010; Quinteros, 2013) and even fewer studies incorporated genital morphology, which proved to be very informative within *Liolaemus* (Quipildor *et al.,* 2018).

Table 1. Divergence times obtained for the clades of the *Liolaemus elongatus* group. (*) Studies based on more inclusive taxonomic groups.

Authors **Clades**	**Schulte (2013)***	**Medina *et al.* (2014)**	**Zheng & Wiens (2016)***	**Medina *et al.* (2017)**	**Esquerré *et al.* (2019)***	**Ruiz *et al.* (2020)**	**Esquerré *et al.* (2022)***
L. elongatus Group	16 Ma	-	12.30 Ma	-	13 Ma	11.50 Ma	15.50 Ma
L. punmahuida Clade	-	-	-	-	2 Ma	4 Ma	4 Ma
L. elongatus-petrophilus Clade	Not recovered	3.75 Ma	10.54 Ma	2.25 Ma	9.50 Ma	10.10 Ma	12.5 Ma
L. petrophilus Clade	Not recovered	-	Not recovered	1.60 Ma	8.20 Ma	7.70 Ma	11 Ma
L. capillitas Clade	Not recovered	Not recovered	6.01 Ma	-	4.50 Ma	4.25 Ma	6 Ma
L. elongatus-kriegi Clade	Not recovered	1.90 Ma	4.74 Ma	1.60 Ma	5.80 Ma	6 Ma	6 Ma
L. kriegi Clade	6 Ma	1.10 Ma	2.93 Ma	1.35 Ma	4 Ma	3 Ma	4 Ma
L. elongatus sensu stricto Clade	Not recovered	-	2.93 Ma	1.30 Ma	4 Ma	4.16 Ma	4.5 Ma

The main aim of this study was to describe a new species of the *Liolaemus elongatus* group, which we recognized as distinct from other members based on morphological characters (scalation, hemipenis, color pattern), and DNA sequences. We inferred a DNA-based phylogeny of the whole group using all available molecular information, including the new taxon described here. Furthermore, we obtained the divergence times of the *L. elongatus* group in a calibrated tree.

Materials and methods

Specimens studied

We studied 84 specimens representing the seven species of the *Liolaemus petrophilus* clade (sensu Avila *et al.,* 2012) and one candidate species. Individuals were collected by hand or noose, euthanized with sodium Pentothal 1%, fixed in 10% formalin, and preserved in 70% ethanol. Field studies did not involve endangered species. We studied the morphological characters commonly included in *Liolaemus* taxonomic studies, such as those described in Laurent (1985), Frost (1992), Etheridge (1993, 1995, 2000), Cei (1986), Lobo and Espinoza (1999), Lobo (2001, 2005), Abdala (2007), Quinteros (2013) and Quipildor *et al.* (2018), which altogether conform a record of more than 200 morphological characters, including scale counts, shape, ornamentation, variation in the disposal of the scales (imbrications), precloacal pores, neck folding, color pattern, life colors, hemipenis, among others. The description of colors in life was made based on photographs taken in the field immediately after capture of individuals. Observations of scalation and body measurements were made using a binocular dissecting microscope (10-40×). All measurements in this study were taken using a digital calliper 0.05 mm (Mitutoyo USA, CD-6”CX, Illinois, USA). The studied specimens are deposited in the Museo Patagónico de Ciencias Naturales “Juan Carlos Salgado” (MPCN), Fundación Miguel Lillo (FML) and the Instituto de Bio y Geociencias del NOA (IBIGEO); they are listed in Appendix S1.

Statistical analyses

To perform our statistical analyses, we considered 91 characters (44 morphometric characters and 47 characters of lepidosis) from 84 adult specimens (see Appendix A) belonging to seven species of *Liolaemus*: *L. austromendocinus* (*n*=17), *L. capillitas* (*n*=15), *L. gununakuna* Avila *et al.* 2004 (*n*=6), *L. parvus* Quinteros *et al.* 2008 (*n*=12), *L. petrophilus* (*n*=7), *L. quinterosi* Ruiz *et al.* 2019 (*n*=5), *L. tulkas* Quinteros *et al.* 2008 (*n*=8) and *Liolaemus* sp. (*n*= 14).

We measured the following morphometric characters: snout-vent length (SVL; from tip of snout to vent), head length (HeL; from tip of snout to posterior edge of auditory meatus), head width (HeW; distance between temporal regions), head height (HeH), distance between orbits (DO), tail length (TL; distance between the cloaca and the tip), base of tail width (TW; at the base of tail in the cloacal region), trunk length (TrL; distance between fore and hind limbs), femur length (FL), tibia length (TiL), foot length (FoL; distance from the tip of fourth toe to ankle), humerus length (HML), humerus width (HMW), radius length (RL), hand length (HdL), auditory meatus height (AMH), auditory meatus width (AMW), neck width (NW), 1st finger length (1stFL), 2nd finger length (2ndFL), 3rd finger length (3rdFL), 4th finger length (4thFL), 5th finger length (5thFL), 1st toe length (1stTL), 2nd tor length (2ndTL), 3rd toe length (3rdTL), 4th toe length (4thTL), 5th toe length (5thTL), eye-tympanum length (ETL; distance between ocular scale and tympanic region), distance between nostrils (DN), subocular scale length (SSL), rostral scale width (RSW), rostral scale height (RSH), mental scale length (MSL), mental scale width (MSW), 4th supralabial scale length (4thSL), 4th lorilabial scale length (4thLL), 5th lorilabial scale length (5thLL), postocular scale length (PoSL), preocular scale length (SPL), auricular scale length (ASL), proportion of scale organs on one side of the body (SOB), relationship between dorsal and lateral body scales (DLB), and relationship between humeral and dorsal body scales (HDS).

We also took classical lepidosis characters using a binocular stereoscope. We counted: supralabial scales (SS), lorilabial scales (LS), infralabial scales (IS), supraocular scales (SOS), frontal scales (FS), scales contact with interparietal (SIn), scales surrounding the nasal (SN), scales between nasal and canthal scale (SNC), scales between rostral and frontal scale (SRF), lorilabials in contact with subocular (LCS), temporal scales (TS), superior ciliary scales (SCS), scale organs in right postrostral (ORP), superciliaries scale (SuS), scales between preocular and lorilabials (SPL), semicircles scale (SeS), postmental scales (PoS), dorsal head scales (DHS), scale organs in left postrostral (OLP), subdigital lamellae on 1st finger (1st FSL), subdigital lamellae on 2nd finger (2nd FSL), subdigital lamellae on 3rd finger (3rd FSL), subdigital lamellae on 4th finger (4th FSL), subdigital lamellae on 5th finger (5th FSL), subdigital lamellae on 1st toe (1st TSL), subdigital lamellae on 2nd toe (2nd TSL), subdigital lamellae on 3rd toe (3rd TSL), subdigital lamellae on 4th toe (4th TSL), subdigital lamellae on 5th toe (5th TSL), scales between 1st postmentals (1st SPo), scales between 2nd postmentals (2nd SPo), scales between 3rd postmentals (3rd SPo), scales between 4th postmentals (4th SPo), scales between subocular and canthal (SSC), scales between subocular and nasal (SSN), scale organs in neck scales (ONS; counted in six scales in the central region of the neck), scale organs on the sides of the body (OTS; counted in six scales on the lateral region of the trunk), scale keels on 4th toe (4th SKT), scales that contact 2nd infralabial (2nd SI), precloacal pores in males (PPM), scales around body (SAB), dorsal scales (DS; between occiput and thighs region), neck scales (NS), gular scales (GS), ventral scales (VS), pygal scales (PS), scales between posterior margin of auditory meatus and antehumeral fold (SNF).

To reduce the number of variables for statistical comparison, a principal component analysis was performed and based on the results, the characters that most contributed to the variability among species were selected. Then, due to the low number of specimens of some species, the statistical analyses were carried out with a non-parametric Kruskal Wallis ANOVA, following procedures described in Conover (1999).

Following previous morphological and taxonomic studies on the sister genus *Phymaturus* (Lobo *et al.,* 2019; Lobo *et al.,* 2021) and recommendations of Chan and Grismer (2021), we performed a regression analysis of each measurement character with body size, and used the residuals of these correlations to remove the possible effect of body size. Moreover, for the analysis of measurements, we only worked with adult specimens; we did not consider juveniles to avoid result biases. Since the number of scales is not influenced by body size, juveniles were included in the analysis of scale count characters. It is worked with the set of individuals without differentiation between sexes due to the small number of samples of some species.

The components of the principal components analysis that explained 75-80% of each subset of characters were retained. Thus, we retained the first four components for scale counts and the first three components for measurement characters. Then, from these components, the characters that most contributed to the variability were chosen for statistical comparisons. The criterion for choosing the variables was a contribution of least 80% to the variability of each component, using as a maximum (100%) the variable with the highest value of each component (Table 2).

Hemipenial morphology

We studied hemipenial morphology of representatives of species of the *Liolaemus petrophilus* clade: *L. austromendocinus, L. parvus*, *L. petrophilus*, *L. quinterosi*, *L. capillitas* (representing the *L. capillitas* subclade) and *Liolaemus* sp. The sample size varied from one to five pairs of hemipenes per species. The specimens studied are listed in Appendix S1. We followed the protocol of Zaher and Prudente (2003) for preparing snake organs, which consisted of removing one of the hemipenes from each fixed specimen through a small incision at the base of the tail. The removed organ was immersed in a 2% KOH solution for 3–5 min or until it became translucent and flexible. The hemipenis was checked and everted manually using forceps to make sure that it was completely everted. Once fully everted, the organ was filled with coloured Vaseline to allow a clear visualisation of ornamentation structures. We obtained digital images of hemipenes in sulcate, asulcate,

Table 2. Components of the PCA that explain between 75–80% of the total variability for morphometric and lepidosis characters.

Lambda	**Value**		**Proportion**		**Cumulative Proportion**	
	Morphometric	Lepidosis	Morphometric	Lepidosis	Morphometric	Lepidosis
1	16.10	16.73	0.40	0.32	0.40	0.32
2	8.90	12.50	0.22	0.24	0.63	0.55
3	7.45	7.53	0.19	0.14	0.81	0.69
4		5.69		0.11		0.80

and lateral views using a camera (Olympus DP25) attached to a stereomicroscope. Terminology of hemipenial character states follows that of Dowling and Savage (1960), Savage (1997), Zaher (1999), Ziegler and Böhme (1999), Zaher and Prudente (2003) and Quipildor *et al.* (2018).

Extraction, amplification and DNA sequencing

DNA extraction from the candidate species was performed using a Qiagen® DNeasy® 96 kit, following the manufacturer's instructions. We sequenced two fragments of mitochondrial genes (12S rRNA [12S ~853bp, 2 individuals] and cytochrome-b [cyt-b, ~800bp, 2 individuals]) and a nuclear gene (a member of the kinesins family 24 [KIF24 ~490 bp, 2 individuals]). The protocols for PCR and DNA sequencing followed Morando *et al.* (2003; 2004) and Portik *et al.* (2010). We used the following primers: 12e (Forward) and tPhe (Reverse) for 12S (Wiens *et al.,* 1999); GLUDGL (Forward) and CB3 3' (Reverse) for Cyt-b (Palumbi, 1996); and KIF24 F1 (Forward) and KIF24 R1 (Reverse) for KIF24.

Both DNA extraction and amplification were performed at the Institute of Diversity and Austral Evolution (IDEAUS-CONICET). All sequences were edited and concatenated using BioEdit v7.2.6 (Hall, 1999). Sequence alignment was performed by running MEGA v7.0.26 and MUSCLE (Kumar *et al.,* 2018) and then checked by eye to maximise identity blocks of sequences. Missing data were coded as "?". Appendix S2 summarises locality data and GenBank accession numbers for the specimens used in this study. In addition, the following sequences obtained from GenBank were included: Avila *et al.* (2004; 2006; 2015), Fontanella *et al.* (2012), Medina *et al.* (2014, 2015, 2017, 2018), Morando *et al.* (2003), and Olave *et al.* (2014).

Divergence time estimates

Tree topology, node ages and substitution rates were simultaneously estimated using Bayesian MCMC (Markov chain Monte Carlo) approach as implemented in BEAST v2.5 (Bouckaert *et al.,* 2019). We used the fossil belonging to the *Eulaemus* clade, which is the earliest record of this subgenus (Albino, 2008) to place a mean prior of 20 Ma on the tree height. A lognormal prior is typically most appropriate for most fossil calibrations (Hedges and Kumar, 2004) because it assumes that the divergence event actually occurred sometime before the appearance of the fossil. In this model, fossils thus represent a hard lower bound and a soft upper bound on a given divergence event. The Yule prior assumes a constant lineage birth rate for each branch in the tree and is considered most suitable for trees describing the relationships between individuals of different species (Ho *et al.,* 2005). An uncorrelated lognormal distributed relaxed clock (UCLD) model was employed, which allows evolutionary rates to vary along branches within lognormal distributions (Drummond *et al.,* 2006). The calibration was conducted for all genes jointly.

Three independent runs of 20 million generations each were performed with sampling every 5000 generations. The three separate runs were then combined (following removal of 10% burn-in) using Log Combiner v2.0 (Drummond and Rambaut, 2007; Rambaut and Drummond, 2008). Adequate sampling and convergence of the chain to stationary distribution were confirmed by inspection of MCMC samples using Tracer v2.0 (Drummond and Rambaut, 2007). The effective sample size (ESS) values of all parameters were greater than 200, which was considered a sufficient level of sampling. The sampled posterior trees were summarised using Tree Annotator v2.0 (Drummond and Rambaut, 2007; Rambaut and Drummond, 2008) to generate a maximum clade credibility tree (maximum posterior probabilities) and calculate the mean ages, 95% highest posterior density (HPD) intervals and posterior probabilities for each node. The recovered topology was visualised with Fig Tree v1.2 (Rambaut and Drummond, 2008). We also calculated the uncorrected average pairwise genetic distances using MEGA 10.2.4 (Kumar *et al.,* 2018).

Results

Statistical analyses

The first three and four principal components explained most of the total variance (Table 2). The analysis of the characters of *Liolaemus* sp. and of the other species that belong to the *L. petrophilus* subclade showed that 59 morphological variables had significant differences ($P < 0.05$). These variables clearly differentiate *Liolaemus* sp. from the rest of the clade, with a total of 21 linear morphometric characters (Table 3) and 38 meristic characters (Table 4).

Phylogeny

We recovered the traditional *Liolaemus elongatus* group composed of five clades; the *L. elongatus kriegi*

Table 3. Summary of statistical values (mean ± standard error) of the of 24 morphometric characters. The ANOVA results obtained from comparisons across species of the Liolaemus petrophilus clade are also indicated. Different capital letters indicate a significant difference between species. Sample size is indicated between parentheses. SVL: snout vent length ; RSW: rostral scale width; PoSL: postocular scale length; TiL: tibia length; TrL: trunk length; HML: humerus length; HMW: humerus width; AMH: auditory meatus height; 4thFL: 4th finger length; 1st toe length; RSH: rostral scale height; MSW: mental scale width; ASL: auricular scale length; 2ndTL: 2nd toe length; 5thTL: 5th toe length; MSL: mental scale length; 4thSL: 4th supralabial scale length; 3rdFL: 3rd finger length; 2ndFL: 2nd finger length; HeH: head height; DO: distance between orbits.

	L. austromendocinus (n=17)		*L. capillitas* (n=11)		*L. gununakuna* (n=6)		*L. parvus* (n=10)		*L. petrophilus* (n=7)		*L. quinterosi* (n=5)		*L. splendidus* sp. nov. (n=11)		*L. tulkas* (n=8)		Test	*p*
SVL	81.14±5.81	BC	74.74±5.65	B	79.16±8.21	BC	59.27±5.43	A	77.64±7.07	B	82.32±5.02	BC	88.18±6.88	C	63.87±5.15	A	H=50.17	<0.0001
RSW	-0.13±0.27	B	-0.09±0.25	BC	0.12±0.34	BCD	-0.04±0.18	BC	-0.13±0.19	AB	-0.60±0.19	A	0.48±0.37	D	0.19±0.23	CD	H=36.03	<0.0001
PoSL	0.08±0.46	ABC	0.14±0.27	BC	0.41±0.35	C	-0.15±0.32	AB	0.13±0.48	ABC	0.16±0.25	BC	-0.41±0.70	A	-0.13±0.10	AB	H=16.00	0.0251
TiL	0.21±1.14	A	-0.13±1.68	A	-1.52±2.08	A	-0.04±1.39	A	-0.60±0.90	A	-0.76±0.47	A	1.83±2.32	B	-0.61±1.49	A	H=18.35	0.0105
TrL	-0.22±2.93	ABC	-2.07±1.66	A	-0.19±2.99	ABCD	0.63±1.88	BCD	-2.30±2.82	AB	-1.32±3.18	ABC	3.03±4.68	D	1.34±5.16	CD	H=16.99	0.0175
HML	-0.76±0.97	A	-0.06±0.92	A	-0.25±0.77	A	0.19±0.87	AB	-0.80±0.64	A	-0.29±1.05	A	1.99±1.56	B	-0.21±0.97	A	H=23.67	0.0013
HMW	-0.55±0.71	A	-0.23±0.41	AB	-0.49±0.76	AB	0.10±0.35	BC	-0.67±0.54	A	-0.36±0.52	AB	1.73±1.16	C	0.16±1.03	AB	H=27.02	0.0003
AMH	-0.11±0.31	A	-0.12±0.33	A	-0.18±0.30	A	-0.10±0.20	A	-0.07±0.20	AB	-0.52±0.41	A	0.44±0.57	BC	0.45±0.40	C	H=24.90	0.0008
4thFL	0.01±0.47	BC	0.25±0.75	BCD	-1.20±3.40	ABC	-0.22±0.56	AB	-0.01±0.97	BCD	-0.98±0.47	A	0.68±0.54	D	0.51±0.33	CD	H=22.07	0.0025
1stTL	0.63±0.48	C	-0.03±0.62	B	0.23±0.56	BC	-0.24±0.48	AB	-0.15±0.59	B	0.46±0.74	BC	-0.91±0.58	A	-0.06±0.40	B	H=33.72	<0.0001
RSH	-0.07±0.14	ABC	0.01±0.11	CD	0.28±0.57	CD	-0.01±0.08	BCD	-0.15±0.17	AB	-0.25±0.10	A	0.16±0.16	D	0.01±0.15	BCD	H=24.42	0.0010
MSW	0.03±0.30	B	-0.21±0.24	AB	0.04±0.73	AB	0.80±0.20	B	-0.25±0.21	A	-0.68±0.31	A	0.47±0.30	C	0.11±0.28	BC	H=33.47	<0.0001
ASL	0.17±0.21	C	0.25±0.17	C	0.00±0.00		-0.05±0.11	AB	0.05±0.37	ABC	0.08±0.35	BC	-0.49±0.27	A	-0.04±0.07	AB	H=33.95	<0.0001
2ndTL	0.08±0.81	BC	-0.08±0.58	ABC	0.48±0.47	C	-0.55±0.47	A	0.17±0.50	BC	-0.10±0.71	ABC	-0.28±0.66	AB	0.57±0.45	C	H=17.85	0.0127
5thTL	0.44±0.76	B	0.54±0.89	B	-0.07±2.51	A	-0.54±0.43	A	-0.13±1.04	AB	-0.80±0.52	A	-0.60±0.72	A	0.49±0.68	B	H=28.81	0.0002
MSL	0.01±0.20	C	0.01±0.16	BC	-0.05±0.47	ABC	-0.09±0.18	AB	-0.06±0.14	BC	-0.01±0.12	BC	0.02±0.20	A	0.13±0.16	C	H=5.83	0.5593
4thSL	0.30±0.45	C	0.24±0.76	BC	-0.19±1.00	ABC	-0.44±0.48	AB	0.25±0.71	BC	0.17±0.87	BC	-0.73±0.77	A	0.39±0.19	C	H=25.10	0.0007
3rdFL	0.09±0.41	C	0.20±0.78	C	-0.08±0.84	ABC	-0.53±0.42	AB	-0.14±0.92	BC	-0.98±0.51	A	0.15±0.52	CD	0.79±0.43	D	H=27.96	0.0002
2ndFL	0.29±0.51	C	0.08±0.68	BC	-0.10±0.52	ABC	-0.37±0.53	AB	-0.48±0.74	AB	-0.73±0.55	A	-0.11±0.33	AB	0.85±0.26	D	H=32.35	<0.0001
HeH	-0.41±0.44	A	0.23±0.71	C	0.34±1.50	ABC	-0.55±0.41	A	0.29±0.49	BC	0.96±0.58	C	-0.33±0.46	AB	0.59±0.59	C	H=31.67	<0.0001
DO	-0.39±0.38	BC	-0.38±0.54	BC	-0.43±0.63	ABC	-0.60±0.37	AB	-1.16±0.39	A	0.39±0.51	CD	1.24±1.40	D	1.49±0.69	D	H=39.65	<0.0001

Table 4. Summary of statistical values (mean ± standard error) of the of 26 lepidosis characters. The non-parametric ANOVA results obtained from comparisons across species of the Liolaemus petrophilus clade are also indicated. Different capital letters indicate a significant difference between species. Sample size is indicated between parentheses. 2nd SI: scales that contact 2nd infralabial; LS: lorilabial scales; LCS: lorilabials in contact with subocular; TS: temporal scales; SCS: superior ciliary scales; OLP: scale organs in left postrostral; ORP: scale organs in right postrostral, ONS: scale organs in neck scales; PPM: precloacal pores in males; SAB: scales around of body; 1st TSL: 1st toe subdigital lamellae; 2nd TSL: 2nd toe subdigital lamellae; 5th TSL: 5th toe subdigital lamellae; 2nd FSL: 2nd finger subdigital lamellae; 3rd FSL: 3rd finger subdigital lamellae; VS: ventral scales; PS: pygal scales; 1st SPo: scales between 1st posmentals; 2nd SPo: scales between 2nd posmentals; 3rd SPo: scales between 3rd postmentals; 4th SPo: scales between 4th postmentals; 5th SPo: scales between 5th postmentals; SNC: scales between nasal and canthal scale; SRF: scales between rostral and frontal scale; SuS: superciliary scales; GS: gular scales; NS: neck scales; SNF: scales between posterior margin of auditory meatus and antehumeral fold; DS: dorsal scales; PoS: postmental scales; SeS: semicircles scales; 4th TSL: toe subdigital lamellae; 4th FSL: finger subdigital lamellae; 1st FSL: 1st finger subdigital lamellae; SS: supralabial scales; SOS: supraocular scales; FS: frontal scales; SN: scales surrounding the nasal.

	L. austromendocinus (n=17)		*L. capillitas* (n=15)		*L. gununakuna* (n=6)		*L. parvus* (n=12)		*L. petrophilus* (n=7)		*L. quinterosi* (n=5)		*L. splendidus* sp. nov. (n=14)		*L. tulkas* (n=8)		Test	*p*
2nd SI	4.18±0.39	B	4.09±0.30	B	4.67±0.82	B	4.00±0.00	B	4.29±0.49	B	4.00±0.00	B	4.00±0.00	B	2.25±0.46	A	H=24.79	<0.0001
LS	7.47±0.87	AB	8.27±2.00	BC	10.50±1.05	D	8.00±0.67	BC	10.00±1.00	D	8.80±0.84	CD	8.73±0.79	CD	6.88±0.35	A	H=37.88	<0.0001
LCS	4.12±0.78	AB	5.27±1.90	BCD	5.67±0.82	D	3.80±0.63	A	5.71±0.49	D	5.40±1.14	CD	4.36±1.03	ABC	3.63±0.52	A	H=28.12	0.0001
TS	10.06±1.09	BC	10.82±0.87	CDE	10.83±1.33	BCDE	10.30±0.95	BCD	11.57±0.53	E	11.40±0.55	DE	9.36±2.01	AB	7.88±0.64	A	H=31.75	<0.0001
SCS	13.88±1.17	B	14.09±1.14	BC	15.33±0.52	CD	16.00±1.05	D	15.00±1.15	BCD	16.20±1.30	D	14.55±1.29	BCD	11.00±0.00	A	H=39.99	<0.0001
OLP	7.53±2.55	AB	6.27±3.13	A	9.83±3.60	BC	7.70±1.34	AB	10.43±3.36	BC	8.80±2.59	ABC	11.55±2.30	C	6.38±1.19	A	H=25.12	0.0006
ORP	6.65±3.39	A	6.45±1.63	A	8.83±3.43	AB	7.60±.2.32	A	10.71±2.87	BC	9.00±1.22	ABC	12.18±1.72	C	7.50±1.20	A	H=31.11	0.0001
ONS	0.87±0.13	AB	0.95±0.11	BC	1.00±0.00	C	1.00±0.00	C	0.93±0.09	BC	0.97±0.08	BC	1.00±0.00	C	0.75±0.09	A	H=25.29	<0.0001
PPM	3.00±0.00	AB	2.71±0.95	AB	3.00±0.00	AB	1.50±0.55	A	4.25±0.96	B	0.00±0.00	A	1.71±0.95	A	0.33±0.58	A	H=26.14	0.0002
SAB	73.41±3.57	C	63.82±5.23	A	76.83±4.79	CD	73.60±3.44	C	80.29±4.31	CD	73.40±3.44	BC	85.27±4.61	D	66.00±1.77	AB	H=55.89	<0.0001
1st TSL	12.41±0.62	BC	11.64±1.03	AB	12.67±0.82	BC	11.60±0.97	AB	12.00±1.53	BC	12.80±0.45	C	12.27±1.42	BC	10.63±0.74	A	H=20.85	0.0018
2nd TLS	17.82±0.88	BC	16.55±0.69	A	17.83±1.33	BC	16.80±0.63	AB	19.14±0.90	C	18.00±1.22	BC	18.45±1.29	C	16.88±1.13	AB	H=30.01	<0.0001
5th TLS	18.94±1.30	B	18.91±1.30	B	18.67±1.37	B	18.70±1.25	B	19.71±0.49	BC	29.20±2.17	C	19.36±1.50	B	12.75±0.89	A	H=36.25	<0.0001
2nd FLS	16.18±0.81	CD	15.45±0.93	ABC	16.50±1.38	CDE	14.80±0.92	AB	16.57±0.53	DE	19.60±0.89	E	15.55±1.04	BCD	14.25±1.04	A	H=36.87	<0.0001
3rd FLS	20.59±1.23	B	20.64±1.36	AB	21.00±2.68	AB	20.20±1.69	AB	21.43±0.79	BC	23.80±1.92	C	21.09±1.14	B	19.00±1.31	A	H=20.74	0.0030
VS	119.00±4.73	B	104.36±7.06	A	121.83±10.53	B	111.60±3.89	A	129.00±7.14	B	108.00±3.81	A	127.82±7.10	B	107.25±5.23	A	H=53.63	<0.0001
PS	16.24±1.35	CD	15.27±1.74	BC	18.33±2.16	DE	15.00±0.94	ABC	19.43±1.40	E	17.20±1.48	CDE	14.27±2.37	AB	12.38±1.85	A	H=42.01	<0.0001
1st SPo	0.00±0.00	A	0.00±0.00	A	0.00±0.00	A	0.00±0.00	A	0.43±0.53	A	0.00±0.00	A	0.00±0.00	A	2.50±0.53	B	H=23.88	<0.0001
2nd SPo	2.12±0.33	A	2.00±0.00	A	1.83±0.41	A	2.00±0.00	A	2.00±0.00	A	2.20±0.45	A	2.09±0.30	A	4.63±0.52	B	H=22.40	<0.0001
3rd SPo	4.47±0.72	AB	4.09±0.94	AB	3.83±0.41	A	4.20±0.42	AB	4.57±0.53	AB	5.00±0.71	BC	4.00±1.00	A	5.88±0.35	C	H=24.62	0.0002
4th Spo	7.00±0.00	C	6.00±0.77	AB	6.50±1.38	BC	6.30±0.67	B	6.14±0.38	AB	6.00±1.22	AB	6.18±1.25	B	5.13±0.83	A	H=24.16	0.0004
5th SPo	8.38±1.04	B	6.50±0.55	A	8.33±0.82	B	7.89±0.93	B	8.71±0.95	B	6.33±0.58	A	7.64±1.03	AB	6.60±0.55	A	H=26.28	0.0002
SNC	2.00±0.00	B	2.00±0.00	B	2.00±0.00	B	2.00±0.00	B	2.00±0.00	B	2.00±0.00	B	1.27±0.65	A	2.00±0.00	B	H=14.70	<0.0001
SRF	5.71±0.59	B	4.91±0.30	A	5.50±0.55	AB	5.50±0.71	AB	6.00±0.00	BC	5.40±0.55	AB	6.82±0.87	C	7.00±0.53	C	H=38.20	<0.0001

SuS	6.82±0.53	B	7.00±0.63	B	7.00±0.63	B	6.90±0.74	B	6.57±0.79	AB	7.00±0.00	B	6.00±1.34	A	6.00±0.00	A	H=18.97	0.0016
GS	43.18±2.90	B	38.36±5.05	A	40.50±4.23	ABC	39.00±1.89	A	43.14±3.76	AB	38.20±2.39	A	54.73±7.75	C	43.13±2.42	B	H=40.74	<0.0001
NS	39.47±2.07	A	37.55±3.27	A	38.50±1.97	A	37.90±2.13	A	36.86±3.53	A	42.00±3.46	AB	55.00±10.17	B	51.88±3.23	B	H=39.81	<0.0001
SNF	29.88±1.65	B	27.27±2.20	A	28.50±2.35	AB	30.10±2.05	B	28.29±1.98	AB	30.80±3.49	BC	41.82±8.47	C	34.25±2.92	C	H=35.88	<0.0001
DS	72.00±3.72	A	74.64±5.33	AB	82.50±6.19	CD	80.10±3.14	C	77.14±3.72	BC	73.00±5.61	AB	89.55±4.82	D	76.25±4.46	ABC	H=43.95	<0.0001
PoS	4.76±0.44	A	4.55±0.52	A	5.00±0.00	A	4.80±0.42	A	5.00±0.00	A	4.60±0.55	A	6.82±0.40	B	4.63±0.52	A	H=31.11	<0.0001
SeS	10.65±1.32	A	10.73±0.90	A	11.17±0.98	AB	11.00±0.47	AB	11.86±0.38	BC	10.80±0.84	AB	13.27±1.19	C	10.13±0.83	A	H=31.75	<0.0001
4th TSL	28.41±2.00	B	27.91±1.45	B	28.33±1.37	B	27.70±1.70	B	31.14±0.90	C	19.00±0.71	A	28.09±2.02	B	26.88±0.64	AB	H=30.54	0.0001
4th FSL	22.71±2.31	B	22.27±1.19	B	24.67±1.21	C	22.20±1.48	B	23.00±0.82	BC	11.20±2.39	A	23.82±2.99	C	22.25±1.04	B	H=30.95	<0.0001
1st FSL	10.65±0.61	CD	10.18±0.40	ABC	10.67±1.21	BCD	9.70±0.82	AB	10.00±0.82	ABC	16.60±2.51	E	11.36±1.21	DE	9.50±0.53	A	H=34.50	<0.0001
SS	7.00±0.87	AB	6.55±0.52	A	7.67±0.82	BC	7.00±0.94	AB	8.43±0.53	C	7.20±0.84	AB	7.73±1.01	BC	7.50±0.53	BC	H=22.16	0.0009
SOS	4.59±0.71	AB	4.18±0.75	A	5.17±0.75	ABCD	5.10±0.74	ABC	5.71±0.49	CD	4.40±0.55	AB	10.82±3.52	D	5.25±0.46	BCD	H=29.23	<0.0001
FS	2.12±0.60	AB	2.82±0.60	BC	2.00±0.63	AB	2.70±0.82	B	2.86±0.69	BC	2.40±0.55	AB	3.73±1.01	C	1.75±0.71	A	H=25.36	<0.0001
SN	6.29±0.59	A	7.18±0.40	BC	6.67±0.82	ABC	7.40±0.52	C	7.29±0.49	BC	6.40±0.89	AB	6.73±0.47	ABC	7.25±0.46	BC	H=22.99	0.0001

clade, formed by the *L. elongatus* sensu stricto clade + *L. kriegi* clade; a clade formed by the *L. petrophilus* clade + *L. capillitas* clade; and the *L. punmahuida* clade (Fig. 1).

Within the *Liolaemus petrophilus* group, we found that the sister clade of *Liolaemus* sp. is the clade formed by (*L. quinterosi L. gununakuna*). At the same time, their sister clade is formed by (*L. austromendocinus* + *L. parvus*). The sister group to all of them is the *L. capillitas* clade together with its eight species (sensu Ruiz *et al*., 2020). Finally, *L. petrophilus* is the sister species to all the remaining species forming the clade.

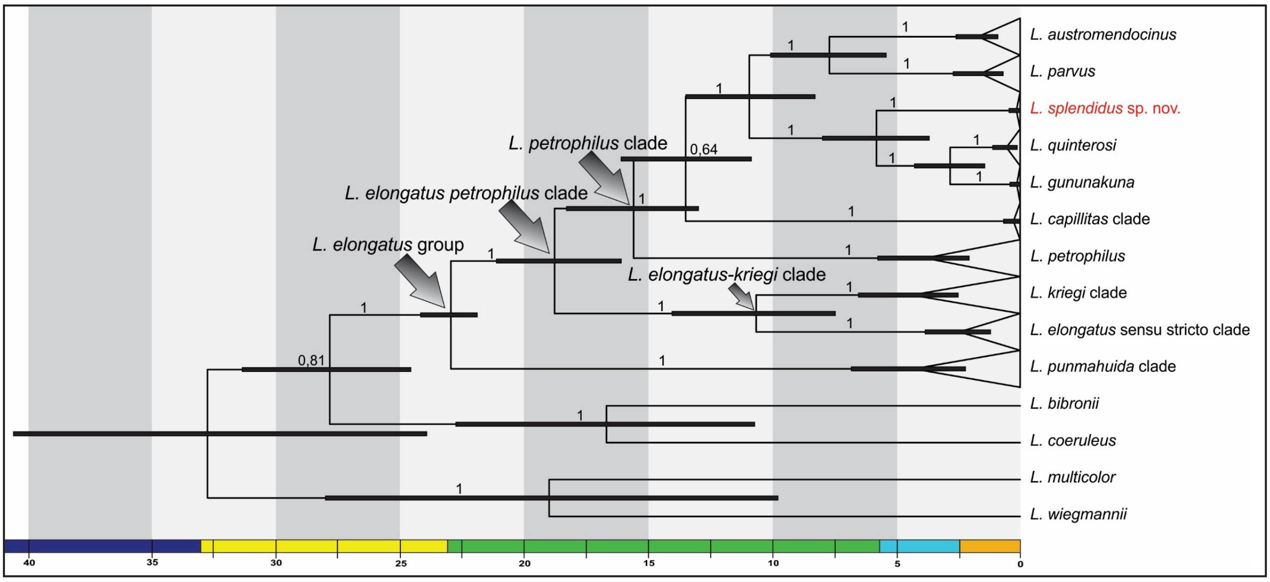

Figure 1. Main clades of the *Liolaemus elongatus* group. Estimates of divergence times for the *L. elongatus* group, under BI topology. Ultrametric tree scaled in Ma. Numbers and horizontal bars on nodes represent posterior probability values and 95% credibility intervals. The different colors of the bar represent the different times. Orange: Pleistocene. Light blue: Pliocene. Green: Miocene. Yellow: Oligocene. Blue: Eocene.

Divergence time estimates

Our results (Fig. 1) show that the *Liolaemus elongatus* group diverged at 23 Ma (95% highest posterior density interval- HPD: 24.5-22) during the Early Miocene. Within this group, the clades originated from the Miocene to the Pleistocene. The *L. punmahuida* clade diverged at 4 Ma (95% HPD: 7-2.3) during the Middle Pliocene. The *L. elongatus-petrophilus* clade would have originated at 19 Ma (95% HPD: 21.5-15.2) during the Early Miocene. The *L. capillitas* clade diverged at 8 Ma (95% HPD: 9.5- 7) during the Late Miocene. The *L. elongatus-kriegi* clade diverged at 11 Ma (95% HPD: 13.5-7.5) during the Middle Miocene. The *L. kriegi* clade originated at 4 Ma (95% HPD: 6.5-2.5) during the Middle Pliocene. The *L. elongatus* sensu stricto clade diverged at 6 Ma (95% HPD: 7.5-5) during the Late Miocene.

Regarding the *Liolaemus petrophilus* clade, our results show that the divergence of the clade occurred during the Middle Miocene around 15 Ma (95% HPD: 18-13). Within the group, the species that belong to the *L. petrophilus* clade originated between the Pliocene and the Pleistocene.

Within the *Liolaemus petrophilus* clade, we found that *L. austromendocinus* and *L. parvus* diverged of their ancestor in common around 1.9 Ma (95%HPD: 2.5-1) and 1.7 Ma (95% HPD: 2.8-0.8) respectively in the Early Pleistocene. *Liolaemus gununakuna* and *L. quinterosi* diverged of their ancestor in common around 0.1 Ma (95%HPD: 0.3-0.01) and 0.5 Ma (95% HPD: 1-0.1) respectively in the Late Pleistocene. *Liolaemus* sp. was separated from the ancestor of *L. gununakuna* and *L. quinterosi* around 0.1 Ma (95% HPD: 0.5-0.01) in the Late Pleistocene. Finally, *L. petrophilus* diverged around 0.5 Ma (95% HPD: 1-0.1) in the Late Pleistocene.

Genetic distances

Genetic distances among cytb sequences within members of the *Liolaemus petrophilus* clade and some species of the *L. elongatus* group are shown in Table 5. *Liolaemus* sp. shows 4.08% distance from

Table 5. Genetic distances of mitochondrial marker (Cyt-b) between the new species (*Liolaemus splendidus* sp. nov.) and related species. The values are expressed as a percentage.

	Liolaemus austromendocinus	*Liolaemus capillitas*	*Liolaemus elongatus*	*Liolaemus gununakuna*	*Liolaemus kriegi*	*Liolaemus parvus*	*Liolaemus petrophilus*	*Liolaemus punmahuida*	*Liolaemus quinterosi*	***Liolaemus splendidus* sp. nov.**
Liolaemus austromendocinus										
Liolaemus capillitas	19.72									
Liolaemus elongatus	18.27	19.27								
Liolaemus gununakuna	10.86	16.07	16.57							
Liolaemus kriegi	17.57	18.24	8.11	13.89						
Liolaemus parvus	9.79	19.53	16.11	10.17	16.39					
Liolaemus petrophilus	18.53	18.77	16.29	16.23	17.98	16.27				
Liolaemus punmahuida	19.87	20.45	15.56	16.77	17.77	18.27	19.70			
Liolaemus quinterosi	10.62	16.66	16.52	1.78	14.76	10.14	16.50	16.72		
***Liolaemus splendidus* sp. nov.**	11.57	16.12	18.56	4.09	15.59	11.23	16.08	17.83	4.08	

L. quinterosi (the shortest distance in the analysis). We found a distance of 4.09% between *Liolaemus* sp. and *L. gununakuna*. The distance between *Liolaemus* sp. and *L. parvus* was 11.23%. The distance obtained between *Liolaemus* sp. and *L. austromendocinus* was 11.57%. *Liolaemus* sp. shows 15.59% distance from *L. kriegi*. We obtained a 16.2% distance between *Liolaemus* sp. and *L. petrophilus*. We found 16.12% distance between *Liolaemus* sp. and *L. capillitas*. The distance obtained between *Liolaemus* sp. and *L. punmahuida* was 17.83%. Finally, we found 18.56% distance between *Liolaemus* sp. and *L. elongatus*.

Hemipenial morphology (Fig. 2)

Hemipenes are cylindrical. The apex is shell-shaped. The asulcate and sulcate faces have calyces. Asulcate face has diffused calyces, homogeneously distributed along the distal region of the organ. On this face it also has a hump-shaped protrusion. Sulcate face has regular calyces and narrow and open sulcus spermaticus, bifurcated at the base of distal lobes of the hemipenes. Apical lobes are conspicuously delimited.

Liolaemus splendidus sp. nov. (Fig. 3)

http://zoobank.org/urn:lsid:zoobank.org:act:76C7B714-FAB3-46DD-8646-05245792202B

Holotype

MPCN-H 503 (Adult male): National Route No. 237, 9 km north of Piedra del Águila, (39°58'54.3''S; 70°02'36''W, 650 m a.s.l.), Collón Curá department, Neuquén province, Argentina. Chafrat P., Flores A., and cols. December 21, 2021.

Paratypes

IBIGEO 5531-5533 (male, female, juvenile respectively): National Route No. 237, 9 km north of Piedra del Águila, (39°58'54.3''S; 70°02'36''W, 638 m), Collón Curá department, Neuquén province, Argentina. Hibbard T., Lobo F., Quipildor M., Slodki D., Valdecantos S. cols. December 8, 2016. MPCN-H-500-502 (female, male, male respectively): National Route No. 237, 9 km north of Piedra del Águila, (39°58'54.3''S; 70°02'36''W, 644 m), Collón Curá department, Neuquén province, Argentina. Chafrat P., Gonzales L., Chafrat H., Medina V., Chafrat C., Chafrat P.J. cols. December 2, 2021. MPCN-H- 504-510 (juvenile, female, male, male, female, male and juvenile respectively): National Route No. 237, 9 km north of Piedra del Águila, (39°58'54.3''S; 70°02'36''W, 650 m), Collón Curá department, Neuquén province, Argentina. Chafrat P., Flores A. cols. December 21, 2021.

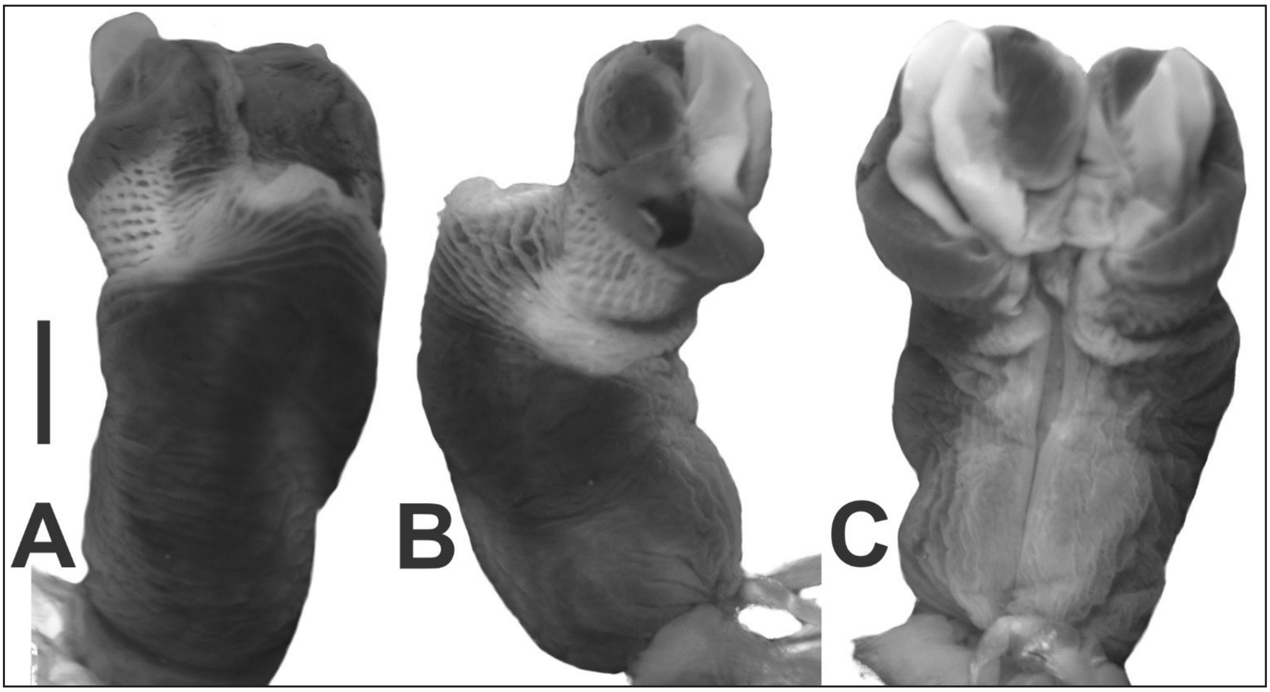

Figure 2. Hemipenial morphology of the *Liolaemus splendidus* sp.nov.(IBIGEO 5531). A: asulcate face, B: lateral face, C: sulcate face. Scale=2mm.

Etymology
The specific epithet "splendidus" is from the Latin 'bright' or 'glittering'. It refers to the predominantly bright green body coloration in this population.

Diagnosis.
Liolaemus splendidus sp. nov. is a large-sized (max. SVL 96.1 mm) member of the *L. elongatus* species group (sensu Avila *et al.*, 2021; Troncoso-Palacios *et al.*, 2021; this study). *Liolaemus splendidus* sp. nov. is distributed in the proximity of the distribution of *L. ceii*, *L. crandalli*, *L. gununakuna*, *L. kriegi*, *L. quinterosi* and *L. shitan* (Fig. 4). Within the *L. petrophilus* clade, the diagnostic characters that differentiate *L. splendidus* sp. nov. from the phylogenetically closest species are the presence of precloacal pores and sexual dichromatism not evident, which separate it from *L. quinterosi*; the larger number of ventral scales, which distinguishes it from *L. gununakuna*; and the max. SVL, which distinguishes it from *L. petrophilus*.

Liolaemus splendidus sp. nov. differs from other species of the *L. elongatus* group in the number of scales around midbody, which in *L. splendidus* sp. nov. (77–95) is lower than in *L. ceii* (102–115) but higher than in *L. capillitas* (58–67), *L. dicktracyi* (60–73), *L. galactostictos* (64–69), *L. heliodermis* (62–69), *L. talampaya* (58–69), *L. tulkas* (63–68) and *L. umbrifer* (58–76). *Liolaemus splendidus* sp. nov. differs from the following eight species: *L. carlosgarini, L. flavipiceus, L. lonquimayensis, L. punmahuida, L. quinterosi, L. riodamas, L. thermarum,* and *L. tregenzai,* males of these species lack precloacal pores (1-3 in *L. splendidus* sp. nov.). Moreover, specimens of *L. splendidus* sp. nov. (max. SVL recorded=96.1 mm) are smaller than specimens of *L. antumalguen* (max. SVL=107.8 mm), *L. austromendocinus* (max. SVL=98.0 mm), *L. buergeri* (max. SVL=108.0 mm), *L. choique* (max. SVL=116.2 mm), *L. gununakuna* (max SVL=97.5 mm), *L. kriegi* (max. SVL=98.0 mm), *L. petrophilus* (max. SVL=97.7 mm), and *L. shitan* (max. SVL=98.7 mm), but larger than specimens of *L. antonietae* (max. SVL=77.6 mm), *L. burmeisteri* (max. SVL=85.2 mm), *L. carlosgarini* (max. SVL=68.8 mm), *L. crandalli* (max. SVL=93.4 mm), *L. galactostictos* (max SVL=81.3 mm), *L. janequeoae* (max SVL=69.6 mm), *L. lonquimayensis* (max SVL=69.7 mm), *L. pikunche* (max. SVL=91.3 mm),

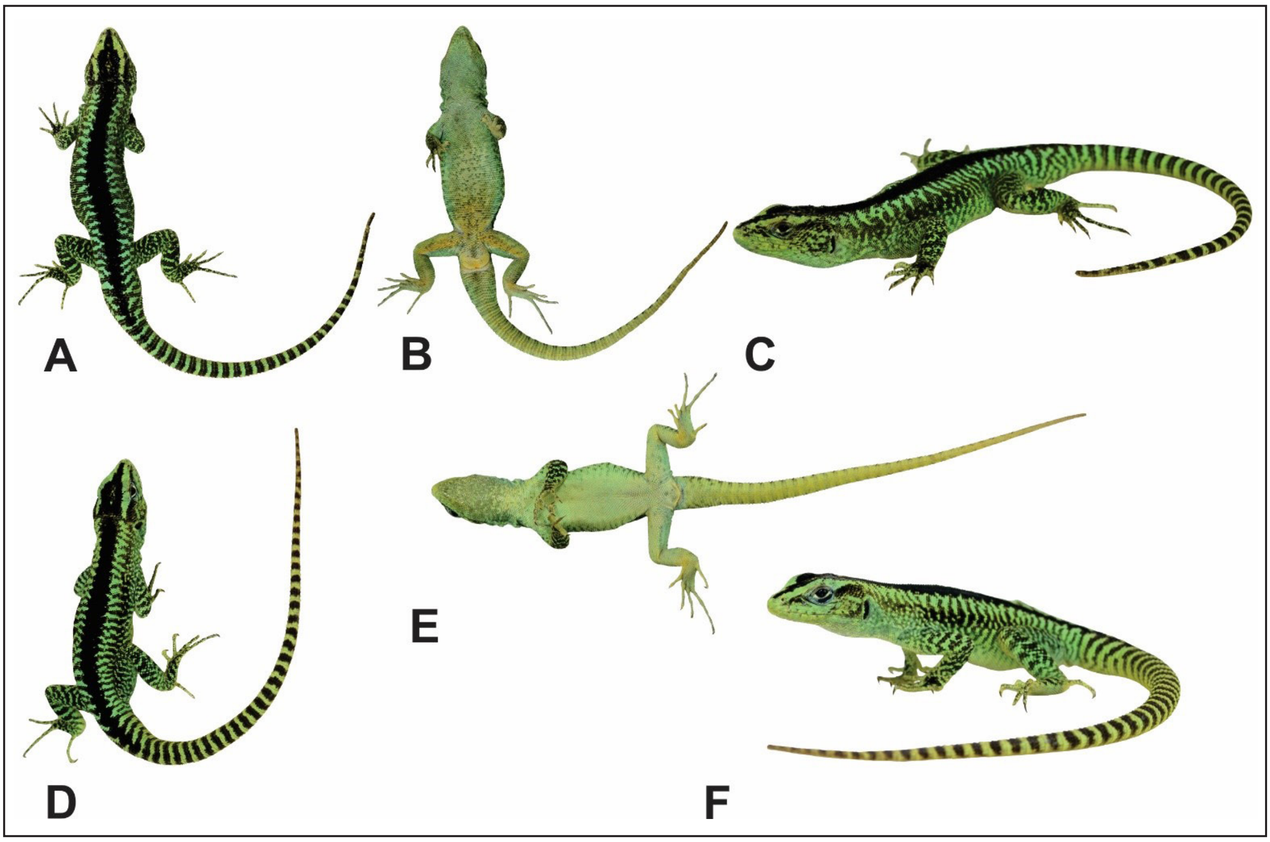

Figure 3. Color in life of *Liolaemus splendidus* sp nov. specimens. A: Male in dorsal view. B: Male in ventral view. C: Male in lateral view. D: Female in dorsal view. E: Female in ventral view. F: Female in lateral view.

L. quinterosi (max. SVL=88.0 mm), *L. scorialis* (max SVL=69.9 mm), *L. smaug* (max. SVL=71.3 mm) *L. tregenzai* (max SVL=90.2 mm), and *L. zabalai* (max SVL=90.3 mm).

The number of dorsal scales in *Liolaemus splendidus* sp. nov. (83–96) is higher than in *L. antumalguen* (70–78), *L. capillitas* (60–73), *L. choique* (65–81), *L. dicktracyi* (64–72), *L. heliodermis* (62–65), *L. galactostictos* (70–79), *L. parvus* (70–81), *L. scorialis* (74–81), *L. talampaya* (64–69), and *L. tulkas* (67–76). The number of scales that contact the second infralabial in *L. splendidus* sp. nov. (4) is higher than in *L. tulkas* (2–3). The number of ventrals in *L. splendidus* sp. nov. (121–145) is higher than in *L. antumalguen* (105–118), *L. burmeisteri* (99–110), *L. capillitas* (96–109), *L. dicktracyi* (98–110), *L. galactostictos* (102–108), *L. gununakuna* (108–112), *L. heliodermis* (109–116), *L. parvus* (96–113), *L. talampaya* (104–108), *L. tulkas* (102–113), and *L. umbrifer* (100–115). Scales of dorsum in *L. splendidus* sp. nov. are lanceolate; this character distinguishes it from *L. buergeri, L. burmeisteri, L. capillitas, L. ceii, L. choique, L. dicktracyi, L. elongatus, L. flavipiceus, L. heliodermis, L. janequeoae, L. kriegi, L. lonquimayensis, L. quinterosi, L. talampaya,* and *L. thermarum*, which have rhomboidal dorsal scales. Sexual dichromatism is absent in *L. splendidus* sp. nov., whereas in *L. heliodermis*, *L. quinterosi*, *L. shitan*, *L. smaug*, *L. tregenzai* and *L. umbrifer* sexual dichromatism is evident. Surface of temporal scales is slightly keeled in *L. splendidus* sp. nov., whereas in *L. tulkas* and *L. carlosgarini,* temporal scales are smooth. Dorsal scales in *L. splendidus* sp. nov. show a strong keel, whereas *L. carlosgarini*, *L. janequeoae*, *L. riodamas*, and *L. tregenzai* exhibit dorsals with only a slight median keel and *L. parvus* has dorsals with only a slight keel or no keel). Dorsal scales in *L. splendidus* sp. nov. present mucron, differing from *L. antonietae*, *L. capillitas*, *L. janequeoae*, *L. scorialis*, *L. tulkas*, *L. umbrifer,* and *L. zabalai* (dorsals without mucron).

Regarding genital morphology, we found subtle differences in the hemipenis length to SVL ratio. The hemipenes of *Liolaemus splendidus* sp nov are proportionally longer (hemipenis length/SVL: 0.12) than in other species of the *L. petrophilus* clade, such as *L. austromendocinus* (0.09), *L. parvus* (0.09), *L. petrophilus* (0.06), and *L. quinterosi* (0.09). Ratios of hemipenes to length and width of proximal, medial and distal regions are higher in *L. splendidus* sp nov. (proximal l/w: 0.32; medial l/w: 0.42, distal l/w: 0.50, respectively) than in *L. austromendocinus* (0.24;0.30;0.42), *L. parvus*, (0.22;0.26;0.40), *L. petrophilus* (0.27;0.31;0.38), and *L. quinterosi* (0.29;0.39;0.43). Larger sample sizes of each species are necessary to confirm these differences.

Several characters regarding color patterns discriminate between species belonging to the *Liolaemus petrophilus* clade (Table 6).

Description of the holotype

Adult male. MPCN-H-503. SVL 96.14 mm. Head length 22.05 mm. Head width 17.30 mm. Head height 9.65 mm. Subocular length 5.34 mm. Auditory meatus taller (4.89 mm) than wide (2.02 mm). Interorbital distance 10.04 mm. Rostral wider (4.32 mm) than tall (1.53 mm). Mental twice wider (5.07 mm) than long (2.14 mm). Trunk length 44.65 mm. Humerus length 12.60 mm, humerus width 6.73 mm, radius length 11.45 mm, manus length 14.24 mm. Femur length 19.78 mm, tibia length 19.60 mm, and foot length 26.71 mm. Tail length 140.19 mm. Base of tail width 13.88 mm.

The dorsal surface of the head is smooth, with 16 scales. Nasal in contact with rostral, surrounded by seven scales, and separated from the canthal by one scale. Six scales between frontal and superciliaries. Five superciliaries. Frontal divided into four scales. Interparietal larger than parietals, in contact with five scales. Semicircles complete. Fifteen supraoculars. Preocular separated from lorilabial row by one scale. Nine slightly keeled temporals. Eight lorilabials, two of them in contact with subocular. Seven supralabials. Six infralabials, the second in contact with four scales. Four scales in contact with mental. Seven posmental scales.

Eighty-seven scales around midbody. Ninety-four scales between occiput and hind limbs. Dorsal scales lanceolate, laminar, imbricate, and keeled. Fifty-seven granular and smooth neck scales. Forty-five scales between posterior margin of auditory meatus and antehumeral fold. Antehumeral, auricular and longitudinal folds evident. Fifty-nine gulars. One hundred thirty ventrals. Thirteen pygal scales. Three precloacal pores. Fourth finger with 24 subdigital lamellae, and fourth toe with 30 subdigital lamellae. Dorsal tail scales laminar with mucron and keel.

Color of the holotype in life (Fig. 3 A-B)

Head yellowish-green with a melanic triangular pattern. Pineal scale yellowish-green with a transversal black line. Lateral region of the head with irregular black spots. Dorsal background light green. Melanic

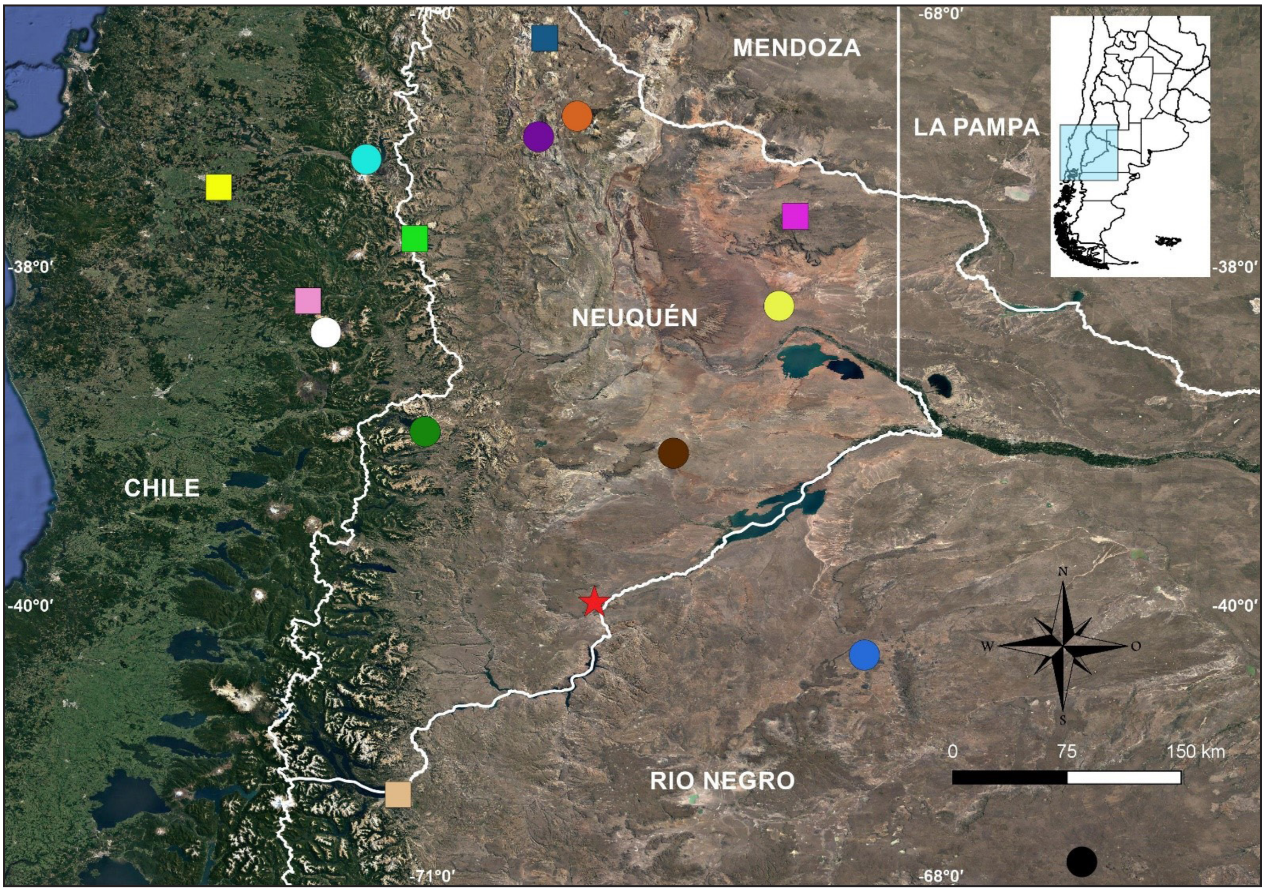

Figure 4. Map of SW Argentina showing the distribution of the *Liolaemus petrophilus* clade. Red star: *L. splendidus* sp. nov. Green circle: *L. ceii*. Fuchsia square: *L. crandalli*. Brown circle: *L. gununakuna*. Pink square: *L. janequeoae*. Beige square: *L. kriegi*. White circle: *L. lonquimayensis*. Black circle: *L. petrophilus*. Orange circle: *L. punmahuida*. Yellow circle: *L. quinterosi*. Turquoise circle: *L. scorialis*. Blue circle: *L. shitan*. Light green square: *L. tregenzai*. Yellow square: *L. zabalai*. Purple circle: *L. burmeisteri*. Petroleum blue square: *L. antumalguen*.

vertebral stripe extends from the head to the base of the tail. The flanks present a pattern of black transversal stripes. The tail presents black rings formed by two rows of scales. The anterior and posterior limbs are light green with black transversal lines. Ventrally, the limbs are light green. The gular region, neck and chest are light green. Abdominal region, including pigal and thighs, up to cloaca is yellowish. Precloacal pores are orange. Ventral region of tail is light green.

Variation

Based on 14 specimens (seven males and seven females, including holotype): SVL of males 84.30 to 96.14 ($\bar{X}$= 90.36; SD = 4.95) and of females 54.12 to 89.64 ($\bar{X}$= 72.60; SD = 16.72). Supralabials 6–9 ($\bar{X}$= 7.50; SD = 1.01). Infralabials 5–8 ($\bar{X}$= 5.88; SD = 0.73). Lorilabials 8–10 ($\bar{X}$ = 8.67; SD = 0.82). Temporals 6–13 ($\bar{X}$= 9.12; SD = 1.81). Number of scales surrounding nasal 6–8 ($\bar{X}$= 6.83; SD = 0.53). Number of scales between canthal and nasal 1–3 ($\bar{X}$ = 1.19; SD = 0.61). Hellmich index or dorsal scales on the head 16 ($\bar{X}$= 16.00; SD = 0.00). Gulars 40–65 ($\bar{X}$= 53.01; SD = 7.88). Scales around midbody 77–95 ($\bar{X}$= 84.73; SD = 4.75). Scales between posterior margin of auditory meatus to antehumeral fold 28–56 ($\bar{X}$= 40.94; SD = 7.97). Neck scales 39–64 ($\bar{X}$= 53.37; SD = 9.88). Dorsal scales 83–96 ($\bar{X}$ = 89.31; SD = 4.76), lanceolate and keeled between occiput and limbs. Ventral scales 121–145 ($\bar{X}$= 126.85; SD = 6.46). Precloacal pores in males 1–3 ($\bar{X}$= 1.51; SD = 0.95). Precloacal pores in females absent. Sexual dichromatism is not evident. Head is yellowish-green with a melanic triangular pattern in the dorsal region. Some specimens present green scales in the melanic region. Side of head with black spots on temporal region and between the nasal and subocular scale. Background color of the dorsal region is light green. The vertical melanic bar extends from the head to the base of the tail. Flank region with transversal melanic stripes. Anterior and posterior limbs are the same color as that of dorsal region, with thin black stripes. Dorsal region of tail is ringed, formed by a complete row of scales and two incomplete rows on the upper and lower parts of the ring. Ventrally, the gular region and chest are green, and some specimens are dark green. The abdominal region ranges from light green to green with some dark scales, forming irregular spots. Pigal region and thighs are yellowish in males and green in females. Cloacal region ranges from pale yellow to light green. Precloacal pores in males are orange. Ventral region of tail ranges from light green to yellowish with faded rings.

Distribution and Natural history

Liolaemus splendidus sp. nov. is an endemic species, whose only known population to date is in its type locality, on the slopes of a basaltic plateau at 650 m a.s.l., located in the south-east of Neuquén Province, Collón Curá department, 8 km north of Piedra del Águila locality, (39º58'54.3''S; 70º02'36''W), on the Argentine Andes foothills (Fig. 4).

The rocky regions of the Olivinic basaltic plateau where this species occurs correspond to the geologic formation known as "Basalto de Santo Tomas", of the lower Pliocene. This formation leans discordantly on the Collon Cura formation, composed of continental sedimentite, which in turn is formed by tuffs, and cinerites from the middle Miocene. These rocks form the piedmont of the plateau (Leanza and Hugo, 1997; Cucchi, 1998).

The vegetation corresponds to an ecotone between the Monte and Patagonian Steppe phytogeographic regions (Fig. 5; Cabrera, 1971). The vegetation associated with the type locality is characteristic of the piedmont of the basaltic plateau due to the presence of typical species of the Patagonian Steppe mixed with Monte species, such as: *Larrea divaricata*, *Larrea nítida, Grindelia chiloensis*, *Senecio filaginoides var. lobulatus, Schinus johnstonii, Ephedra ochreata, Atriplex lampa*, *Maihuenia patagónica*, *Prosopis alpataco, Hyalis argentea* var. *latisquama, Lycium chilense, Chuquiraga erinacea*, *Prosopis denudans var. patagonica*. On the other hand, the vegetation on the slope and upper region of the plateau is typical of the "Western District" of the "Patagonian Phytogeographic region" (León *et al*., 1998): steppe vegetation of shrubs and grasses, with the occurrence of *Azorella prolifera*, *Prosopidastrum globosum*, *Verbena tridens*, *Pappostipa* sp., *Festuca* sp., *Poa* sp., *Adesmia trijuga*, *Colliguaja integerrima*, *Anarthrophyllum strigulipetalum*, and *Calceolaria biflora*. (Burkart, 1964; Cabrera, 1971; Roig, 1987; Arbo, 1999; Gandullo *et al*., 2004; 2016; Riveros *et al*., 2011).

Liolaemus splendidus sp. nov. is a saxicolous and robust species, with remarkable sexual dimorphism: males are larger than females (SVL: Male 96.14 mm - Female 89.03 mm), while the size relationships also change in the head (width, length and height), the torso and the extremities. Weight is up to 19.5 g in adult females and up to 22.0 g. in adult males. We do not have reproduction data.

Based on the observation of faeces, the diet is predominantly insectivorous. We found a great content of ants of the genera *Acromyrmex* (*A. lobicornis* and *A. lundi), Camponotus* sp., *Pogomomyrmex* sp., *Solenopsis* sp., followed, to a lesser degree, by *Hemiptera indet, Diptera* spp., *Colepotera*, Coccinellidae, *Adalia bipunctata*., Hydrophilidae, *Hydrophilus* sp., Tenebrionidae *and Araneae indet*. Among Coleoptera, we identified Buprestidae, mainly *Lasionota (Lasionota bruchi* and *Lasionota alternans)* and *Agrilus* spp. The diet also included large amounts of vegetation, with presence of Molle (*Schinus johnstonii*) seeds as well as other plant remains.

The new species is abundant and easy to observe. They were observed always between 10:30 in the morning and 6:30 in the evening, with an activity peak between 11:30 in the morning and 1:00 in the afternoon, and another from 4 in the afternoon and 6:30 in the evening; between these peaks, little activity was observed, since they avoid extreme solar radiation. We observed an apparent social stratification, with dominant males and adult females being found generally in the upper part of the plateau, and juveniles and subadults inhabiting the lower part. The dominant males and females descended to the lower part of the plateau towards midday, where they intermingled with the juveniles. Then they returned to the upper part after about 5:00 in the afternoon. This behaviour could be related to feeding and social patterns. They are elusive and tend to hide away under a rock when approached at 5 m distance. After a few minutes, they came out again from their hiding place, displaying head bobbing movements that denote communication.

Their coloration is similar to lichens that are present on the rocks where they inhabit, a feature that would reduce the rate of predation by raptor birds.

Liolaemus splendidus sp. nov. is sympatric with *Liolaemus* cf. *bibronii*, *Liolaemus darwinii* (Bell, 1843), *Liolaemus gracilis* (Bell, 1843), *Homonota*

Table 6. Body pattern and coloration of species of the *Liolaemus petrophilus* clade.

	***Liolaemus splendidus* sp. nov.**	***Liolaemus austromendocinus***	***Liolaemus capillitas***	***Liolaemus gununakuna***	***Liolaemus parvus***	***Liolaemus petrophilus***	***Liolaemus quinterosi***
Head color	Green with black lines	Brown/Black	Brown/Black	Iridescent Yellow	Brown	Brown/ Black	Brown/ Black
Body color	Green/Black	Brown	Brown/Black	Iridescent Yellow	Brown/ Dark brown	Brown/ Yellowish	Brown/ White
Dorsal body pattern	Vertebral band and transverse bars	Indistinct	Indistinct	Transverse bars	Dark scales on vertebral region and flanks	Transverse bars	White dots over vertebral region
Tail rings	Present	Slightly marked to present	Absent	Present	Slightly marked to absent	Present	Present
Sexual Dichromatism	Absent	Absent	Absent	Absent	Absent	Absent	Present
Cloacal region color (males)	Yellowish green	Yellowish	Bright red	Yellowish green	Yellowish	Light grey with dark spots	Yellowish

horrida (Burmeister, 1861) and *Homonota darwinii* (Boulenger, 1885).

Discussion

Evidence and phylogeny

Since it was first diagnosed by Cei (1974) until its current redefinition (Medina *et al.,* 2018; Troncoso-Palacios *et al.,* 2018; Avila *et al.,* 2021 and this study), the *Liolaemus elongatus* group has suffered many changes in its taxonomic composition and several phylogenetic hypotheses have been proposed. In fact, the species, which initially belonged to the *L. elongatus* group, were included in at least three monophyletic groups (*L. elongatus*, *L. kriegi*, and *L. petrophilus* clades), and sometimes four (including the *L. punmahuida* clade). The relationship of these three groups varies in the different proposals. Our results are congruent with those of Morando *et al.* (2003), Avila *et al.* (2004), Medina *et al.* (2014), Escobar-Huerta *et. al.* (2015), Troncoso-Palacios *et al.* (2016; 2018) and Ruiz *et al.* (2020); all of these works suggest that the *L. elongatus* clade is sister to the *L. kriegi* clade, and these species form a clade that is sister to the *L. petrophilus* clade (Fig. 1). In all those studies, including the present one, the group that is sister to those clades is the *L. punmahuida* clade (Avila *et al.,* 2015), formed by *L. punmahuida* and *L. flavipiceus*.

In this study, we present morphological (lepidosis, coloration, hemipenis, Table 3, 4 and 6) and molecular evidence (Fig. 1; Table 5) that supports the species status of *Liolaemus splendidus* sp. nov., a species that belongs to the *L. petrophilus* clade within the *L. elongatus* group. According to observations of Quipildor *et al.* (2018), who described hemipenial morphology of several *Liolaemus* species, the morphology of *L. splendidus* sp. nov. is congruent with the patterns observed within the *Liolaemus* sensu stricto subgenus, due to the presence of calyces in the sulcate face, as well as an elongated margin of the distal apex. Furthermore, our observations are consistent with those in the species of the *L. elongatus* group in the presence of a valve-shaped ornamentation, calyces with thin borders, and ornamentation distributed on the upper third of the hemipenis. According to our morphological and molecular evidence of the *L. elongatus* group, *L. splendidus* sp. nov. is phylogenetically close to *L. quinterosi*. This is reflected in the morphology of the copulating organ, since our observations for *L. splendidus* sp. nov. are similar to findings described by Ruiz *et. al.,* (2019) for *L. quinterosi*: a lump-shaped protuberance on the asulcate face, which has a curvature towards the apex region of the hemipenis, and the same number of stems in the sulcate region. On the other hand, the hemipenes of *L. splendidus* sp. nov. can be clearly distinguished from those of the remaining species of the *L. petrophilus* clade in that it is longer and more robust than in the other species (*L. austromendocinus*, *L. parvus*, *L. petrophilus* and *L. quinterosi*). This could be due to the rapid evolu-

Figure 5. Environment where *Liolaemus splendidus* sp.nov. is distributed.

tion of genital characters in relation to non-genital ones. Recent studies suggest that characters related to copulatory organs evolve more rapidly than other morphological characters, possibly due to the pressure of sexual selection acting on them (Klaczko *et al.*, 2015; Klaczko *et al.*, 2017; Klaczko and Stuart, 2015; Quipildor *et al.*, 2021).

We compared the coloration and the patterns within the *Liolaemus elongatus* group (Table 6) and found that, in general, species have earth tones (mostly greys and browns) with some white stripes or scales (Ruiz, 2020). The group members that have more striking coloration are *L. petrophilus* (only some populations), and *L. gununakuna*, with a yellowish coloration on the back. Notably, all species of *L. elongatus* with highly contrasting coloration belong to the *L. petrophilus* group. Furthermore, the peculiar bright green coloration of *L. splendidus* sp. nov. is noticeable

Our results recovered the relationship of *Liolaemus austromendocinus* as sister to *L. parvus*, which is congruent with other works (Avila *et al.*, 2012; Escobar-Huerta *et al.*, 2020). In this work, besides recovering *L. splendidus* sp. nov. within the *L. petrophilus* clade, we incorporated the sequences of *L. quinterosi*, recovering a clade formed by *L. gununakuna*, sister to *L. quinterosi* and *L. splendidus* sp. nov. as sister to the latter two; in turn, this clade is sister to a clade formed by *L. austromendocinus* and *L. parvus*. Furthermore, we recovered the subclade of *L. capillitas* as sister to the previous clades and finally *L. petrophilus* as sister to all the other species in the clade. The latter topology is congruent with the results of Ruiz et *al.* (2020).

Divergence times

In the last decade, few systematic and biogeographic studies have included the *Liolaemus elongatus* group or some of its representatives. Some studies focused on the group (Medina *et al.*, 2014, 2017; Ruiz *et al.*, 2020), whereas others were conducted at large taxonomic scales (Schulte, 2013; Zheng and Wiens, 2016; Esquerré *et al.*, 2019; Esquerré *et al.*, 2022). The results of this work partly agree with the previously mentioned studies. As for those that do agree, the divergence time obtained in the present work for the *L. punmahuida* clade is 4 Ma, an age that exactly coincides with that reported by Ruiz *et al.* (2020) and Esquerré *et al.* (2022). Regarding the divergence time of the *L. kriegi* clade, our 4 Ma result agrees with results reported by Esquerré *et al.* (2019; 2022) and is within the HPD reported by Schulte (2013), Zheng and Wiens (2016) and Ruiz *et al.* (2020), of 6, 2.93, and 3 Ma for the clade, respectively. Regarding the ages that do not agree with any previous study or are outside the HPD, our finding in the *L. elongatus* group (23 Ma) presents a minimum difference of approximately 7 Ma from the work of Schulte (2013). The *L. elongatus petrophilus* clade (19 Ma) and the *L. petrophilus* clade (15 Ma) have a difference of only between 6–4 Ma with respect to the ages obtained by Esquerré *et al.* (2022). The *L. elongatus kriegi* clade (11 Ma) differs from results of Ruiz *et al.* (2020) by 5 Ma. The *L. capillitas* clade (8 Ma) only differs from the results obtained by Zheng and Wiens (2016) and Esquerré *et al.* (2022) by 2 Ma. Finally, the *L. elongatus* sensu stricto clade differs by only 1.5 Ma from the results of Esquerré *et al.* (2022).

These differences in node ages may be due to differences in methodology and in datasets among studies. As for the concordances in divergence time of the *Liolaemus punmahuida* clade, the works of Ruiz *et al.* (2020) and Esquerré *et al.* (2022) reported the same results as those obtained in this study, probably because all works used the same fossil for calibration. This congruence can also be observed in the divergence time of the *L. kriegi* clade and the times obtained by Esquerré *et al.* (2019; 2022), who used the same fossil. The differences found in this work from other works are probably due to different taxonomic scales of analysis (Schulte 2013; Zheng and Wiens, 2016) and/or in the calibration datum employed (Medina *et al.*, 2014; 2017).

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Appendix 1

Specimens studied. MPCN: Museo Patagónico de Ciencias Naturales "Juan Carlos Salgado". FML: Fundación Miguel Lillo. IBIGEO: Instituto de Bio y Geociencias del NOA. MLP.S: Museo de La Plata.

Species	Num. Cols	Latitude	Longitude	Statistical analyses	Hemipenis
L. austromendocinus	MCN 2022	34°44´10”S	68°34´53”W	X	
	MCN 2027	34°49´29”S	70°00´32”W	X	
	MCN 2028	34°49´29”S	70°00´32”W	X	
	MCN 2029	34°49´29”S	70°00´32”W	X	
	MCN 2030	34°49´29”S	70°00´32”W	X	
	MCN 3686	35°55´44”S	68°32´36”W		X
	MCN 3691	35°55´44”S	68°32´36”W		X
	MCN 3698	35°55´44”S	68°32´36”W	X	X
	MCN 3702	35°55´44”S	68°32´36”W	X	
	MCN 3703	35°55´44”S	68°32´36”W	X	

	MCN 3704	35°55´44”S	68°32´36”W	X	
	MCN 3705	35°55´44”S	68°32´36”W	X	
	MCN 3706	35°55´44”S	68°32´36”W	X	
	MCN 3707	35°55´44”S	68°32´36”W	X	
	MCN 3708	35°55´44”S	68°32´36”W	X	
	MCN 3709	35°55´44”S	68°32´36”W	X	
	MCN 3710	35°55´44”S	68°32´36”W	X	
	MCN 3711	35°55´44”S	68°32´36”W	X	X
	MCN 3712	35°55´44”S	68°32´36”W	X	
L. capillitas	FML 01229-11	27°20´15”S	66°23´06”W	X	
	FML 01794-2	28°53’38”S	67°14’52”W	X	
	FML 01794-6	28°53’38”S	67°14’52”W	X	
	FML 01229-20	27°20´15”S	66°23´06”W	X	
	FML 01229-16	27°20´15”S	66°23´06”W	X	
	FML 01794-7	28°53’38”S	67°14’52”W	X	
	FML 01794-8	28°53’38”S	67°14’52”W	X	
	FML 01794-19	28°53’38”S	67°14’52”W	X	
	FML 01794-21	28°53’38”S	67°14’52”W	X	
	FML 01914-15	27°21’05”S	67°20’19”W	X	
	FML 01914-15	27°21’05”S	67°20’19”W	X	
	FML 01914-31	27°21’05”S	67°20’19”W	X	
	FML 01794-9	28°53’38”S	67°14’52”W	X	
	IBIGEO 5835	27°20’41”S	66°22´36”W	X	X
	IBIGEO 5836	27°20’41”S	66°22´36”W	X	
	IBIGEO 5837	27°20’41”S	66°22´36”W	X	
	MCN 2135	27°17’00”S	67°26´00”W		X
L. gununakuna	FML 21537	39°02’55”S	70°16´36”W	X	
	FML 22375	39°02’32”S	70°18´27”W	X	
	MACN 37685	38°32’59”S	70°20´27”W	X	
	MACN 37686	38°32’59”S	70°20´27”W	X	
	MLP.S 2352	39°06’00”S	69°34´37”W	X	
	MLP.S 2353	39°06’00”S	69°34´37”W	X	
L. parvus	MCN 2888	31°11’21”S	69°42´15”W	X	
	MCN 2889	31°11’21”S	69°42´15”W	X	
	MCN 2890	31°11’21”S	69°42´15”W	X	
	MCN 3632	31°11’21”S	69°42´15”W		X
	MCN 4006	29°39’34”S	68°51´31”W	X	
	MCN 4007	29°39’34”S	68°51´31”W	X	
	MCN 4008	29°39’34”S	68°51´31”W	X	
	MCN 4009	29°39’34”S	68°51´31”W	X	
	MCN 4049	29°39’34”S	68°51´31”W	X	
	MCN 4050	29°39’34”S	68°51´31”W	X	
	MCN 4070	29°39’34”S	68°51´31”W	X	
	MCN 4074	29°39’34”S	68°51´31”W	X	
	MCN 4081	29°39’34”S	68°51´31”W	X	
	IBIGEO 6085	32°52’36”S	68°33´35”W		X
	IBIGEO 6103	32°52’36”S	68°33´35”W		X
L. petrophilus	MCN 1347	43°50’51”S	69°04´19”W		X

	MACN 11683	40°43’10”S	69°50´05”W	X	
	MACN 11684	40°43’10”S	69°50´05”W	X	
	MACN 11685	40°43’10”S	69°50´05”W	X	
	MACN 11686	40°43’10”S	69°50´05”W	X	
	MLP.R 5400	41°19’47”S	69°33´59”W	X	
	MLP.R 5401	41°19’47”S	69°33´59”W	X	
	MLP.R 5402	41°19’47”S	69°33´59”W	X	
L. quinterosi	FML 30504	38°13´51”S	68°57´13”W	X	
	FML 30505	38°13´51”S	68°57´13”W	X	
	FML 30506	38°13´51”S	68°57´13”W	X	
	FML 30507	38°13´51”S	68°57´13”W	X	X
	FML 30508	38°13´51”S	68°57´13”W	X	
L. splendidus	MPCH-H-500	39°58´54”S	70°02´36”W	X	
	MPCH-H-501	39°58´54”S	70°02´36”W	X	
	MPCH-H-502	39°58´54”S	70°02´36”W	X	
	MPCH-H-503	39°58´54”S	70°02´36”W	X	
	MPCH-H-504	39°58´54”S	70°02´36”W	X	
	MPCH-H-505	39°58´54”S	70°02´36”W	X	
	MPCH-H-506	39°58´54”S	70°02´36”W	X	
	MPCH-H-507	39°58´54”S	70°02´36”W	X	
	MPCH-H-508	39°58´54”S	70°02´36”W	X	
	MPCH-H-509	39°58´54”S	70°02´36”W	X	
	MPCH-H-510	39°58´54”S	70°02´36”W	X	
	IBIGEO 5531	39°58´54”S	70°02´36”W	X	X
	IBIGEO 5532	39°58´54”S	70°02´36”W	X	
	IBIGEO 5533	39°58´54”S	70°02´36”W	X	
L. tulkas	FML 18316	27°43’12”S	66°58’33”W	X	
	FML 18317	27°43’12”S	66°58’33”W	X	
	FML 18318	27°43’12”S	66°58’33”W	X	
	FML 18319	27°43’12”S	66°58’33”W	X	
	FML 18320	27°43’12”S	66°58’33”W	X	
	FML 18321	27°43’12”S	66°58’33”W	X	
	FML 18322	27°43’12”S	66°58’33”W	X	
	FML 18323	27°43’12”S	66°58’33”W	X	
	IBIGEO 5813	27°48’00”S	68°13’00”W		X

Appendix 2

Accession numbers of the specimens used in this study.

Species	Num. Cols	Cyt-b	12s	KIF24	Latitude	Longitude
L. austromendocinus	LJAMM-CNP 2716	AY173838.1	AY173907.1	MG660175	34°59´29”S	68°37´24”W
	LJAMM-CNP 5147	AY367815.1	AY367843.1	MG660176	36°49’05”S	68°59’09”W
	LJAMM-CNP 10574	MG660005	MG660055	MG660177	34°32´20”S	69°15’56”W
	MCN 3698				35°55'44"S	68°32'36"W
L. buergeri	LJAMM-CNP 5294	KJ494165.1	KJ493986.1	KP121232.1	36°43’41”S	70°37’30”W

L. capillitas	LJAMM-CNP 47100	AY173657.1	AY173913.1	-	27°26´00”S	66°20´00”W
	LJAMM-CNP 2788	AY173555.1	MG660051	MG660168	27°25´14”S	66°24´50”W
L. elongatus	LJAMM-CNP 3715	KY127905.1	KP121215.1	KP121231.1	44°31’02”S	69°11’26”W
	LJAMM-CNP 5532	KY127990	KY127492	KY127597	40°19'31"S	69°26'18"W
L. flavipiceus	LJAMM-CNP 7906	KP121330.1	MG660056	KP121241.1	35°58'47"S	70°23'29"W
L. gununakuna	LJAMM-CNP 2690	AY173545.1	AY173903.1	MG660173	39°06'40"S	69°34'29"W
	LJAMM-CNP 4443	MG660004	MG660054	MG660174	39°12'18"S	70°03'56"W
	LJAMM-CNP 10403	MG660003	MG660053	MG660172	39°06'40"S	69°34'29"W
L. kriegi	LJAMM-CNP 2613	KJ494186.1	KP121213.1	KP121227.1	38°53’12”S	70°58’42”W
	LJAMM-CNP 5562	KJ494190.1	KJ493993.1	KP121233.1	40°34’04”S	69°44’59”W
L. parvus	LJAMM-CNP 2704	AY173610.1	AY173905.1	-	29°22´00”S	69°28´00”W
	LJAMM-CNP 2706	AY173611.1	-	MG660170	29°22´00”S	69°28´00”W
	BYU 47106	AY367809.1	AY173906.1	MG660171	32°59´00”S	69°20´00”W
L. petrophilus	LJAMM-CNP 5481	JN846994.1	MG660049	MG660164	42°22’36”S	67°34’00”W
	LJAMM-CNP 6982	KP121326.1	KP121216.1	KP789577.1	39°44’02”S	68°28’44”W
	LJAMM-CNP 11355	KP789552.1	KP121211.1	MG660166	40°33’41”S	67°51’36”W
L. punmahuida	LJAMM-CNP 2626	MG660007	MG660058	MG660179	37°06’16”S	70°08’27”W
L. quinterosi	MPCN 436				38°13’51”S	68°57’13”W
	MPCN 437				38°13’51”S	68°57’13”W
L. splendidus	IBIGEO 5531				39º58'54"S	70º02'36"W
	IBIGEO 5532				39º58'54"S	70º02'36"W
Outgroup						
L. multicolor	LJAMM-CNP 12006	KF968893	KF969085	KF968144	22°41’00”S	65°43’00”W
L. bibronii	LJAMM-CNP 8212	MG660009.1	MG660059.1	MG660180.1	41°17´04”S	66°28´25”W
L. coeruleus	LJAMM-CNP 978	MG660016.1	MG660062.1	MG660186.1	38°54’05”S	70°48’57”W
L. wiegmannii	LJAMM-CNP 4040	DQ237342.1	KF969173.1	KF968228.1	38°38´00”S	62°18´00”W

Trabajo

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New morphology data and geographic distribution expansion of *Leposternon mineiro* Ribeiro, Silveira & Santos-Jr, 2018 (Squamata, Amphisbaenia, Amphisbaenidae)

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ABSTRACT
We provide new morphological and geographic distribution data for *Leposternon mineiro*. Fifteen specimens of *L. mineiro* were analyzed (six specimens of the type series and nine additional specimens), in addition to 404 specimens of other species of the genus, used for identification and comparison. Meristic characters, morphometrics, head scale patterns and dentition matched the previously published diagnosis of the species, but we also found some variations compared to the type series. We expanded the ranges of the following diagnostic characters of *L. mineiro*: snout-vent length 221–380 mm, tail length 11.5–23.4 mm, 252–300 dorsal postpectoral half-annuli, 252–280 ventral postpectoral half-annuli, 14–16 caudal annuli, 26–37 dorsal segments and 24–39 ventral segments in a midbody annuli, and 2–4 precloacal pores. The new record from the municipality of Cristalina, State of Goiás, extends the geographic distribution of the species by 148 km west of nearest record.

Key words: Amphisbaenian

RESUMO
Nós fornecemos novos dados morfológicos e de distribuição geográfica para *Leposternon mineiro*. Foram analisados quinze espécimes de *L. mineiro* (seis espécimes da série-tipo e nove exemplares adicionais), além de 404 exemplares de outras espécies do gênero, utilizados para identificação e comparação. Caracteres merísticos, morfométricos, padrões de escamas da cabeça e dentição corresponderam com a diagnose da espécie publicada anteriormente, mas encontramos algumas variações em comparação à série-tipo. Nós ampliamos as variações dos seguintes caracteres diagnósticos de *L. mineiro*: comprimento rostro-cloacal de 221–380 mm, comprimento da cauda de 11,5–23,4 mm, 252–300 meios anéis pós-peitorais dorsais, 252–280 meios anéis pós-peitorais ventrais, 14–16 anéis caudais, 26–37 segmentos dorsais e 24–39 segmentos ventrais em um anel do meio do corpo e 2–4 poros pré-cloacais. O novo registro do município de Cristalina, Estado de Goiás, amplia a distribuição geográfica da espécie em 148 km a oeste do registro mais próximo."

Palavras Chave: Ansfibênios

Introduction

The genus *Leposternon* is a monophyletic group of South American amphisbaenids (Mott and Vieites, 2009; Ribeiro, 2010; Ribeiro *et al*., 2011). The species of this genus can be easily identified by the presence of a

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relatively large and robust body, head dorsoventrally compressed, nostrils opening on the ventral surface of snout, suture connecting each nostril to the edge of mouth, nasal shield absent, rostronasal shield followed by a sequence of one to five enlarged shields along the dorsal surface of head, gular portion without segmental cover, more than two dermal annuli per vertebrae, tail very short with rounded tip, and autotomic site absent on tail (Ribeiro *et al.*, 2008). Currently, *Leposternon* is composed of 11 species, with the following geographic allocation: Brazil (10 spp.), Argentina (two spp.), Uruguay (one sp.), Paraguay (one sp.), and Bolivia (one sp.) (Ribeiro *et al.*, 2018). Of these species, five have pre-cloacal pores [*L. cerradensis* Ribeiro, Vaz-Silva & Santos-Jr, 2008; *L. kisteumacheri* Porto, Soares & Caramaschi, 2000; *L. maximus* Ribeiro, Nogueira, Cintra, Silva Jr. & Zaher, 2011; *L. mineiro* Ribeiro, Silveira & Santos-Jr, 2018; and *L. polystegum* (Duméril *in* Duméril & Duméril, 1851)]. It is likely that these pores are responsible for the secretion of products from the epidermal glands, and that both in amphisbaenians and other groups of lizards, they are related to both reproductive activities and to other behaviors, such as inter and intra-specific communication (Antoniazzi *et al.*, 1993; Jared *et al.*, 1999; Ribeiro, 2010; Ribeiro *et al.*, 2018).

Leposternon mineiro was originally described based on six specimens, being split apart from the other species of the group by a combination of characters that include the size and shape of the cephalic shields, the number of half-annuli of the body and dentition form. So far, the species is known only for the municipalities of João Pinheiro (type locality) and Buritizeiro, both in the State of Minas Gerais, Brazil. It is the most recently described species in the genus and the sixth member of the genus from the Brazilian Cerrado, the others being *L. cerradensis*, *L. infraorbitale* (Bertold, 1859), *L. maximus*, *L. microcephalum* Wagler in Spix, 1824, and *L. polystegum* (Ribeiro *et al.*, 2018).

During the faunal monitoring and rescue activities at the Batalha Hydroelectric Power Plant, on the border between the municipalities of Cristalina (State of Goiás) and Paracatu (State of Minas Gerais), near the banks of the São Marcos River, nine specimens of *Leposternon mineiro* were collected and later deposited in the Herpetological Collection of the Pontifícia Universidade Católica de Goiás. These new specimens have characters that slightly modify the existing description and diagnosis of the species, as well as representing a new geographic record that extends the distribution of the species westwards of the previously known range.

Materials and methods

Fifteen specimens of *Leposternon mineiro* were analyzed [the type series (six specimens) and nine additional specimens)] from the following collections (acronyms of scientific collections between parentheses): Herpetological collection of the Centro de Estudos e Pesquisas Biológicas, Pontifícia Universidade Católica de Goiás, Goiânia, Brazil (CEPB); Herpetological Collection of the Universidade de Brasília, Brasília, Brazil (CHUNB); and Museu Nacional, Rio de Janeiro, Brazil (MNRJ). To compare *L. mineiro* with congeneric species (other 10 species), 404 specimens were used (Appendix). Morphological terminology follows Ribeiro *et al.* (2008). The images of illustrated specimen were produced with a stereomicroscope with an attached camera. Morphometric measurements were performed following Ribeiro *et al.* (2011). Bilateral variation is reported as right/left. The dorsal and ventral postpectoral half-annuli were counted on the right side. For the diagnosis of the species, we used a combination of 31 characters, including pre-cloacal pores, pholidosis, morphometric data, tooth form and number, premaxillary foramina, and coloration in preservative. The geographic distribution was based in Ribeiro *et al.* (2018) and from additional specimens (new sample).

Results

With the new sample of *Leposternon mineiro* morphological character data were complemented (see Table 1) and the diagnosis of the species was revised, as presented below.

1. Updated diagnosis

Leposternon mineiro can be distinguished from *L. bagual* Ribeiro, Santos-Jr & Zaher, 2015, *L. microcephalum*, *L. infraorbitale*, *L. octostegum* (Dúmeril *in* Dúmeril & Dúmeril, 1851), *L. scutigerum* (Hemprich,1820), and *L. wuchereri* (Peters, 1879) by presence of precloacal pores (*versus* absent) and pectoral portion with many shields, mostly small and diamond-shaped (*versus* pectoral shields with an irregular form, and dermal annuli not regularly arranged). Additionally, *L. mineiro* differs from

Table 1. Pholidosis, dentition, and morphometric data of *Leposternon mineiro*. Pholidoses: PPO = precloacal pores, PEC = pectoral shields, SL = supralabial, IL = infralabial, DOA = dorsal postpectoral half-annuli, VEA = ventral postpectoral half-annuli, TA = tail annuli, DS = dorsal segments in midbody half-annulus, VS = ventral segments in midbody half-annulus, COL = coloration of adult specimens in preservative. Skull: PMXT = premaxilla teeth, MXT = maxillar teeth, DT = dentary teeth, FPMX = foramina of the premaxilla, SVL = snout-vent length, TL = tail length. Morphometric data (expressed as proportions): RL/HL = rostronasal length/head length, RW/HW = rostronasal width/maximun width of head, AZL/HL = azygous shield length/head length, AZW/HW = azygous shield width/posterior height of head, PFL/HL = prefrontal length/head length, PFW/HW = prefrontal width/head width, PFH/PFW = prefrontal length/prefrontal width, FSL/HL = frontal suture length/head length, TPL/HL = temporal length/head length, MB/SVL = midbody diameter/snout-vent length, TL/SVL = tail length/snout-vent length. In parenthesis number of specimens, means, and standard deviation, respectively. N/A = not applicable. N/AN = not analyzed.

Characters	**Type series**	**Present study**	**General**
PPO	2–4	2	2–4
PEC	Many shields, mostly small and diamond-shaped	Many shields, mostly small and diamond-shaped	Many shields, mostly small and diamond-shaped
SL	2/2	2/2	2/2
IL	3/3	3/3	3/3
FPMX	Absent	Absent	Absent
COL	Cream White	Cream White	Cream White or yellow
PMXT	5	N/AN	5
MXT	3/3	N/AN	3/3
DT	6/6	N/AN	6/6
DOA	277–300 (5, 290.6, 9.2)	252–275 (9, 264.8, 7.3)	252–300 (15, 274.4, 14.0)
VEA	270–280 (5, 275.0, 4.4)	252–267 (9, 261.2, 5.4)	252–280 (15, 266.0; 7.93)
TA	15-16 (4, 15.2, 0.4)	14–16 (9, 15.3, 0.8)	14–16 (15, 15.4, 0.7)
DS	26–30 (5, 28.2, 1.5)	33–37 (9, 34.9, 1.5)	26–37 (15, 32.2; 3.7)
VS	24–39 (5, 29.3, 5.3)	28–36 (9, 32.5, 2.7)	24–39 (15, 31.3, 4.0)
SVL	221–358 (5, 304.8, 50,9)	273–380 (9, 332.3, 36,3)	221.0–380.0 (14, 322.0, 42.4)
TL	11,5–20,5 (5, 17.3, 3,1)	18.0–23.3 (9, 20.3, 1,8)	11.5–23.4 (14, 19.3, 2.9)
AZL/HL	36.2–40.6 (6, 38.6, 1.9)	31.5–36.4 (9, 34.4, 1.9)	29.8–40.6 (15, 36.1, 3.0)
AZW/HW	27.4–40.5 (6, 32.1, 4.7)	15.2–27.6 (9, 22.9, 4.4)	22.7–40.6 (15, 36.1, 4.8)
PFL/HL	36.8–40.2 (6, 38.6, 1.5)	22.9–30.8 (9, 26.4, 2.6)	19.2–32.1 (15, 26.0, 4.4)
PFW/HW	24.5–35.8 (6, 28.4, 4.6)	26.5–34.0 (9, 29.5, 2.6)	24.5–29.5 (15, 29.5, 3.4)
FSL/HL	19.2–23.7 (6, 21.2, 1.5)	19.8–25.6 (9, 21.5, 2.0)	20.0–25.7 (15, 22.7, 1.6)
RL/HL	24.4–28.7 (6, 26.7, 1.8)	23.2–30.8 (9, 31.4, 2.5)	23.3–30.8 (15, 26.8, 2.1)
RW/HW	57.5–72.1 (6, 64.4, 5.5)	53.6–60.7 (9, 56.9, 2.4)	55.8–72.1 (15, 61.7, 4.6)
TPL/HL	24.8–29.9 (6, 27.7, 2.3)	21.1–29.0 (9, 24.9, 3.0)	22.6–29.9 (15, 26.4, 2.7)
MB/SVL	2.2–2.6 (5, 2.4, 0.2)	2.7–3.4 (9, 3.0, 0.2)	2.2–3.4 (14, 2.8, 0.4)
TL/SVL	5.2–6.5 (5, 5.7, 0.5)	5.3–6.6 (9, 6.1, 0.4)	5.2–6.0 (14, 6.0, 0.5)

unpored *Leposternon* species by the following combination of morphological characters: ***Pholidosis and body coloration***: two supralabials (*versus* three in *L. infraorbitale* and one in *L. octostegum*); three infralabials (*versus* one in *L. octostegum* and *L. scutigerum*; two in *L. infraorbitale* and *L. microcephalum*); rostronasal and azygous shields in contact (*versus* separated in *L. infraorbitale* and *L. microcephalum*); 252–300 dorsal postpectoral half-annuli (*versus* > 352 in *L. octostegum*); 250–280 ventral postpectoral half-annuli (*versus* < 230 in *L. bagual* and *L. microcephalum*; and > 340 in *L. octostegum*); 14–16 tail annuli (*versus* < 14 in *L. bagual*, *L. infraorbitale*, *L. microcephalum*, *L. octostegum*, and *L. wuchereri*); 26–37 dorsal segments in midbody half-annulus (*versus* 16–19 in *L. wuchereri*); 24–39 ventral segments in midbody half- annulus (*versus* 16–21 in *L. wuchereri*); adult specimens in preservative with dorsal portion of body whitish (*versus* yellow in *L. infraorbitale* and *L. scutigerum*; dark brown in *L. ba-*

gual and *L. microcephalum*). ***Skull*** (CEPB 1846): five premaxillary teeth (*versus* seven in *L. microcephalum*; one in *L. octostegum* and *L. scutigerum*), three maxillary teeth (*versus* four in *L. bagual*, *L. infraorbitale*, and *L. microcephalum*; five in *L. scutigerum*); six dentary teeth (*versus* five in *L. octostegum* and *L. scutigerum*; four or five in *L. wuchereri*); premaxillary dorsal and ventral foramina absent (*versus* present in *L. bagual*, *L. infraorbitale*, *L. microcephalum*, and *L. wuchereri*; details in Gans and Montero, 2008 and Ribeiro, 2010). ***Morphometrics***: rostronasal width 55.8–72.1% of maximum width of head (*versus* rostral wider in *L. scutigerum*); azygous shield length 31.5–40.6% of head length (*versus* azygous longer in *L. wuchereri*); azygous shield width 22.7–40.6% of maximum width of head (*versus* azygous wider in *L. octostegum* and *L. wuchereri*); prefrontals length 34.9–41.8% of head length (*versus* shorter in *L. bagual*, *L. microcephalum*, and *L. wuchereri*); prefrontals width 24.5–35.8% of maximum width of head (*versus* prefrontals wider in *L. bagual* and *L. wuchereri*); frontals length of suture 20.0–25.7% of head length (*versus* frontals longer in *L. wuchereri*); temporal length 22.6.1–29.9% of head length (*versus* temporal shorter in *L. wuchereri* and *L. bagual*); midbody width 2.2–3.4% of snout–vent length (*versus* midbody wider in *L. infraorbitale*); and tail length 5.2–6.5% of snout–vent length (*versus* tail shorter in *L. octostegum*) (Table 1).

Leposternon mineiro differs from other species of the genus with precloacal pores in having two supralabials in each size of the mouth (*versus* three in *L. polystegum*) and three infralabials (*versus* two in *L. cerradensis*); rostronasal and azygous shields in contact (*versus* separated by an irregular shield in *L. polystegum*); azygous shield with anterior margin almost straight (*versus* anterior margin rounded in *L. kisteumacheri*); 252–300 dorsal postpectoral half-annuli (*versus* > 305 in *L. maximus*); 252–280 ventral postpectoral half-annuli (*versus* > 300 in *L. cerradensis* and *L. maximus*); adult specimens in preservative with dorsal portion of body whitish (*versus* yellow in *L. polystegum*); three maxillary teeth (*versus* two in *L. polystegum*, four in *L. cerradensis* and *L. kisteumacheri*); six dentary teeth (*versus* five in *L. polystegum*); rostronasal length 20.3–23.1% of maximum length of head (*versus* rostronasal longer in *L. cerradensis*); azygous shield length 31.5–40.6% of head length (*versus* azygous shorter in *L. polystegum*) and 22.7–40.6% of maximum width of head (*versus* azygous wider in *L. polystegum*); prefrontals length 34.9–41.8% of head length (*versus* prefrontals shorter in *L. maximus* and *L. polystegum*); frontals length suture 20.0–25.7% of head length (*versus* frontals longer in *L. polystegum*); midbody width 2.2–3.4% of snout–vent length (*versus* narrower midbody width in *L. maximus*); and tail length 5.2–6.6% of snout–vent length (*versus* tail shorter in *L. polystegum*).

2. Characterization of species (based on 15 specimens, including the type series)

Leposternon mineiro can be characterized by present 2/2 supralabials; 3/3 infralabials; supraocular present (n = 1) and absent (n = 14); snout–vent length 221–380 mm ($\bar{X}$ = 322.5 mm ± 42.4; n = 14); head length 7.1–10.9 mm ($\bar{X}$= 9.3 mm ± 1.2; n = 15), representing 2.7–3.4% of snout-vent length ($\bar{X}$= 2.9 ± 0.2; n = 14); tail length 11.5–23.4 mm ($\bar{X}$= 19.3 ± 2.9; n = 14), representing 5.2–6.6% of snout-vent length ($\bar{X}$= 6.0 ± 0.5; n = 14); midbody diameter 5.8–11.3 mm ($\bar{X}$= 9.0 mm ± 1.9; n = 15), representing 2.2–3.4% of snout-vent length ($\bar{X}$= 2.8 ± 0.4; n = 14); tail diameter (fifth annuli) 5.8–10.7 mm ($\bar{X}$= 8.8 mm ± 1.3; n = 14), representing 2.3–3.2% of snout-vent length ($\bar{X}$= 2.7 ± 0.2; n = 14); anterior head height 1.4–4.6 mm ($\bar{X}$ = 2.3 mm ± 0.9; n = 15), representing 26.1–36.7% of posterior head height ($\bar{X}$ = 28.9 ± 3.1; n = 13); posterior head height 4.9–8.7 mm ($\bar{X}$ = 7.0 mm ± 1.3; n = 13); anterior head width 2.2–4.0 mm ($\bar{X}$ = 3.2 mm ± 0.4; n = 15), representing 36.3–55.3% of posterior head width ($\bar{X}$ = 44.3 ± 5.9; n = 15); and posterior head width 5.1–9.0 mm ($\bar{X}$ = 7.2 mm ± 1.2; n = 15) (Table 1).

Rostronasal approximately as high (1.4–2.7 mm) as long (1.8–3.3 mm), with high representing 66.7–98.9% of length ($\bar{X}$ = 83.1 ± 8.6; n = 15); relatively wide, with length representing 50.1–61.7% ($\bar{X}$ = 56.2 ± 3.3; n = 15) and height 37.6–56.7% ($\bar{X}$= 46.7 ± 5.5; n = 15) of width, in contact with the first supralabials laterally, and with prefrontals and azygous shield posteriorly.

Azygous shield longer than wide, with width representing 53.8–80.0% of length ($\bar{X}$ = 63.2 ± 7.9; n = 15), anterior portion almost as wide as posterior portion in specimens CEPB 1844 and 1845 with the shield in rectangular shape, while in the other specimens the anterior edge is less wide than the posterior edge, with the shield having a more elongated trapezium shape, the two sides of azygous shields form a straight line in contact with the rostronasal anteriorly, and prefrontals and supraocular laterally (see Fig. 1), and frontals posteriorly. Prefrontals almost rectangular, longer than wide, with width representing 49.0–69.2% of length ($\bar{X}$ = 60.1 ± 7.1; n = 15), in contact with the first supralabials and oculars laterally, and with frontals and temporals posteriorly.

Frontals almost hexagonal, almost as wide (1.6–2.5 mm, $\bar{X}$ = 2.0 ± 0.3; n = 15) as long (1.6–2.7 mm suture length, $\bar{X}$ = 2.0 ± 0.3; n = 15; 1.6–3.4 mm longer length,$\bar{X}$ = 2.4 ± 0.6; n = 15), in contact at midline, with temporals laterally, and with parietals posteriorly. Parietals almost rectangular, relatively small (0.7–1.3 mm suture length, $\bar{X}$ = 1.0 ± 0.2; n = 14), representing 7.1–13.7% of head length ($\bar{X}$ = 10.6 ± 1.5; n = 14), arranged in a row of four shields between the temporals, except in specimen CEPB 1842, which has two parietals, in contact with temporals laterally, and first dorsal half-annulus posteriorly.

Temporals irregular, relatively long, with length representing 22.6–29.0% of head length ($\bar{X}$ = 26.5 ± 2.8; n = 15), in contact with prefrontals anteriorly, oculars and postoculars laterally, and first dorsal half-annulus posteriorly. Supraocular shield absent, except in specimen CEPB 1844 on right side of head. Oculars irregular or almost quadrangular, representing 14.3–20.0% of head length ($\bar{X}$ = 17.1 ± 1.4; n = 15), with a larger posterior margin, in contact with the first supralabials anteriorly, second supralabials laterally, and postoculars posteriorly. Eyes visible, placed posteriorly in the superior portion of the ocular shield. Postoculars almost triangular, in contact with the first postsupralabials laterally.

Two supralabials, the first longer and taller than second, second representing 32.8-66.2% of length of the first ($\bar{X}$ = 52.0 ± 9.7; n = 15) and 49.5–74.9% of height of the first ($\bar{X}$ = 63.5 ± 7.6; n = 14). First supralabial largest, with 2.3–5,4 mm length ($\bar{X}$ = 3.2 mm ± 0.8; n = 15) and 1.2–2.1 mm height ($\bar{X}$ = 1.8 mm ± 0.3; n = 15), in contact with rostronasal anteriorly, prefrontals laterally, and oculars and second supralabials posteriorly. Second supralabial, relatively small, almost rectangular, with 1.1–2.0 mm length ($\bar{X}$ = 1.6 mm ± 0.3; n = 15) and 0.9–1.5 mm height ($\bar{X}$ = 1.2 mm ± 0.2; n = 15), in contact with ocular laterally and postsupralabials posteriorly. Two and three rectangular postsupralabials. Three infralabials, first smaller, almost triangular, representing 4.2–9.0% of head length($\bar{X}$ = 6.4 mm ± 2.4; n = 13), in contact with mental anterolaterally, postmental laterally and second infralabials posteriorly, except the specimens CEPB 1843 and 1847 that have the anterior edges fused with the mental; second largest, irregular, relatively narrow, anterior margin similar to the width of the first infralabials and narrower posterior margin, representing 32.6–41.5% of head length ($\bar{X}$ = 37.1 ± 3.1; n = 15), 4.0–8.0 times longer ($\bar{X}$ = 5.5 ± 1.1; n = 13) and 1.3–3.9 times·wider ($\bar{X}$ = 2.2 ± 0.7; n = 15) than first infralabials, and 1.6–2.9 times longer ($\bar{X}$ = 2.0 ± 0.4; n = 9) and 0.3–0.8 times·wider ($\bar{X}$ =0.6 ± 0.2; n = 9) than third infralabials, in contact with postmental and malars laterally and third infralabial

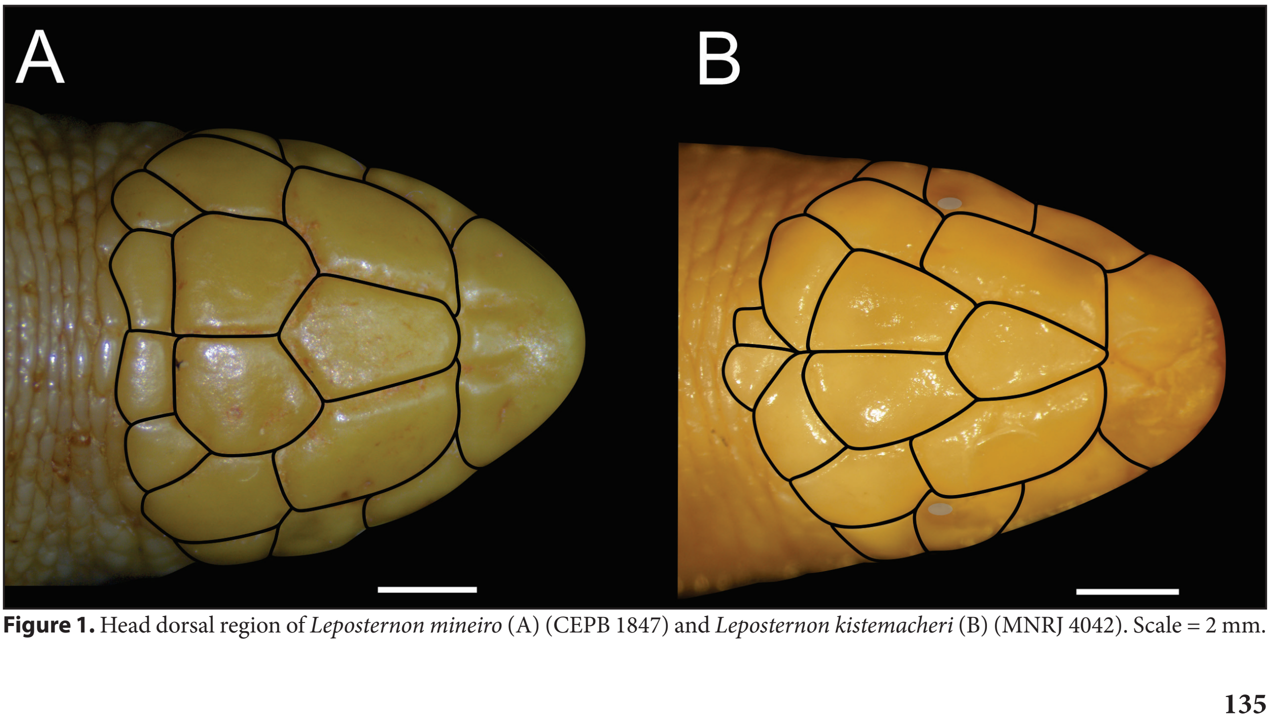

Figure 1. Head dorsal region of *Leposternon mineiro* (A) (CEPB 1847) and *Leposternon kistemacheri* (B) (MNRJ 4042). Scale = 2 mm.

posteriorly; third infralabials relatively small, slightly rectangular, contact lateral malar laterally.

Mental with anterior margin larger and smaller than posterior margin, with variation in shape and form, in contact with postmental posteriorly. Specimen CEPB 1840 has trapezoidal-shaped anterior mental, in a form similar to the holotype, with the anterior portion smaller than the posterior portion, contacting the first infralabial and malar laterally, and the postmental posteriorly; CEPB 1839, 1841, 1842, 1845 and 1846 have the mental with the anterior portion in the shape of a semicircle, followed by a trapezoid, with the anterior widths of the mental relatively larger than the posterior ones; CEPB 1843 has the anterior edges of the two infralabials and the anterior mental fused together; CEPB 1844 has fused anterior and posterior mentals; CEPB 1847 has the two infralabials fused and forming a single shield that contacts the second infralabial laterally, and the anterior mental posteriorly; and CEPB 1839 and 1847 have an rectangular shaped azygous shield that is wider than it is long, contacting the anterior mentalis anteriorly, the posterior mentalis posteriorly and the first malar laterally.

Body with 14–17 anterior half-annuli ($\overline{X}$ = 15.2 ± 0.9; n = 15); 252–300 dorsal postpectoral half-annuli ($\overline{X}$ = 274.2 ± 14.6; n = 14); 252–280 ventral postpectoral half-annuli ($\overline{X}$ = 266.4 ± 8.1; n = 14); 3/5 lateral half-annuli; 14–16 tail annuli ($\overline{X}$ = 15.4 ± 0.7; n = 14); 26–37 dorsal segments per half-annulus in midbody ($\overline{X}$ = 32.2 ± 3.7; n = 15); 24–39 ventral segments per half-annulus in midbody ($\overline{X}$ = 31.3 ± 4.0; n = 15); fifth tail annulus with 37–47 segments ($\overline{X}$ = 41.8 ± 2.3; n = 15). Anal flap semicircular with 10–19 cloacal segments; one precloacal pore at each side of the cloaca. Most of the pectoral scales diamond-shaped, with the central scales larger and laterals smaller; pectoral portion with 9.0–14.3 mm of length ($\overline{X}$ = 11.6 ± 1.7; n = 15), representing 3.2–4.3% of snout-vent length ($\overline{X}$ = 3.7 ± 0.3; n = 14). Lateral grooves separating the body into dorsal and ventral portions, lateral grooves absent on tail, and dorsal groove present.

3. Geographic distribution

Leposternon mineiro is known from the northwest region of the Brazilian State of Minas Gerais (municipalities of Buritizeiro and João Pinheiro), and for the municipality of Cristalina, State of Goiás (the record presented here). All known locations occur the Cerrado domain, within the São Francisco River Basin and on the left bank of the São Marcos River near the Batalha hydroelectric plant (Fig. 2). Based on existing geographic records, the sample collected in the municipality of Cristalina (17°6'42.36"S; 47°22'29.31"W) extends the distribution of the species by 148 kilometers to the west (Fig. 2) and is the first record of this species for the State of Goiás.

Discussion

The new sample from *Leposternon* from State of Goiás contains some data that diverge from the type series (Table 1): numbers of dorsal and ventral half-annuli, dorsal segments, ratio of prefrontal width and head width, ratio of the diameter of the midbody and snout-vent length, and color pattern. The variables ratio of azygous length to head length and ratio of azygous width to head width are at the limit of variation between populations, with a small margin of overlap (31.5–36.4 vs. 36.2–40.2, and 15.2–27.6 vs. 27.4–40.5, respectively). For the cephalic shields, the presence of a supraocular on the left side of the head was identified, with variation in the number of parietals from 2–4 (vs. 2 of the type series). Geographical variation is commonly observed in squamate reptiles, but the causes of these variations are still topics of investigation, and both environmental and geographic features are likely to be involved. For snake species, geographic variations identified mainly in pholidotic characters from different populations were for many years considered to be related to environmental conditions (temperature, humidity, etc.) during specimen development (Fox, 1948; Alexander and Gans, 1966). However, studies with recent phylogeographic evidence have been correlated such between-population variations with ancient geographic events of habitat fragmentation (Grazziotin *et al.*, 2006). Despite the morphological differences in some characters between the population from the municipality of Cristalina (representing the western-most known distribution of the species) and the population of the São Francisco River basin (the eastern-most), due to the relatively small scale variations in limits of the characters mentioned above (except for the color pattern), we have opted here for a conservative approach, and consider that the observed differences represent geographic variation within the species instead treat the new specimens as an separate species. Additionally, studies involving a greater number of specimens and molecular data might contribute

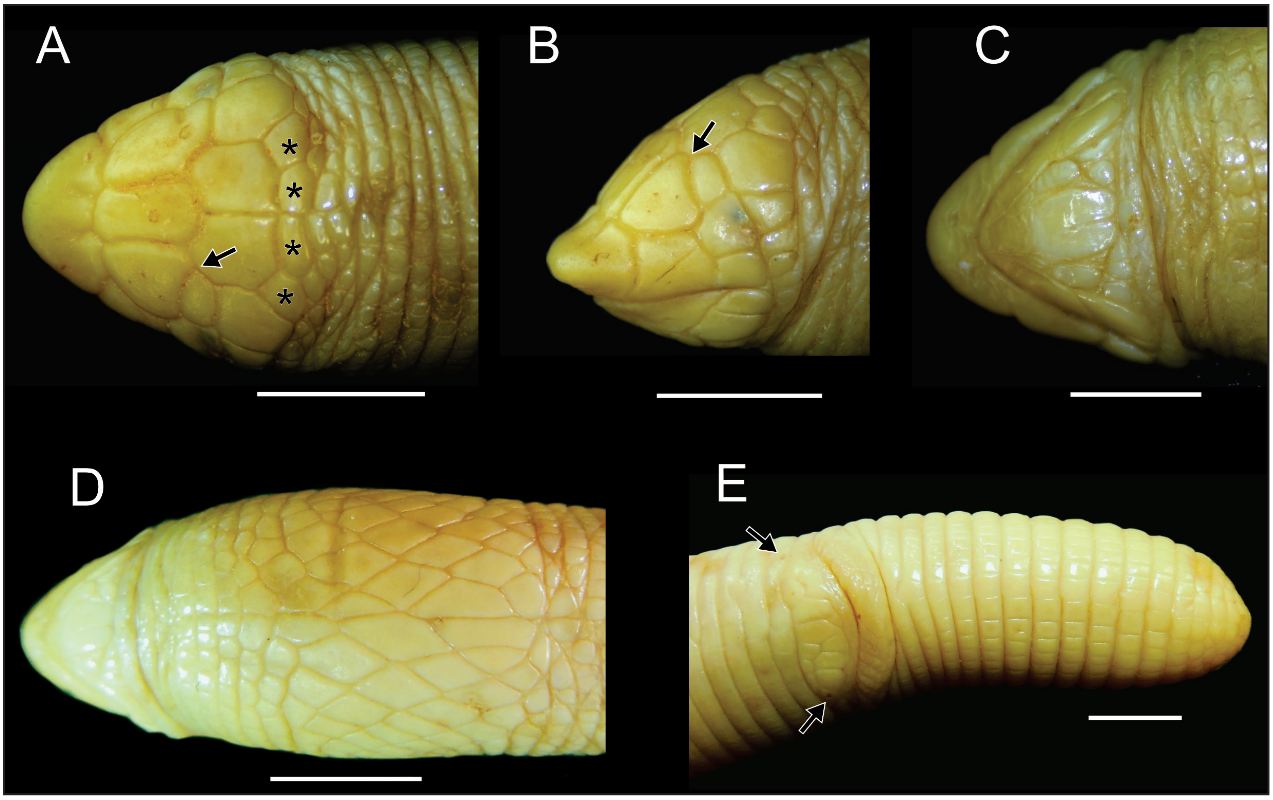

Figure 2. Specimen of *Leposternon mineiro* (CEPB 1844) from municipality of Cristalina, State of Goiás, Brazil. Head region in (A) dorsal, (B) lateral and (C) ventral views. (D) Pectoral region and (E) cloacal and ventral region of the tail. Black arrow in (A) and (B) indicate the presence of a supraocular on the left side; asterisks indicate the specimen's four parietal scutes; and black arrows in (E) indicate the location of pre-cloacal pores. Scale = 5 mm.

further to the clarification of the taxonomic status of the Cristalina population.

Leposternon mineiro can be distinguished from the other species of the genus according to the diagnosis presented in the results. However, with *L. kisteumacheri*, it presents overlapping values of dorsal and ventral postpectoral half-annuli and shares equal numbers of cephalic shields. *L. mineiro* can be easily distinguished from *L. kisteumacheri* by the shape and size of the azygous, as in *L. mineiro* the anterior edge of the azygous is straight, while in *L. kisteumacheri* this edge is curved and anteriorly projected (Fig. 3). More quantitatively, the number of teeth is different in the two species (see diagnosis).

According to Costa *et al.* (2020), one of the main deficits in biodiversity data in the 21st century is the lack of information on species distributions, which therefore constitutes one of the global priorities for reptile conservation. In Brazil, most municipalities (ca. 90%) do not have any records of amphisbaenians (Colli *et al.*, 2016), and 23 amphisbaenian species (28.1% of the national species richness) are represented only by the type series, and/or specimens from the type locality. Such this lack of detailed knowledge of species distributions is an obstacle to the identification of the risks of species extinction and the definition of priority areas for the conservation. The sample analyzed in the current study expands the knowledge of *L. mineiro* both terms of diagnostic data and for that on geographic distributions. In the most recent extinction risk assessment for the Brazilian fauna (“Livro Vermelho da Fauna Brasileira Ameaçada de Extinção”, 2018) organized by the Chico Mendes Institute, 14 species of amphisbaenians were categorized as being Data Deficient (DD), four as Near Threatened (NT) and seven as Endangered (Vulnerable and Endangered). *Leposternon mineiro* was not included in that study because it was in the process of being described. Currently, the distribution is still restricted to three localities within the Cerrado domain and encloses about 7,000 square kilometers including many well-sampled areas, all in use by mechanized agriculture. In addition, one of the records comes a site that was being inundated by the construction of the Batalha Hydroelectric Power Plant, a strongly impacted envi-

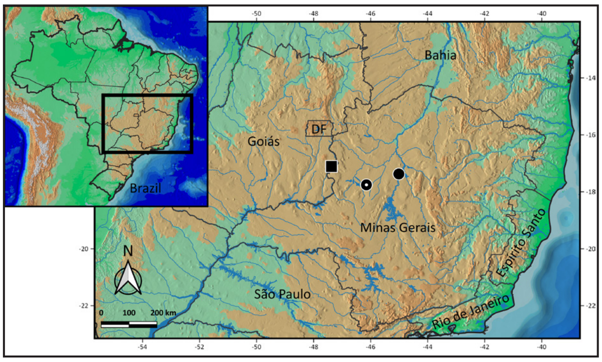

Figure 3. Geographic distribution of *Leposternon mineiro*. Circles represent the locations given in Ribeiro *et al.* (2008) (type locality is the symbol with a white dot in the center) and the square represents the new record for the municipality of Cristalina, Goiás, on the border with the state of Minas Gerais.

ronment. These environmental characteristics place the species in risk of extinction, and it is therefore likely to be categorized as such in future ICMBio assessments.

Acknowledgments

We are very grateful to all the herpetological collection curators and curatorial staff for allowing us to examine the specimens under their care; and to Ricardo Montero for comments and suggestions. This study was funded by CNPq, through the program to support research projects and training human resources for biological taxonomy – PROTAX 22/2020, process number 441967/2020-5 CNPq. JPE and SR thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, for funding partially this study (CNPq; 131058/2021-5). JPE received a master studentship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES.

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Appendix 1. Analyzed specimens

Scientific collections: Centro de Estudos e Pesquisas Biológicas da Universidade Católica de Goiás, Goiânia, Brazil (CEPB); Coleção Herpetológica da Universidade de Brasília, Brasília, Brazil (CHUNB); Coleção Herpetológica da Universidade Federal do Mato Grosso, Cuiabá, Brazil (UFMT); Coleção Zoológica de Referência of Universidade Federal de Mato Grosso do Sul, Campus de Corumbá, Corumbá, Brazil (CEUCH); Coleção Zoológica da Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (ZUFRJ); Facultad de Ciências de la Universidad de la República, Montevideo, Uruguay (ZVC–R); Fundación Miguel Lillo, Tucumán, Argentina (FML); Museo Nacional de História Natural do Paraguay, Assunción, Paraguay (MNHNP); Museu de Ciências Naturais da Pontifícia Universidade Católica de Minas Gerais, Belo Horizonte, Brazil (MCN–R); Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil (MCP); Museu de Ciências Naturais of Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil (MCN); Museu de História Natural de Capão da Imbuia, Curitiba, Brazil (MHNCI); Museu Nacional / Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Museu Paraense Emílio Goeldi, Belém, Brazil (MPEG); Museu de Zoologia da Universidade Estadual de Santa Cruz, Ilhéus, Brazil (MZUESC); Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP); Museum Für Naturkunde, Berlin, Germany (ZMB); Zoologisches

Museum, Berlin, Germany (ZSM); Muséum National d'Histoire Naturelle, Paris, French (MNHN).

Leposternon cerradensis (n = 20) — BRAZIL: GOIÁS: APORÉ: CEPB 5377, 5378; MNRJ 16111 (paratype); MZUSP 96347 (holotype), MZUSP 96348, MZUSP 98036 (paratype).

Leposternon kisteumacheri (n = 5) — BRAZIL: BAHIA: Jequié: MZUSP 8929; MINAS GERAIS: Januária: MZUSP 6674; Manga: MNRJ 4041 (holotype), 4042, 4044 (paratype).

Leposternon maximus (n = 71) — BRAZIL; GOIÁS: MAMBAÍ: MZUSP 99189, MZUSP 99194, MZUSP 99195, MZUSP 99198 (paratype); BURITINÓPOLIS: MZUSP 99198 (paratype); MINAS GERAIS: FORMOSO: MZUSP 93158 (holotype).

Leposternon mineiro (n = 15) — BRAZIL: MINAS GERAIS: BURITIZEIRO: CHUNB 44482; MNRJ 15489; MNRJ 15490; MNRJ; 15766; MNRJ 17795; BALANÇA; MNRJ 16198 (holotype); CRISTALINA: CEPB 1841; CEPB 1844; CEPB 1840; CEPB 1839; CEPB 1845; CEPB 1843; CEPB 1846; CEPB 1847; CEPB 1842.

Leposternon polystegum (n = 15) —BRAZIL: ALAGOAS: Piranhas: MZUSP 79410, MZUSP 79411, MZUSP 79414, MZUSP 79416, MZUSP 79417; MARANHÃO: Nova Vida: MPEG 11678; Paruá: MPEG 11500, MPEG 11504, MPEG 11756, MPEG 11757; Paraquéu: MPEG 11502, MPEG 11755; PARÁ: Belém: MPEG 198, MPEG 199; TOCANTINS: Lajeado: MZUSP 94293.

Leposternon infraorbitale (n = 109) —BRAZIL: ACRE: Rio Branco: MZUSP 6387; GOIÁS: Aporé: MZUSP 99233–99242; Aragarças: MPEG 1187; BAHIA: Barra do Choça: MZUESC 5852; Boa Nova: MZUESC 2285, MZUESC 4546; Igrapiúna: MZUESC 4858, MZUESC 4865, MZUESC 5110, MZUESC 5111, MZUESC 5953; Ilhéus: MZUESC 4765, MZUESC 4777; Itabuna: MZUSP 78803; Itacaré: MCP 18180, MZUESC 3939; Itapebi: MZUESC 3274, MZUESC 3276; Santo Antônio de Jesus: MZUSP 57768; Ubaitaba: MCP 18175; MATO GROSSO: Araputanga: UFMT 3029, UFMT 3320, UFMT 3324, UFMT 3825, UFMT 3835, UFMT 3838, UFMT 3951, UFMT 3956, UFMT 3959; Campos Novos dos Parecis: UFMT 3466, UFMT 3471, UFMT 3477; Chapada dos Guimarães: UFMT 370, UFMT 2465, UFMT 2466; Cuiabá: UFMT 3473; Jauru: UFMT 3258–3260, UFMT 3264, UFMT 3322, UFMT 3325, UFMT 3326, UFMT 3332, UFMT 3836, UFMT 3837, UFMT 3952–3955, UFMT 3957, UFMT 3958, UFMT 3960; Nortelândia: UFMT 2915, UFMT 2917; Vale de São Domingos: CEUCH 2177, CEUCH 2179–2186, UFMT 3295, UFMT 3376, UFMT 3401, UFMT 3412, UFMT 3413, UFMT 3451, UFMT 3500, UFMT 3507, UFMT 3524, UFMT 3526, UFMT 3534, UFMT 3552, UFMT 3556, UFMT 3611–3613, UFMT 3617, UFMT 3618, UFMT 3632, UFMT 3633, UFMT 3636–3639; DISTRITO FEDERAL: Brasília: MZUSP 47708, 88123; MINAS GERAIS: Cataguases: MCNR 1026, MCNR 1027; Indianópolis: MCNR 207; Paraopé: MNRJ 1782, MZUSP 13752; Unaí: MCNR 1027; MNRJ 10936; PARÁ: MZUSP 95681; RONDÔNIA: Espigão do Oeste: MHNCI 7347; UFAC 1804.

Leposternon microcephalum (n = 173) —ARGENTINA: FML 1291; BUENOS AIRES: MNHN 488 (holotype de *Lepidosternon phocaena*); CHACO: Departamento de San Fernando: FML 13544; CORRIENTES: MCN 6068; Departamento Barrio Lomas: FML 15903, FML 15904; SALTA: Departamento Orán: FML 2756, FML 14902; SANTA FÉ: Departamento La Capital: FML 1291. FORMOSA: Departamento de Laishi: FML 11312, FML 11313, FML 11318, FML 11319. BOLÍVIA: BUENA VISTA: Santa Cruz: FMNH 35666, FMNH 35667. BRAZIL: BAHIA: Boa Nova: MZUESC 4547; ESPIRÍTO SANTO: Alfredo Chaves: MZUSP 1943; Santa Leopoldina: MZUSP 6514, MZUSP 6515; Santa Tereza: MZUSP 8811, MZUSP 17448, MZUSP 17449, MZUSP 17451; São José do Calçado: MZUSP 93705; GOIÁS: Luziânia: CHUNB 49955; Minaçu, UHE Serra da Mesa: MZUSP 85220–85224; MATO GROSSO DO SUL: Anastácio: MZUSP 73315; Anaurilândia: MZUSP 16, ZUFRJ 1490; Bataiporã: MZUSP 88860; MINAS GERAIS: MZUSP 6464; Alto Jequitibá: MZUSP 95034; Belo Horizonte: ZUFRJ 797; Cataguases: MCNR 671, MCNR 708; Juiz de Fora: MZUSP 77036; Ouro Branco: MZUSP 6463; Perdões: MCNR 471; Recreio: MNRJ 11280; Sereno: MZUSP 6615; Uberlândia: MZUSP 4638; Viçosa: MZUSP 6560; PERNAMBUCO: Fernando de Noronha: MZUSP 7691. PARANÁ: Adrianópolis: MHNCI 3064; Antonina: MHNCI 11303, MZUSP 3464, MZUSP 3465; Guaraqueçaba: MHNCI 7744; Matinhos: MHNCI 957, MHNCI 2832, MHNCI 9685; Mirador: MHNCI 8275; Morretes: MHNCI 1397, MHNCI 6329; Paranaguá: MHNCI 4134, MZUSP 1265; Pontal do Paraná: MHNCI 5853; Santa Izabel do Ivaí: MHNCI 8263; São José dos Pinhais: MHNCI 7654, MHNCI 7736; Tapira: MHNCI 8272; RIO DE JANEIRO: ZMB 1395 (holotype of *Lepidosternon maximiliani*), ZMB 1396 (holotype of *Lepidosternon petersi*); Campo Grande: ZUFRJ 1676; Duque de Caxias: MZUSP 6394, MZUSP 6397–6399; Floriano: MZUSP 6578; Manguinhos: MZUSP 7677, MZUSP 8284; Miguel Pereira: MZUSP 65390; Rio de Janeiro: MZUSP 2426, MZUSP 2676, MZUSP 13762, ZSM 3150 (holótipo); Araquari: MHNCI 7265, MZUSP 7395. SANTA CATARINA: Corupá: MZUSP 1249, MZUSP 6466, MZUSP 6487, MZUSP 6488, MZUSP 6518; Florianópolis: MZUSP 67046, UFRJ 1003; Ilha do Arvoredo: MZUSP 67047; Itapoá: MHNCI 1400. SÃO PAULO: MZUSP 77039; Alecrim: MZUSP 6610; Aparecida do Norte: MZUSP 77538; Assis: MZUSP 77038; Campo limpo: MZUSP 89660; Candido Motta: MZUSP 6577; Cotia: MZUSP 77021; Diadema: MZUSP 77524; Embu: MZUSP 77020; Engenheiro Marsillac: MZUSP 77534; Forte do Itapuí: MZUSP 77030; Guararema: MZUSP 6640, MZUSP 77537; Guarujá: MZUSP 89391; Ilha da Queimada: MZUSP 77031, MZUSP 77032; Ilha dos Alcatrazes: MZUSP 6496; Itirapina: MZUSP 6593; Java: MZUSP 6602–6604; Jundiaí: MZUSP 77533; Juquitiba: MZUSP 77539; Marília: MRT 11982–11984; Miracatu: MZUSP 77526; Paratei do Meio: MZUSP 77024; Pedro Toledo: MZUSP 77029; Peruíbe: MZUSP 77026, MZUSP 81402; Piquete: MZUSP 1252; Piracicaba: MZUSP 6559, MZUSP 77041; Regente Feijó: MHNCI 3665; Registro: MZUSP 77528; Ribeirão Pires: MZUSP 6561, MZUSP 77525; Rosana: MZUSP 95612; Santo Amaro: MZUSP 77017–77019; Santo Anastácio: MZUSP 6592; São Bernardo do Campo: MZUSP 89803; São Carlos: MZUSP 6554, MZUSP 77536; São Lourenço do

Turvo: MZUSP 6493; São Paulo: MZUSP 11959, MZUSP 77011–77016, MZUSP 77037, MZUSP 77042, MZUSP 77514–77516, MZUSP 77520, MZUSP 77521, MZUSP 89140; São Sebastião: MZUSP 6525; Taubaté: MZUSP 87545; Tupã: MZUSP 77043; Ubatuba, Ilha do mar Virado: MHNCI 7238, MZUSP 77027, MZUSP 77028, MZUSP 78431. PARAGUAY MNHNP 9446; MNHNP 10378; DEPARTAMENTO CENTRAL: MNHNP 5111; Norte de Nemby: MNHNP 7671; Asunción: MNHNP 8468, MZUSP 28386 (lectotype of *Lepidosternon latifrontalis*); Colônia Nueva Italia: FMNH 42290; Fernando de la Mora: MNHNP 10926; San Lorenzo: MNHNP 5106, MNHNP 5109, MNHNP 5110. URUGUAY: SALTO: ZVC–R 2016, ZVC–R 5906.

Leposternon octostegum (n = 4) —BRAZIL: BAHIA: Camaçari: MCP 18192–18193, MCP 96349; Salvador: MZUSP 96350.

Leposternon scutigerum (n = 5) —BRAZIL: RIO DE JANEIRO: Rio de Janeiro: MNRJ 7186, MNRJ 12452; MZUSP 2519, MZUSP 7075; ZUFRJ 289.

Leposternon wuchereri (n = 4) —BRAZIL: ESPÍRITO SANTO: Santa Tereza: MZUSP 8812; São Mateus: MNRJ 3892; BAHIA: Santa Clara: MCNR 279, 280.

Trabajo

Cuad. herpetol. 37 (2): 141-160 (*2023*)

Estudios acústicos en poblaciones argentinas de *Leptodactylus* (Anura, Leptodactylidae): revisión histórica y datos adicionales

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ABSTRACT
The first systematized acoustic studies in Argentinean anuran populations began in the 1960s with the contributions made by Avelino Barrio. Since then, with some exceptions, the knowledge about the vocalizations of local species has had little development. *Leptodactylus* is a neotropical frog genus with 83 recognized species, 14 of which are found in Argentina. In this context, and with the aim of contribute to the acoustic knowledge about the anuran species that occur in Argentina, a review of the vocalizations of *Leptodactylus* species was addressed, with emphasis in local populations. Additionally, this information is compared with new acoustic data for *L. bufonius*, *L. elenae*, *L. fuscus*, *L. gracilis*, *L. laticeps*, *L. latinasus*, *L. luctator*, *L. macrosternum* and *L. podicipinus* obtained in populations from Corrientes, Chaco and San Juan provinces. Moreover, some terms used in previous studies are reviewed in order to standardize the terminology applied to the calls of *Leptodactylus* species. The absence of descriptions about vocalizations from topotypic populations is also noted, of which *L. elenae*, *L. laticeps*, and *L. luctator* have their type locality in Argentina. Studies on acoustic variation in anuran amphibians, whether for taxonomic or geographic variation purposes, can be considered incipient in Argentina, and therefore, there are still an important information gap. Comparisons of the acoustic variables of the advertisement call between different populations allow us to understand how the acoustic variables vary according to the environmental or geographical factors. Furthermore, the recordings of other types of calls contribute to increase the information about the acoustic repertoires and have a comprehensive knowledge about aspects of the natural history and behavior of the species. Finally, it is important to highlight the need for additional acoustic studies on anuran amphibians from Argentina, especially due to the continuous alteration or loss of their habitats, and consequently of the acoustic diversity and soundscape.

Key words: Advertisement Calls; Amphibians; Argentina; Bioacoustics; Vocalizations

RESUMEN
Los primeros estudios acústicos sistematizados en poblaciones argentinas de anuros comenzaron en la década de 1960 con las contribuciones de Avelino Barrio. Desde entonces, con algunas excepciones, el conocimiento acerca de las vocalizaciones de especies locales ha tenido un escaso desarrollo. El género de ranas neotropicales *Leptodactylus* incluye 83 especies, 14 de ellas presentes en Argentina. En este contexto, y con el fin de relevar los estudios acústicos en representantes de este género, se recopilaron los principales estudios sobre sus vocalizaciones, con énfasis en poblaciones locales. Adicionalmente, se compara esta información con nuevos datos acústicos para *L. bufonius*, *L. elenae*, *L. fuscus*, *L. gracilis*, *L. laticeps*, *L. latinasus*, *L. luctator*, *L. macrosternum* y *L. podicipinus* obtenidos a partir de poblaciones de las provincias de Corrientes, Chaco y San Juan. Complementariamente, se revisan algunos términos utilizados en estudios previos con el fin de estandarizar la terminología aplicada a los cantos de las especies de *Leptodactylus*, y se identifican, además, aquellas que carecen de descripciones de sus vocalizaciones en poblaciones topotípicas, de las cuales *L. elenae*, *L. laticeps* y *L. luctator* poseen su localidad tipo en Argentina. Los estudios de variación acústica en anfibios anuros, ya sea con fines taxonómicos o de variación geográfica, pueden considerarse incipientes en Argentina, y por lo tanto, existen todavía importantes vacíos de información. La comparación de las variables acústicas de los cantos de advertencia entre diferentes poblaciones permite comprender de qué manera

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éstas varían en función de los factores ambientales o geográficos. Además, el registro de otros tipos de cantos contribuye a incrementar el conocimiento sobre el reportorio acústico y tener un conocimiento integral sobre aspectos de la historia natural y del comportamiento de las especies. Finalmente, es importante destacar la necesidad de estudios acústicos adicionales en anfibios anuros de Argentina, especialmente debido a la continua alteración o pérdida de sus hábitats y por consiguiente, de la diversidad acústica y del paisaje sonoro.

Palabras Clave: Bioacústica; Anfibios; Vocalizaciones; Cantos de Advertencia; Argentina

Introducción

Una de las características más conspicuas en el comportamiento de los anfibios anuros es la emisión de vocalizaciones, las cuales permiten la comunicación entre individuos de una misma especie, e incluso entre individuos de especies diferentes (Gerhardt, 1994). Estas vocalizaciones constituyen, en realidad, un amplio repertorio vocal, en el que pueden identificarse diferentes tipos de cantos de acuerdo al contexto en el que son emitidos. Una revisión reciente identificó entre las vocalizaciones de anuros, cantos con funciones reproductivas, agresivas, defensivas, e incluso relacionadas con la alimentación (Toledo *et al.*, 2015; Kölher *et al.*, 2017).

Entre las vocalizaciones con funciones reproductivas se encuentran los cantos de advertencia, uno de los más escuchados entre los anuros durante la época reproductiva. Dado que presentan características propias en las distintas especies (es decir, son especie-específicos) se consideran claves en el reconocimiento de las especies y de la pareja (Blair, 1964; Ryan, 1988; Ryan y Rand, 1993). En la mayoría de los casos los cantos son utilizados por los machos, para atraer a las hembras o reunir a otros machos en grupos de canto o agregaciones. Debido a estas particularidades, son empleados como un carácter diagnóstico en la identificación de especies y son una herramienta imprescindible en estudios taxonómicos (Angulo y Reichle, 2008).

En general, los estudios acústicos en anuros argentinos son escasos, y merecen destacarse aquellos publicados en la década de 1960 por Avelino Barrio (*e.g.*, Barrio, 1962; 1964; 1965; 1966). Los aportes de Barrio representan los primeros estudios sistematizados para poblaciones argentinas en los que se incluyen valores cuantitativos de las variables temporales y espectrales, además de representaciones gráficas (principalmente espectrogramas). A pesar de las limitaciones técnicas de la época, los datos y las figuras de los espectrogramas se aproximan bastante a lo que pueden obtenerse actualmente con equipos y programas modernos de análisis de sonidos. Además de su enorme valor histórico, estos registros y espectrogramas son los únicos disponibles actualmente para ciertas poblaciones y especies locales. Más recientemente, aunque con algunas excepciones, la mayoría de las caracterizaciones acústicas acompañan las descripciones de nuevas especies. Incluso, para muchas especies presentes en Argentina se conocen sus características acústicas, sólo a partir de datos obtenidos en otras poblaciones de países vecinos, en ocasiones geográficamente muy alejadas y con identificación taxonómica todavía incierta.

Leptodactylus es un género de ranas neotropicales con 83 especies reconocidas actualmente (Frost, 2022). En Argentina están presentes 14 de ellas, en su mayoría, especies de amplia distribución. Una síntesis de las principales características acústicas de todas las especies de *Leptodactylus* puede ser consultada en de Sá *et al.* (2014).

Para poblaciones argentinas de *Leptodactylus*, los primeros aportes que incluyen valores de las variables temporales y espectrales y espectrogramas fueron brindados por Barrio (1965; 1966; 1973). Posteriormente, una cinta casete con muestras de cantos de varias especies (incluyendo algunas de *Leptodactylus*), junto a un catálogo con información sobre las grabaciones y una serie de figuras de los espectrogramas fueron publicados por Straneck (1992) y Straneck *et al.* (1993), respectivamente. En el catálogo mencionado no se incluyen descripciones de los valores temporales ni espectrales, si bien pueden deducirse de los espectrogramas graficados. Más tarde, algunas especies de *Leptodactylus* de poblaciones del sur de Córdoba fueron caracterizadas acústicamente por Salas *et al.* (1998). Entre las descripciones más recientes pueden referirse aquellas para *L. furnarius* (Baldo *et al.*, 2008), el par de especies crípticas *L. apepyta* y *L. mystacinus* (Schneider *et al.*, 2019), y *L. elenae* (Silva *et al.*, 2020). La única especie cuyas caracterizaciones acústicas aún no fueron descriptas formalmente en poblaciones argentinas son *L. labyrinthicus* y *L. laticeps*.

El objetivo de este trabajo es contribuir al conocimiento de las vocalizaciones de anuros argentinos del género *Leptodactylus*, y aportar información para futuros estudios taxonómicos, de variaciones geográficas o de comportamiento. Para ello, se brinda una lista actualizada de la literatura disponible sobre las vocalizaciones de las 14 especies de *Leptodactylus* presentes en Argentina, en especial aquella asociada a poblaciones locales. Adicionalmente, se compara esta información con nuevos datos acústicos obtenidos a partir de poblaciones procedentes de las provincias de Corrientes, Chaco y San Juan.

Materiales y métodos

Se recopiló información bibliográfica sobre descripciones acústicas de las especies de *Leptodactylus* presentes en Argentina. La búsqueda se focalizó en las poblaciones argentinas, aunque se incluyeron las de otros países que comprenden la distribución de cada especie. Específicamente, se hizo énfasis en la literatura en cuyas descripciones se incluyen valores de variables temporales y espectrales. Adicionalmente, la información recabada es complementada con la re-descripción del canto de varias especies, los cuales fueron obtenidos en diversas salidas de campo utilizando un grabador digital M-Audio Micro-TrackII o Marantz PMD 222, en ambos casos, acoplado a un micrófono direccional Sennheiser ME-66 con modulador K6. Se utilizó una tasa de muestreo de 44 kHz y 16 bits de resolución, y al finalizar cada grabación se registró la temperatura ambiente y el porcentaje de humedad relativa del aire (HRA) usando un termohigrómetro digital TFA. Las grabaciones se editaron y analizaron con el programa Raven Pro 1.4, 32-bit versión (Bioacoustics Research Program 2011). Las variables acústicas temporales se midieron en oscilogramas y las variables espectrales en espectrogramas con la siguiente configuración: ventana tipo Hann, de 512 puntos, superposición del 50% y resolución de 512 puntos (DFT), contraste 50% y brillo 50%. La frecuencia dominante se midió utilizando la opción pico de frecuencia. Oscilogramas y espectrogramas de cada una de las especies fueron generadas utilizando el programa Seewave v.1.6 (Sueur *et al.*, 2008) en la plataforma R v.3.6.0 (R Development Core Team 2019) con la siguiente configuración: ventana de Hanning, superposición del 90% y resolución de 512 puntos (FFT).

Las variables acústicas se midieron siguiendo a Köhler *et al.* (2017). De las descripciones ya existentes, los parámetros temporales fueron estandarizados a milisegundos (ms), mientras que las espectrales, a Hertz (Hz).

Los registros analizados en este trabajo se encuentran incorporados a la Fonoteca Zoológica de la Universidad Nacional del Nordeste (FZ-UNNE) y los datos correspondientes (localidades, coordenadas, fecha y temperatura) están detallados en el Apéndice I. Ejemplares de referencia asociados con algunos registros se encuentran depositados en la Colección Herpetológica “Blanca Beatriz Álvarez” de la Universidad Nacional del Nordeste (UNNEC).

Resultados

Las referencias bibliográficas sobre vocalizaciones para las 14 especies de *Leptodactylus* presentes en Argentina son presentadas en la Tabla 1, discriminadas entre poblaciones locales y de otros países. A continuación, se caracterizan acústicamente otras poblaciones argentinas de *Leptodactylus* procedentes de las provincias de Corrientes, Chaco y San Juan (Apéndice 1), pertenecientes a nueve de estas especies:

1) *Leptodactylus bufonius* Boulenger, 1894. Localidad tipo: Asunción, Paraguay. Dos tipos de cantos se describen para esta especie. Un canto simple (considerado aquí como el canto de advertencia), el cual consiste en notas cuya duración varía entre 115 y 300 ms y son emitidas regularmente a una tasa promedio de 56 notas/min. Presenta un pico de frecuencia dominante promedio de 1499 Hz, y el rango de frecuencia se ubica entre 1007 y 2028 Hz (Tabla 2, Fig.1). El segundo tipo de canto está compuesto por grupos de tres notas. Los grupos de notas duran en promedio 284 ms, y son emitidos con una tasa de 40 cantos/min. Dentro de cada grupo, las notas son emitidas con una tasa de 310–482 notas/min. De las tres notas, la segunda o central presenta una menor duración y un pico de frecuencia dominante menor (Tabla 3; Fig.1).

2) *Leptodactylus elenae* Heyer, 1978. Localidad tipo:

Tabla 1. RDescripciones disponibles de cantos de advertencia de especies de *Leptodactylus* presentes en Argentina (se discriminan poblaciones locales y otras que comprenden el rango de distribución de las distintas especies).

Especies	Poblaciones argentinas		Otras poblaciones	
	Localidades	Referencias	Localidades	Referencias
L. apepyta	Formosa: Las Lomitas; Tucumán: El Ceibal y El Cadillal	Schneider *et al.* (2019)		
L. bufonius	Salina Grande (límite entre las provincias de Córdoba y La Rioja) La Rioja Embarcación (Salta) Chancaní (Córdoba) Corrientes (Corrientes)	Barrio (1965) Philibosian *et al.* (1974). Straughan y Heyer (1976) Straneck (1992), Straneck *et al.* (1993) Stănescu *et al.* (2022)	Paraguay: Filadelfia Bolivia: Cordillera (Santa Cruz)	Heyer y Scott (2006) Schalk y Leavitt (2017)
L. elenae	Villa Ángela (Chaco). Parque Nacional Iguazú (Misiones) Resistencia (Chaco)	Barrio (1965) Straneck (1992), Straneck *et al.* (1993) Silva et al (2020)	Brasil: Mato Grosso Bolivia: Puerto Almacén (Santa Cruz) Paraguay: Itapúa	Silva *et al.* (2020) Márquez *et al.* (1995) Heyer *et al.* (1996), Heyer y Heyer (2002)
L. furnarius	RN Nº 12 y RP Nº 3 (Candelaria, Misiones)	Baldo *et al.* (2008)	São Paulo Mato Grosso Minas Gerais	Sazima y Bokermann (1978), Baldo *et al.* (2008) Heyer y Heyer, (2004) Giaretta y Kokubum (2004)
L. fuscus	Villa Ángela y Gral. Pinedo (Chaco) Embarcación (Salta) Parque Nacional Iguazú (Misiones).	Barrio (1965) Straughan y Heyer (1976), Heyer y Reid (2003) Straneck (1992)	Colombia: Villavicencio; Brasil: Manaos; Bolivia; Guayana Francesa Costa venezolana. Brasil: Boraceia Bolivia: Valle de Satja (Cochabamba) Paraguay, Brasil, Bolivia, Colombia, Panamá, Trinidad y Tobago, Surinam, Guayana Francesa Brasil: Floriano (Piauí)	Heyer (1978) Lescure (1972) Rivero (1971) Heyer *et al.*(1990) Márquez *et al.* (1995) Heyer y Reid (2003) Lima *et al.*, (2018)

L. gracilis	Ingeniero Maschwitz (Buenos Aires) Martínez (Buenos Aires) Bajos del Cazador (Escobar, Buenos Aires). Córdoba	Barrio (1965, Heyer (1978), Barrio (1973) Straneck (1992), Straneck *et al.* (1993) Salas *et al.* (1998)	Brasil: Santa Catarina y Rio Grande do Sul Bolivia: Guadalupe (Santa Cruz)	García-Pérez y Heyer (1993) Köhler y Lötters (1999)
L. labyrinthicus	-	-	Brasil: Mato Grosso, Minas Gerais, Sao Paulo, Paraíba Bolivia: Puerto Almacén (Santa Cruz) Venezuela	Heyer (2005) Márquez *et al.* (1995) Rivero y Esteves (1969)
L. laticeps	Reserva Natural Formosa	Schaefer *et al.* (datos no publicados)	Paraguay: Filadelfia	Heyer y Scott (2006)
L. latinasus	Punta Lara, La Plata e Ingeniero Maschwitz (Buenos Aires), Rosario y Colonia Las Toscas (Santa Fe), y Villa Ángela y cercanías de Resistencia (Chaco). Buenos Aires: Escobar: Bajos del Cazador Salta: Embarcación Córdoba Buenos Aires: Punta Lara	Barrio (1965) Straneck (1992), Straneck *et al.* (1993) Straughan y Heyer (1976), Heyer y Juncá (2003) Salas *et al.* (1998), Basso y Basso (1992)		
L. luctator	Santa Fe: Helvecia Entre Ríos: Colón: Parque Nacional El Palmar Córdoba	Barrio (1966) Straneck (1992), Straneck*et al.* (1993) Salas *et al.* (1998)	Brazil: Araguari y Uberlândia (Minas Gerais); Piatã (Bahia)	Magalhães *et al.* (2020)
L. macrosternum	Santa Fe: Helvecia	Barrio (1966)	Brasil: Araguari (Minas Gerais) Brasil: Macaíba (Rio Grande do Norte) Brasil: Araguari (Minas Gerais); Macaíba (Rio Grande do Norte)	Heyer y Giaretta (2009) y Camurugi *et al.* (2017) Magalhães *et al.* (2020)

L. mystacinus	Córdoba: Santa Rosa de Calamuchita Córdoba: Calamuchita: Pozo Verde Córdoba Misiones: Colonia Victoria y Oberá; Santa Fe: Rosario; Buenos Aires: Ramallo; Entre Ríos: localidades no precisadas; Córdoba: Pozo Verde; Corrientes: Bella Vista	Barrio (1965) Straneck (1992), Straneck *et al.* (1993) Salas *et al.* (1998) Schneider *et al.* (2019)	Uruguay: Montevideo; Brasil: São Paulo, Bahia, Rio Grande do Sul, Santa Catarina, Minas Gerais y Goiás Brasil: Rio de Janeiro Paraguay: Itapúa Brasil: Uberlândia (Minas Gerais)	Schneider *et al.* (2018) Abrunhosa *et al.*(2001) Heyer *et al.* (2003) Oliveira-Filho y Giaretta (2008)
L. plaumanni	Misiones: Bernardo de Irigoyen. Misiones: Parque Provincial Urugua-í	Barrio (1973), Heyer(1978), Cardoso (1985) Straneck (1992), Kwet *et al.* (2001)	Brasil: São Francisco de Paula y Canela (Rio Grande do Sul),Rancho Queimado (Santa Catarina)	Kwet *et al.* (2001)
L. podicipinus	Santa Fe Misiones: Parque Nacional Iguazú	Barrio(1965) Straneck (1992), Straneck *et al.* (1993)	Paraguay: Itapúa; Brasil: São Paulo, Rondônia, y Amazonas Bolivia:, Valle de Sajta (Cochabamba) Brasil: Goiás Brasil: São Paulo Brasil: Limeira do Oeste, Araporã, Ituiutaba, Monte Alegre de Minas, Uberlândia (Minas Geras); Itaguaçu (Bahia); Costa Marques, Vilhena (Rondônia); Cuiabá (Mato Grosso)	Heyer (1994) Márquez *et al.* (1995) Guimarães *et al.* (2001) Silva *et al.* (2008) Gazoni *et al.* (2021)

Tabla 2. Principales parámetros temporales y espectrales del canto de advertencia de poblaciones argentinas de *Leptodactylus*. Para cada especie se informa el número de individuos/cantos analizados, y para cada variable se presenta el promedio, el desvío estándar y el rango.

	Tasa de canto (notas/min)	**Duración de la nota (ms)**	**Duración de la internota (ms)**	**Pico de Frecuencia Dominante (Hz)**	**Límite inferior de la frecuencia dominante (Hz)**	**Límite superior de la frecuencia dominante (Hz)**	**Ancho de banda de la frecuencia dominante (Hz**
L. bufonius (canto simple) 7/147	55,8 ± 5,5 (49,8-62,8)	195,9 ± 52,9 (115-300)	844,6 ± 83,08 (449-1655)	1498,8 ± 121,8 (1313-1723)	1087,6 ± 37,3 (1007-1166)	1798,8 ± 143,1 (1619-2028)	711,2 ± 160,4 (528-1021)
L. elenae 3/68	94,4 ± 15,2 (83,6-111,9)	234,0 ±16,8 (208-264)	409,8 ±84,9 (204-813)	1343,2±45,3 (1292,0-1378,1)	733,5 ± 25,8 (689,1-757,6)	1588,1 ±31,1 (1523,8-1630,9)	854,6 ± 45 (773,1-929,6)
L. fuscus 9/176	45,6 ±6,1 (37,4-52,7)	280,5 ± 37,2 (212-350)	1072,1±194,1 (463-2721)	2040,9±103,2 (1808,8-2325,6)	821,6±66,5 (656,2-982,7)	2576±193,2 (2237,9-3150,0)	1754,6±224,7 (1455,6-2493,8)
L. gracilis 2/38	176,2 ± 15,3 (165,4-187,1)	48,7±10,2 (38-61)	293,0±39,9 (212-420)	1734,7±145,0 (1550,4-1894,9)	796,9±138,2 (655,3-942,5)	2256,2±103,2 (2133,9-2392,6	1459,3±241,4 (1209,2-1714,7)
L. laticeps 2/30	28,9 ± 7,5 (23,6-34,2)	263,0±14,8 (233-282)	1889,0±443,3 (133,0-1174,0)	1122,3±2,7 (1119,7-1125,0)	705,8±14,0 (672,9-723,7)	1423,6±29,8 (1371,3-1499,7)	717,8±38,3 (647,6-807,5)
L. latinasus 5/125	188,9±54,1 (100,8-254,4)	50,3±1,5 (45-72)	231,1±51,5 (156-463)	3570,9±123,0 (3101-3876)	2943,4±10,8 (2623-3083)	4057,3±141,5 (3.518-4.321)	1085,7±178,9 (697-1451)
L. luctator 5/50	44,6±10,1 (32,2-55,9)	322,1±37,6 (235-392)	1082,0±438,3 (471,02522,0)	275,6±34,8 (258,4-344,0)	126,6±23,6 (73-157)	420,6±35,2 (350,6-472,2)	294,0±56,3 (205,5-393,1)
L. macrosternum							
Gruñido corto (*grunt*) 1/9		104,1±8,4 (90-115)		392,4±45,4 (344,5-430,7)	268,6±27,4 (214,6-316,1)	981,0±155,7 (678,3-1156,5)	574,2±155,3 (258,4-775,2)
Gruñido largo (*growl*) 1/1		383		516,8			
Trino (*trill*) 1/5		509,8±86,6 (363-571)		706,3±38,5 (689,1-777,2)	513,1±47,1 (468,2-576,9)	949,3±43,9 (881,2-997,1)	241,2±38,5 (172,3-258,4)
L. podicipinus 4/133	173,1±94,2 (81,0-293,9)	32,3±4,9 (19-42)	405,7±238,5 (133,0-1174,0)	2899,7±91,0 (1636,5-3100,8)	1216,6±167,8 (840,3-1667,4)	3438,0±224,13126,0-(3937,3)	2221,3±292,6 (1687,4-2762,9)

Embarcación (Salta, Argentina). El canto consiste en un silbido de 208–264 ms de duración, emitido con una tasa de 83 a 111 notas/min. La frecuencia fundamental coincide con la frecuencia dominante, con un pico promedio de 1343 Hz. Desde el inicio de la nota (con una frecuencia mínima de 734 Hz) se observa una suave modulación ascendente, que al llegar al último cuarto o quinto, muestra una fuerte modulación ascendente, con forma sigmoidea, y que rápidamente se aplana hasta el final de la nota donde alcanza una frecuencia máxima de 1588 Hz (Tabla 2, Fig. 2). Por arriba de la frecuencia dominante, uno o dos armónicos extras con energía acentuadas fueron observados. No se identificaron pulsos, la amplitud aumenta suavemente hasta alcanzar su pico en el último cuarto o quinto (coincidiendo con la modulación acentuada en la frecuencia), y luego decae rápidamente.

3) *Leptodactylus fuscus* (Schneider, 1799). Localidad tipo: Surinam. El canto de advertencia consiste en un silbido corto (212–350 ms) emitido con una tasa promedio de 46 notas/min. La frecuencia es marcadamente ascendente en la mayor parte de la nota, con un breve y débil descenso en la parte final (los últimos 30–40 ms); al inicio del canto la frecuencia mínima varía entre 656 y 983 Hz, en la parte final alcanza entre 2238 y 3150 Hz. La frecuencia fundamental coincide con la frecuencia dominante, y presenta un pico entre los 1809–2326 Hz. Los cantos presentan una estructura armónica, formada por al menos tres bandas más, la segunda de mayor energía y las restantes dos, débilmente marcadas (Tabla 2, Fig.3).

4) *Leptodactylus gracilis* Duméril y Bibron, 1840. Localidad tipo: Montevideo, Uruguay. El canto de advertencia consiste en notas de corta duración (38–61 ms) emitido con una tasa promedio de 176 notas/min. La frecuencia fundamental coincide con la frecuencia dominante, la cual presenta un pico entre los 1550 y 1895 Hz. Esta banda asciende rápidamente a lo largo de la nota, desde los 797 Hz, al inicio, hasta los 2256 Hz, al final. Por encima de esta banda se observan de dos a tres bandas armónicas adicionales, de baja intensidad (Tabla 2, Fig.4).

5) *Leptodactylus laticeps* Boulenger, 1918. Localidad tipo: Santa Fe, Argentina. El canto consiste en notas simples con un rango de duración de 233–282 ms y emitidas con una tasa promedio de 29 notas/min. La frecuencia dominante (=fundamental) presenta

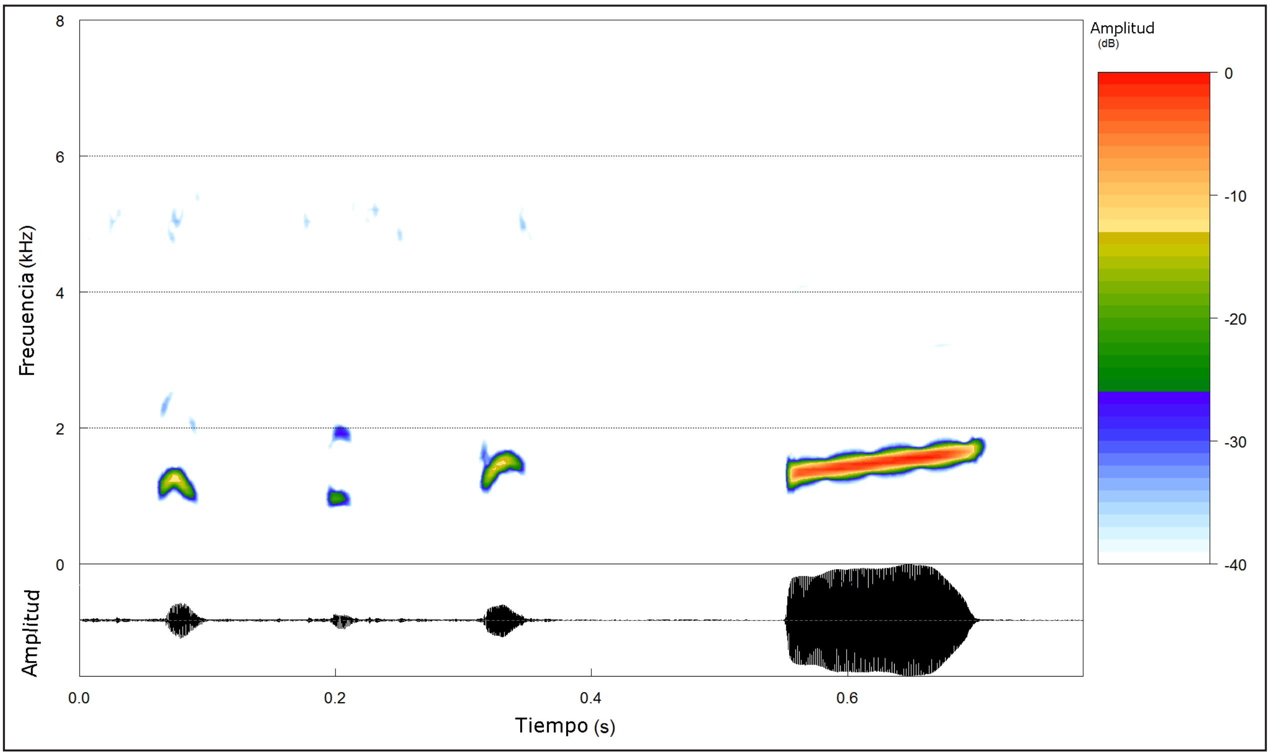

Figura 1. Vocalizaciones de *Leptodactylus bufonius*. Las primeras tres notas corresponden a un canto compuesto: FZ UNNE 0586 (Ea. Iberá, Mercedes, Corrientes), y la siguiente a un canto simple: FZ UNNE 0108 (UNNEC 13926, Felipe Yofre, Corrientes). Espectrograma (arriba) y oscilograma (abajo). El intervalo entre ambos tipos de notas es arbitrario.

un pico promedio de 1122Hz, ocupa un ancho de banda promedio de 718 Hz (Tabla 2, Fig. 5), y una suave modulación ascendente a lo largo de la nota. No se distinguen pulsos.

6) *Leptodactylus latinasus* Jiménez de la Espada, 1875. Localidad tipo: Montevideo, Uruguay.

El canto de advertencia consiste en notas de corta duración (45–72 ms) emitidas con una tasa

Tabla 3. Parámetros temporales y espectrales del canto compuesto de un macho de *L. bufonius* (N =22 cantos). FZ-UNNE 0586: Mercedes, Ea. Iberá (Corrientes); 21,6°C. Se informa el promedio, el desvío estándar y el rango.

	1° Nota	**2° Nota**	**3° Nota**
Duración de la nota (ms)	34,6 ±3,8 (28-43)	27,2 ±7,8(11-39)	35,6 ± 15,0 (19-94)
Pico de frecuencia dominante (Hz)	1241,1 ± 101,8 (1033,6-1378,1)	927,9 ± 37,0 (861,3-947,5)	1397,7 ± 59,1 (1292-1464,3)
Límite inferior de la frecuencia dominante (Hz)	869,1 ± 34,5 (822-940,3)	785,2 ± 18,2 (724,1-805,9)	1069,5 ± 58,674 (959-1215,3)
Límite superior de la frecuencia dominante (Hz)	1430,4 ± 107,0 (1265,3-1603,8)	1100,2 ± 63,3 (961,8-1184,7)	1599,9 ± 77,482 (1442,4-1756,9)
Ancho de banda de la frecuencia dominante (Hz	561,33 ± 89,196 (419,1-677)	314,94 ± 53,731 (205,2-386,8)	530,37 ± 118,41 (227,1-773,7)
	1°Internota	2°Internota	Intercanto
Duración (ms)	95,1 ± 7,1 (86-116)	91,3 ± 4,5 (82-100)	1335,9 ± 300,6 (916-2369)

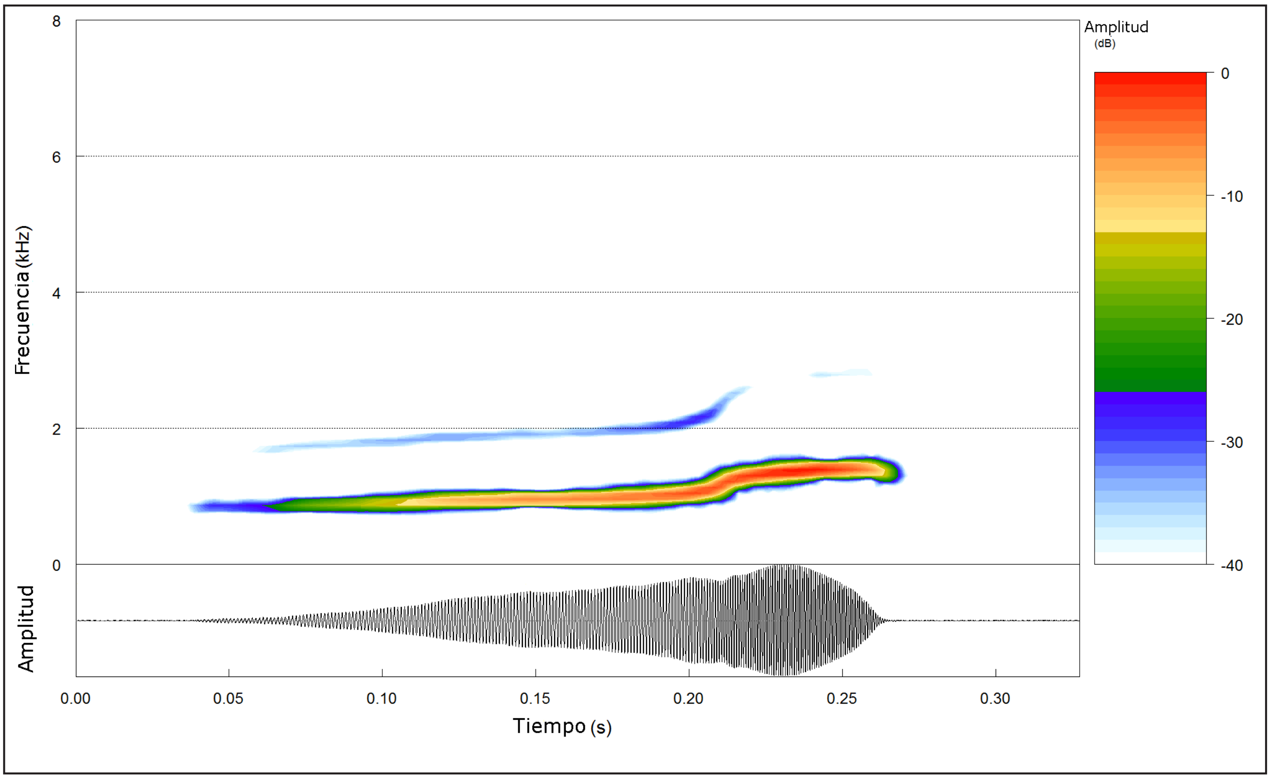

Figura 2. Canto de advertencia de *Leptodactylus elenae*. FZ UNNE 0749: B° Yecohá, Corrientes capital; 21,6°C. Espectrograma (arriba) y oscilograma (abajo).

promedio que varía entre 101 y 254 notas/min. La frecuencia dominante (=fundamental) tiene un pico entre los 3101 y 3876 Hz (Tabla 2, Fig.6); presenta una suave modulación ascendente a lo largo de la nota, en promedio, desde los 2943 Hz hasta los 4057 Hz. No se distinguen pulsos y se observa una marcada modulación ascendente hasta el primer cuarto de la nota, que luego desciende suavemente hasta el final de su emisión. Se distinguen además cuatro armónicos adicionales por encima de la frecuencia fundamental.

7) *Leptodactylus luctator* Hudson 1892. Localidad tipo: Villa Elvira, La Plata (Buenos Aires, Argentina). Los cantos analizados en este trabajo fueron emitidos por machos que se encontraban vocalizando en el centro de nidos de espuma. Están compuestos por notas no pulsadas cuya amplitud aumenta gradualmente hasta aproximadamente el 80 % de su longitud. La duración de la nota varía entre 235–392 ms y son emitidas a una tasa de 32–56 notas/minuto. El pico de frecuencia dominante (=fundamental) varía entre 258–344 Hz y no se distinguen bandas armónicas (Tabla 2, Fig.7).

8) *Leptodactylus macrosternum* Miranda-Ribeiro 1926. Localidad tipo: Salvador (Bahia, Brasil). Tres tipos de cantos fueron identificados: gruñidos largos (“growls”), gruñidos cortos (“grunts”) y trinos (“trills”). Los registros analizados incluyen un gruñido largo y una serie de gruñidos cortos combinados con trinos (Fig. 8, Tabla 2). El gruñido largo registrado presentó una duración de 383 ms, está compuesto por 18 pulsos que son emitidos a una tasa de 47 pulsos/s y presenta una frecuencia dominante de 516,8 Hz. Uno o dos gruñidos cortos son emitidos entre los trinos. Los gruñidos cortos tienen una duración de 90–115 ms con una modulación de amplitud muy baja, que comprende un aumento hasta el segundo cuarto de su longitud. La frecuencia dominante varía entre 344–431 Hz. Los trinos tienen una duración de 363–571 ms, están compuestos por 11–15 pulsos separados por intervalos de silencio, y son emitidos a una tasa aproximada de 26 notas/s. La amplitud de las notas aumenta suavemente hasta el último cuarto de su longitud. El pico de frecuencia dominante varió entre 689–777 Hz.

9) Leptodactylus podicipinus Cope, 1862. Localidad tipo: “Paraguay”. El canto de advertencia consiste en notas de corta duración, de 19–42 ms, emitidas con una tasa promedio de 173 notas/min. No se reconocen pulsos, pero la primera mitad del canto presenta una importante modulación de amplitud que determina dos picos distintivos. La frecuencia fundamental coincide con la frecuencia dominante, con un pico promedio de 2900 Hz. Existe una pronunciada modulación de frecuencia a lo largo de la nota, que asciende desde los 1216 Hz al inicio, hasta los 3438 Hz al final (Tabla 2, Fig. 9). Uno de los individuos analizados exhibió dos patrones de emisión de notas, de manera “aislada” y “en pares”. La nota “aislada” y la primera del par tienen una duración similar, mientras que la segunda del par es ligeramente menor que las anteriores.

Discusión

El repertorio vocal de muchas especies de anuros incluye una gran variedad de tipos de cantos con diferentes funciones, y para algunas especies, este repertorio es aún poco conocido, o sus funciones necesitan ser confirmadas. Entre los cantos más conocidos se encuentran los cantos de advertencia. Las vocalizaciones que se consideran como tal en especies de *Leptodactylus*, con algunas excepciones como *L. petersii* (Gazoni *et al.*, 2021), tienden a ser relativamente estereotipadas en estructura, y están compuestas por un único tipo de nota (Carvalho *et al.*, 2022).

En *Leptodactylus*, cuatro grupos de especies fueron propuestos por de Sá *et al.* (2014): los grupos de *L. latrans*, de *L. fuscus*, de *L. melanonotus* y de *L. pentadactylus*. Todos los grupos están representados en Argentina, y para la mayoría de las 14 especies cuya distribución incluye este país, se conocen sus cantos de advertencia en poblaciones locales, excepto para *L. labyrinthicus* (del grupo de *L. pentadactylus*). En Argentina, la distribución de *L. labyrinthicus* está restringida a la provincia de Misiones y a una pequeña región del NE de la provincia de Corrientes, sus registros son escasos y su categoría de conservación es Vulnerable (Vaira *et al.*, 2012). Datos acústicos de esta especie están disponibles para poblaciones de Brasil (Tabla 1).

La mayoría de las especies de *Leptodactylus* presentes en Argentina pertenecen al grupo de *L. fuscus.* En este trabajo se re-describen los cantos de nuevas poblaciones de *L. bufonius*, *L. elenae*, *L. fuscus*, *L. gracilis*, *L. laticeps* y *L. latinasus*. En *L. bufonius* se conocen dos tipos de cantos. De acuerdo a lo mencionado anteriormente, el canto simple constituido por una nota única corresponde al canto de advertencia, mientras que los cantos formados por tres notas posiblemente representan un canto de cortejo, dado que su emisión está asociada con un inusual comportamiento de cortejo (Faggioni *et al.*, 2017). El canto de advertencia de las poblaciones

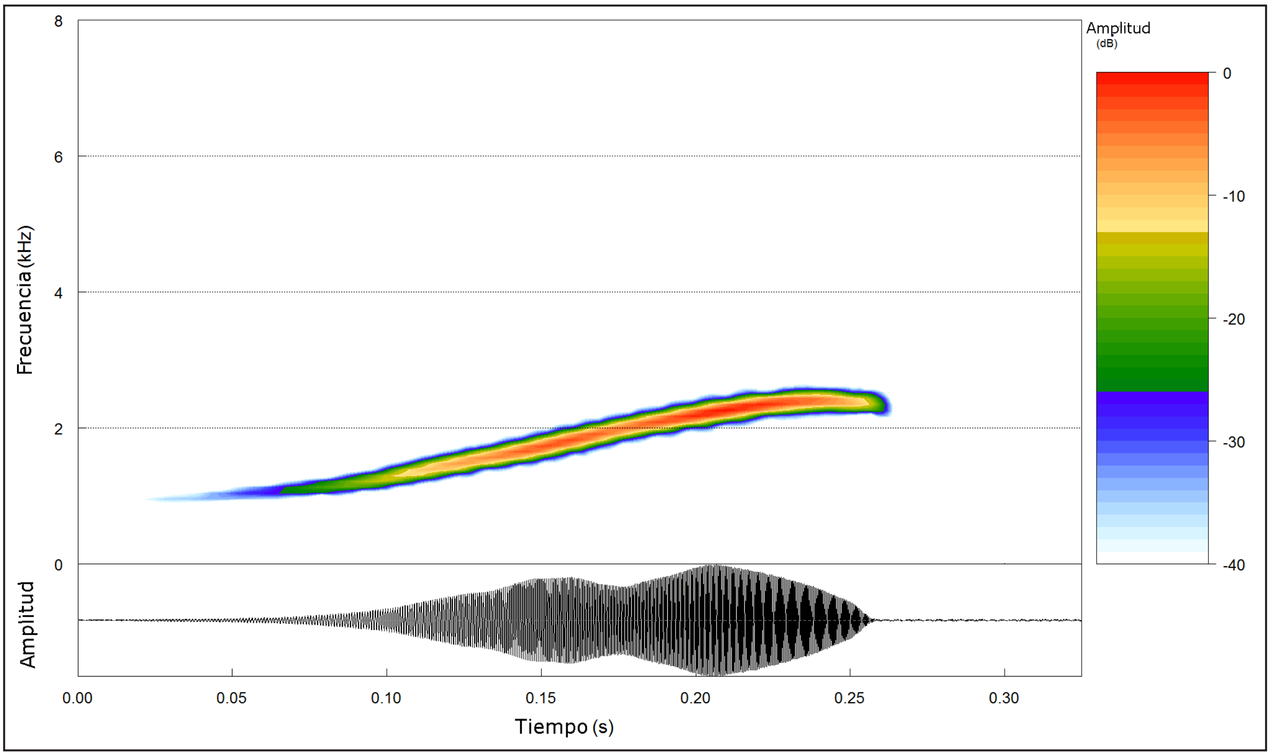

Figura 3. Canto de advertencia de *Leptodactylus fuscus* (UNNEC 13932). FZ UNNE 0652: Estancia San Antonio P., Alvear, Corrientes; 24°C. Espectrograma (arriba) y oscilograma (abajo).

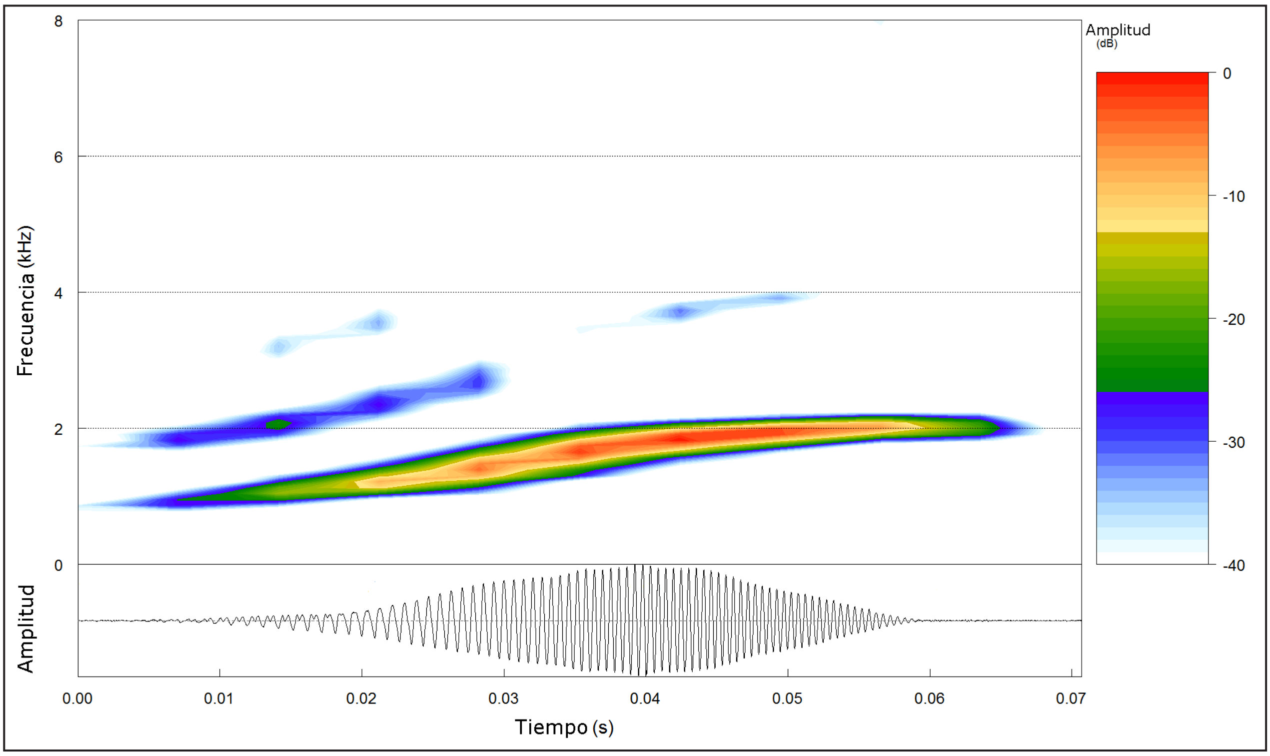

Figura 4. Canto de advertencia de *Leptodactylus gracilis.* FZ UNNE 0157: Parque Nacional Mburucuyá, Corrientes; 20,5°C. Espectrograma (arriba) y oscilograma (abajo).

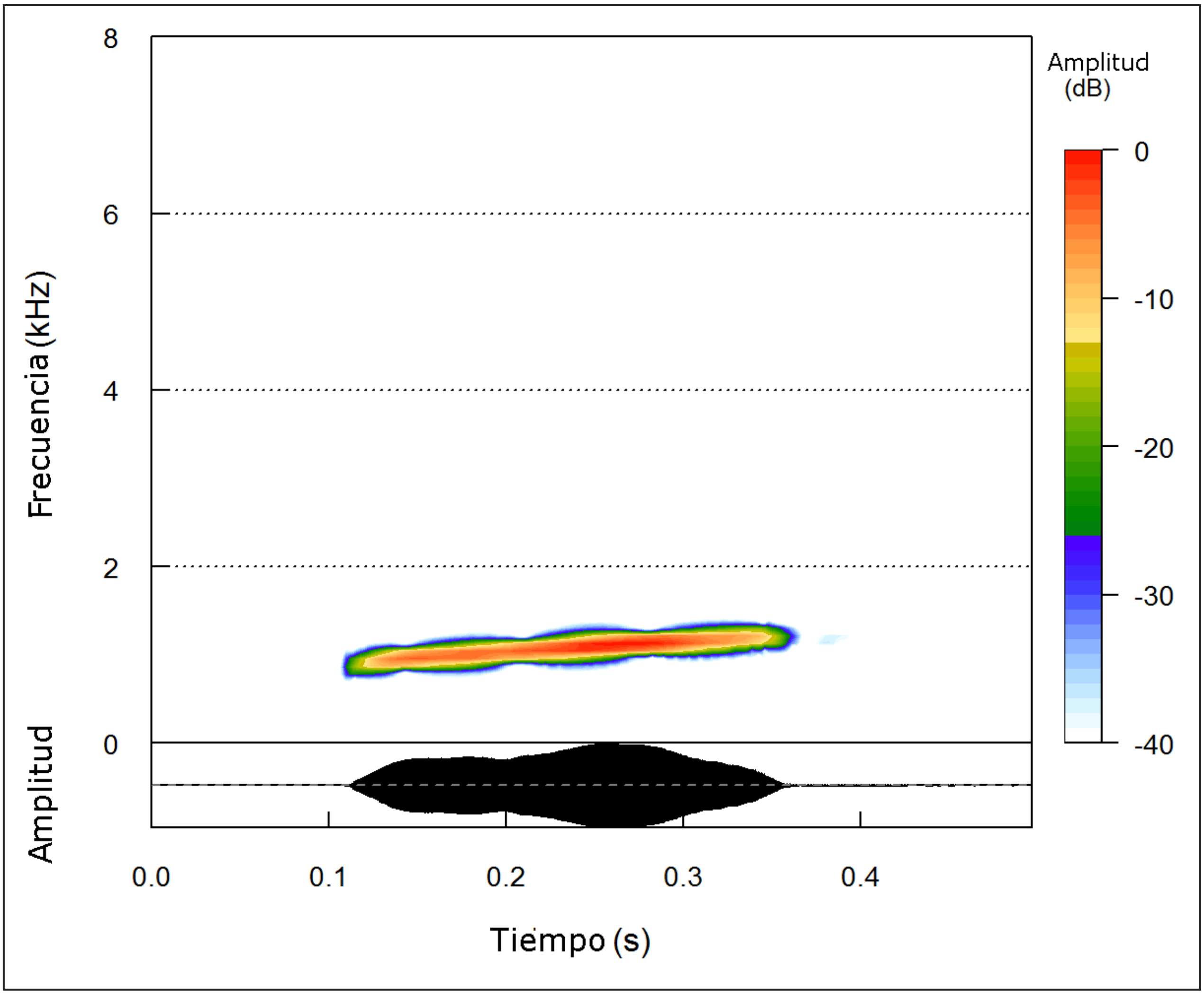

Figura 5. Canto de advertencia de *Leptodactylus laticeps* (UNNEC 13637). FZ UNNE 0840: Miraflores, Chaco; 17,3°C. Espectrograma (arriba) y oscilograma (abajo).

estudiadas aquí presenta características similares a aquellas publicadas por Barrio (1965) y Stănescu *et al.* (2022) para otras poblaciones argentinas. En esta última publicación se describe un patrón en forma de "T" en el oscilograma, y la presencia de algunas bandas armónicas. Las poblaciones de Bolivia estudiadas por Schalk y Leavitt (2017), presentan dos picos de amplitud en el oscilograma y se diferencian además de otras poblaciones por presentar una tasa de canto (38 notas/min) menor y una duración de la nota (500 ms) relativamente mayor. Variaciones en los parámetros temporales en el canto de los anuros pueden ser explicadas por factores ambientales como la temperatura, con cuyo aumento la duración de las notas se acorta y la tasa de repetición aumenta. Sin embargo, a una temperatura más alta, en comparación con otros estudios, se registraron notas de mayor duración y una tasa de repetición más baja. Existen también diferencias en los cantos compuestos; la duración de los grupos de notas es ligeramente mayor (390 ms) y se emiten con una tasa baja (16 notas/min). A su vez, las notas que conforman los grupos son de mayor duración (130 ms) y su pico de frecuencia dominante promedio, más alto (1750Hz) (Schalk y Leavitt, 2017). Ante estas variaciones, es importante mencionar que *L. bufonius* posee una baja estructuración genética (Brusquetti *et al.*, 2019), por lo cual, en este caso, las diferencias acústicas entre diferentes poblaciones pueden ser asignadas a variaciones geográficas, o incluso artefactos de grabación.

En Argentina, el canto de advertencia de *L. elenae* se conoce para poblaciones de Villa Ángela y Resistencia, Chaco (Barrio, 1965; Silva *et al.*, 2020). Los parámetros temporales y espectrales son similares con las poblaciones de Corrientes analizadas en

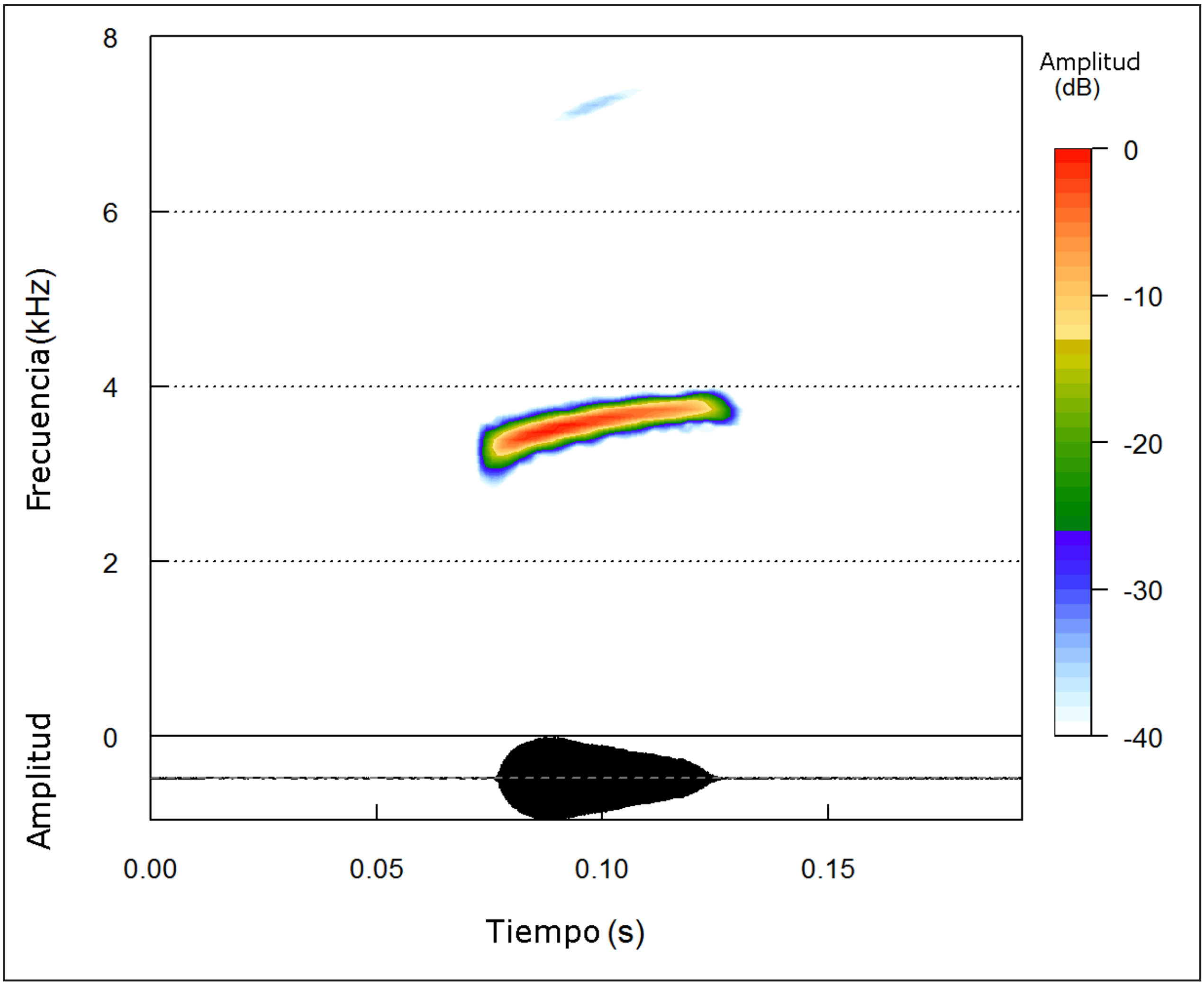

Figura 6. Canto de advertencia de *Leptodactylus latinasus*. FZ UNNE 0751: B°Yecohá, Corrientes Capital; 29°C. Espectrograma (arriba) y oscilograma (abajo).

este trabajo así como también al de otras poblaciones de Bolivia (De la Riva, 1993; Márquez *et al.,* 1995) y Paraguay (Heyer *et al.,* 1996; Heyer y Heyer, 2002).

Leptodactylus fuscus es una especie ampliamente distribuida en Sudamérica y datos moleculares sugieren que constituye un complejo de varias especies (Wynn y Heyer, 2001; Camargo *et al.*, 2006). Sin embargo, aunque acústicamente fueron encontradas algunas variaciones acústicas entre distintas poblaciones, éstas no coinciden con linajes genéticos ni geográficos (Heyer y Reid, 2003). En Argentina, cantos de poblaciones de la provincia de Chaco (descriptas bajo el nombre de *L. sibilator*) fueron analizados por Barrio (1965), y de la provincia de Salta (Embarcación), por Straughan y Heyer (1976). La naturaleza pulsátil en la población de Embarcación (Salta) coincide con la distribución del clado B propuesto por Camargo *et al.* (2006), diferenciándose de otras poblaciones, como las estudiadas aquí (sin pulso ni estructura pulsátil), asignadas al clado C. Diferentes poblaciones asignadas al clado B, particularmente aquellas procedentes de Bolivia, exhiben importantes variaciones acústicas (Márquez *et al.;* 1995; Heyer y Reid, 2003) que dejan en evidencia la necesidad de estudios complementarios que permitan reevaluar y contrastar los datos acústicos a partir de registros estandarizados, que eviten, por ejemplo, variaciones por equipos de grabación utilizados u otros artefactos de grabación.

El canto de las poblaciones de *L. latinasus* analizados en este trabajo es similar al de otras poblaciones (Barrio, 1965; Straughan y Heyer, 1976; Salas *et al.*, 1998), excepto por la tasa de canto. Esta variación ya fue previamente notada por Heyer y Juncá (2003), atribuyéndolos a los contextos de grabación en el que fueron registrados los ejemplares.

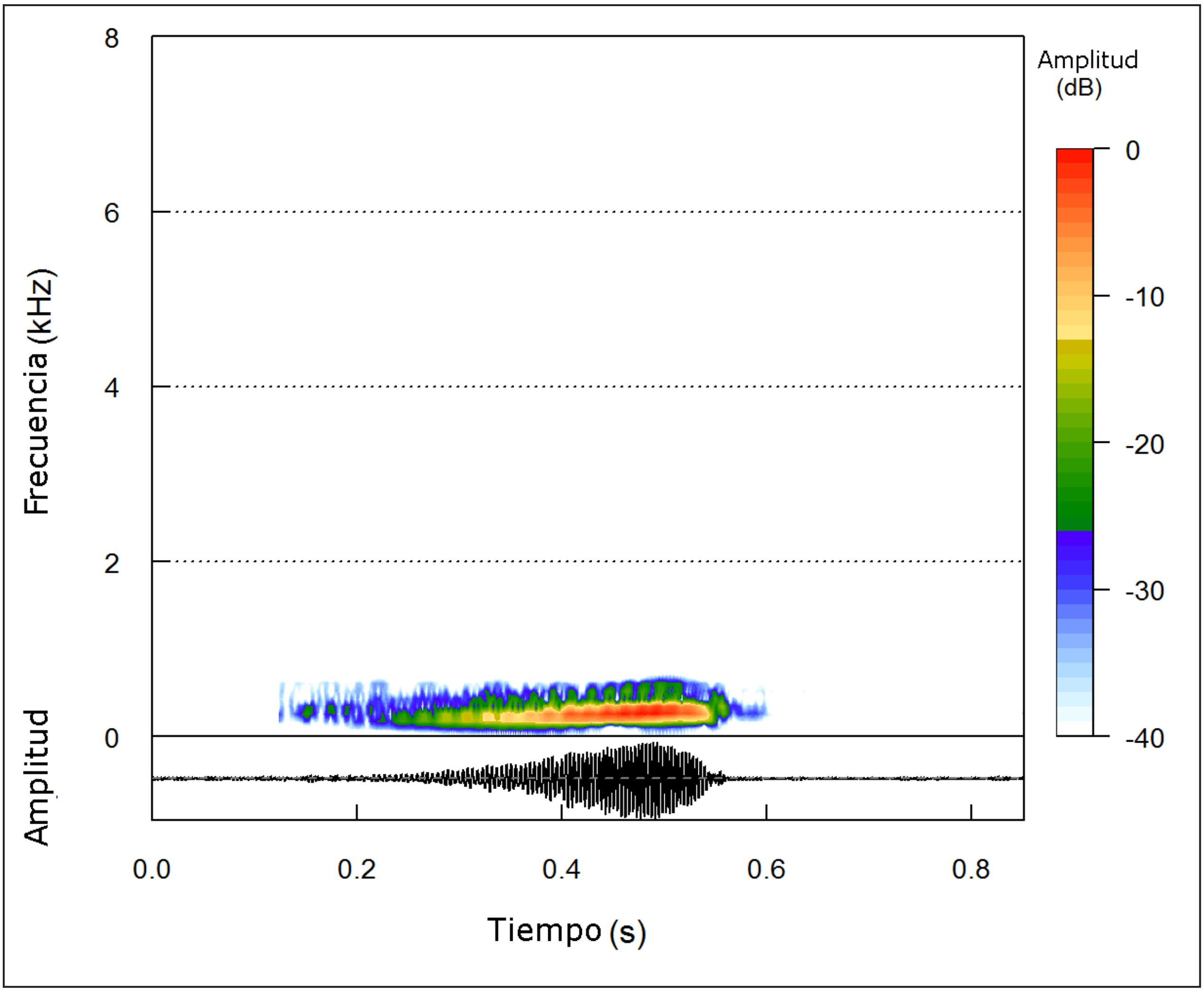

Figura 7. Canto de advertencia de *Leptodactylus luctator*. FZ UNNE 0819: Paso de la Patria (Corrientes);25°C. Espectrograma (arriba) y oscilograma (abajo).

Al igual que en *L. latinasus*, el canto de *L. gracilis* es también similar con otras poblaciones previamente estudiadas (Barrio, 1965; Heyer, 1978; García-Pérez y Heyer, 1993; Salas *et al.*, 1998).

El canto de advertencia de *L. laticeps* registrados en este trabajo es similar al descripto para la población de Filadelfia, Paraguay (Heyer y Scott, 2006). Se ha sugerido que su canto se asemeja al de *L. bufonius*, probablemente como parte de un mecanismo de predación en forma de señuelo auditivo para atraer a las hembras de *L. bufonius* (Heyer y Scott, 2006), sin embargo, esta hipótesis no ha sido probada formalmente. Cuando esta idea fue propuesta *L. laticeps* era agrupado en el grupo fenético de *L. pentadactylus*, pero posteriormente *L. laticeps* fue incluido en el grupo de *L. fuscus* (de Sá *et al.*, 2014), con lo cual la similitud entre los cantos no es algo inesperado.

El grupo de *L. latrans* está representado en Argentina por *L. macrosternum* y *L. luctator*. Barrio (1966) caracterizó los cantos de ambas especies en poblaciones de este país y diferenció al par de especies crípticas, entonces conocidas bajo los nombres de *L. chaquensis* y *L. ocellatus*. Recientemente, el estado taxonómico de ambas especies fue revisado por Magalhães *et al.*(2020), quienes revalidaron *L. macrosternum* y *L. luctator.* Los parámetros acústicos para la nueva población de *L. luctator* analizada en este trabajo son similares a los mencionados por Barrio (1966) y Magalhães *et al.* (2020), excepto por la ausencia de bandas armónicas. Los machos analizados en este estudio se encontraban vocalizando en el centro de nidos ya formados, por lo que futuros estudios deberían contrastar estos cantos con el de machos que no se encuentren asociados a nidos y analizar posibles diferencias de acuerdo al contexto.

Con respecto a *L. macrosternum*, tres tipos de cantos son reconocidos (Magalhães *et al.,* 2020). Los gruñidos largos y trinos ya fueron registrados previamente en poblaciones argentinas por Barrio (1966), por lo que los gruñidos cortos registrados en este estudio resultan novedosos. Descripciones detalladas de estos tipos de vocalizaciones fueron brindadas para poblaciones de Brasil (Heyer y Giaretta, 2009; Camurugi *et al.*, 2017; Magalhães *et al.,* (2020). En general, los parámetros temporales y espectrales son similares, pero una ligera diferencia de 200 Hz en el pico de frecuencia dominante fue encontrada en las poblaciones argentinas (500 vs 700 Hz). Dado el número bajo de vocalizaciones registradas en nuestro trabajo, no pudimos evaluar la frecuencia con la que son emitidas estas vocalizaciones. Sin embargo, se ha informado que los tres tipos de notas son emitidos con una tasa altamente variable, en forma de notas simples, en secuencias de una misma nota (principalmente gruñidos largos o trinos) o en combinaciones de más de un tipo de nota; y como los gruñidos largos o "growls" son los más frecuentes en contextos reproductivos, estos representarían al canto de advertencia (Camurugi *et al.,* 2017). Estudios complementarios con datos detallados del contexto permitirán una mejor comprensión de las funciones de los diferentes tipos de notas registradas en esta especie.

Leptodactylus podicipinus es el único representante del grupo de *L. melanonotus* en Argentina, y los pocos datos acústicos para poblaciones locales fueron aportados por Barrio (1965). Otras poblaciones fueron caracterizadas acústicamente (Heyer, 1994; Márquez *et al.,* 1995; Guimarães *et al.*, 2001; Silva *et al.*, 2008), sin embargo, la identidad taxonómica de algunas de ellas debe ser revisada dado que varias

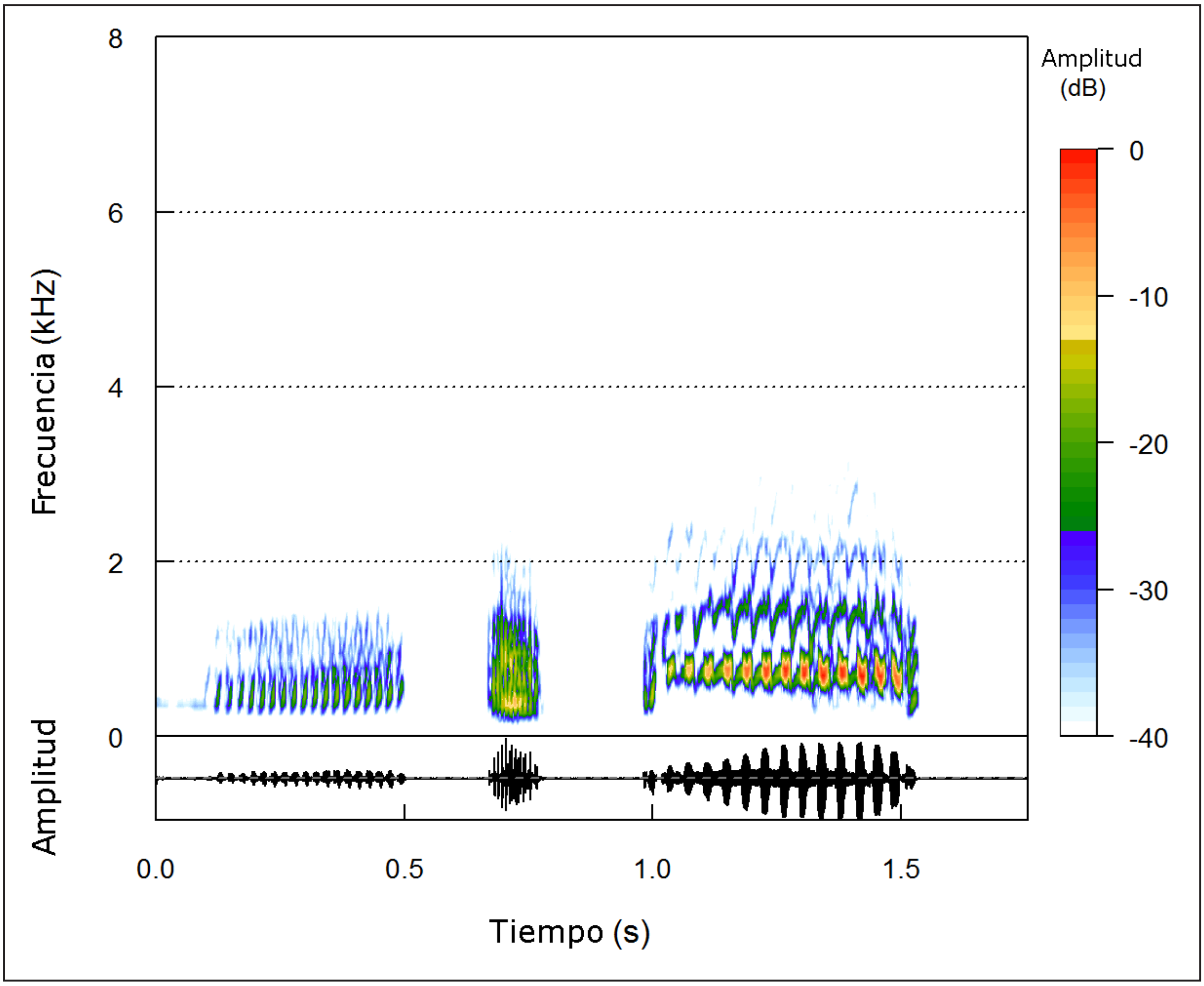

Figura 8. Vocalizaciones de *Leptodactylus macrosternum*. Dos gruñidos cortos y un trino. FZ UNNE 0680: Miraflores (Chaco); 29,7°C. Espectrograma (arriba) y oscilograma (abajo).

especies fueron recientemente descriptas o revalidadas (Gazoni *et al.*, 2021; Carvalho *et al.*, 2022). Las características de las poblaciones analizadas en este trabajo coincide con el patrón "gota de agua ("*water drop*") propuesto por Carvalho *et al.* (2022). En cuanto a la parte espectral, la mayoría de los estudios coinciden con la presencia de una frecuencia con modulación ascendente pronunciada. Adicionalmente, se menciona una estructura armónica compleja en la que durante los primeros 20 ms del canto, la frecuencia está enfatizada a los 1156 Hz, y en la parte final a los 2335 Hz. En la mayoría de los casos conocidos, la frecuencia dominante se ubica en la parte final del canto, sin embargo, en ocasiones, puede coincidir con la frecuencia más baja de la parte inicial, e incluso ubicarse en la parte inicial (Carvalho *et al.*, 2022, este estudio). Estos picos de intensidad en diferentes partes del canto explican, sin dudas, parte de las variaciones encontradas entre diferentes estudios y pueden ser consecuencia de diferentes equipos utilizados en el registro de las vocalizaciones u otros factores como la distancia a la que se grabó el ejemplar. El control de las condiciones de grabación, así como la estandarización de los equipos de grabación deben ser considerados especialmente en estos casos. Estas variaciones pueden ser explicadas también por una característica de los miembros del grupo de *L. melanonotus*: su amplio repertorio vocal, en comparación con otros *Leptodactylus* (Carvalho *et al.*, 2022). En consecuencia, el contexto de las grabaciones es elemental para reconocer posteriormente las funciones de diferentes tipos de cantos o patrones, como podría ser el caso de la emisión de notas de manera aislada y en pares registrado en un individuo analizado en este trabajo.

Luego de contrastar algunas variaciones entre poblaciones, es importante considerar que los estudios acústicos en especies de *Leptodactylus* comenzaron en la década de 1960, por lo que en varios de ellos se incluyeron términos que hoy resultan obsoletos, existen artefactos de registro o errores conceptuales, especialmente sobre los pulsos y los armónicos. Una estructura pulsada en estudios previos fue mencionada (ambiguamente) para cantos de *L. bufonius*, *L. fuscus*, *L. gracilis* y *L. podicipinus* (Barrio, 1965; Straughan y Heyer, 1976; Heyer, 1978; Salas *et al.*, 1998), definida sobre la base de modulaciones irregulares y débiles de amplitud, sin intervalos de silencio. De acuerdo a Köhler *et al.* (2017), un canto con ligeras modulaciones debe ser clasificado como pulsátil, en contraste con aquellos cantos con fuertes modulaciones de amplitud, e intervalos de silencio, donde los pulsos están bien definidos. En *Leptodactylus*, pulsos completos pueden observarse

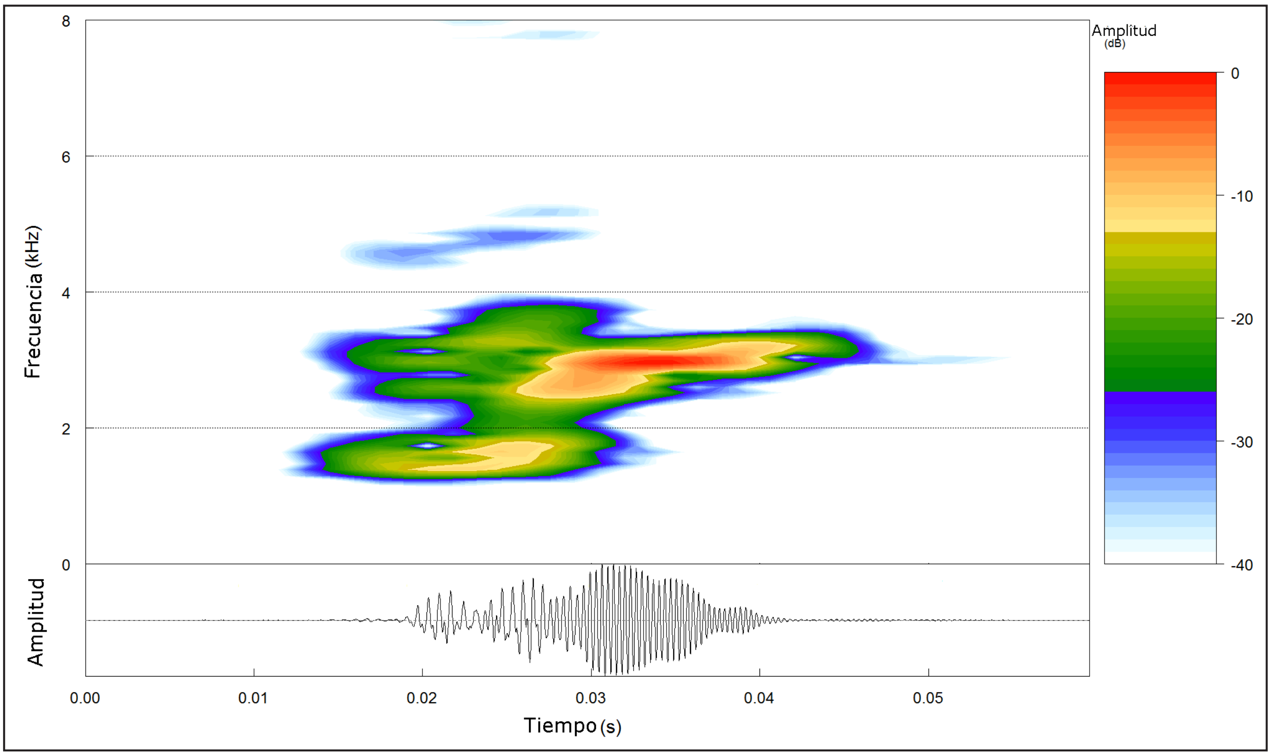

Figura 9. Canto de advertencia de *Leptodactylus podicipinus*. FZ UNNE 0758: B° Yecohá, Corrientes Capital; 28°C. Espectrograma (arriba) y oscilograma (abajo).

solamente en algunos representantes del grupo de *L. melanonotus* como *L. riveroi* (Carvalho *et al.*, 2022) o del grupo de *L. latrans* como *L. macrosternum* (Magalhães *et al.*, 2020, este estudio).

En *L. fuscus* y en *L. podicipinus*, además de la banda dominante, se ha mencionado una banda de frecuencia fundamental, muy baja (entre los 85–500 Hz en *L. fuscus* y a los 138,5 Hz en *L. podicipinus*) (Barrio, 1965; Márquez *et al.,* 1995). Dado que la frecuencia fundamental es la misma que la dominante en todas las especies de *Leptodactylus* (Carvalho *et al.*, 2002), frecuencias más bajas no deben representar armónicos, y estas bandas más bajas que la dominante, posiblemente representen un artefacto de grabación.

En otro sentido, las vocalizaciones de anuros constituyen un componente central en estudios de taxonomía integrativa, y son fundamentales para la identificación de especies crípticas. En este argumento, la caracterización del canto de poblaciones topotípicas puede servir de referencia para futuros estudios taxonómicos, o bien de variabilidad geográfica. Para el caso de *Leptodactylus*, de las especies con localidad tipo en Argentina se conocen las vocalizaciones de ejemplares topotipos para *L. mystacinus* y *L. apepyta* (Schneider *et al.,* 2019), restando aún datos para *L. luctator*, *L. elenae* y *L. laticeps*. Adicionalmente, de las especies presentes en Argentina, se desconocen también cantos topotipos de *L. bufonius*, *L. gracilis*, *L. latinasus*, *L. macrosternum* y *L. podicipinus*.

La comparación de las variables acústicas de los cantos de advertencia entre diferentes poblaciones, así como el registro de otros tipos de cantos contribuyen sustancialmente a comprender de qué manera las variables acústicas pueden variar o no en función de los factores ambientales o geográficos y a profundizar el conocimiento sobre aspectos de la historia natural y del comportamiento de las especies. En Argentina, los estudios de variación acústica en anfibios anuros pueden considerarse incipientes y por lo tanto, existe todavía un importante vacío de información. Para muchas especies locales sus vocalizaciones aún se desconocen, y de aquellas conocidas, en la mayoría de los casos, están focalizados en los cantos de advertencias (los más frecuentemente oídos), y en contextos taxonómicos. Otros tipos de vocalizaciones con funciones diferentes al canto de advertencia son emitidos con menor frecuencia, y por lo tanto, son menos registrados y conocidos, excepto que los estudios estén focalizados en alguna especie en particular y en la diversidad de sus vocalizaciones. La falta de información en este sentido contrasta con el aporte significativo que las vocalizaciones pueden brindar para comprender distintos aspectos relacionados con la historia natural o el comportamiento de las especies.

Finalmente, este trabajo pretende revalorizar los estudios acústicos en anfibios anuros de Argentina y promover un mayor desarrollo de esta disciplina en el país. A partir de la identificación de algunos vacíos de información se busca, además, fomentar acciones tendientes a registrar acústicamente la mayor cantidad posible de poblaciones de anuros, teniendo en cuenta que muchos ambientes son alterados con el consiguiente desplazamiento o pérdida de la anuro fauna local, y con ello, parte de la diversidad acústica.

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Apéndice 1. Registros analizados y hospedados en la Fonoteca Zoológica de la Universidad Nacional del Nordeste (FZ UNNE). Ejemplares de referencia: UNNEC.

Leptodactylus bufonius. FZ-UNNE 0106 (UNNEC 13925): Felipe Yofre (Corrientes): 29°06'54"S, 58°20'21"O; 24°C; 21/feb/2012 (20:40); FZ-UNNE 0108 (UNNEC 13926): Felipe Yofre (Corrientes): 29°06'54"S, 58°20'21"O; 23,3°C; 21/feb/2012 (21:22); FZ-UNNE 0674: Campo Doña Irma, Miraflores (Chaco): 25°34'512"S, 61° 1'15"O; 28,6°C; 07/feb/2021 (22:15); FZ-UNNE 0962-0965: Los Rincónes (San Juan): 30°11'52"S, 67°44'59"O; 21,9-23,2°C; ene/2007; FZ-UNNE 0586: Ea. Iberá, Mercedes (Corrientes): 28°43'02"S, 57°25'40"O; 21,6°C; 10/oct/2018 (00:25).

Leptodactylus elenae. FZ-UNNE 0097 (UNNEC 13927), 0098: Campus UNNE, Corrientes Capital (Corrientes): 27°28'05"S, 58°46'57"O; 23,6°C; 22/nov/2011 (19:40-19:58); FZ-UNNE 0749: B° Yecohá, Corrientes Capital (Corrientes): 27°25'44"S, 58°42'33"O; 29,5°C; 27/ene/2021 (22:01).

Leptodactylus fuscus. FZ-UNNE 0612, 0616 (UNNEC 13928) y 0622 (UNNEC 13929): acceso a Garruchos, Santo Tomé (Corrientes): 28°08'44"S, 55°41'41"O; 23,1-24,9°C; 11/oct/2018 (19:57-21:06); FZ-UNNE 0635 (UNNEC 13930) y 0646 (UNNEC 13931): Ea. San Antonio P, Alvear (Corrientes): 28°56'02"S, 56°24'18"O; 18,3°C; 13/feb/2019 (21:30-22:36); FZ-UNNE 0637, 0642,0649, 0652 (UNNEC 13932): Ea. San Antonio P, Alvear (Corrientes): 28°56'02"S, 56°24'18"O; 24,4-26-4°C; 14/feb/2019 (20:23-21:43).

Leptodactylus gracilis. FZ-UNNE 0036: Ea. El Socorro, Mercedes (Corrientes): 28°39'53"S, 57°21'39"O; 23,1°C; 05/mar/2009 (21:30); FZ-UNNE 0157: PN Mburucuyá, Mburucuyá (Corrientes): 28°00'46"S, 58°01'43"O; 20,5°C; 12/oct/2012 (19:11).

Leptodactylus laticeps. FZ-UNNE 1214: Fuerte Esperanza (Chaco): 25°04'13"S, 61°36'53"O; 26,7°C; 01/feb/2014 (00:33); FZ-UNNE 0838 (UNNEC 13637): Campo Doña Irma, Miraflores (Chaco): 25°34'43"S, 61°00'50"O; 17,3°C; 20/nov/2021 (23:55).

Leptodactylus latinasus. FZ-UNNE 0743, 0746 y 0751: B° Yecohá, Corrientes Capital (Corrientes): 27°25'44"S, 58°42'33"O; 29-30°C; 27/01/2021 (21:21-22:18); FZ-UNNE 0662(UNNEC 13933): Campo Doña Irma, Miraflores (Chaco): 25°34'42"S, 60°59'40"O; 30,5°C; 06/feb/2020 (23:00); FZ-UNNE 0604: Ea. El Socorro, Mercedes (Corrientes): 28°41'21"S, 57°26'02"O; 16,5°C; 10/oct/2018 (21:08).

Leptodactylus luctator. FZ-UNNE 0806, 0807, 0816, 0868, 0872: Ea. San Pedro, Paso de la Patria (Corrientes): 27°18'47"S, 58°31'13"O; 25°C; 15/nov/2021 (22:50-23:10).

Leptodactylus macrosternum. FZ-UNNE 0679, 0680: Campo Doña Irma, Miraflores (Chaco): 25°34'512"S, 61° 1'15"O; 28°C; 7/feb/2021 (22:42).

Leptodactylus podicipinus. FZ-UNNE 0053 (UNNEC 13934): Ea. El Socorro, Mercedes (Corrientes): 28°39'56"S, 57°21'41"O; 25,5°C; 02/mar/2010 (21:05); FZ-UNNE 0094: Campus UNNE, Corrientes Capital (Corrientes): 27°28'05"S, 58°46'57"O; 27,7°C; 22/nov/2011 (18:20); FZ-UNNE 0756 y 0758: B° Yecohá, Corrientes Capital, (Corrientes): 27°25'44"S, 58°42'33"O; 28°C; 27/ene/2021 (22:40-22:42).

Trabajo

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Ecologia térmica de anuros da Caatinga, Nordeste do Brasil

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ABSTRACT
Thermal ecology of anurans in the Caatinga, Northeastern Brazil. Anurans regulate their body temperature through external heat sources. In this sense, the microhabitat used can influence this physiological process. Thus, the objective of this study was to verify the body temperature of anuran species in the Caatinga domain and to correlate the possible variations to the different microhabitats used. For this, active searches were carried out during the day and night shifts, between August 2019 and May 2021. The frogs were captured manually to measure the cloacal temperature and then released. Substrate and air temperatures were also checked for associations with body temperature. In total, 420 frogs were captured, and the body temperature was obtained from 147 individuals of five species in the rainy season (*Leptodactylus macrosternum*, *Scinax x-signatus*, *Rhinella granulosa*, *Rhinella diptycha* and *Pithecopus gonzagai*), and from 273 individuals of four species in the dry season (*L. macrosternum*, *S. x-signatus*, *R. granulosa* and *R. diptycha*). In general, the highest body temperature averages (28.4-29.3ºC) were observed in the rainy season for *L. macrosternum*, *R. granulosa* and *R. diptycha*. The most preferred microhabitats of anurans were soil and water. Finally, even though an association between body temperature and air temperature was found, substrate temperature proved to be the most important source of thermal regulation for most anuran species investigated.

Key words: Bufonidae; Hylidae; Leptodactylidae; Semiarid region; Body temperature.

RESUMO
Os anuros regulam sua temperatura corpórea por meio de fontes externas de calor. Nesse sentido, o micro-hábitat utilizado pode influenciar nesse processo fisiológico. Desta forma, o objetivo deste estudo foi verificar a temperatura corpórea de espécies de anuros no domínio Caatinga e correlacionar as possíveis variações aos diferentes micro-hábitats utilizados. Para isso foram realizadas buscas ativas nos turnos diurno e noturno, entre agosto de 2019 e maio de 2021. Os anuros foram capturados manualmente para aferição da temperatura cloacal e soltos em seguida. As temperaturas do substrato e do ar também foram verificadas para associações com a temperatura corpórea. No total, 420 anuros foram capturados, sendo obtida a temperatura corpórea de 147 indivíduos de cinco espécies no período chuvoso (*Leptodactylus macrosternum*, *Scinax x-signatus*, *Rhinella granulosa*, *Rhinella diptycha* e *Pithecopus gonzagai*), e de 273 indivíduos de quatro espécies no período seco (*L. macrosternum*, *S. x-signatus*, *R. granulosa* e *R. diptycha*). De um modo geral, as maiores médias de temperatura corpórea (28,4–29,3ºC) foram observadas no período chuvoso para *L. macrosternum*, *R. granulosa* e *R. diptycha*. Os micro-hábitats de maior preferência dos anuros foram o solo e a água. Finalmente, ainda que tenha sido encontrada associação da temperatura corpórea com a temperatura do ar, a temperatura do substrato demonstrou ser a fonte mais importante na regulação térmica para a maioria das espécies de anuros investigadas.

Palavras-chaves: Bufonidae; Hylidae; Leptodactylidae; Região semiárida; Temperatura corpórea.

Introdução

Os anuros são animais ectotérmicos, por serem incapazes de regular de forma endógena a temperatura corpórea, dependendo assim de fontes externas para ajustá-la (Duellman e Trueb, 1986). Ou seja, esses animais controlam a temperatura corpórea por meio de ajustes comportamentais (permanência em abrigos, exposição ao sol e seleção de temperaturas), fisiológicos (aclimatação e resfriamento por

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evaporação) e da relação térmica com o ambiente (Brattstrom, 1963). Esse último caso, particularmente, envolve perda e ganho de calor por convecção e condução, radiação térmica do ambiente e perda de calor por evaporação que auxiliam na termorregulação (Brattstrom, 1963; Bovo, 2015).

A termorregulação em anfíbios é um processo complexo, visto a necessidade de manutenção da umidade na pele para garantir as trocas gasosas, podendo ser comprometida pela economia de água (Brattstrom, 1979). Mesmo considerando a possibilidade de termorregulação comportamental, ao buscar diferentes ambientes térmicos, os anfíbios são muito específicos nas exigências de seu habitat, principalmente devido ao controle do balanço hídrico, que pode se sobrepor aos esforços para encontrar uma temperatura ótima (Oromí *et al.*, 2010). Nesse sentido, a maioria dos anfíbios apresenta tendência termoconformista, ou seja, a temperatura corpórea está intimamente relacionada às temperaturas do ar e do substrato (Lambrinos e Kleier, 2003). Apesar desses conhecimentos, informações que visam compreender as relações térmicas que se estabelecem na termorregulação de anuros são escassos, sobretudo em regiões de clima semiárido (Sanabria *et al.*, 2003; Navas *et al.*, 2008; Sanabria e Quiroga, 2019).

A Caatinga ocupa uma área de cerca de 912.529 Km^2, o equivalente a 11% do território nacional (Silva *et al.*, 2017). A região apresenta clima semiárido, com altas temperaturas, baixa umidade relativa, evapotranspiração elevada e índices pluviométricos irregulares concentrados em um período curto do ano (Ab'saber, 1974). Embora este domínio morfoclimático seja caracterizado por condições inóspitas e por um ciclo anual de chuva bastante imprevisível (Souzareis, 1976), abriga alta riqueza de espécies de anfíbios anuros, sendo atualmente representada por 98 espécies (Garda *et al.*, 2017).

Para as áreas de Caatinga *stricto sensu*, caracterizadas pela vegetação típica do semiárido brasileiro, adaptadas à forte sazonalidade das chuvas e às altas temperatura (Souzares, 1976; Ab'saber, 2003), os estudos da herpetofauna receberam mais atenção dos pesquisadores na última década (Garda *et al.*, 2013; Cavalcanti *et al.*, 2014; Pedrosa *et al.*, 2014; Magalhães *et al.*, 2015; MUNIZ *et al.*, 2016; Garda *et al.*, 2017). Contudo, essas localidades ainda estão entre as regiões mais pobremente estudadas no Brasil, principalmente em relação a anurofauna (Garda *et al.*, 2017). Por essa razão, existem várias lacunas sobre ecologia e biologia desse grupo, principalmente com relação a adaptação desses animais sob condições de temperatura elevada e baixa umidade relativa (Silva *et al.*, 2017).

Nesta perspectiva, o presente estudo amplia o entendimento da biologia e do comportamento termorregulatório em espécies habitantes do semiárido brasileiro. Objetivou-se assim, identificar a temperatura corpórea média de atividade em espécies de anuros da Caatinga. Além disso, verificou-se a relação da temperatura corpórea dos anuros com as temperaturas do substrato e do ar em diferentes intervalos do período de atividade.

Material e Métodos

O estudo foi realizado em 10 áreas que estão sob influência do Projeto de Integração do Rio São Francisco com as Bacias Hidrográficas do Nordeste Setentrional (PISF), todas localizadas no estado de Pernambuco (Fig. 1). A coleta de dados ocorreu entre agosto de 2019 e maio de 2021, compreendendo os períodos sazonais seco (entre junho e dezembro) e chuvoso (entre janeiro e maio). No período seco, as amostragens foram realizadas em duas áreas de monitoramento no Eixo Leste (PML; 217 km de extensão) e duas no Eixo Norte (PMN; 260 km de extensão) do PISF denominadas: PML02 (Floresta), PML08 (Custódia), PMN03 (Cabrobó) e PMN06 (Salgueiro). No período chuvoso os dados foram coletados em seis áreas, quais sejam: PML08 (Custódia), PML09 (Custódia), PML10 (Floresta), PMN06 (Salgueiro), PMN08 (Salgueiro) e PMR02 (Monitoramento Ramal do Agreste: Sertânia). As áreas do PISF abrangem as Depressões Sertanejas Meridional e Setentrional. Estas apresentam paisagem típica da Caatinga: com planície baixa, relevo predominante suave-ondulado, com elevações residuais, clima semiárido, e vegetação que varia de arbustiva a arbórea (Velloso *et al.*, 2002). Além das áreas do PISF, nós também coletamos dados térmicos dos anuros no *campus* de Ciências Agrárias (CCA) da Universidade Federal do Vale do São Francisco (UNIVASF) no município de Petrolina (Fig. 1). O campus encontra-se geologicamente no Submédio São Francisco, na ecorregião da Depressão Sertaneja Meridional. Apresenta clima semiárido e vegetação do tipo Caatinga hiperxerófila, caracterizada pela abundância de cactáceas, árvores de pequeno porte, arbustos que perdem as folhas no período seco, e o solo é predominantemente arenoso

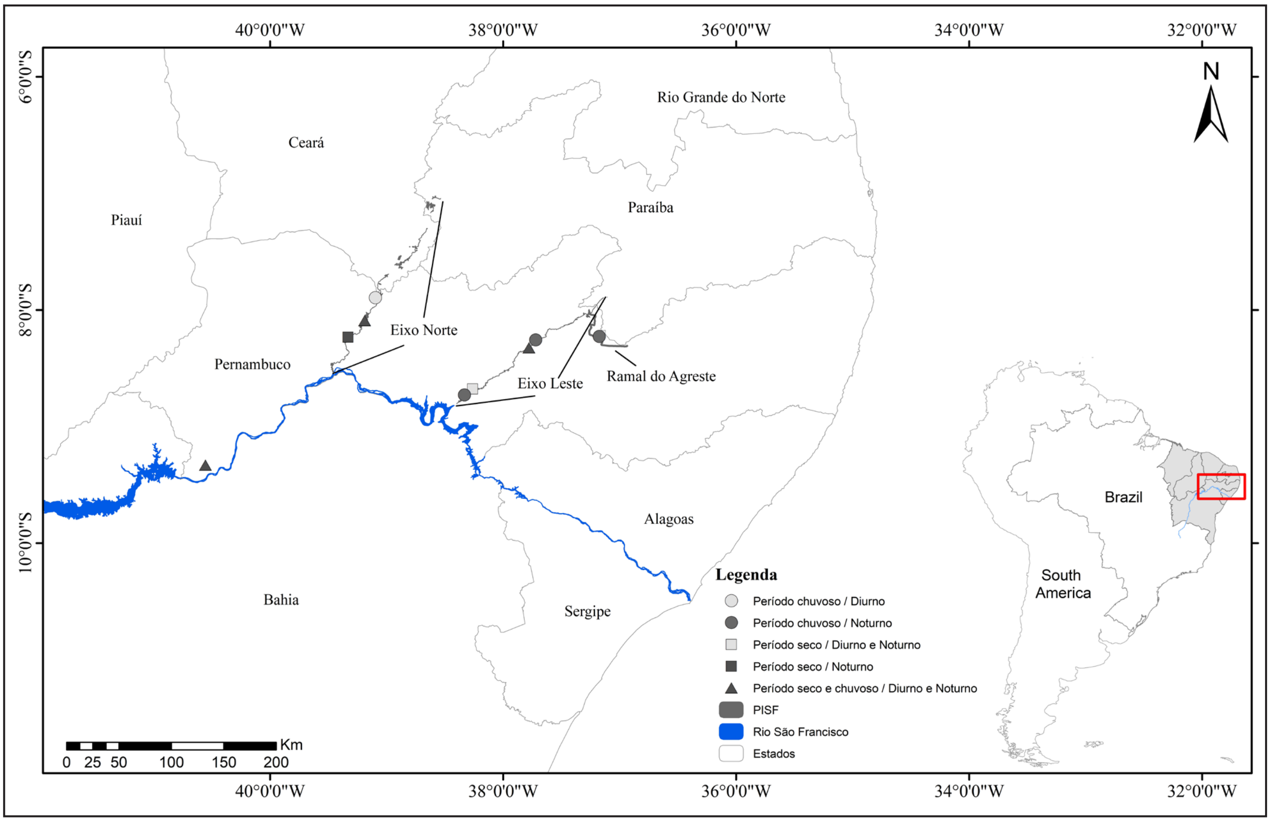

Figura 1. Mapa do Brasil, representando o estado de Pernambuco e a localização das áreas de estudo. Fonte: Leandro Oliveira, 2023.

(Oliveira *et al.*, 2009).

A busca ativa por anuros foi realizada em transectos durante três dias consecutivos em cada área de amostragem, nos períodos diurno (08:00 às 12:00 horas) e noturno (18:00 às 22:00 horas). As capturas manuais foram realizadas nos corpos d'água (reservatórios, riachos e poças temporárias) utilizados pelos anuros. Assim, foram coletados dados térmicos de cinco espécies de anuros que habitam a Caatinga: *Rhinella diptycha*, *R. granulosa*, *Leptodactylus macrosternum*, *Scinax x-signatus* e *Pithecopus gonzagai*. Essas espécies foram escolhidas por serem abundantes e facilmente encontradas na área de estudo (Rodrigues, 2003; Garda *et al.*, 2013), possibilitando a coleta de um grande volume de dados.

Para obtenção dos dados, a temperatura corpórea (cloacal) dos anuros foi aferida com o auxílio de um sensor de temperatura (Instrutherm® modelo S-02K) acoplado a um termo-higrômetro digital portátil (precisão de 0,1°C; Instrutherm® modelo HT-300). Somente foram consideradas as temperaturas cloacais obtidas em até 30 segundos de investida no procedimento de captura do anuro. Feita a captura, imediatamente foram aferidas as temperaturas cloacal, do substrato e do ar. Para a temperatura do ar foi considerada uma distância de 3 cm do substrato (Sanabria *et al.*, 2003). Nesse momento também foi registrada a presença do anuro nos turnos diurno ou noturno e o micro-habitat utilizado. Após os procedimentos os animais foram soltos nos mesmos locais de encontro. Ressalta-se que cada local foi visitado apenas uma vez para evitar pseudorréplicas dos dados obtidos para cada espécie.

A temperatura corpórea de cada espécie foi determinada pela média das temperaturas corpóreas registradas para todos os indivíduos capturados. O efeito das temperaturas do substrato e do ar sobre as temperaturas corpóreas foi analisado por meio de uma regressão múltipla no programa PAST versão 2.17 (Hammer *et al.*, 2001).

Para verificar se houve diferença nas médias de temperatura corpórea dos anuros entre os períodos seco e chuvoso, assim como entre os turnos noturno e diurno foi utilizado o teste t de Student. Para testar a diferença no uso de micro-hábitats entre os períodos chuvoso e seco foi utilizado o teste do Qui-quadrado (χ^2). Nas análises estatísticas, o nível de significância adotado foi de 5% e os valores estatísticos foram expressos como média e desvio padrão. A normalidade dos dados foi verificada com o teste de Shapiro-Wilk.

Aspectos éticos e legais
O presente estudo foi desenvolvido sob autorizações do IBAMA (nº 94/2014 e 95/2014, com renovações) e aprovação da Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal do Vale do São Francisco – UNIVASF (nº 0006/270619).

Resultados

De um total de 420 anuros foi aferida a temperatura de 147 indivíduos no período chuvoso e 273 no período seco. *Leptodactylus macrosternum* foi a espécie com maior número de indivíduos (n = 197), seguido de *S. x-signatus* (n = 68), *R. granulosa* (n = 71), *R. diptycha* (n = 65) e *Pithecopus gonzagai* (n = 15).

Para todas as espécies de anuros, a temperatura corpórea média geral (24,2–29,3ºC), assim como as temperaturas médias nos períodos seco (24,2–25,8ºC) e chuvoso (25,6–29,3ºC), e nos turnos diurno (23,3–34,2ºC) e noturno (24,0–29,5ºC) encontram-se descritas na Tabela 1.

As temperaturas corpóreas, no geral, apresentaram diferenças significativas entre os períodos seco e chuvoso para as espécies *L. macrosternum* (t = 1,6; p <0,05; n = 197), *R. diptycha* (t = 1,6; p <0,05; n = 65) e *R. granulosa* (t = 1,6; p <0,05; n = 75), sendo mais altas no período chuvoso. Por outro lado, a temperatura de *S. x-signatus* não apresentou diferença significativa entre os períodos seco e chuvoso (t = 1,6; p = 0,42; n = 68).

Durante o estudo apenas *R. granulosa*, *R. diptycha* e *L. macrosternum* foram registrados no período diurno. Esses anuros foram encontrados, em sua maioria, em fendas no solo do leito seco de poças temporárias. Independente da consideração de períodos seco e chuvoso, *Rhinella granulosa* (t = 1,6; p = 0,01; n = 75) e *L. macrosternum* (t = 1,7; p= 0,01; n = 197) apresentaram diferença significativa nas temperaturas corpóreas entre os turnos diurno e noturno, sendo as maiores temperaturas verificadas no período diurno. Enquanto *R. diptycha* não apresentou diferença significativa (t = 2,3, p = 0,49, n = 65).

Com relação à temperatura dos micro-hábitats, no período seco as médias variaram de 24,3°C a 26,5°C para o substrato e de 23,7° a 26,2°C para o ar (Fig. 2). No período chuvoso, as médias de temperatura do substrato oscilaram entre 25,4°C e 28,4°C e as do ar de 25,4°C a 28,3°C (Fig. 3). Os valores de temperatura não diferiram entre os componentes substrato e ar para as espécies *R. granulosa*, *R. diptycha*, *P. gonzagai* e *S. x-signatus*.

Tanto no período seco quanto no chuvoso, as temperaturas corpóreas dos anuros (exceto *R. granulosa*) foram mais associadas às temperaturas do substrato (maior coeficiente r^2) (Tabela 2). Nos dois casos em que as temperaturas corpóreas dos anuros também foram associadas às temperaturas do ar (*R. diptycha* no período seco e *S. x-signatus* no período chuvoso) as temperaturas do substrato ainda tenderam a exercer maior efeito sob as temperaturas corpóreas dos anuros (Tabela 2). *Pithecopus gonzagai*, com coleta de dados térmicos exclusivamente no período chuvoso, não apresentou associações

Tabela 1. Temperatura corpórea (Tc) geral e nos turnos diurno e noturno para os anuros capturados em áreas da Caatinga, nos períodos seco (s) e chuvoso (c), entre agosto de 2019 e maio de 2021. Os dados são apresentados na forma média ± desvio padrão. O traço (—) representa ausência de registro de temperatura. Letras minúsculas e maiúsculas na Tc geral indicam diferença estatística significativa entre períodos seco e chuvoso em cada espécie. Não houve análise comparativa entre Tc diurno e Tc noturno, separadamente nos períodos seco e chuvoso, devido ao baixo tamanho amostral do turno diurno.

Espécie	Período	Tc geral (°C)	Tc diurno (°C)	Tc noturno (°C)
Leptodactylus macrosternum	s	24,2 ± 2,2 (n=153) a	27,0 ± 2,6 (n=11)	24,0 ± 2,0 (n=142)
	c	29,3 ± 1,7 (n=44) A	34,2 (n=1)	29,2 ± 1,5 (n=43)
Rhinella granulosa	s	25,7 ± 2,3 (n=47) a	27,3 ± 0,7 (n=10)	25,3 ± 2,4 (n=37)
	c	28,4 ± 1,7 (n=28) A	27,5 ± 1,3 (n=15)	29,5 ± 1,7 (n=13)
Rhinella diptycha	s	25,0 ± 1,8 (n=30) a	23,3 (n=1)	25,1 ± 1,9 (n=29)
	c	28,4 ± 2,4 (n=35) A	28,0 ± 4,1 (n=3)	28,5 ± 2,3 (n=32)
Scinax x-signatus	s	25,8 ± 2,6 (n=43) a	—	25,8 ± 2,6 (n=43)*
	c	25,6 ± 2,3 (n=25) a	—	25,6 ± 2,3 (n=25)*
Pithecopus gonzagai	s	—	—	—
	c	28,2 ± 2,6 (n=15)	—	28,2 ± 2,6 (n=15)*

significativas das temperaturas corpóreas com as temperaturas ambientais.

Ainda com relação ao substrato utilizado pelos anuros, durante o período seco as espécies *L. macrosternum*, *R. granulosa* e *S. x-signatus* foram mais frequentemente encontrados no solo (Figs. 4A, 4C e 4D). Já *R. diptycha* foi capturado com maior frequência dentro d'água (Fig. 4B). Enquanto no período chuvoso *L. macrosternum* continuou com maior frequência no solo (Fig. 4A), assim como *R. diptycha* (Fig. 4B). Diferente do observado no período seco, *R. granulosa* teve preferência por estar dentro d'água (Fig. 4C), *S. x-signatus* por superfícies rochosas (Fig. 4D) e *P. gonzagai* por estratos arbustivos (Fig. 4E).

Apenas *L. macrosternum* não apresentou diferença no uso de micro-hábitats entre os períodos seco e chuvoso (χ^2 = 4,14; p = 0,52). Já *R. diptycha* (χ^2 = 12,38; p = 0,01), *R. granulosa* (χ^2 = 23,92; p <0,05) e *S. x-signatus* (χ^2 = 22,79; p = 0,001) apresentaram diferença no uso dos micro-hábitats entre os períodos sazonais seco e chuvoso.

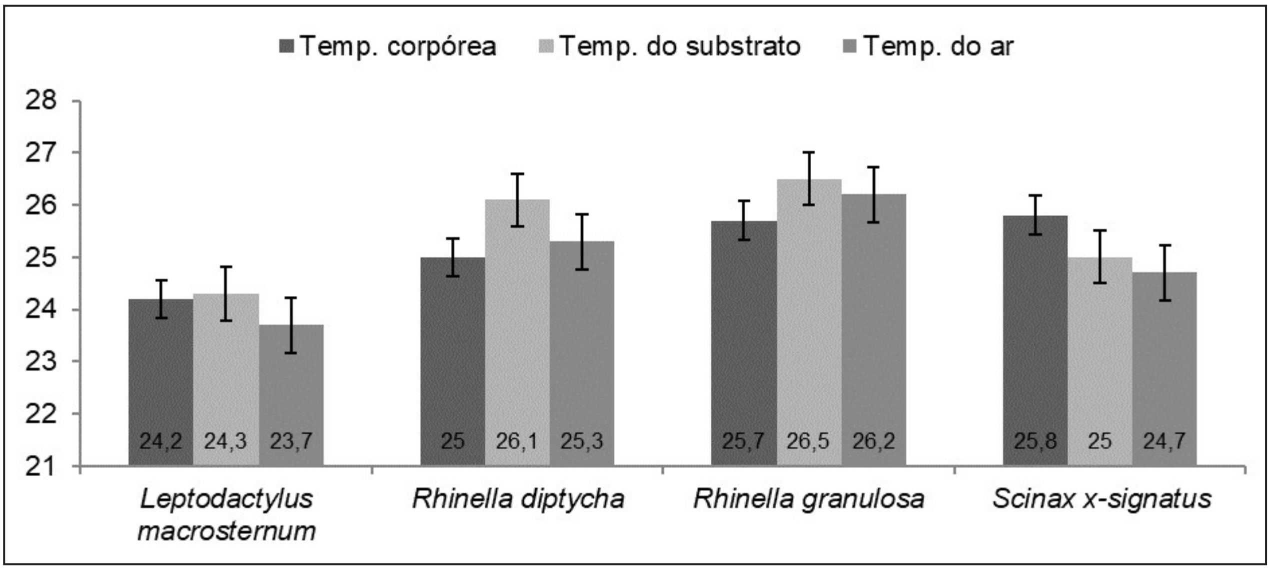

Figura 2. Temperatura média (°C) do corpo, do substrato e do ar nos micro-hábitats utilizados pelos anuros no período seco.

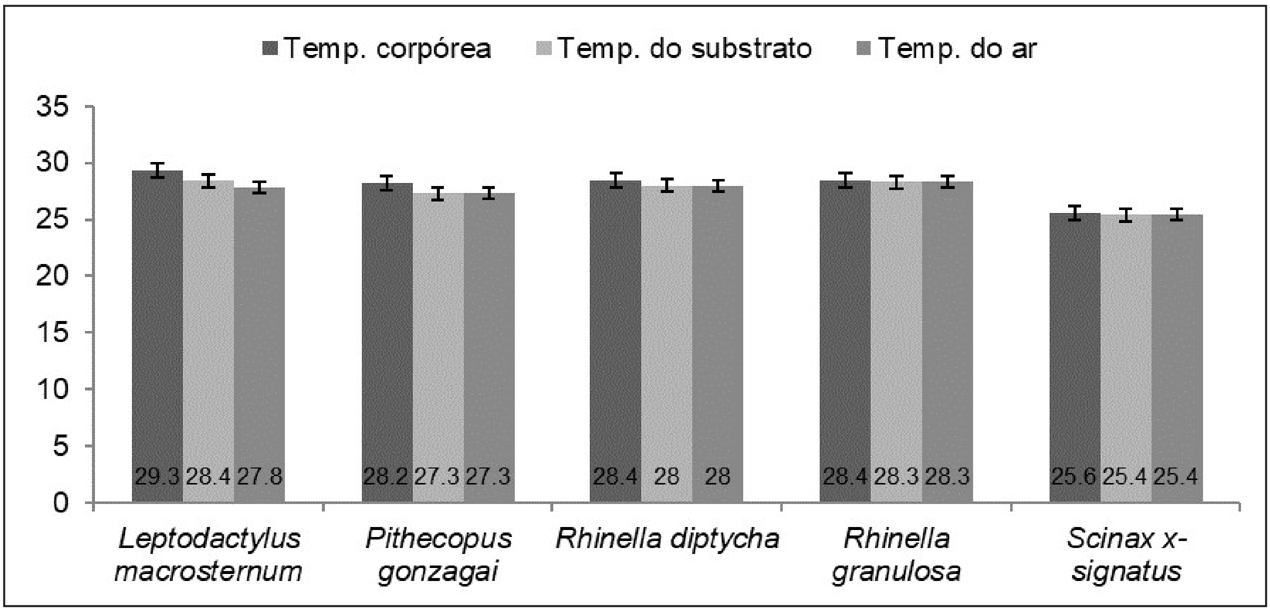

Figura 3. Temperatura média (°C) do corpo, do substrato e do ar nos micro-hábitats utilizados pelos anuros no período chuvoso.

Tabela 2. Associação das temperaturas corpóreas dos anuros com a temperaturas do substrato (Ts) e do ar (Ta), nos períodos seco (s) e chuvoso (c) em áreas da Caatinga, entre agosto de 2019 e maio de 2021. Asterisco (*) representa associação significativa entre a temperatura corpórea dos anuros e as temperaturas ambientais (substrato e ar).

Espécie	Período	Associação com Ts	Associação com Ta
Leptodactylus macrosternum	S	(r2= 0,64, p = 0,0001, n = 153)*	(r2 = 0,48, p = 0,07, n = 153)
	C	(r2 = 0,46, p = 0,01, n = 44)*	(r2 = 0,39, p = 0,42, n = 44)
Rhinella diptycha	S	(r2 = 0,18, p = 0,0009, n = 30)*	(r2 = 0,05, p = 0,008, n = 30)*
	C	(r2 = 0,68, p = 0,03, n = 35)*	(r2 = 0,64, p = 0,56, n = 35)
Rhinella granulosa	S	(r2 = 0,61, p = 0,0001, n = 47)*	(r2 = 0,46, p = 0,29, n = 47)
	C	(r2 = 0,33, p = 0,13, n = 28)	(r2 = 0,27, p = 0,68, n = 28)
Scinax x-signatus	S	(r2 = 0,70, p = 0,0001, n = 43)*	(r2 = 0,53, p = 0,37, n = 43)
	C	(r2 = 0,87, p = 0,002, n = 25)*	(r2 = 0,85, p = 0,007, n= 25)*
Pithecopus gonzagai	C	(r2 = 0,78, p = 0,76, n = 15)	(r2 = 0,81, p = 0,15, n = 15)

Discussão

Nossos resultados, no geral, mostram que a amplitude de temperatura corporal média nas espécies de anuros (24,2–29,3°C) encontra semelhança com aquela estimada para outras populações de anuros de regiões áridas, em especial bufonídeos e leptodactilídeos (Sanabria e Quiroga, 2019). Essa faixa de temperatura parece ser muito determinada pela interação entre preferências de micro-hábitat, a sazonalidade e a hora do dia em que a atividade dos anuros ocorre. Por exemplo, a prevenção de altas temperaturas por *Scinax x-signatus* e *Pithecopus gonzagai*, no período seco e diurno na Caatinga, contrasta com os dados obtidos para *Leptodactylus macrosternum* e os bufonídeos (*R. diptycha* e *R. granulosa*) os quais foram menos restritivos à atividade sob tais condições climáticas desidratantes, o que poderia ser justificado como um mecanismo para atingir suas temperaturas corporais adequadas.

A maioria dos anuros que ocorrem em condições aparentemente desfavoráveis da Caatinga, especialmente no período de seca, tem mecanismos para se proteger da dessecação na superfície do solo, tais como, se enterrando, acumulando ureia nos fluidos corporais e formando casulos (Navas *et al.*, 2004; Varjão e Ribeiro, 2018). Altas temperaturas ambientais têm efeitos diretos no comportamento e na fisiologia dos anfíbios (Rome *et al.*, 2002). Nossos dados revelam um maior número de anuros com temperatura corpórea aferida durante a noite o que indica que estes animais, em termos comportamentais, passam o dia em abrigos para evitar as altas temperaturas, e se tornam ativos à noite. Em termos fisiológicos, a pele úmida dos anfíbios pode aumentar a taxa de perda de água por evaporação o que é uma das principais causas de perda de energia calórica (Shoemaker *et al.*, 1992; Bovo, 2015). Nesta perspectiva, a troca de calor por exposição direta aos raios solares não é interessante para os anuros. De fato, a heliotermia foi relatada em poucos anuros, tais como em *Anaxyrus boreas* e *Rhinella spinulosa* (Lillywhite *et al.*, 1973; Lambrinos e Kleier, 2003). Os anuros do presente estudo, no período diurno (principalmente *R. granulosa*), utilizaram fendas no solo do leito seco de poças temporárias. De acordo com Navas et al. (2004), estes locais de refúgio ainda podem reservar umidade necessária para que os anuros evitem a perda de água por evapotranspiração.

Visto que a termorregulação dos anfíbios fora da água é desafiadora (Köhler *et al.*, 2011), o comportamento seletivo de temperatura nos anfíbios é muitas vezes sobreposto por esforços para se manter hidratado (Tracy *et al.*, 1993). Neste estudo, os anuros tiveram preferência por ambientes próximos a corpos d’água. E uma das estratégias para manter a hidratação e uma temperatura corporal constante é a escolha de um micro-hábitat apropriado (Seebacher e Alford, 2002). Nesse sentido, os micro-hábitats ocupados pelos anuros, neste estudo, parecem refletir esse compromisso.

A temperatura corpórea da maioria das espécies de anuros estudadas apresentou associação significativa com a temperatura do substrato, exceto *R. granulosa* e *P. gonzagai* no período chuvoso. A respeito disso, para *R. granulosa* um anuro terrícola, a temperatura do substrato ainda apresentou tendência a exercer maior efeito sobre sua temperatura corpórea, ainda que tenha havido maior uso de micro-hábitats dentro d’água em detrimento ao

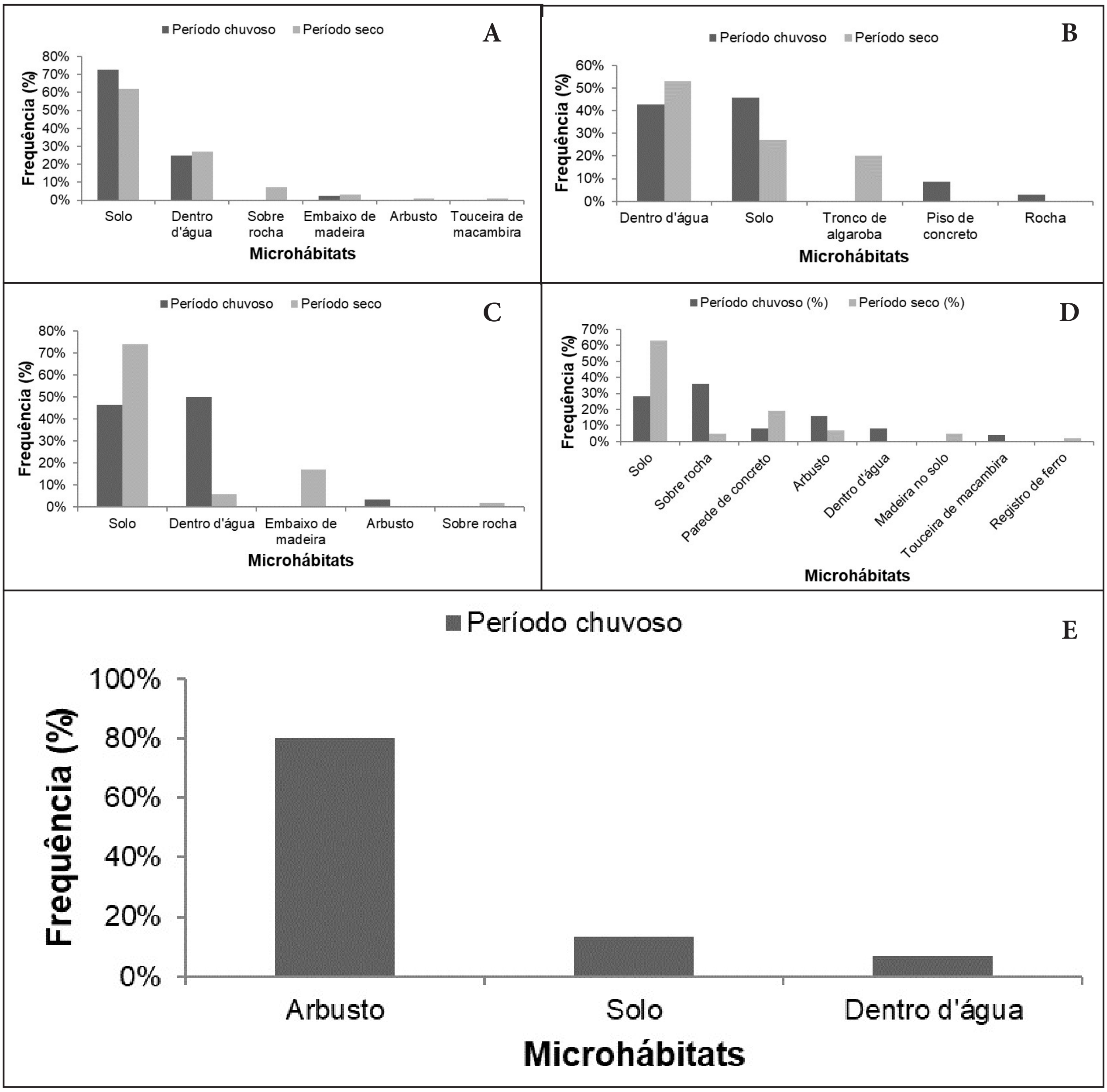

Figura 4. Micro-hábitats utilizados pelos anuros durante os períodos chuvoso e seco. (A) *Leptodactylus macrosternum*, (B) *Rhinella diptycha*, (C) *Rhinella granulosa*, (D) *Scinax* x-*signatus*, (E) *Pithecopus gonzagai* (dados obtidos apenas para o período chuvoso). Notas: "macambira" é uma planta da família das bromeliáceas. "Algaroba" é uma árvore da família das leguminosas. "Registro de ferro" se trata de um objeto de natureza antrópica.

solo. Em contrapartida, o anuro noturno *P. gonzagai* teve sua temperatura corpórea com tendência a um maior efeito da temperatura do ar, o que confere com seu hábito arborícola, sob influência de convecções de ar na regulação térmica.

A associação com a temperatura do ar foi significativa apenas em *R. diptycha* no período seco e em *S. x-signatus* no período chuvoso. Para *R. diptycha*, que representa anuros de grande tamanho corporal (medindo de 230 a 250 mm de comprimento rostro-cloacal, quando adultos; Carvalho-e-Silva *et al.* 2015), é possível que esse fator tenha favorecido a maior distância em altura do solo, e influenciado nessa relação térmica com o ar, o que merece investigação futura. Para *S. x-signatus* o uso de micro-hábitats verticais também favorece a ação das convecções de ar na regulação térmica. No entanto, para essas duas espécies, a associação com o substrato ainda foi maior (dado o maior coeficiente r^2, Tabela 2). Isto sugere que essas espécies de anuros, e as demais reportadas nessa relação, apresentam uma termorregulação tigmotérmica, que é a obtenção de calor por meio da condução. Visto que o ar apresenta menor condutividade de calor em relação aos substratos, a temperatura corpórea tende a ser mais influenciada pela temperatura do substrato (Zug *et al*., 2001). Um padrão observado em outros anuros, tais como em *Rhinella arenarum* (Sanabria *et al*., 2003).

Variações sazonais em temperatura corpórea têm sido registradas em animais ectotérmicos (Zug *et al*., 2001; Ribeiro e Freire, 2010). De acordo com esses estudos, elas podem ser influenciadas pelas distintas condições térmicas e pelas fontes de calor ambiental de cada estação, que resultam em um processo de aclimatação para cada espécie manter a temperatura do corpo dentro da faixa apropriada para desempenho comportamental e ecológico. *Leptodactylus macrosternum*, *R. granulosa* e *R. diptycha* apresentaram temperaturas corpóreas mais elevadas no período chuvoso. Esse cenário corrobora com os estudos que reportam a influência do gradiente térmico ambiental na variação da temperatura dos anuros (Sanabria *et al*., 2003; Sanabria e Quiroga, 2019).

Conclusão

O presente estudo ajuda a sanar uma grande lacuna de informação sobre a ecologia térmica dos anuros brasileiros, explorando a relação entre a temperatura do corpo e as temperaturas ambientais de cinco espécies comuns, que ocorrem no bioma Caatinga. A variação sazonal das temperaturas corpóreas, significativamente mais altas no período chuvoso para a maioria das espécies, o espectro de micro-hábitats utilizados, e as faixas de temperaturas ambientais exploradas, cuja temperatura do substrato exerceu maior influência na temperatura corpórea dos anuros, são resultados importantes deste estudo.

Altas temperaturas ambientais têm efeitos diretos sobre o comportamento e a fisiologia dos anfíbios. Em um cenário atual de emergência climática, cujo aquecimento global está causando um aumento na temperatura global em ritmo acelerado, e um aumento de eventos climáticos extremos com implicações ecológicas reconhecidas, resultados de caráter analítico como os deste estudo, podem auxiliar na identificação de como os efeitos de mudanças climáticas, assim como nas mudanças antropogênicas globais, como o uso de agrotóxicos, têm impacto sobre a fisiologia térmica de anfíbios.

Em termos de perspectiva para novos estudos, os desafios aqui encontrados e enfrentados para a coleta de dados térmicos dos anuros, em especial no período de estiagem, em áreas de amostragem muito distantes umas das outras, mostram a possiblidade de realizar abordagens comparativas com populações de anuros de outras áreas geográficas, assim como, em ambientes mais heterogêneos do bioma Caatinga.

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Trabajo

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Body Size, Age and Growth Pattern of the most represented anurans in Inselbergs of northeastern Argentina

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ABSTRACT
The decline of biodiversity is an urgent concern that raises significant alarm today. Several species vanish without their biology being understood, or sometimes, even without awareness of their existence. Due to the significance that the Inselbergs has to biodiversity and nature, as they have been recognized as genuine evolutionary laboratories, they present a unique opportunity to enhance our understanding of biologically unexplored species. Our study presents the first information regarding the body size, age and growth pattern of *Scinax fuscovarius*, *Odontophrynus asper*, and *Melanophryniscus atroluteus,* the three most frequently encountered anuran species in the Inselbergs outcrops of northeastern Argentina. We have demonstrated that the variations in body size cannot be attributed to the effect of the specific hill where the species inhabit. We discovered that sexual dimorphism in age and the analyzed morphological variables is expressed differently in the three examined species. We also demonstrated that the observed differences in body size between populations of *M. atroluteus* of Inselbergs and the Atlantic Forest of Argentina can be attributed to differences in age at sexual maturity and growth patterns. However, we believe that other proximate mechanisms should be taken into account, such as the effects of the environment and inter- and intra-specific competition experienced during the larval stages, for a better understanding of the population differences in adult body size and age. Our findings will assist in the evaluation of species management and conservation strategies within their habitat.

Key words: Inselbergs, Paraje Tres Cerros, Body size, Age, Anurans.

RESUMEN
El declive de la biodiversidad es una preocupación urgente que suscita gran alarma hoy en día. Varias especies desaparecen sin que se comprenda su biología o, a veces, incluso sin que se sepa de su existencia. Debido a la importancia que los Inselbergs tienen para la biodiversidad y la naturaleza, ya que han sido reconocidas como auténticos laboratorios evolutivos, presentan una oportunidad única para mejorar nuestra comprensión de especies biológicamente inexploradas. Nuestro estudio presenta la primera información relativa al tamaño corporal, edad y patrón de crecimiento de *Scinax fuscovarius*, *Odontophrynus asper*, and *Melanophryniscus atroluteus,* las tres especies de anuros más frecuentemente encontradas en los afloramientos de los Inselbergs del noreste de Argentina. Hemos demostrado que las variaciones en el tamaño corporal no pueden atribuirse al efecto del cerro específico donde habitan las especies. Descubrimos que el dimorfismo sexual en edad y en las variables morfológicas analizadas se expresa de manera diferente en las tres especies examinadas. También demostramos que las diferencias observadas en el tamaño corporal entre las poblaciones de *M. atroluteus* de los Inselbergs y del Bosque Atlántico de Argentina pueden atribuirse a diferencias en la edad a la madurez sexual y en los patrones de crecimiento. Sin embargo, creemos que deben tenerse en cuenta otros mecanismos próximos, como los efectos del ambiente y la competencia inter e intraespecífica experimentada durante las fases larvarias, para comprender mejor las diferencias poblacionales en el tamaño corporal y la edad de los adultos. Nuestros hallazgos ayudarán a evaluar las estrategias de gestión y conservación de las especies dentro de su hábitat.

Palabras Clave: Inselbergs, Paraje Tres Cerros, Tamaño corporal, edad, Anuros.

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Introduction

Body size is perhaps the most important trait with the greatest implications throughout their life cycle (Atkinson, 1994; Woodward *et al.*, 2005; White *et al.*, 2007). This is because body size is related to the fitness of an animal, due to its close association with longevity, fecundity, metabolic rate and tolerance to environmental stress, among others (Calder, 1984; Schmidt-Nielsen, 1984). Anurans show an indeterminate growth pattern, with continuous growth, which asymptotizes once sexual maturity is reached (Hemelaar, 1998; Smirina, 1994; Halliday and Tejedo, 1995). Consequently, differences found in adult size of anurans are, in most cases, dependent on environmental and/or genetic factors that cause variations in the growth pattern between the post-metamorphic phase and reproductive maturity (e.g. Halliday and Verrell, 1988; Esteban and Sanchiz, 2000). Thus, one of the main objectives for evolutionary and systematic biologists is the detection and description of these patterns of inter and intra-specific variation, and the inference of processes that help to understand them (Cracraft, 1989). In addition, the need to increase our knowledge of these processes becomes even more relevant if we consider that in a given isolate ecosystem, these patterns of variation may lead to the evolution of endemic species, or even to the formation of new species (Endler, 1977; Foster and Endler, 1999).

*Inselberg*s (from the Germanic "island-mountain") are a type of isolated ecosystem on the mainland, found in many parts of the world, constituted by a diverse set of isolated rock habitats and recognized as biogeographic islands (Bornhardt, 1900; Porembski and Barthlott, 2000; Fitzsimons and Michael, 2017). In these ecosystems, the distinctive combination of environmental conditions (such as microclimate and soil) that sharply contrast with those in the surrounding matrix often gives rise to ecologically unique habitat features (Coor *et al.*, 1993; Porembski and Barthlott, 2000; Burke, 2003). Many authors agree that these isolated ecosystems represent centers of biodiversity concentration, refuges for threatened flora and fauna, as well as favorable scenarios for endemic phenomena and conservation of relictual populations (Porembski *et al.*, 1998; Porembski and Barthlott, 2000; Fredericksen *et al.*, 2003; Cajade *et al.*, 2013a). In addition, due to their isolation and particular environmental characteristics, these ecosystems represent true evolutionary laboratories where species experience evolution in isolation (Endler, 1977; Foster and Endler, 1999; Barthlott and Porembski, 2000; Porembski, 2007; Cajade *et al.*, 2013a; Argoitia *et al.*, 2021; Piñeiro *et al.*, 2021; Piñeiro, 2022).

In northern Argentina, three isolated Inselbergs located in the east-central region of Corrientes province (Fig. 1) have been explored through an interdisciplinary program of several projects to study their biodiversity (Meregalli, 1998; Ravenna, 2003, 2009; Cajade *et al.*, 2013a,b; Odriozola, 2014; Isler, 2016; Ojanguren-Afilastro *et al.*, 2017; Gervazoni, 2017; Fandiño *et al.*, 2017; Nadal *et al.*, 2018; Oliva and Panizza, 2019; Ellis *et al.*, 2020; Argoitia *et al.*, 2021; Piñeiro *et al.*, 2021; Courtis *et al.*, 2022). Recently, Piñeiro *et al.* (2021) described the herpetofauna diversity that inhabit there, where recorded 19 rare species, seven threatened species and two endemic species, and discussed the role of the Inselbergs in amphibians and reptiles conservation, contributing to its valorization. In addition, they emphasizing the need for further study help evaluate species and conservation strategies in the natural reserve where they live.

In order to increasing the knowledge of the life history traits of the amphibians that live there, and contribute to their conservation, we studied the body size, age structure and whether Sexual Size Dimorphism (SSD) existed, in the three most represented anurans in Inselbergs outcrops of northeastern Argentina (Piñeiro, 2022). We also analyzed if this dimorphism could be explained by proximate mechanisms such as differences in growth patterns. This will increase our knowledge of the life history traits of these amphibians, which help evaluate species management and conservation strategies in the natural reserve where they live. In addition, we tested whether there were differences in body size and age between the *Melanophryniscus atroluteus* populations of the Inselberg and the Atlantic Forest of Argentina. Finally, we discussed the possible causes that might determine it.

Materials and methods

Study site and Species

We carried out fieldwork in the Inselbergs Nazareno (29° 0.6′ 26.51" S, 56° 55′ 56.90" W, 179 m a.s.l., 83 ha) and Chico (20° 0.6′ 45.74" S, 56° 55′ 7.78" W, 148 m a.s.l., 34 ha), two out of the three hills located in the east-central region of Corrientes province,

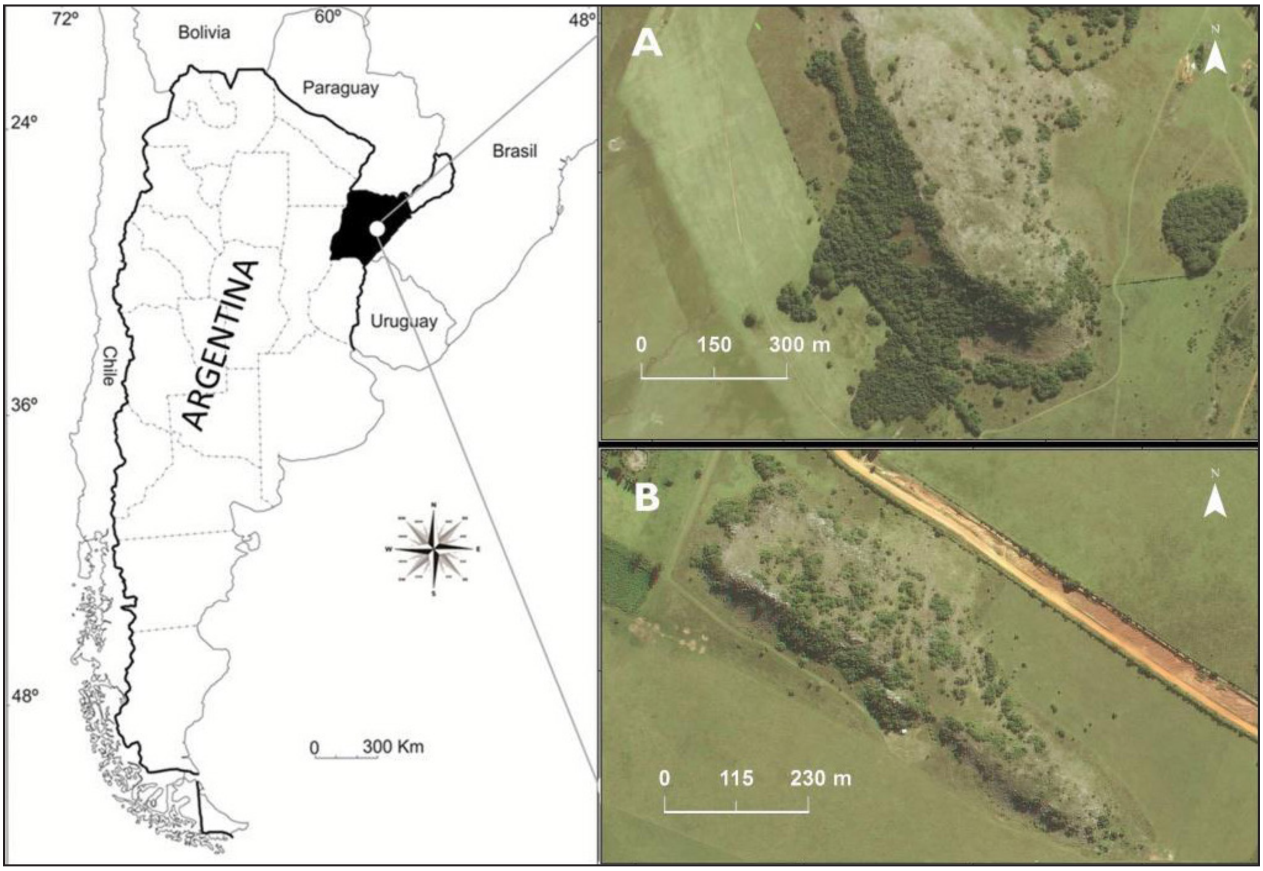

Figure 1. Isolated rocky outcrops of Paraje Tres Cerros, Corrientes, Argentina. View of the Nazareno (a) and Chico (b) hills.

Argentina (Fig. 1). The study area is located within the Espinal district, one of the subdivisions of the Pampeana province, corresponds to the Neotropical biogeographic region (Arana *et al.,* 2021). The climate and topography of the study site is detailed in Piñeiro *et al.,* (2021). The three most represented anuran species in the Inselbergs (Piñeiro, 2022), analyzed in the present study, were: Scinax fuscovarius, "Snouted-tree frog" is a species of frog of the Hylidae family, very common in arboreal and peri-domestic habitats. During the breeding season it is found in permanent water bodies such as streams, cutwaters and temporary ponds. The males of this species vocalize on the ground or on rocks at the water's edge, perched on herbaceous and arbustive vegetation, as well as on tree branches or fallen trunks. It´s distributed in various ecoregions in Argentina, Bolivia, Brazil, Paraguay, Uruguay (Zaracho *et al*., 2012). *Odontophrynus asper*, "Common Lesser Escuerzo" is a species of toad of the Odontophrynidae family. This species has terrestrial and fossorial habits. It lives in forests and grassland, temporary and semi-permanent vegetated lagoons, in caves and urban canals. They bury themselves and remain hidden most of the year. Males vocalize from the water among the vegetation. It´s distributed in Atlantic forest and humid Chaco ecoregions in Argentina, Brazil, Paraguay, Uruguay (Zaracho *et al*., 2012). *Melanophryniscus atroluteus*, "Uruguay Redbelly Toad" is a small-sized toad species belonging to the Bufonidae family. This terrestrial species exhibits breeding activity that takes place from mid-autumn (April) and extends until mid-spring (late September-October), characterized by explosive events triggered by rainfall over the course of two or three consecutive days (Marangoni and Baldo, 2023). It inhabits grasslands between patches of forest. It has a defense behavior that consists of displaying the soles of the feet and the palms of the hands before a potential predator (*unken reflex*). It´s distributed in a humind Chaco ecoregions in Argentina, Brazil, Paraguay, Uruguay (Zaracho *et al*., 2012).

Body size and Sexual size dimorphism
Sampling methods

We analyzed de body size and sexual size dimorphism using individuals obtained of two different sampling methods. On the one hand, we analyzed

132 individuals (*S. fuscovarius* n = 82, *O. asper*n = 37 and *M. atroluteus* n = 13), caught using pitfall traps and coverboards during a previous study conducted from April 2015 to April 2017 in Nazareno and Chico Hills (see Piñeiro, 2022, for a detailed description). On the other hand, in addition to the individuals caught using pitfall traps, we also measured 55 individuals of *M. atroluteus* collected by hand in a reproductive event on August 27 2017 (Table 1). This event occurred in a temporary pond (10 to 20 cm deep) located between the Nazareno and Chico Hills (29º 0.6' 28.8" S, 56º 55' 22.8" W), filled immediately after heavy rains. In this reproductive event of *M. atroluteus*, we collected either calling males or pairs in amplexus, during chorusing mornings between 10:00 and 12:00 h, to ensure that all individuals were mature.

Measurements and tissue samples.

The procedures described below were carried out on all individuals of the three species, regardless of the capture method used. Individuals were firstly sexed taking into account secondary sexual characteristics. Then, the following measurements were taken in the sample place of the capture according to Duellman (1970). We measured the snout-vent length (SVL), head width (HW) and tibia-fibula length (TFL), using a digital calliper (0.1 mm precision). Also, we measured body mass (BM) using an electronic balance MH-500g/0.01g. Moreover, we individually-marked all individuals by using toe-clipping codes (Donnelly and Guyer, 1994), and the toes stored in 70% ethanol at room temperature for age estimation through skeletochronology (see below). In addition, this marks also allowed us to test the existence of migrations between populations. Finally, individuals were released back into their habitats. The sampling followed the general guidelines proposed by the Dirección de Recursos Naturales of the Corrientes province, Argentina, under project code PI 12F007 of the Laboratorio de Herpetología, of the Universidad Nacional del Nordeste.

Skeletochronology

We used clipped toes from 121 mature individuals for age estimation through skeletochronology (*S. fuscovarius* n = 43, *O. asper* n = 25 and *M. atroluteus* n = 53). We preferably use the third toe of the right leg in case it has been cut off during individual marking. Otherwise, we use any toe that has been clipped to avoid additional injuries to the individuals. We followed the standard methods in skeletochronology (e.g., Smirina, 1972; Halliday and Verrel, 1988, Castanet and Smirina, 1990; Sinch *et al.,* 2015), with minor modifications proposed by Marangoni (2006). Clipped toes were washed in water for 30 minutes, decalcified in 5% nitric acid for 1–3 hours, afterward dehydrated, paraffin-embedded, sectioned using a rotation microtome (Arcano RMT-30) at 14–16 μm, and stained with Harris hematoxylin. We took digital images of those cross-sections where the size of the medullar cavity was at its minimum and the periosteum was at its maximum, using a microscope (Leica Model DM500) and the software Leica LEAD Technologies Inc.V1.01. Cross-sections were observed and measured using the computer package Image-Pro Plus version 4.5 (Media Cybernetics 1993–1994, Silver Spring, Maryland, USA) and calibrated using a standard micrometer. Two independent observers (JMP and FM) recorded the presence/absence of the line of metamorphosis and counted the LAGs. In those individuals with no remnant of the line of metamorphosis, we estimated the degree of resorption by osteometrical analysis (Sagor *et al.*, 1998; Tomašević *et al.*, 2008). We distinguished annual growth marks (i.e., LAGs *sensu stricto*) from non-annual ones (i.e., irregular interruptions during short periods of inactivity), using the method described in Sinsch *et al.* (2007). We computed the bone size following the methods of Hemelaar (1985): the longest and shortest perpendicular axes of each LAG were measured, in each of two diaphyseal sections per specimen examined. Afterwards, axis measurements were multiplied together and the square root of the product calculated (average diameter of each LAG). This procedure was done for the LAGs in each of two diaphyseal sections per specimen.

Age-related parameters

We computed the following age-related parameters for each sex (*sensu* Leskovar *et al.*, 2006): (1) age at maturity was estimated as: a) the minimum number of LAGs counted in those breeding individuals from reproductive events (calling males or pairs in amplexus of *M. atroluteus*); or b) we inferred the age of sexual maturity by observing the bone growth pattern in the cross-sections in individuals from pitfall traps and coverboards (individuals of *S. fuscovarius* and *O. asper*). We considered the distance between two LAGs to be an indicator of individual growth at a given age, and a pattern of decreasing intervals between LAGs after a few years is thought to indicate the onset of sexual maturity, with resources being reallocated from growth to reproduction (Smirina, 1994). (2) longevity: the maximum number of LAGs counted in breeding individuals; (3) potential reproductive lifespan: the difference between longevity and age at maturity; (4) median lifespan: median of age distribution; (5) size at maturity: the average snout-vent length of all first breeders with the minimum number of LAGs.

Growth patterns

We used nonlinear estimation in STATISTICA 8 statistical package (StatSoft Inc. 2007, Tulsa, USA) to compute von Bertalanffy growth equation(von Bertalanffy, 1938): $S_t = S_m - (S_m - S_0)\ e^{-k\,(t - t0)}$

where t = number of growing seasons experienced (age); t_0 = age at metamorphosis (proportion of the growing season already elapsed at metamorphosis); S_t = average body size after having experienced t growing seasons; S_m = average maximal body size; S_0 = average body size at metamorphosis; k = growth coefficient, defining the slope of the growth curve, measuring the exponential rate of approach to S_m. In order to obtain the size at metamorphosis (S_0, se above) and thus to fit the growth model (von Bertalanffy, 1938), were collected clutches of *S. fuscovarius*, *O. asper* and *M. atroluteus* in temporary pools formed after heavy rains in the study area. The clutches (in stages below 20 according to Gosner, 1960) were maintained at a constant density and temperature and a 12:12 photoperiod until the reabsorption of the tail (45-46 Gosner stage), when were measured and weighed. We measured the SVL and weight from 24 metamorphs random selected of *S. fuscovarius*, 10 of *O. asper* and 36 *M. atroluteus*, which were used to fit the von Bertalanffy growth model and estimated growth parameters (VBgPs) by nonlinear least squares regression. The metamorphs were housed in the Laboratorio de Investigación en Diversidad, Ecología y Conservación de Vertebrados (LABIDECOV, Universidad Nacional del Nordeste), Corrientes province, Argentina, under acronym LABIDECOV-16 (*S. fuscovarius*), LABIDECOV-15 (*O. asper*) and LABIDECOV-14 (*M. atroluteus*).

Statistical analyses

All variables were log-transformed in order to achieve normality. We tested all data for normality and homoscedasticity using ShapiroWilk and Levene tests and chose the statistic tests accordingly. We did not find differences in either body size measurements between *M. atroluteus* from pitfalls and reproductive events (*P* value found = 0.896), and we therefore subsequently pooled the data sets from both capture methods to analyze the body size. We used multi- and univariate analyses of variance to test for differences in the body size variables and age-related parameters measured between hills and sexes within species. Means were compared using post-hoc Scheffé multiple comparison test, at α = 0.05 (Scheffé, 1953). We also assessed the sexual size dimorphism (SSD) for each body measurement using the sexual dimorphism index (SDI), following Lovich and Gibbons (1992): SDI = mean $\text{size}_{\text{larger sex}}$/mean $\text{size}_{\text{smaller sex}}$, with the result arbitrarily defined as positive when females are larger than males, and negative when males are larger. We used linear regressions to test the association between body size and age. We used a two-sample Mann-Whitney test to check for differences in mean age between males and females. We used data of body traits, age and growth included in a previous study by Marangoni and Baldo (2023), to test whether there were differences in body size between the *M. atroluteus* populations of the Inselberg (present study) and the Atlantic Forest of Argentina (Marangoni and Baldo, 2023). All statistical analyses were performed using the STATISTICA 8.0 statistical package (StatSoft Inc. 2007, Tulsa, USA).

Results

Body size and sexual size dimorphism

A MANOVA on SVL, BM, TFL and HW in the three species studied, revealed that there was no significant effect of the Hills where the individuals were collected (*S. fuscovarius*: Wilk's $\lambda = 0.884$, $F_{4,69} = 2.259$, $P = 0.072$; *O. asper*: Wilk's $\lambda = 0.663$, $F_{4,23} = 66.574$, $P < 0.05$ and *M. atroluteus*: Wilk's $\lambda = 0.585$, $F_{4,8} = 1.419$, $P = 0.312$). We therefore subsequently pooled the data sets from both Hills to analyze the body size and sexual size dimorphism. The descriptive statistics of body traits measured and SDI in the three species are presented in Table 1.

Univariate ANOVAs showed significant effects of sex on SVL, TFL and BM for *M. atroluteus* (Table 1). We did not observed a regular pattern in SDI among species. This showed negative values in some cases, indicating that the males were larger than the females in certain variables. However, in other cases, the values were positive, indicating the reverse situation, with the females being larger than the males (Table 1). The body size showed signifi-

Table 1. Body traits: snout-to-vent length (SVL), head width (HW), tibio-fibula length (TFL), body mass (BM) and bone size (BS) of male and female of the three species studied. SDI = sexual dimorphism index. Δ = Individual from pitfall traps pooled with those from reproductive event. ∞ The sample sizes are those in which skeletochronology was applied (see Table 3). The asterisks compare sexual size dimorphism (SSD) within species: * = $P<0.01$, ** = $P<0.001$, ns = not significant. All values are means ± 1 SD..

Species/ Body Traits	***Scinax fuscovarius* (n = 82)**				***Odontophrynus asper* (37)**				***Melanophryniscus atroluteus* (68Δ)**		
	Male (n=31)	Female (n=23)	Juvenile (28)	SDI	Male (n=11)	Female (n=16)	Juvenile (10)	SDI	Male(n=41Δ)	Female (n=27Δ)	SDI
SVL	38.96 ± 4.85	37.78 ± 4.31 ns	30.93 ± 4.24	-1.03	40.58 ± 4.31	39.68 ± 3.04 ns	34.55 ± 4.75	-1.02	21.91 ± 1.89Δ	22.49 ± 1.33*Δ	1.03
HW	13.03 ± 1.61	12.54 ± 1.37 ns	10.20 ± 1.30	-1.25	17.89 ± 1.22	18.16 ± 1.29 ns	15.63 ± 2.13	1.01	6.83 ± 0.52Δ	6.66 ± 0.41 NSΔ	-1.02
TFL	18.87 ± 2.46	18.22 ± 2.36 ns	14.79 ± 2.39	-1.04	11.31 ± 1.24	11.08 ± 1.10 ns	10.16 ± 1.80	-1.02	6.67 ± 0.60Δ	5.86 ± 0.26 **Δ	-1.14
BM	4.59 ± 1.81	4.01 ± 1.36 ns	2.12 ± 0.91	-1.14	9.09 ± 2.49	9.11 ± 2.19 ns	5.89 ± 2.40	1.00	1.27 ± 0.37Δ	1.70 ± 0.65*Δ	1.34
BS∞	278.51 ± 48.08∞	275.33 ± 54.42∞ ns		-1.01	279.33 ± 46.76	313.54 ± 41.57 ns		1.12	202.68 ± 19.84	200.19 ± 21.01 ns	-1.02

Table 2. Relationships between body size: snout-to-vent length (SVL), body mass (BM), bone size (BS) and age, in the three species studied. All variables were log-transformed.

Specie/sex	**Regression equation**	**r2**	**p**	**Regression equation**	**r2**	**p**
Scinax fuscovarius		Male (n = 23)			Female (n = 20)	
SVL:BS	y = 7.967 - 0.646*x	0.206	**0.029**	y = 6.175 - 0.160*x	0.008	0.708
BM:BS	y = 5.861 - 0.172*x	0.159	0.059	y = 5.570 + 0.020*x	0.001	0.887
SVL:Age	y = 2.577 - 0.371*x	0.032	0.414	y = 1.974 - 0.288*x	0.006	0.745
BM:Age	y = 1.274 - 0.034*x	0.003	0.806	y = 1.033 - 0.076*x	0.004	0.797
Odontophrynus asper	Male (n = 11)		Female (n = 14)			
SVL:BS	y = 3.788 + 0.495*x	0.092	0.366	y = 4.234 + 0.409*x	0.415	0.056
BM:BS	y = 5.083 + 0.247*x	0.199	0.446	y = 5.247 + 0.223*x	0.142	0.184
SVL:Age	y = -7.185 + 2.281*x	0.296	0.084	y = -6.223 + 1.975*x	0.095	0.284
BM:Age	y = 1.051+ 0.091*x	0.004	0.851	y = 0.099 + 0.425*x	0.038	0.505
Melanophryniscus atroluteus	Male (n = 30)		Female (n = 23)			
SVL:BS	y = 5.256 + 0.002*x	0.001	0.852	y = 5.045 + 0.011*x	0.019	0.521
BM:BS	y = 5.287 + 0.059*x	0.028	0.371	y = 5.246 + 0.094*x	0.032	0.405
SVL:Age	y = -4.465 + 1.871*x	0.188	**0.002**	y = -3.912 + 1.698*x	0.147	**0.011**
BM:Age	y = 1.3456 - 0.0788*x	0.008	0.555	y = 1.6733 - 0.5504*x	0.178	**0.005**

cant positive relationship with bone size only in *S. fuscovarius* (Table 2).

Age-related parameters

All sections showed well-defined lines of arrested growth (LAGs) in the periosteal bone and were relatively easy to count to assess individual age (Fig. 2). Endosteal resorption never prevented the age estimation. The line of metamorphosis was visible in 78.8% of the total samples, considering the three species. But even, in those specimens in which the resorption line was visible (25.2%), and the line of metamorphosis was replaced during the endosteal bone modeling, the inner LAG was never completely removed. Annuli (Peabody, 1958) were easily distinguishable from actual LAGs, because they always stained more faintly and were often broader than true LAgs. The demographic age-related parameters of the three species studied are summarized in Table 3, and the age structure of adults in Fig. 2. Males of *S. fuscovarius* and *O. asper* were older than females, conversely females tended to be older in *M. atroluteus* (Table 3), although significant dimorphism in mean age was only found in *S. fuscovarius* (Mann-Whitney, U = 138,5; P = 0.025). There were not intra and inter-specific differences in age at maturity (1-2 LAGs) and longevity (5-6 LAGs) (Table 3). The most frequent observed age class was represented by 3-4 years old individuals, indicating that most of the active animals were in their second or third reproductive year (Table 3 and Fig. 3). Age was significantly correlated to SVL in males and females and to BM in females of *M. atroluteus* (Table 2).

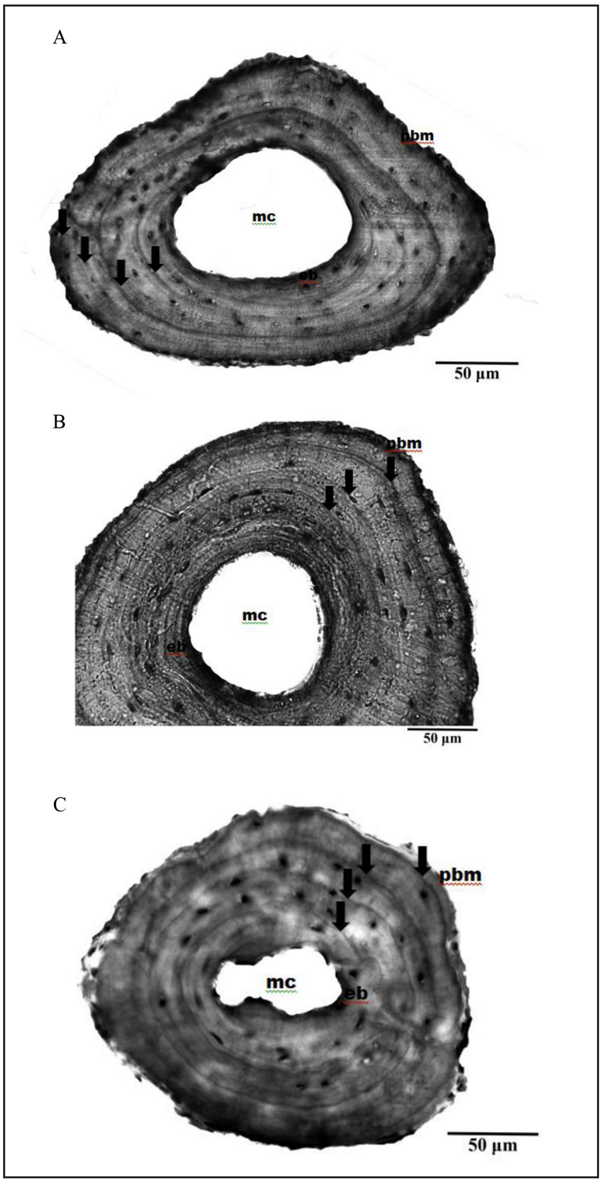

Figure 2. Cross sections of phalanges stained with Ehrlich's hematoxylin of (A) *Scinax fuscovarius* (male, 35.82 mm, 4.32 g, 5 years), (B) *Odontophrynus asper* (female, 40.34 mm, 8.55 g, 5 years) and (C) (female, 20.72 mm, 1.27 g, 4 years). An arrowhead indicates the lines of arrested growth (LAGs), medullar cavity (mc), endosteal bone (eb), periosteal bone margin (pbm).

Growth patterns

The patterns of growth for body size fits well with a von Bertalanffy's growth model (Table 4 and Fig. 3). The asymptotic average snout-vent length (SVL_{max}) was slightly higher in males of *S. fuscovarius* and *O. asper,* conversely were estimated bigger females in *M. atroluteus* (Table 4). The growth coefficient (k) value was higher in females of *M. atroluteus* and *O. asper*, whereas it was higher in males of *S. fuscovarius* (Table 4). However, no difference was *significant* in either SVL_{max} or k between sexes (lowest value obtained being P = 0.135).

Inselbers (ISB) vs. Atlantic Forest of Argentina (AFA) populations

M. atroluteus from the ISB were smaller and youngers than AFA ones (Fig. 4) and, they reached the age at maturity one year before in average (Table 3 and Fig. 5, see also Appendix A, to compare data from Marangoni and Baldo, 2023). The patterns of growth for body size fits well with a von Bertalanffy's growth model (r^2 = 0.987, n = 89 and r^2 = 0.979, n = 48, ISB and AFA respectively; Fig. 5). The asymptotic average snout-vent length (SVL_{max}) and growth rate coefficient (k) were lower in ISB than AFA population.

Table 3. Features of populations life history traits of male and female of the three species studied. AM = age at maturity: Δ = estimated by bone growth pattern of individuals from pitfall traps and coverboards; * = lowest age class recorded in reproductive events (calling males or pairs in amplexus); PRLS = potential reproductive lifespan; SVL = snout-vent length; BM = body mass.

Species/Sex (N)	Mean± SD [LAGs]	Mode [Frequency]	Median Lifespam [yr]	AM [LAGs]	Longevity [LAGs]	PRLS [yr]	SVL at AM X ± SD mm [n]	BM at AM X ± SD mm [n]	Maximum Size SVL-BM mm/g [at age in LAGs]
Scinax fuscovarius									
Male (23)	3.52 ± 0.95	3 (12)	3	2Δ	5	3	39.54 ± 0.23 (n = 2)	4.17 ± 0.37 (n = 2)	47.31 - 7.59 (3 - 3)
Female (20)	2.80 ± 1.24	2-3 (7)	2	1Δ	6	5	36.69 ± 5.42 (n = 2)	3.88 ± 2.24 (n = 2)	43.47 - 6.72 (4 - 2)
Odontophrynus asper									
Male (11)	3.73 ± 0.33	4 (6)	4	1Δ	5	4	34.88 (n =1)	8.35 (n = 1)	48.53 - 13.17 (4 - 5)
Female (14)	3.14 ± 0.35	3-4 (4)	3	1Δ	5	4	36.84 ± 1.02 (n = 2)	6.96 ± 0.61 (n = 2)	44.76 - 12.94 (4 - 2)
Melanophryniscus atroluteus									
Male (30)	3.0 ± 1	3 (3)	3	2*	6	4	20.87 ± 1.82 (n = 3)	1.21 ± 0.08 (n=3)	25.83 - 2.1 (3-4)
Female (23)	3.87 ± 1.01	4 (9)	4	2*	6	4	24.36 (n = 1)	1.97 (n=1)	25.92 - 2.45 (3-3)

Discussion

The slow pace of species description in relation to extinction rates means that several species disappear without their biology being known or without even having been aware of their existence (Mora *et al.*, 2011). In the last 20 years, several studies have demonstrated the importance of inselbergs in biodiversity and nature conservation (Fitzsimons and Michael, 2017; Michael and Lindenmayer, 2018). These are a unique opportunity to increase knowledge about biologically unexplored species, especially considering that inselbergs are regarded as true evolutionary laboratories with a wide variety of unique microhabitats, where species can undergo evolutionary processes in isolation (Barthlott and Porembski, 2000; Porembski, 2007; Cajade *et al.*, 2013a). Our study provides the first information about the body size and age structure of the three most commonly found anuran species in the Inselbergs outcrops of northeastern Argentina. We demonstrated that differences in body size cannot be attributed to an effect of the hill where the species inhabit. We found that sexual dimorphism in age and the morphological variables analyzed, is expressed differently in the three species examined. We also demonstrated that the observed differences in body size between populations of *M. atroluteus* of Inselberg and the Atlantic Forest of Argentina can be attributed to differences in age at sexual maturity and growth patterns. Thus, our results will contribute to a better understanding of the life history characteristics of these amphibians, which in turn can aid in the assessment of species management and conservation strategies within their natural reserve habitat. The loss of biodiversity is a pressing issue that generates significant concern today.

Body size and sexual size dimorphism

The body size of different species inhabiting isolated environments, such as islands or island-like systems (e.g. Inselbergs), can be affected by a combination of ecological and genetic factors (Van Valen, 1973; Baeckens, 2020; Lomolino, 2005), which has been demonstrated in many taxa including amphibians (Roth, 1990; Raia and Meiri 2006; Herridge and Lister, 2012; Montesinos, 2012; Rebouças *et al.*, 2018). The effect of resource availability may determine for example a smaller body size (insular dwarfism), due

Table 4. Estimated parameters from von Bertalanffy's equation, for body growth (SVL) of the three species studied. SVL_{max} = average maximal body size (mm), k = growth coefficient, defining the shape of the growth curve, r^2 = model fit. All values are means ±1 SE (CI 95%).traps and coverboards; * = lowest age class recorded in reproductive events (calling males or pairs in amplexus); PRLS = potential reproductive lifespan; SVL = snout-vent length; BM = body mass.

Estimated parameters/ species	SVL_{max} ± SE	k	r^2
Scinax fuscovarius			
Male (n = 23)	38.54 ± 0.79 (37 – 40.12)	2.14 ± 0.52 (0.72 – 2.51)	0.954
Female (n=11)	37.05 ± 0.76 (35.52 – 38.57)	1.23 ± 0.35 0.32 – 2.14	0.958
Odontophrynus asper			
Male (n = 11)	41.51 ± 1.14 (39.11 – 43.91)	1.16 ± 0.43 0.26 – 2.08	0.974
Female (n=14)	40.16 ± 0.77 (38.56 – 41.75)	2.08 ± 0.67 (0.69 – 4.48)	0.979
Melanophryniscus atroluteus			
Male (n = 30)	22.41 ± 0.38 (21.65 – 23.17)	0.96 ± 0.15 (0.65 – 1.27)	0.990
Female (n=23)	23.18 ± 0.37 (22.44 – 23.93)	1.13 ± 0.35 (0.44 – 1.86)	0.993

to competition for limited access to food resources, among other causes. In these isolated environments natural selection may act more intensively and rapidly. This can result in specific adaptations to body size to make the best use of resources and environmental conditions (Anderson and Handley Jr., 2002; Keogh *et al.*, 2005). Conversely, without predation pressure, due to the lack of predators in isolated environments, individuals may grow larger and reach a larger size (island gigantism or insular gigantism) (Lomolino, 1985; Barahona *et al.*, 2009; Herczeg *et al.*, 2009). In addition, in small, isolated populations, genetic drift may play an important role. Changes in body size may occur due to random mutations and lack of genetic exchange with larger populations (Pardo *et al.*, 2005; Wang *et al.*, 2014). One of the aims of our study was to test whether the Inselbergs outcrops of northeastern Argentina, which form an island-like system (see below), have an impact on the life history traits of the three most abundant anuran species in these environments. Our results revealed that the hills did not have a significant effect on the analyzed morphometric variables, indicating that differences in body size cannot be attributed to the specific hill where the species inhabit. However, we observed a reduction in body size in the populations from the Inselbergs when compared to the populations of *S. fuscov*arius studied by Goldber *et al.* (2018). These authors found that the populations of *S. fuscovarius* varied from east to west and less from south to north, with frogs being largest in the northwestern populations. Interestingly, the authors also found that individuals of both sexes from the three studied regions of *S. fuscovarius* have a larger body size than the populations from the Inselbergs. Goldber *et al.* (2018) suggest that factors primarily related to the life history are mainly driving the geographical variation observed in *S. fuscovarius.* Furthermore, the authors observed that the degree of sexual size dimorphism was also greater in the western populations (Appendix A). However, we do not observe sexual size dimorphism in the populations from the Inselbergs. The sexual size differences in our study were very small and statistically not significant, which could be explained by the absence of significant differences between sexes both growth rates and age at sexual maturity (see following sections). Additionally, larger and older *O. asper* individuals than those found in the Inselbergs were observed in other populations analyzed by Otero *et al.* (2001). (Appendix A). These results lead us to consider the remaining question: is there complete isolation among the inselberg amphibian populations such that ecological and genetic factors would generate these observed differences in body size in the absence of gene flow and if so, was there enough time for it to happen? Individually-marks allow us confirms the absence of flow between Inselbergs populations, since we did not find individuals with a mark from one population to another (Piñeiro, 2022). This also was confirmed in our previous mark-recapture studies made in *Homonota taragui*, a critically endangered gecko endemic to the Inselbergs (Courtis *et al.*, 2022). Thus, we might suggest that specific processes, such as selection or genetic drift, might be acting in the hills in the absence of gene flow. However are needed studies primarily aimed at confirming whether there is isolation of the hill populations in relation to the surrounding matrix populations and those throughout the species' distribution range, as well as deeper studies to determine the role of the evolutionary processes that are taking place in the Inselbergs amphibians of northern Argentina.

Age-related parameters and growth patterns

The study of life history traits, including age structure, size at sexual maturation, and growth rate, is crucial for understanding the evolutionary ecology of isolated populations. Island dwarfism and gigantism syndromes go beyond size differences and encompass significant changes in these traits. Our study showed no significant differences in age-related parameters between the Inselberg spe-

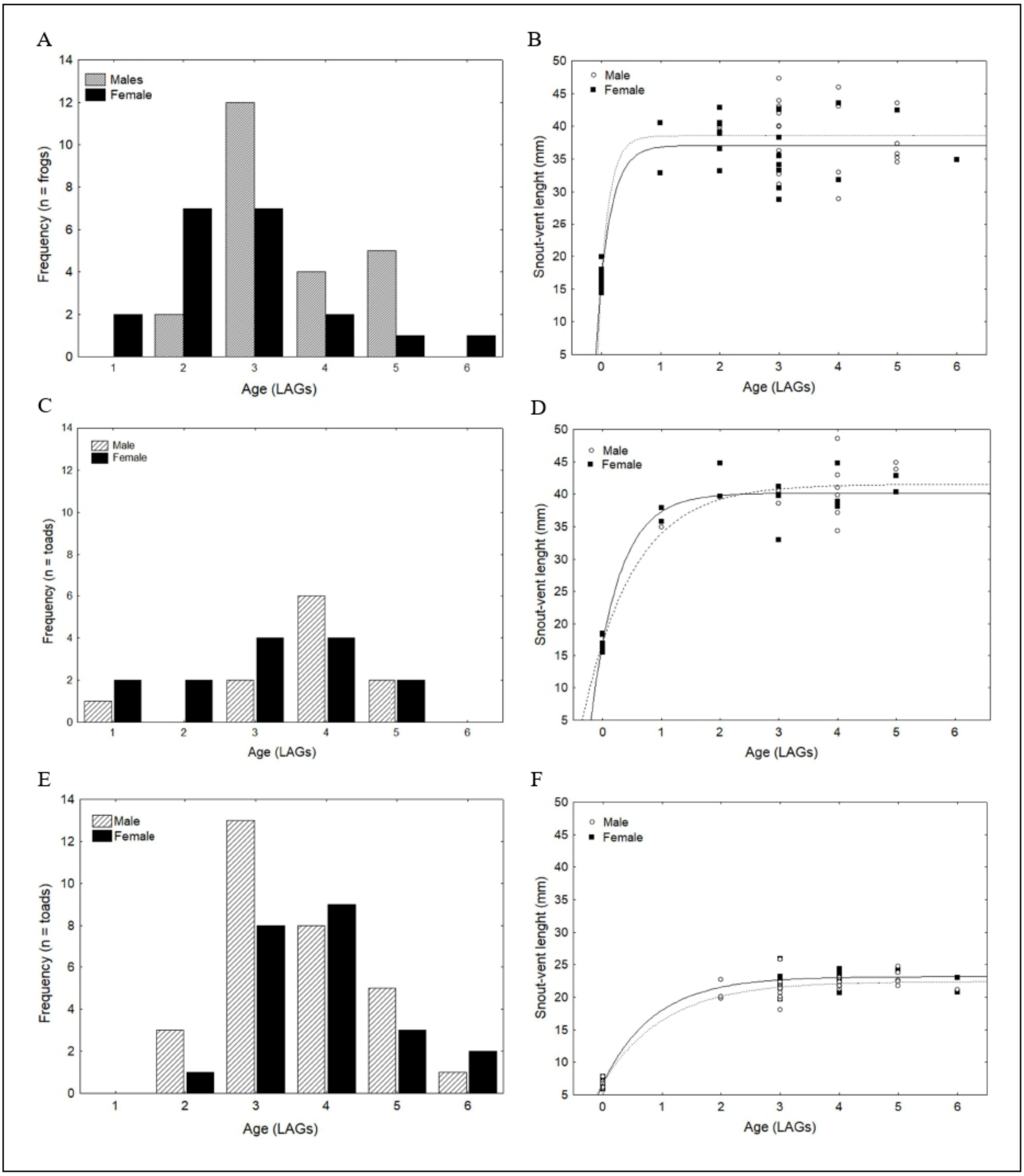

Figure 3. Age structure and growth curves fitted to the von Bertalanffy model, for body growth (SVL) of *Scinax fuscovarius* (A-B), *Odontophrynus asper* (C-D), and *Melanophryniscus atroluteus* (E-F).

cies. Similar ages at sexual maturity and longevity were found in males and females of *O. asper* and *M. atroluteus*, which align with the findings in others species within the same genera or family previously studied (see Appendix A). Nevertheless, females of *S. fuscovarius* matured one year earlier than males, and they are longer-lived than males. There are no other studies on age parameters conducted on *S. fuscovarius* to be discussed, however, differences in longevity were also observed in *Melanophryniscus rubriventris* (males/females = 10/4 years, Quinzio, 2003) and *Rhinella achalensis* (males/females = 8/11 years, Sinsch *et al*., 2001) and *M. atroluteus* from de Atlantic Forest of Argentina (Marangoni and Baldo, 2023). Differences in ages of sexual maturity and longevity have been explained in terms of differences in growth rates and energy allocation in *R. achalensis* and other bufonids (Sinsch *et al*., 200; Marangoni *et al*., 2021; Marangoni and Baldo, 2023). In addition, differential mortality due to the significant energy investment by females in gamete production, which could shorten their life expectancy, was suggested in *M. rubriventris* (Quinzio, 2003). Our results suggest that there is no effect on the variables related to age due to the relative isolation and the potential absence of gene flow among the populations in the Inselbergs. Likewise, we consider our findings to be very preliminary, and more exhaustive studies will be necessary. This includes increasing the number of Inselbergs species and distant populations of the same species, allowing for better comparisons and analysis of the parameters studied. In addition, when analyzing differences in any life history traits between populations, we must analyze other causal sources that may be involved even in lack of genetic isolation (Hyeun-Ji, 2020).

Inselbergs (ISB) vs. Atlantic Forest of Argentina (AFA) populations

Variation in body size and age-related parameters in animals is influenced by many contingent factors, which may lead to intra and inter-population variations at small or large-spatial scales (Bergmann, 1847; James, 1970; Endler, 1977). This phenomenon has been well-documented in amphibians, with the major factors studied being latitude, altitude, and longitude (Bidau *et al*., 2011; Goldberg *et al*., 2018; Yu *et al*., 2019; Hou *et al*., 2023), terrestrial environment (Gomez-Mestre and Tejedo, 2005; Marangoni *et al*., 2008), temperature and precipitation (Bidau

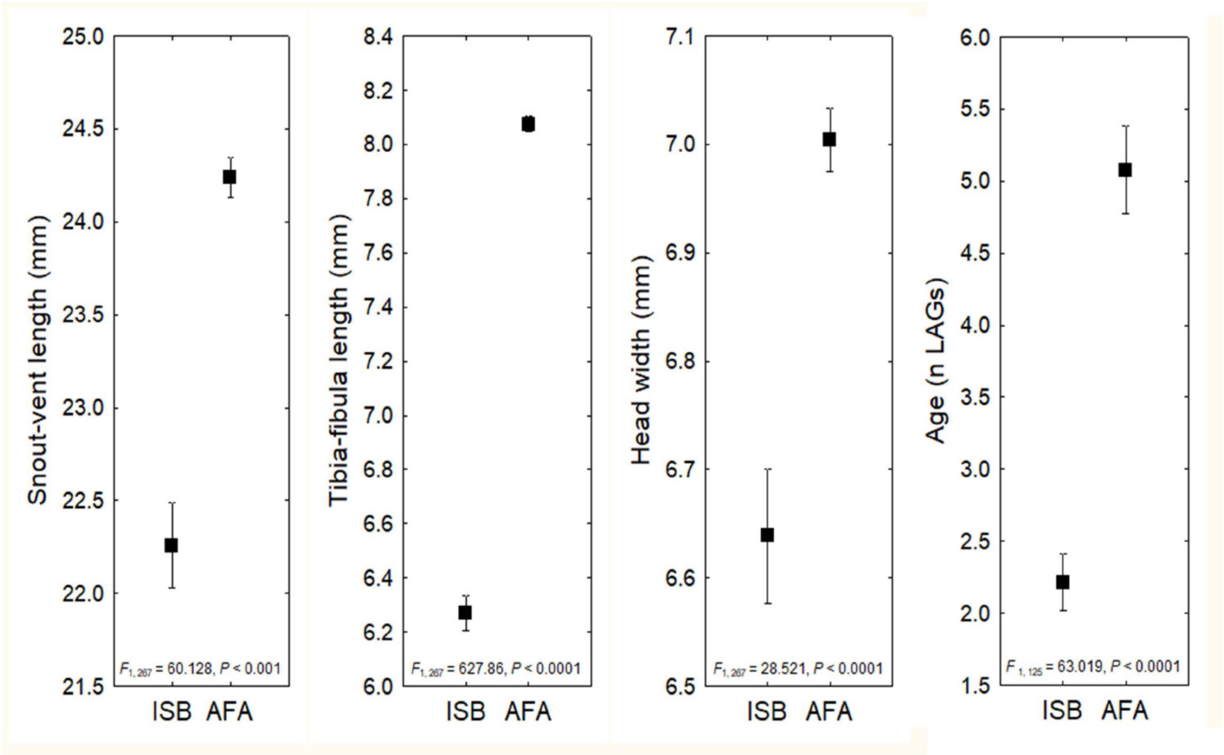

Figure 4. Body size and age differences between Inselberg (ISB, present study) and Atlantic Forest of Argentina (AFA, Marangoni and Baldo, 2023) populations of *Melanophryniscus atroluteus*. Vertical bars denote ± standard errors.

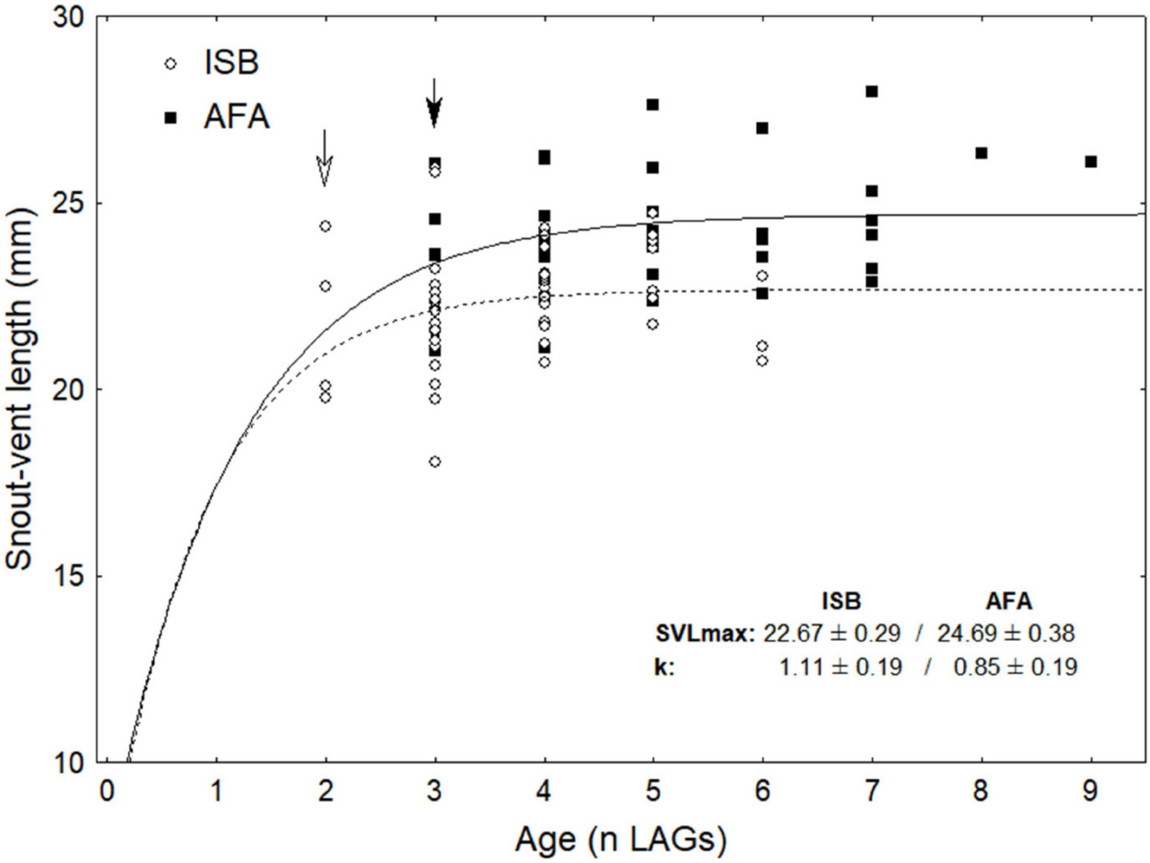

Figure 5. Growth pattern differences between Inselberg (ISB, present study) and Atlantic Forest of Argentina (AFA, Marangoni and Baldo, 2023) populations of *Melanophryniscus atroluteus*. Growth curves fitted to the von Bertalanffy model, for body growth (SVL). Arrows (white = ISB and black = AFA) denote the age at maturity. The parameter estimated by the model (SVLmax and k) are mean ± standard errors.

et al., 2011; Goldberg, 2018; Jiang *et al.*, 2022), food availability (Leips and Travis, 1994; Tracy, 1999), predators risk (Gómez, 2019), abundance (Green and Middleton, 2013), among others. *M. atroluteus* from the ISB showed significant differences in body size, age, and growth compared to the AFA populations analyzed by Marangoni and Baldo (2023). On average, they were smaller, younger, have a shorter lifespan, experience faster growth rates, and reach maturity earlier than the AFA populations (Fig. 4). We suggest that these differences in adult body size could be explained by analyzing the growth pattern and age of sexual maturity. ISB populations demonstrate accelerated growth, reaching sexual maturity earlier, which is associated with a commitment to attaining a smaller adult body size (Fig. 5). This growth pattern, linked to a specific life strategy (Shine, 1989, 1990), has been documented in numerous amphibian species (Halliday and Verrell, 1988; Marangoni *et al.*, 2021). However, we also propose two alternative mechanisms that could account for the divergence of the ISB and AFA populations of *M. atroluteus*. It is since the size and growth performance of terrestrial juveniles may be better explained by the selective force driving phenotypic differentiation during the larval stage, rather than by terrestrial conditions (e.g., Altwegg, 2003). These two mechanisms are not mutually exclusive and could complement each other in shaping the observed differences. One of them is related to the specific environment where reproductive activity and larval development occur. Reproductive activity was recorded in both populations during autumn-winter, but it took place in a lentic environment (temporary ponds) in the ISB population, whereas it occurred in a lotic environment (streams) in the AFA population. On the other hand, the second's aspect is associated with inter-specific relationships within their habitat.

While we did not observe other amphibian species reproducing synchronously at the study site with *M. atroluteus* in the ISB population, the AFA population reproduces in synchrony with two other species of Melanophryniscus (*M. devincenzii* and *M. krauczuki*). As a result, it is common to observe larvae of the three species living in sympatry. Moreover, we frequently observed fighting or calling interactions among males of *M. atroluteus* in active defense of their calling sites. In addition, we also observed several other amphibian species, such as *Psedupaludicola falcipes*, *Physalaemus riograndensis*, *Leptodactylus gracilis*, *Limnomedusa macroglossa*, *Physalaemus cuvieri*, *Physalaemus* aff. *albonotatus* and *Rhinella azarai*, reproducing synchronously at the same sites throughout most of the breeding season or once the spring rains began (Marangoni and Baldo, 2023). The variation in body shapes due to the interaction between environment and phenotype has recently been studied in 17 species of *Melanophryniscus*, which inhabit different habitats (Severgnini *et al.*, 2021). The researchers found that pond-dwelling tadpoles exhibited a higher rate of body shape evolution compared to stream-dwelling tadpoles, although the difference was not statistically significant. Additionally, the authors suggest that differences in the abundance of predators and competitors between environments, could lead to phenotypic variation and the selection of specialized body shapes (see also Huang *et al.*, 2020). Thus, we propose that the differences in adult morphometric traits between ISB and AFA populations could also be explained as carryover effects of the environments and inter- and intra-specific competition experienced during the larval stages (Werner, 1986; Reques and Tejedo, 1997).

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Species-populations/Sex	Meand AgeLAGs (n)			AM			Longevity			Mean SVLmm (n)			Source
	Male	Female	♂+♀	Male	Female	♂+♀	Male	Female	♂+♀	Male	Female	♂+♀	
Hylidae													
Boana puchella	3.07 ± 0.7 (15)	3.93 ± 1.07* (14)		2	3		4	7		37.06 ± 4.57 (15)	43.6 ± 4.4* (14)		1
Boana puchella (AR)			2.60 ± 0.51 (17)			2			3			40.26 ± 2.39 (17)	2
Boana puchella (LA)			3.29 ± 0.85 (19)			2			5			46.34 ± 2.97 (19)	2
Boana puchella (RC)			2.96 ± 0.76 (27)			2			4			43.27 ± 3.27 (27)	2
Boana cordobae	na	na		2	2		5	5		48.01 ± 4.99 (39)	51.27 ± 5.06* (21)		3
Boana punctata	3.46 ± 0.78 (13)	3.25 ± 0.9 ns (24)		2	2		5	5		32.03 ± 3.27 (13)	29.57 ± 3.4* (24)		1
Boana raniceps	3.77 ± 0.87 (22)	3.66 ± 1.5 ns (6)		3	2		6	6		58.44 ± 4.64 (22)	56.53 ± 6.28 ns (6)		1
Nyctimantis siemersi	3.52 ± 0.13 (40)	3.87 ± 0.17 ns (16)		2	3		5	5		69.17 ± 3.56 (59)	74.19 ± 4.14* (28)		4
Ololygon berthae	na	na		1	2		3	3		18.95 ± 1.14 (15)	21.7 ± 1.71* (12)		5
Scinax fuscovarius	3.52 ± 0.95 (23)	2.80 ± 1.24* (20)		2	1		5	6		38.96 ± 4.85 (31)	37.78 ± 4.3 ns (23)		6
Scinax fuscovarius (East)	na	na		na	na		na	na		43.65 2.58	44.35 1.91	43.92 2.35	7
Scinax fuscovarius (Center)	na	na		na	na		na	na		43.87 2.52	44.48 2.99	44.11 2.73	7
Scinax fuscovarius (West)	na	na		na	na		na	na		46.60 2.93	48.62 3.70	47.77 3.41	7
Cicloramphidae													
Odontophrynus americanus	3.73 ± 0.33 (11)	3.14 ± 0.35 ns (14)		1	1		5	5		40.58 ± 4.31 (11)	39.68 ± 3.04 ns (16)		6
Odontophrynus asper (4n)			4.19 ± 0.9 (37)			2			6			47.81 ± 2.21 (37)	8
Odontophrynus cordobae (2n)			3.94 ± 1.02 (34)			2			7			47.2 ± 2.97 (34)	8
Odontophrynus (Hybrids, 3n)			3.76 ± 0.92 (34)			2			6			46.36 ± 2.58 (34)	8

Odontophrynus cf. *barrioi*	2.05 ± 0.20 (20)	2.20 ± 0.21 ns (18)	1	1	3	4	51.46 6 4.64 (20)	52.42 6 4.13 ns (20)	9
Bufonidae									
Melanophryniscus atroluteus (ISB)	3.0 ± 1 (30)	3.87 ± 1.01 ns (23)	2	2	6	6	21.91 ± 1.89 (41)	22.49 ± 1.33* (27)	6
Melanophryniscus atroluteus (AFA)	4.56 ± 1.25 (18)	5.55 ± 1.61* (20)	3	3	7	9	23.63 ± 1.18 (148)	25.76 ± 1.4* (66)	10
Melanophryniscus devincenzii	5.27 ± 1.16 (15)	5.33 ± 0.90 ns (15)	3	4	7	7	23.49 ± 1.16 (67)	27.45 ± 1.54* (30)	10
Melanophryniscus krauczuki	3.06 ± 0.9 (17)	3.5 ± 0.99 ns(18)	2	2	5	5	20.88 ± 1.21 (94)	23.52 ± 1.44* (23)	10
Melanophryniscus rubriventris	4.03 ± 1.53 (32)	3.50 ± 0.52 ns (16)	3	3	10	4	38.75 ± 2.03 (157)	42.76 ± 3.45* (89)	11
Rhinella achalensis	5.27 ± 1.72 (91)	4.89 ± 1.57 ns (114)	1	1	8	11	57.97 ± 6.75 (91)	54.59 ± 6.8* (114)	12
Rhinella arenarum (C1)	1.6 ± 0.7 (15)	1.7 ± 0.6 ns (3)	1	1	3	2	93.8 ± 11.9 (15)	98.3 ± 1.8 ns (3)	13
Rhinella arenarum (C2)	2.2 ± 0.9 (16)	2.7 ± 1.2 ns (3)	1	2	4	4	101.5 ± 7.9 (16)	112.9 ± 3.5* (3)	13
Rhinella arenarum (C3)	1.9 ± 0.8 (15)	2 ± 1.4 ns (3)	1	2	3	3	92.3 ± 12.1 (15)	110.5 ± 4.28* (3)	13
Rhinella arenarum (UL)	2.9 ± 0.9 (14)	3.3 ± 1.2 ns (3)	1	2	4	4	93.5 ± 5.5 (14)	100.8 ± 6.0 ns (3)	13
Rhinella arenarum (SM)	2.7 ± 0.7 (28)	2.9 ± 0.9 ns (14)	1	1	5	4	100.6 ± 7.7 (28)	108.4 ± 9.9* (14)	13
Rhinella arenarum (2000)	2.4 ± 0.9 (105)		1		6		99.4 ± 8.8 (105)		14
Rhinella arenarum (2008)	3.0 ± 0.7 (21)	2.6 ± 0.9 ns (12)	2	2	5	4	101.5 ± 7.1 (39)	108.6 ± 9.6* (15)	14
Rhinella arenarum (2000)	2.4 ± 0.9 (105)		1		6		99.4 ± 8.8 (105)		14
Rhinella arenarum (2008)	3.0 ± 0.7 (21)	2.6 ± 0.9 ns (12)	2	2	5	4	101.5 ± 7.1 (39)	108.6 ± 9.6* (15)	14

Appendix A : AM = age at maturity. Sources: 1 = Fassetta, 2023; 2 = Baraquet *et al.*, 2021; 3= Otero *et al.*, 2017; 4 = Cajade *et al.*, 2013; 5 = Lezcano 2020; 6 = present study; 7 = Goldberg *et al.* 2018; 8= Otero *et al.*, 2021; 9 = Quiroga *et al.*, 2015; 10 = Marangoni and Baldo 2023; 11 = Quinzio 2003; 12 = Sinsch *et al.*, 2001; 13 = Bionda *et al.*, 2018; 14 = Bionda *et al.*, 2015. Descriptive statistics are given as mean ± standard deviation or error (see sources) and sample size (n). Significant differences in SVL and mean age between males and females ($P < 0.05$) are marked with *. ns = not significant. na = not analyzed. ∞ = SVLmax estimated by the von Bertalanffy growth equation.

Trabajo

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Composition, richness, abundance, and association of anuran fauna with the flooded habitats in the Ariri district, eastern Amazon

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ABSTRACT
Local inventories provide primary key information on diversity and distribution of species for conservation purposes. Here we describe the composition and conservation status of anuran fauna in flooded habitats eastern Amazon and evaluate to preference and the level of association of species with the flooded habitats in a poorly known area of the flooded forest present in the Ariri district, state of Amapá, North Brazil. Four nocturnal sampling events were carried out during the rainy season: beginning of the rainy season (March-May 2014 and December 2014-February 2015), mid rainy season (June-August 2014), and end of rainy season (September-November 2014), using visual encounter survey. We identified 29 anuran species. According to flooded habitat preference, the highest richness of species was found in high várzea (22 species, 75.9%, *H'* = 3.091), and 16 species (55.2%) was recorded with high association with flooded habitats. Including all flooded habitats, a greater number of constant species were found, followed by accessory and accidental species. Species accumulation curve showed a tendency toward stabilization of species richness only in the end of rainy season. Our study provides important data on the local anuran fauna and the presence of species typical of flooded and non-flooded habitats demonstrates a certain degree of similarity between species composition, reinforcing the importance of flooded habitats for the preservation of anurans of the Amazonia Forest in north Brazil.

Key words: Anurans; Inventories; Floodplain forests; Conservation; Natural history; Biology.

Introduction

Amazonian flooded forests are riverside areas of high rates of productivity that are flooded during the rainy season (Junk *et al.,* 2012), playing a key role in the regional biodiversity (Ramalho *et al.,* 2018). Representing between 3-4% of the Amazon basin, floodplains include a mosaic of habitats, ranging from the lotic mainstem river to slow-moving channels, ponds, and seasonally flooded fields and forests (Ward *et al.,* 2002). These habitats provide sites for aquatic and semi-aquatic taxa, including invertebrates and vertebrates, that exhibit adaptations and life cycles synchronized to match seasonal flood pulses (Ocock *et al.,* 2014).

This might be especially true for anurans. Many species that inhabit flooded forests (Ramalho *et al.,* 2016; Moraes *et al.,* 2022) in one or more stages of their life cycle, are usually present fidelity to their habitats regulated by the flood pulse (Ramalho *et al.,* 2018). However, despite the ecological importance of Amazonian flooded forests, these habitats are globally threatened by deforestation, fires, hydroelectric dam, invasive species, and pollution (Tockner and Stanford, 2002; Fearnside *et al.,* 2021). The maintenance of these habitats is one of the primary factors determining the presence of anuran species with adaptations to survive in this type of flooded forest and who need these environments to complete the reproductive cycles (Duellman and Trueb, 1994).

The flooded habitats present in the eastern Amazon are poorly known area in the Brazilian

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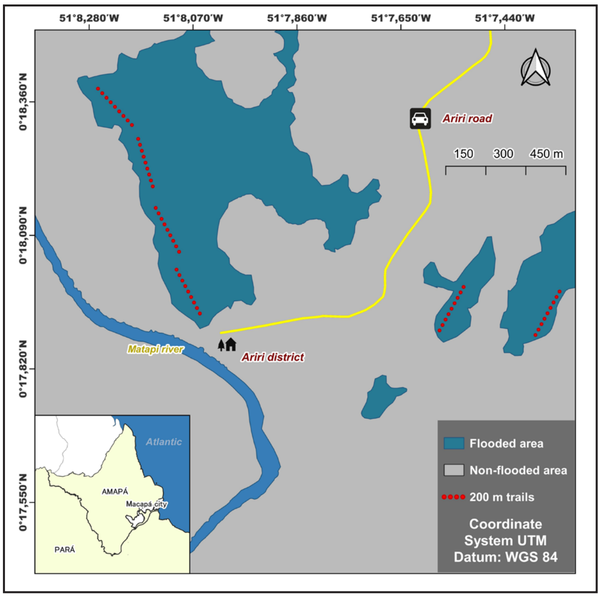


Figure 1. Map showing the study site in the Ariri district, eastern Amazon, municipality of Macapá, Amapá state, Brazil. An illustration of how transects were laid out. Illustrated is the 50 m. distance between the beginning of the first transect and the non-flooded edge.

Amazonia, with several gaps in the knowledge of richness, composition, and geographical distribution of anuran fauna. Some studies on anurans in flooded areas have already been made in Central Amazonia (Waldez *et al.*, 2013; Ramalho *et al.*, 2016; Debien *et al.*, 2019; Moraes *et al.*, 2022), however, these studies in the eastern Amazon are largely underestimated and scarce (Corrêa *et al.*, 2015; Corrêa *et al.*, 2020), with several studies concentrated only in upland forest "*terra firme*" and protected areas (Benício and Lima, 2017; Silva e Silva and Costa-Campos, 2018; Pedroso-Santos *et al.*, 2019; Costa-Campos *et al.*, 2022). In the present study we investigated the composition and conservation status of anuran fauna in flooded habitats in eastern Amazon and evaluated the preference and the level of association of species with the flooded habitats.

Materials and methods

2.1. Study site

The study was undertaken riverside communities known locally as Ariri (0°17'57"N, 51°7'47"W) located north of the municipality of Macapá, Amapá state, in the eastern Brazilian Amazon. The area is flooded by black-water river of the Matapi river basin and is composed of Amazonian savanna with large areas of gallery forest and flooded forest (Silva *et al.*, 2016). The climate is Equatorial (Am) following Koppen's classification (Alvares *et al.*, 2013). The annual accumulated rainfall was 2261.6 mm. with an annual average temperature ranged of 24 °C to 32.1 °C (NHMET, 2022). The region is going through an intense urbanisation process and anthropogenic occupation, whereby most of its forest cover was affected.

2.2. Field procedures

Three sampling sites were selected for data collection. In each study site, a 200 m rectangular transect were built starting at a random point (Rödel and Ernst, 2004).

Equal numbers of transects (6) were surveyed during the diurnal (11:00-16:30h), crepuscular (18:00-19:00h) and nocturnal (19:30-22:00h) periods (see Fig. 1).

Each transect was searched for five days each during the rainy season: beginning of the rainy season (March-May 2014 and December 2014-February 2015), mid rainy season (June-August 2014), and end of rainy season (September-November 2014). The samplings were based on the Visual Encounter Survey (VES) method of Crump and Scott (1994), and Auditory Survey (Zimmerman, 1994), resulted in a total sampling effort of 460 person-hours.

The species were classified according to flooded habitat preference (Junk *et al.*, 2012) in aquatic macrophytes (habitats with presence of aquatic vegetation *Nymphoides indica* (L.) Kuntze and *Salvinia auriculata* Aubl), low várzea (habitats that spend much of the year flooded), high várzea (habitats subjected to shorter flooding periods), and non-flooded (habitats no influenced by the flood pulse - upland forest "*terra firme*") and numbered according to the level of association with the flooded habitats in three groups (Moraes *et al.*, 2022): (1) Amazonian species with geographic distribution encompassing other habitats adjacent to the flooded forest; (2) Amazonian species absent or rare in Amazonian flooded forests; and (3) Amazonian species typical from the flooded forest.

2.3. Data analysis

We analyzed the distribution of the species abundance, using rank abundance curves or Whittaker plots (Whittaker, 1965). Species are ranked in descending order from the highest number to the lowest and then the species are plotted in sequence numbering from the highest to the lowest along the X-axis. The logged transformed number of individuals by using log10 format is plotted at the Y-axis (Magurran, 2011). The curve formed in the plot will follow either four main patterns of rank-abundance curve: log-normal, log-series, broken stick, and geometric series. We analyzed these plots in PAST 2.17 (Hammer *et al.,* 2001).

The frequency of species occurrence and flooded habitat preference was classified according to the constancy index (Dajoz, 1983), which allowed its presence to be considered constant (present in ≥ 50% of samples), accessory (present in 25% ≤ C ≤ 50% of samples), or accidental (present in ≤ 25% of samples).

We generated rarefaction curves based on abundance to assess our sampling efficiency and examine the differences in species diversity across different sampling periods using the iNEXT package of R version 4.3.1 (R Development Core Team, 2020). We plotted four rarefaction/extrapolation curves, with confidence intervals, corresponding to three orders (q = 0, 1, 2) of Hill numbers (Chao *et al.,* 2014) to compare amphibian species diversity between the four sampling periods (and combinations) using 95% confidence intervals based on a 200 bootstrap replications method. The importance of the abundance distribution increases with increasing Hill order. For q=0, the Hill number is the richness, for q=1, it is the (exponential) Shannon entropy and for q=2, it is the inverse Simpson index.

We also estimated species diversity using the Shannon–Wiener index, based on richness and the abundance of species found in each rainy season and flooded habitats. To test for differences among each rainy season and flooded habiats in species diversity of anurans, we performed one-way analysis of variance (ANOVA) coupled with Tukey post-hoc test, using the software PAST 2.17 (Hammer *et al.,* 2001).

Results

3.1. Species composition

We identified 29 anuran species that belong to 14 genera and six families: Aromobatidae (1 species); Bufonidae (3), Hylidae (15), Leptodactylidae (7), Microhylidae (1), and Pipidae (1) (Fig. 2; Fig. 3; Table 1). *Lysapsus bolivianus* was the most abundant species, representing 9.8% of all specimens collected, followed by *Leptodactylus leptodactyloides* (8.4%) and *Sphaenorhynchus carneus* (7.9%).

The Shannon–Wiener index in the study area was similar in each rainy season, ranging from *H'* = 2.534 in the beginning of the rainy season to *H'* = 2.957 in the end of rainy season (Table 1). Species diversity differed significantly among rainy season (ANOVA: $F_{5.36} = 4.74$; p = 0.004), with differences significant (p = 0.017; p = 0.001) between beginning of the rainy season (March-May 2014) and mid rainy season (June-August 2014), and mid rainy season (June-August 2014) and beginning of the rainy season (December 2014-February 2015), respectively.

For the flooded habitats, the higher values of the Shannon–Wiener index was at higher várzea (*H'* = 3.091) and low várzea (*H'* = 3.045) (Table 2). Species diversity differed significantly among flooded habitats (ANOVA: $F_{7.17} = 7.35$; $p < 0.001$), with differences being significant between aquatic macrophytes and low várzea (p = 0.002), and aquatic macrophytes and high várzea ($p < 0.001$).

According to flooded habitat preference, the highest richness of species was found in high várzea (22 species, 75.9%) followed by low várzea (21 species, 72.4%) (Fig. 4). The most species (16 species, 55.2%) was recorded with high association with flooded habitats (Table 2).

3.2. Constancy of occurrence index and rank abundance curve

According to the constancy of occurrence index, the presence of 17 species was constant (58.6%), seven were accessory (24.2%), and five (17.2%) should be considered accidental (see Table 1). Considering the flooded habitat preference, the registered species at low várzea and high várzea were constant (72.4% and 75.9%, respectively), in non-flooded was accessory (44.8%), and in aquatic macrophytes was accidental (27.6%).

The rank abundance curve of frogs shown the broken stick pattern ($\chi2= 15.1$, $p= 0.94$). The presence of dominant species was detected in this curve, with many accidental species represented by singletons (single individual) and doubletons (two individuals). *Scinax fuscomarginatus* and *Pipa pipa* were the singletons and *Scinax x-signatus* and *Trachycephalus typhonius* were the doubleton (Fig. 5).

3.3. Species accumulation curve

Our abundance-based rarefaction curves appeared to reach an asymptote (Fig. 6), indicating that our overall sampling effort, in different seasons was sufficient and that additional species are expected with increased sampling. The results of Hill numbers rarefaction curves revealed that there were slight

Figure 2. Anuran fauna recorded in flooded forest in the Ariri district, eastern Amazon: A) *Allobates femoralis*; B) *Rhinella major*; C) *R.* gr. *margaritifera*; D) *R. marina*; E) *Dendropsophus haraldschultzi*; F) *D. leucophyllatus*; G) *D. walfordi*; H) *Boana lanciformis*; I) *B. raniceps*; J) *Lysapsus bolivianus*; K) *Pseudis paradoxa*; L) *Scinax boesemani*; M) *S. fuscomarginatus*; N) *S. garbei*; O) *S. ruber*.

Figure 3. Anuran fauna recorded in flooded forest in the Ariri district, eastern Amazon: A) *Scinax x-signatus*; B) *Sphaenorhynchus carneus*; C) *S. lacteus*; D) *Trachycephalus typhonius*; E) *Pseudopaludicola boliviana*; F) *Adenomera hylaedactyla*; G) *Leptodactylus fuscus*; H) *L. leptodactyloides*; I) *L. macrosternum*; J) *L. mystaceus*; K) *L. petersii*; L) *L. podicipinus*; M) *Elachistocleis helianneae*; N) *Pipa pipa*.

Table 1. Abundance and constancy of occurrence per species of anurans recorded in flooded forest in eastern Amazon, municipality of Macapá, Amapá state, Brazil, during the beginning of the rainy season (March-May 2014 and December 2014-February 2015), mid rainy season (June-August 2014), and end of rainy season (September-November 2014). The samplings were based on the Visual Encounter Survey (VES) and Auditory Survey (AS). Constancy index, Aci = Accidental; Ace = Accessory; Cons = Constant.

Family/Species	**March-May/2014**		**June-August/2014**		**September-November/2014**		**December/2014-February/2015**		**Constancy index**	**Total**
	VES	AS	VES	AS	VES	AS	VES	AS		
Aromobatidae										
Allobates femoralis (Boulenger, 1884)	2	1	0	0	0	0	0	0	Aci	3
Bufonidae										
Rhinella major (Müller & Helmich, 1936)	8	0	5	0	3	0	4	0	Cons	20
Rhinella gr. *margaritifera*	0	0	2	0	5	0	0	0	Ace	7
Rhinella marina (Linnaeus, 1758)	2	0	2	0	4	0	4	0	Cons	12
Hylidae										
Dendropsophus haraldschultzi (Bokermann, 1962)	0	0	4	0	2	0	0	0	Ace	6
Dendropsophus leucophyllatus (Beireis, 1783)	0	0	3	2	2	0	0	0	Ace	7
Dendropsophus walfordi (Bokermann, 1962)	9	0	5	0	9	5	8	0	Cons	36
Boana lanciformis (Cope, 1871)	0	0	6	0	4	0	0	0	Ace	10
Boana raniceps (Cope, 1862)	4	0	7	0	8	0	4	0	Cons	23
Lysapsus bolivianus Gallardo, 1961	5	2	12	4	8	3	10	4	Cons	48
Pseudis paradoxa (Linnaeus, 1758)	0	2	5	3	0	2	0	4	Cons	16
Scinax boesemani (Goin, 1966)	0	0	2	0	4	0	1	0	Cons	7
Scinax fuscomarginatus (A. Lutz, 1925)	0	0	0	0	0	0	1	0	Aci	1
Scinax garbei (Miranda-Ribeiro, 1926)	2	0	3	0	0	0	0	0	Aci	5
Scinax ruber (Laurenti, 1768)	5	0	3	0	7	0	2	0	Cons	17
Scinax x-signatus (Spix, 1824)	0	0	2	0	0	0	0	0	Aci	2
Sphaenorhynchus carneus (Cope, 1868)	17	1	13	2	4	0	2	0	Cons	39
Sphaenorhynchus lacteus (Daudin, 1800)	4	2	8	3	3	0	0	2	Cons	22
Trachycephalus typhonius (Linnaeus, 1758)	0	0	0	0	2	0	0	0	Aci	2
Leptodactylidae										
Pseudopaludicola boliviana Parker, 1927	0	0	18	0	9	8	2	0	Cons	37
Adenomera hylaedactyla (Cope, 1868)	6	2	3	3	5	0	3	0	Cons	22
Leptodactylus fuscus (Schneider, 1799)	6	1	15	7	5	0	2	2	Cons	38
Leptodactylus leptodactyloides (Andersson, 1945)	0	0	23	0	13	0	5	0	Cons	41

Leptodactylus macrosternum Miranda-Ribeiro, 1926	0	0	1	0	5	0	4	0	Cons	10
Leptodactylus mystaceus (Spix, 1824)	0	0	4	1	2	0	0	0	Ace	7
Leptodactylus petersii (Steindachner, 1864)	6	0	10	3	3	0	0	0	Cons	22
Leptodactylus podicipinus (Cope, 1862)	5	0	12	4	2	4	0	0	Cons	27
Microhilydae										
Elachistocleis helianneae Caramaschi, 2010	0	0	1	0	2	0	0	0	Ace	3
Pipidae										
Pipa pipa (Linnaeus, 1758)	0	0	1	0	0	0	0	0	Aci	1
Species richness	15	26	24	16	---	---				
Shannon-Wiener diversity (H')	2.534	2.918	2.957	2.538	---	---				

differences in observed species richness (q = 0) and diversity (q = 1 e q = 2) across sampling periods.

Considering all 29 species, the number of species ranged from 15 (March-May 2014) to 16 (December 2014-February 2015) at the beginning of the rainy season to 26 in the middle of the rainy season (June-August 2014), and 24 species at the end of the rainy season (September-November 2014) (Fig. 7). The greatest number of individuals (202) was recorded in the mid of the rainy season.

Discussion

The anuran fauna composition in the study area resembles those reported in other studies that were carried out in flooded forest areas in the Central Amazonia (e.g., Höld, 1977; Waldez *et al.,* 2013; Ramalho *et al.,* 2016; Moraes *et al.,* 2022). The data showed that the most representative families in terms of species richness were Hylidae and Leptodactylidae. This result is similar to those of other studies in areas of flooded forest and floating meadows (Upton *et al.,* 2014; Böning *et al.,* 2017). We found a high diversity index caused by a heterogeneously distributed of anurans among the different flooded habitats. This promotes a high species turnover along the flooding gradient and increases regional species diversity (Moraes *et al.,* 2022). Arboreal and cryptozoic species such as *Trachycephalus thyphonius* and *Elachistocleis helianneae* may be detected only when they aggregate for reproduction, after heavy rains. Similarly, species strongly associated with aquatic habitats, such as *Lysapsus bolivianus*, *Pseudis paradoxa*, *Sphaenorhynchus carneus*, *S. lacteus* and *Pipa pipa*, may be absent from areas that periodically dry out.

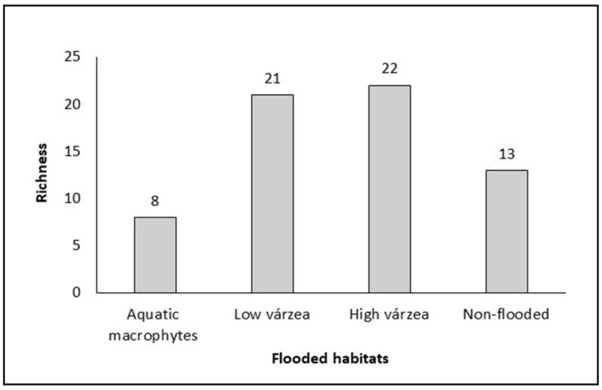


Figure 4. Richness of anuran fauna recorded in flooded forest in the Ariri district, eastern Amazon, according to flooded habitat preference.

Species abundance varied significantly between the rainy season, with most species being more abundant in the mid rainy season (June-August 2014), and end of rainy season (September-November 2014), months with lower precipitation. At these two seasons, although the accumulation of rain (256.1 mm) was twice lower than at the beginning of the rainy season (579.3 mm), the average temperature was higher. In general, anurans in neotropical regions tend to be more abundant in months with higher accumulation of rain and with higher temperatures, as they are clearly important factors driving the reproductive activity (Duellman and Trueb, 1994; Schalk and Saenz, 2016).

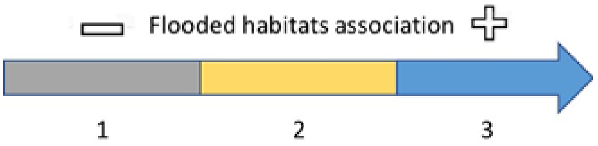
The higher abundance and the presence of anuran species exclusive recorded in the months of lower rainfall, might be related to the lek system found in almost all anuran assemblages, which consequently leads to a high aggregation of species and individuals (Wells, 2007). It is important to highlight that, despite having months with lower precipitation, the flooded habitats remain with water, which can favor the occurrence of anuran species not recorded in the periods with the highest rainfall. On the other hand, the higher abundance of anuran species may be related the influence of diversity structuring mechanisms on the anuran assemblages (Ramalho *et al.,* 2016), the high diversity of macrophytes (Mormul *et al.,* 2013) or the association between plant species and anuran species (Höld, 1977; Upton *et al.,* 2014).

All species considered abundant in the flooded habitats have a widely distributed across Amazonia (Frost, 2022). Except for *Adenomera hylaedactyla* and *Allobates femoralis*, all species were recorded exclusively in flooded habitats. This may be attributed to the association of these species to non-flooded (*terra firme* forests) that are not influenced by seasonal flood pulses (Waldez *et al.,* 2013; Ramalho *et al.,* 2016).

The index of constancy of occurrence showed that a low number of species classified as accidental, because the species *S. fuscomarginatus* and *Pipa pipa* were recorded only in one sample (singletons). Among the species considered accidental are those with explosive breeding (*T. thyphonius* and *E. helianneae*), which reproduce for a few days, often at high densities (Wells, 1977; Sousa and Costa-Campos, 2021), a dependent species of the non-flooded forest (*A. femoralis*), species recorded in association with the bromeliad (*S. fuscomarginatus*, *S. garbei* and *S. x-signatus*), and species strictly aquatic (*P. pipa*), which

Table 2. Flooded habitat preference per species of anurans recorded in flooded forest in the Ariri district, eastern Amazon, municipality of Macapá, Amapá state, Brazil. The colors show the level of association of anurans recorded with the flooded habitats.

Species	Flooded habitat preference			
	Aquatic macrophytes	Low várzea	High várzea	Non-flooded
Adenomera hylaedactyla (Cope, 1868)				▨
Allobates femoralis (Boulenger, 1884)				▨
Rhinella gr. *margaritifera*			▨	▨
Rhinella major (Müller & Helmich, 1936)			▨	▨
Rhinella marina (Linnaeus, 1758)		▨	▨	▨
Scinax ruber (Laurenti, 1768)		▨	▨	▨
Trachycephalus typhonius (Linnaeus, 1758)		▨	▨	▨
Leptodactylus fuscus (Schneider, 1799)			🟨	🟨
Leptodactylus macrosternum Miranda-Ribeiro, 1926		🟨	🟨	🟨
Leptodactylus mystaceus (Spix, 1824)			🟨	🟨
Pseudopaludicola boliviana Parker, 1927			🟨	🟨
Scinax fuscomarginatus (A. Lutz, 1925)			🟨	
Scinax x-signatus (Spix, 1824)			🟨	
Boana lanciformis (Cope, 1871)	🟦	🟦		
Boana raniceps (Cope, 1862)	🟦	🟦	🟦	
Dendropsophus haraldschultzi (Bokermann, 1962)	🟦	🟦	🟦	
Dendropsophus leucophyllatus (Beireis, 1783)		🟦	🟦	
Dendropsophus walfordi (Bokermann, 1962)		🟦	🟦	
Elachistocleis helianneae Caramaschi, 2010		🟦	🟦	
Leptodactylus leptodactyloides (Andersson, 1945)		🟦	🟦	🟦
Lysapsus bolivianus Gallardo, 1961	🟦	🟦		
Leptodactylus podicipinus (Cope, 1862)		🟦	🟦	
Leptodactylus petersii (Steindachner, 1864)		🟦	🟦	
Pseudis paradoxa (Linnaeus, 1758)	🟦	🟦		
Scinax boesemani (Goin, 1966)		🟦	🟦	🟦
Scinax garbei (Miranda-Ribeiro, 1926)		🟦	🟦	
Sphaenorhynchus carneus (Cope, 1868)	🟦	🟦	🟦	
Sphaenorhynchus lacteus (Daudin, 1800)	🟦	🟦		
Pipa pipa (Linnaeus, 1758)	🟦	🟦		
Species richness	8	21	22	13
Shannon-Wiener diversity (H')	2.079	3.045	3.091	2.565



are more difficult to capture with the methodologies used in this study.

The results obtained from the rarefaction curve suggest that the species composition has stabilized in the end of rainy season (September-November 2014), indicating that sampling was sufficient to record most species present in the area, and that new sampling efforts are unlikely to add further species to this species composition. On the other hand, the rarefaction curves in the beginning and middle of the rainy season did not stabilize. These differences are related to the record of rare species in some samples (e.g., *Allobates femoralis*, *Pipa pipa*, *Scinax fuscomarginatus*, *S. garbei* and *S. x-signatus*), which

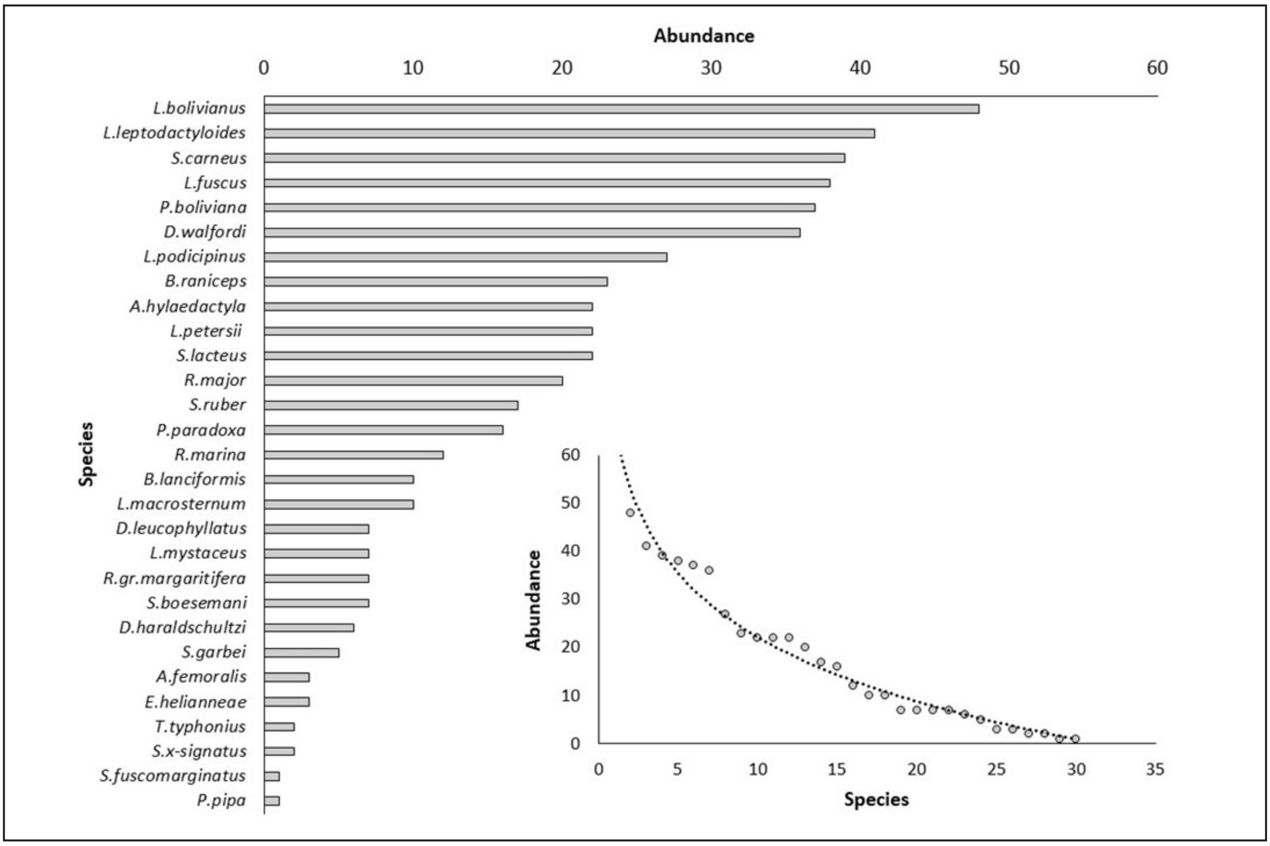


Figure 5. Whittaker diagram showing the relative abundance of the 29 anurans species recorded in flooded forest in the Ariri district, eastern Amazon. Bars represent relative abundance (%), numbers the total abundance of individuals of each species collected and observed.

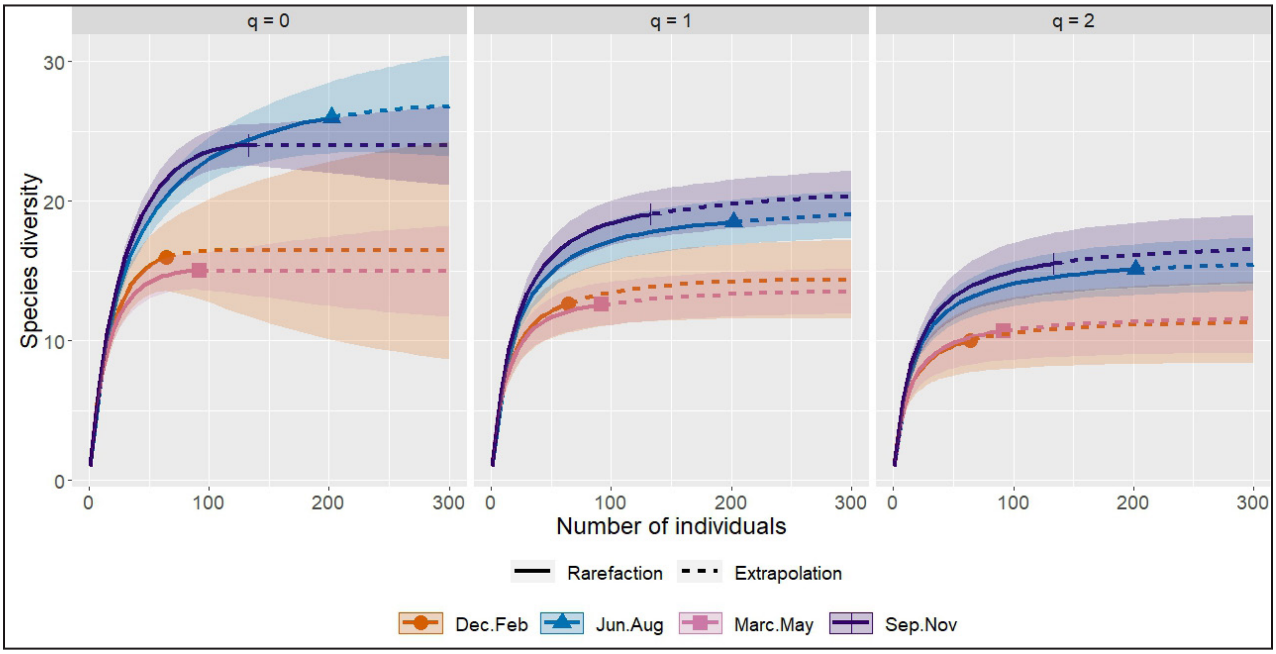


Figure 6. Comparison of the diversity of anuran species in different seasons through rarefaction solid lines and extrapolation dotted lines based on the number of individuals of the anurans species. Species diversity was estimated using Hill numbers: q = 0 (anuran species richness), q = 1 (exponential of Shannon's entropy index) and q = 2 (inverse of Simpson's concentration index).

agrees with the data found by Menin *et al.* (2008).

Although we could assess only the present-day pattern of species composition in the flooded habitats, the urbanization may have decreased species richness and abundance due to habitat degradation caused by anthropogenic activities (Fearnside, 2005), resulting in negligence in the protection and adequate conservation actions of the flooded habitats.

In this sense local inventories provide primary key information on diversity and distribution of species for conservation purposes, and the presence of species typical of flooded and non-flooded areas demonstrates a certain degree of similarity between species composition, reinforcing the importance of flooded habitats for the preservation of anurans of the Amazonia Forest in north Brazil.

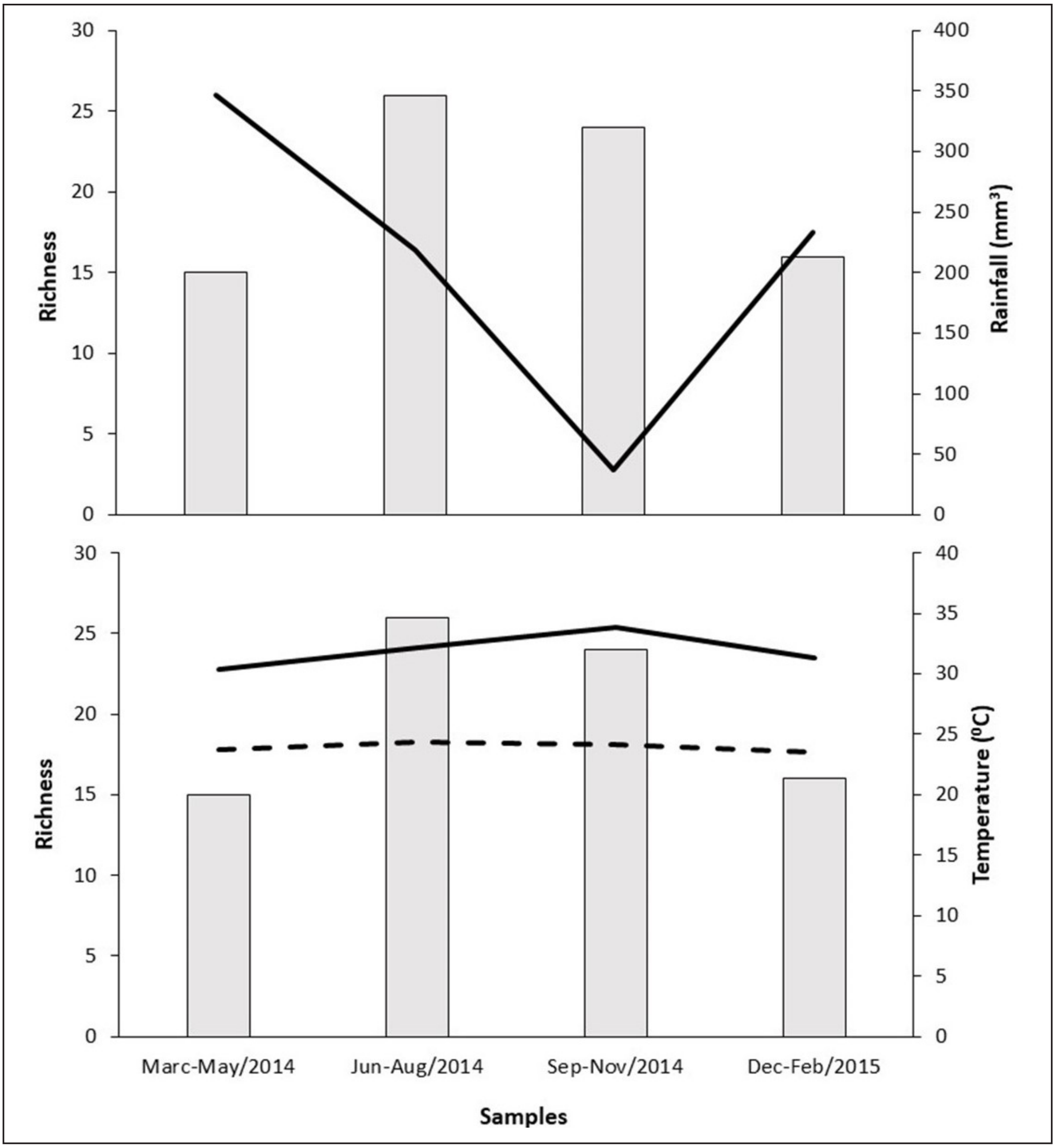


Figure 7. Correlation of anuran fauna richness recorded in an flooded forest in the Ariri district, eastern Amazon, with (A) rainfall (mm3) and temperature (°C).

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Comparing leaf litter anuran diversity in two habitats of an Atlantic Forest area in Rio de Janeiro State, Southeastern Brazil

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ABSTRACT

We compared species richness, relative abundance, density (ind/100 m^2) and biomass per hectare (g/ha) of leaf-litter anurans between forest and restinga habitats in an insular Atlantic Forest area in southeastern Brazil. The local assemblage of leaf litter anurans was composed by nine species (eight of them occurring in the forest and six in the restinga), of which the most abundant were *Ischnocnema parva* and *Adenomera marmorata* in the forest, and *I. parva* and *Dendrophryniscus lauroi* in the restinga. The estimated local density of leaf-litter anurans was 11.7 ind/100m^2 (biomass per hectare = 848.4 g/ha) in forest and 7.3 ind/100m^2 in restinga (biomass per hectare = 262.8 g/ha). Anuran species richness, estimated densities and biomass in the restinga were lower than that of the forest, probably due to the less favorable environmental conditions of restingas for amphibians.

Key Words: Amphibian survey; Frog; Ilha Grande island; Restinga; Tropical forest.

The Brazilian Atlantic Forest is considered one of the world's 36 biodiversity hotspots, having irreplaceable threatened areas with very high species richness and rates of endemism (Mittermeier *et al.*, 2011; CEPF, 2016). This biome is important with respect to amphibian diversity and conservation (Hrdina and Romportl, 2017) because approximately 625 amphibian species are known to occur within its limits, and most of them are endemic (Rossa-Feres *et al.*, 2017). Nevertheless, new species are still being steadily described from this biome (e.g., Cruz *et al.*, 2019; Silva *et al.*, 2020; Nunes *et al.*, 2021).

While assemblages of leaf litter frogs have been relatively well-studied in forests within the Atlantic Forest domain (e.g., Giaretta *et al.*, 1999; Dixo and Martins, 2008; Siqueira *et al.*, 2009; Santos-Pereira *et al.*, 2011; Vagmaker *et al.*, 2020), they have been less studied in other ecosystems within this biome, such as the restingas, which are open coastal habitats formed by sand dunes covered by herbaceous, shrubby and low arboreal vegetation (Suguio and Tessler, 1984; Araújo, 1992). Nevertheless, studies in restinga areas have increased in recent years, expanding the knowledge about amphibian assemblages from this ecosystem (e.g., Oliveira & Rocha, 2015; Gondim-Silva *et al.*, 2016; Mageski *et al.*, 2017; Oliveira *et al.*, 2017, 2020; Carmo *et al.*, 2019; Martins *et al.*, 2019). In general, anuran species composition in restinga assemblages represents a subset of the species available in the forests that border the Brazilian coast (Rocha *et al.*, 2008; Silva *et al.*, 2008), though some species are apparently endemics of the restinga ecosystem (Carvalho-e-Silva *et al.*, 2000; Peloso *et al.*, 2012; Cardozo *et al.*, 2018).

In the Atlantic Forest of the state of Rio de Janeiro, 201 amphibian species are known to occur, including 197 anurans and four caecilians, with a high rate of endemism (Dorigo *et al.*, 2018). Ilha Grande is a large continental island at the southern coast of the state of Rio de Janeiro that is home to 34 anuran species (Rocha *et al.*, 2018). Most of the published data about the composition and structure of anuran assemblages in this island have originated from its forested areas (Rocha *et al.*, 2000, 2001, 2018; Van

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Sluys *et al.*, 2007; Bittencourt-Silva and Silva, 2013; Goyannes-Araújo *et al.*, 2015), which comprise most of the island's vegetation cover (Alho *et al.*, 2002). Herein, we compare the leaf litter anuran assemblages between forest and restinga habitats in Praia do Sul State Biological Reserve, on the southern coast of Ilha Grande, including data on species composition and relative abundance. We expected to record lower species richness and abundance of leaf litter anurans in restinga in comparison to the forest, since the restinga is an open and relatively xeric ecosystem characterized by sandy soils with high salt concentration (Franco *et al.*, 1984; Suguio and Tessler, 1984). In addition, we predicted that most leaf litter anuran species found in the restinga would also occur in the forest.

Ilha Grande is a continental island of ca. 19,000 ha on the southern coast of Rio de Janeiro State, southeastern Brazil. The present study was carried out within the Praia do Sul State Biological Reserve (Reserva Biológica Estadual da Praia do Sul, hereafter RBEPS; 3,502 ha), located at the seaward side of the island, with fieldwork being done in both forest (23° 10' 25"S, 44° 18' 45" W) and restinga (23° 10' 29" S, 44° 17' 56" W) habitats (see Rocha *et al.*, 2018). The climate on Ilha Grande is wet and warm, with average annual temperature of 21°C and annual rainfall of 2,200 mm (INEA, 2013).

Surveys were carried out in forest (at 10-100 m a.s.l.) and restinga (near sea level) areas during the wet season (September and October 2012 and October 2013), to eliminate potential differences caused by seasonal effects. Habitats were not sampled simultaneously, that is, our team sampled the habitats on alternate days in each field expedition. We sampled leaf litter frogs using 4 x 4 m quadrats (plot sampling; Jaeger and Inger, 1994), located at least 50-m apart, using 40 plots (quadrats) in each habitat type (640 m^2), totaling 1280 m^2 of sampled area. Total number of plots was divided between years (20 plots by habitat in each year) and none of the plots was established at exactly the same sampling point of a previous one. The corners of each plot were marked with wooden stakes and the area was enclosed with a 75 cm high soft mesh fence that was buried into or attached to the ground. After sunset, 3-4 people carefully searched each plot on their hands and knees (side by side) for about half an hour using headlamps. During the searches, leaves, branches and stones were overturned using hand rakes, and rock crevices and fissures among tree roots were also checked for frogs.

All anurans encountered within a given plot were identified to species level, weighed (to the nearest 0.2 g using Pesola®) and most of them were released after the crew finished searching the area. Individuals collected during the study were euthanized with a topical anesthetic gel (lidocaine 5%), fixed in 10% formalin solution, and preserved in 70% ethylene alcohol. We estimated the overall density of anurans per unit of forest floor (frogs/100 m^2) by dividing the total number of anurans found by the total area sampled in each habitat (640 m^2) and multiplying the result by 100 (see Allmon, 1991). The total biomass (g) and the biomass of leaf litter anurans per hectare (g/ha) were also extrapolated. We followed Haddad et al. (2013) to define each anuran species' reproductive mode.

The comparison of species richness between areas was undertaken by inference of overlapping 95% confidence intervals (MacGregor-Fors and Payton, 2013) for individual-based rarefaction curves performing 1,000 randomizations without replacement using the program EstimateS 9.1.0 (Colwell, 2013). Moreover, we compared alpha diversity measures between areas using "true diversity" (Jost, 2006) computed in the R package *hillR* (Li, 2018), using Shannon entropy. We calculated Hill-Shannon diversity considering that it responds strongly to both very high and very low rarity values, emphasizes neither rare nor common species, and has been considered a good choice for characterizing variation in biodiversity (Roswell *et al.*, 2021). Species composition similarity between areas was quantified through the Sørensen index (Magurran and McGill, 2011).

Voucher specimens of all anuran species were deposited at the amphibian collection of Museu Nacional (MNRJ), Universidade Federal do Rio de Janeiro (Appendix 1).

We recorded nine leaf litter anuran species during the study at the RBEPS, with eight of them being recorded in forest and six in restinga (Table 1; Fig. 1). The nine species recorded by us represent half of the 18 species known to occur at the RBPS (see Rocha *et al.*, 2018). Most of those species have direct development with terrestrial eggs or use lentic water bodies for reproduction (Nunes-de-Almeida *et al.*, 2021). Most of the species that we did not record were either arboreal or rheophilic, and thus unlikely to be found on the leaf litter. However, two of the unrecorded species (*Ischnocnema* cf. *guentheri*

Table 1. Reproductive modes (RM), number of individuals (NI), estimated density (frogs/100m2; between parenthesis), total biomass (g) and biomass per hectare (g/ha) recorded for each species of anuran recorded in the leaf litter of two Atlantic Rainforest areas (forest and restinga) within the Praia do Sul State Biological Reserve, Ilha Grande, in southeastern Brazil. Reproductive modes (according to Haddad and Prado 2005): 1 = Eggs and exotrophic tadpoles in lentic water; 2 = Eggs and exotrophic tadpoles in lotic water; 6 = Eggs and exotrophic tadpoles in water in tree holes or aerial plants; 8 = Eggs and endotrophic tadpoles in water in tree holes or aerial plants; 11 = Foam nest floating on pond; exotrophic tadpoles in ponds; 23 = Direct development of terrestrial eggs; 28 = Foam nest on the humid forest floor; subsequent to flooding, exotrophic tadpoles in ponds.

Species	Forest			Restinga		Total NI (frogs/100m2)
	RM	NI (frogs/100m2)	B (g)(g/ha)	NI (frogs/100m2)	B (g)(g/ha)	
Brachycephalidae						
Ischnocnema bolbodactyla (Lutz, 1925)	23	1 (0.2)	0.3 (4.7)			1 (0.1)
Ischnocnema parva (Girard, 1853)	23	39 (6.1)	11.6 (181.3)	15 (2.3)	5.9 (92.2)	54 (4.2)
Bufonidae						
Dendrophryniscus lauroi Miranda-Ribeiro, 1926 *a*	8	1 (0.2)	0.1 (1.6)	15 (2.3)	2.8 (43.8)	16 (1.3)
Rhinella ornata (Spix, 1824)	1/2	1 (0.2)	9.1 (142.2)			1 (0.1)
Craugastoridae						
Haddadus binotatus (Spix, 1824)	23	5 (0.8)	13.9 (217.2)			5 (0.4)
Hylidae						
Scinax sp. (gr. *perpusillus*)	6			1 (0.2)	0.1 (1.6)	1 (0.1)
Leptodactylidae						
Adenomera marmorata Steindachner, 1867	32	22 (3.4)	12.6 (196.9)	5 (0.8)	2.7 (42.2)	27 (2.1)
Physalaemus signifer (Girard, 1853)	11/28	4 (0.6)	5.7 (81.9)	1 (0.2)	1.0 (15.6)	5 (0.4)
Microhylidae						
Chiasmocleis lacrimae Peloso, Sturaro, Forlani, Gaucher, Motta and Wheeler, 2014	1	2 (0.3)	1.0 (15.6)	10 (1.6)	4.7 (73.4)	12 (0.9)
Total		75 (11.7)	54.3 (848.4)	47 (7.3)	17.2 (268.8)	122 (9.5)

a – Listed in Rocha et al. (2018) as *Dendrophryniscus brevipollicatus* (see Cruz *et al.*, 2019).

and *Leptodactyus flavopictus*) are actually ground-dwellers, and their absence in our plot samples may suggest that they are locally rare.

Individual-based rarefaction curves from forest and restinga showed that species richness of restinga fell within the 95% confidence interval of expected species richness of forest based on random subsamples of 47 individuals (Fig. 2), rejecting the first of our predictions, i.e., that the forest assemblage would be richer than that of the restinga. The forest assemblage had strong dominance by a small number of species and a long 'tail' of rare species, whereas the restinga assemblage had minor variation in rarity, and lower species richness (Fig. 3). We found a lower value of true diversity for the forest assemblage (3.9) than for the restinga assemblage (4.8). Diversity metrics summarize rank-abundance distributions to enable quantitative comparisons; in this way, true diversity measures the mean rarity of the species in the sample, and the community consisting of species that are, on average, rarer has higher diversity (Roswell *et al.*, 2021).

Index of similarity between forest and restinga was 0.7, with five shared species (Table 1). The species recorded in only one habitat type presented low abundances [forest: *Ischnocnema bolbodactyla*, N = 1; *Rhinella ornata*, N = 1; *Haddadus binotatus*, N = 5; restinga: *Scinax* sp. (gr. *perpusillus*), N = 1]. As we expected, almost all species recorded in the restinga were also sampled in the forest (with only one exception). Indeed, anuran species recorded in restinga areas usually have a wide geographic distribution and tend to also occur in other ecosystems of the Atlantic Forest domain (Carvalho-e-Silva *et al.*, 2000; Van Sluys *et al.*, 2004; Bastazini *et al.*, 2007). *Scinax* sp. (gr. *perpusillus*) was found by us only in the restinga,

Figure 1. Individuals representing the nine species recorded during fieldwork within the Praia do Sul State Biological Reserve, Ilha Grande, in southeastern Brazil. A) *Ischnocnema bolbodactyla*; B) *Ischnocnema parva*; C) *Dendrophryniscus lauroi*; D) *Rhinella ornata*; E) *Haddadus binotatus*; F) *Scinax* sp. (gr. *perpusillus*); G) *Adenomera marmorata*; H) *Physalaemus signifer*; I) *Chiasmocleis lacrimae*. Photos by F. B. S. Telles (A, F) and M. Santos-Pereira (B, C, D, E, G, H, I).

and it has also been recorded in other coastal areas near the RBEPS (Rocha *et al.*, 2018), probably being a species typical of that type of habitat. Because most anurans found by us at low altitudes inside the forest generally have direct development or use lentic water bodies for reproduction (Haddad and Prado, 2005; Nunes-de-Almeida *et al.*, 2021), these species can find favorable microclimatic conditions available on the leaf litter of restinga environments (e.g., Telles *et al.*, 2012; Carmo *et al.*, 2019). The availability of spawning sites, especially wetland habitats, is known to affect species composition in restinga areas (Oliveira *et al.*, 2017).

Despite *Ischnocnema bolbodactyla*, *Rhinella ornata* and *Haddadus binotatus* having been found only in the forest during our study, they have also been recorded in some open areas at Ilha Grande (Rocha *et al.*, 2018), and in other restinga areas of Southeastern Brazil (e.g., Carvalho-e-Silva *et al.*, 2000; Van Sluys *et al.*, 2004; Telles *et al.*, 2012; Carmo *et al.*, 2019). Thus, because these species had low abundance (or low detectability) locally, especially *I. bolbodactyla* and *R. ornata*, and both studied areas constitute an environmental continuum, the similarity of leaf litter anuran species composition between habitats at the RBEPS is probably even greater than our estimates suggest.

In the forest, 75 individual frogs were sampled, with *Ischnocnema parva* (N = 39; density = 6.1 ind/100 m^2) and *Adenomera marmorata* (N = 22; density = 3.4 ind/100 m^2) being the most abundant species, together accounting for *ca*. 80% of the individuals recorded in that habitat. In another forest area on Ilha Grande, at the Pico do Papagaio mountain, these two species were also the most common, representing *ca*. 65% of the individuals sampled in the leaf litter (Goyannes-Araújo *et al.*, 2015). In forested areas near the Dois Rios village, these species were also among the most abundant leaf litter anurans, together with *Brachycephalus didactylus* and *Cycloramphus* (= *Zachaenus*) *parvulus* (Rocha *et al.*, 2001; Van Sluys *et al.*, 2007). These anurans may be more widely distributed within the forest in Ilha Grande because they are not dependent on water bodies for reproduction (Haddad and Prado, 2005; Haddad *et al.*, 2013). For example, individuals of *A. marmorata* make foam nests in subterranean constructed chambers, where eggs are deposited and endotrophic tadpoles complete development (Haddad and Prado, 2005). The genus *Ischnocnema* belongs to the Brachycephaloidea superfamily (*sensu* Padial *et al.*, 2014), whose members lay eggs on the forest floor and have direct development. Direct-developing anurans are abundant in the leaf litter community of different areas in Neotropical leaf litter anuran assemblages (e.g., Scott 1976; Lieberman 1986; Fauth *et al.*, 1989; Giaretta *et al.*, 1999), possibly due to their independence from water bodies for reproduction.

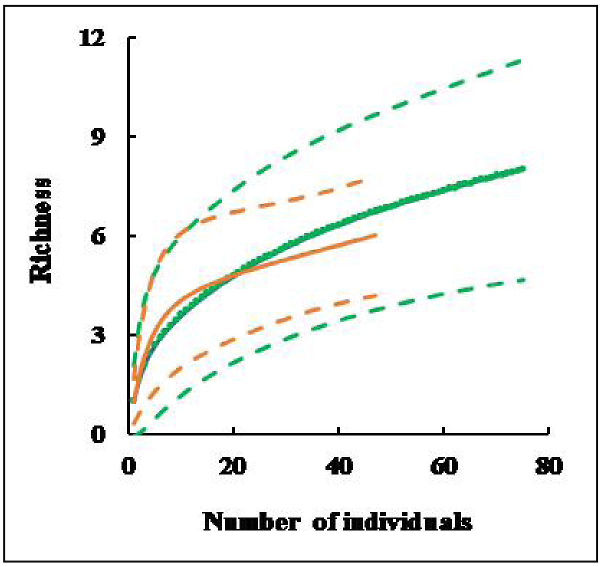

Figure 2. Individual-based species-rarefaction curves, with respective 95% confidence intervals (dotted lines), of two leaf litter anuran assemblages sampled in forest (in green) and restinga (in orange) within the Praia do Sul State Biological Reserve, Ilha Grande, in southeastern Brazil.

In the restinga, 47 individuals were collected, with *I. parva* and *Dendrophryniscus lauroi* (N = 15; density = 2.3 ind/100m^2 in both cases) as the most abundant species, followed by *Chiasmocleis lacrimae* (N = 10; density = 1.6 ind/100m^2). *Ischnocnema parva* (a species with direct development and terrestrial eggs) and *D. lauroi* (which deposits eggs in phytotelmata, where endotrophic tadpoles develop; Cruz *et al.*, 2019) comprised *ca*. 65% of all anurans sampled in that environment. Phytotelm-breeding anurans such as *D. lauroi* occur mainly in bromeliads, which are important in the restinga ecosystem as they are used as shelter, feeding, breeding, and courtship sites for several anuran species (e.g., Bastazini *et al.*, 2007; Silva *et al.*, 2008; Telles *et al.*, 2012). Bromeliads are common and widely distributed in the restinga at RBEPS (Araújo and Oliveira, 1988), which explains the relatively high local abundance of *D. lauroi*. During our study, we found *D. lauroi* and the treefrog *Scinax* sp. (gr. *perpusillus*) in the leaf litter, but we do not know if they were actually dwelling on the forest floor (e.g., for foraging) or were just moving between perches.

The total biomass of leaf litter anurans in the forest was 54.3 g (extrapolated anuran biomass per hectare = 848.4 g/ha), with *H. binotatus* (13.9 g; 217.2 g/ha), *A. marmorata* (12.6 g; 196.9 g/ha) and *I. parva* (11.6 g; 181.3 g/ha) presenting the greatest biomass (Table 1). The total biomass of leaf litter anurans in the restinga was 17.2 g (extrapolated anuran biomass per hectare = 262.8 g/ha), with *I. parva* (5.9 g; = 92.2 g/ha) and *Chiasmocleis lacrimae* (4.7 g; = 73.4 g/ha) presenting the greatest biomass values (Table 1).

The estimated anuran density in the forest floor was high compared to some other studies of leaf litter-frog assemblages from various Atlantic Forest areas in Rio de Janeiro State (e.g., Rocha *et al.*, 2007, 2011, 2013; Almeida-Gomes *et al.*, 2008, 2010; Siqueira *et al.*, 2011a). Although the density estimates were near between the two types of habitats at the RBEPS (total density of 11.7 ind/100 m^2 in forest and of 7.3 ind/100m^2 in restinga), the estimated anuran biomass per unit of restinga (262.8 g/ha) was almost one-third of that of the forested area (848.4 g/ha). This may indicate that the forest assemblage tends to contain more larger individuals than that of the restinga, although more data are needed to confirm this. The estimated anuran biomass per unit of area in forest ecosystems from other sites of Rio de Janeiro State, in the municipalities of Guapimirim (938.4 g/ha; Rocha *et al.*, 2011) and Cachoeiras de Macacu (684.2 g/ha; Siqueira *et al.*, 2009), as well as another area in Ilha Grande (1150 g/ha; Rocha *et al.*, 2001), was close to what we found in the forest at RBEPS (848.4 g/ha). The latter value was also very similar to the one estimated by for an Atlantic Forest area in the state of Paraná, in southern Brazil (842.4 g/ha), although the estimated frog density in that area was only 3.7 ind/100m^2 (Santos-Pereira *et al.*, 2011).

All species found during our study are considered Atlantic Forest endemics, which was expected given the high degree of endemism for anurans in this biome (Haddad *et al.*, 2013; Rossa-Feres *et al.*, 2017). The record of *Scinax* sp. is referable to an apparently undescribed species of the *S. perpusillus* group (Bittencourt-Silva and Silva, 2013; Rocha *et al.*, 2018), which has so far been only reported from Ilha Grande and is possibly endemic to that island. In terms of conservation, most species recorded at the RBEPS are categorized as Least Concern (IUCN, 2021), except for *Chiasmocleis lacrimae*, which is considered "Endangered" (Pimenta and Peixoto, 2004). However, this species may need an update in its conservation status, as it has not been reassessed for more than 15 years and, since then, its northernmost populations have been found to represent distinct species (Tonini *et al.*, 2014; Forlani *et al.*, 2017), thus reducing the extent of the geographic distribution of *C. lacrimae*. None of the species found in the present survey is listed as threatened in the Red Book of Threatened Brazilian Fauna (ICMBio/MMA, 2018).

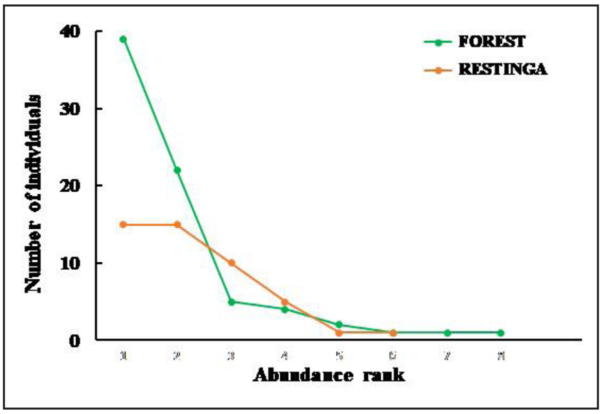

Figure 3. Observed rank-abundance distributions for the frog samples from two habitats (forest, in green, and restinga, in orange) within the Praia do Sul State Biological Reserve, Ilha Grande, in southeastern Brazil.

Although we have found higher absolute values of species richness in forest than in restinga, both rarefaction curves and true diversity indexes showed that the restinga assemblage contains a relatively high anuran species diversity. Anuran biomass production was more than three times smaller in restinga than in forest, which may suggest that productivity does not explain differences in species diversity between habitats, and that abiotic factors probably mediate the positive interrelationship between species diversity and ecosystem functioning.

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Appendix I

Voucher specimens of species collected in the present study deposited at the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ).

BRACHYCEPHALIDAE: *Ischnocnema bolbodactyla*: MNRJ 89141; *Ischnocnema parva*: MNRJ 93869-72, 93882. BUFONIDAE: *Dendrophryniscus lauroi*: MNRJ 93863-66, 93880; *Rhinella ornata*: MNRJ 93876. CRAUGASTORIDAE: *Haddadus binotatus*: MNRJ 93867-68, 93881. HYLIDAE: *Scinax* sp. (gr. *perpusillus*): MNRJ 93883. LEPTODACTYLIDAE: *Adenomera marmorata*: MNRJ 93860-62, 93878-79; *Physalaemus signifer*: MNRJ 93873-75. MICROHYLIDAE: *Chiasmocleis lacrimae*: MNRJ 93884-90.

Nota

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Análisis morfológico de un caso de polimelia en *Rhinella dorbignyi* (Anura: Bufonidae)

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ABSTRACT
Morphological analysis of a polymelia case in *Rhinella dorbignyi* (Anura: Bufonidae). This note describes the morphology of a supernumerary left hind limb in an adult female of *Rhinella dorbignyi* found in Atalaya (Magdalena, Buenos Aires). The case was studied through radiographs of the specimen in life, and once dead, the morphological approach included dissection to observe the appendicular muscles and diaphanization with double staining to observe the skeleton. The notable features of the supernumerary limb are: (1) absence of finger I, (2) shorter length of all portions of the limb and less robustness than in a normal hind limb, (3) anomalous junction with the pelvic girdle by means of a well marked femoral head with limb-type features associated to bipedal gait, (4) femur without muscles, and (5) tibia and fibula completely fused in midline. In short, the supernumerary limb is a reduced duplication of the left hind limb.

Key Words: Garden Toad; Ectopic Limbs; Abnormalities.

RESUMEN
Esta nota describe la morfología de un miembro posterior izquierdo supernumerario en una hembra adulta de *Rhinella dorbignyi* hallada en Atalaya (Magdalena, Buenos Aires). El caso se estudió mediante radiografías del ejemplar vivo, y una vez muerto, se realizó un abordaje morfológico que incluyó disección para observación de musculatura apendicular y diafanización con doble tinción para observar los rasgos esqueléticos. Se destaca del miembro supernumerario: (1) ausencia de dedo I, (2) menor longitud de todas las porciones del miembro y menor robustez que en un miembro posterior normal, (3) unión anómala con la cintura pélvica mediante una cabeza femoral bien marcada con las características de miembros asociados a marcha bípeda, (4) fémur sin músculos y, (5) tibia y fíbula completamente fusionados en la línea media. En definitiva, el miembro supernumerario es una duplicación, reducida, del miembro posterior izquierdo.

Palabras claves: Sapito de Jardín; Miembros Ectópicos; Anormalidades.

La polimelia es la presencia de miembros supernumerarios y ha sido reportada para todos los grandes grupos de tetrápodos: anfibios (Das y Mohanty-Hejmadi, 2000; González-Fernández *et al*., 2004; Duque-Amado *et al*., 2020), reptiles (Cupul-Magaña *et al*., 2014), aves (Anderson *et al*., 1985; Lleonart *et al*., 2010) y mamíferos (García-Espinosa *et al*., 2002; Montes *et al*., 2012; Araujo *et al*., 2019).

La infección con parásitos, altos índices de endogamia, exposición a contaminantes ambientales y a niveles elevados de radiación ultravioleta se han propuesto como posibles causas de esta anormalidad (Huchzermeyer, 2003; Barragán-Ramírez y Navarrete-Heredia, 2011; Velo-Antón *et al*., 2011; Rothschild *et al*., 2012). Se ha asumido que la polimelia disminuye la probabilidad de supervivencia en individuos afectados (Araujo *et al*., 2019) ya que puede causar alteraciones en la locomoción normal, en la búsqueda de alimento (Sparling *et al*., 2010), evasión de predadores (Johnson *et al*., 1999) y en el proceso reproductivo (Lleonart *et al*., 2010).

La presencia de polimelia en anuros ha sido documentada desde el siglo XIX (Rothschild *et al*., 2012) siendo uno de los primeros registros el de un ejemplar de *Pelobates fuscus* con un miembro extra sobre su tórax. Los reportes siguientes abarcan múltiples familias y llegan incluso a mencionar casos de individuos con hasta 10 miembros extra (Session y Ruth, 1990; Lannoo, 2009; Rothschild *et al*., 2012). En Argentina existen pocos reportes

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de polimelia: la primera observación realizada fue hecha en *Rhinella arenarum* (como *Bufo arenarum*) por Marelli (1942) quien reportó un individuo con dos miembros supernumerarios, dato no publicado y citado por Gaggero (1959) quien también reporta un individuo de la misma especie con un miembro extra. Peri y Williams (1998) hallaron un individuo de *Pseudis platensis* con dos miembros posteriores extra; Fabrezi (1999) reportó una larva de *Lepidobatrachus llanensis* con un miembro anterior duplicado asociado a infección por trematodos y; finalmente, Peltzer *et al.* (2011) y Lajmanovich *et al.* (2012) reportaron individuos de *Rhinella dorbignyi* (como *R. fernandezae*) y *R. arenarum* con miembros supernumerarios, sugiriendo una asociación con exposición a agroquímicos.

El objetivo de esta nota es reportar el hallazgo y describir la morfología de un miembro posterior izquierdo supernumerario en *R. dorbignyi* con énfasis en la organización de sus componentes morfológicos.

Una hembra adulta de *R. dorbignyi* fue hallada en una zanja peridomiciliaria, durante la primera semana de octubre del año 2018 en la localidad de Atalaya (partido de Magdalena, provincia de Buenos Aires, Argentina). Al capturarla pudimos constatar la presencia de un miembro extra situado entre sus patas traseras (Fig. 1). Su peso al momento de la captura era 19,88 g y su longitud hocico cloaca 6,53 cm. El resto del aspecto físico era normal para la especie. El animal fue trasladado al laboratorio para su estudio con permiso del Ministerio de Asuntos Agrarios de la Provincia de Buenos Aires (acta 22500-41820/17 - disposición nº 73). Allí se constató, en una primera inspección, que el miembro supernumerario constaba de cuatro dedos y era más corto y delgado respecto a los miembros posteriores normales. También se observó, que si bien, el miembro extra no participaba de la locomoción, tampoco resultaba un obstáculo para la misma.

Mediante radiografías del ejemplar vivo se pudo observar estilopodio y zeugopodio de apariencia normal y para el autopodio pudo verificarse la presencia de metatarsos y falanges, sin más detalle. La radiografía también reveló que el miembro extra presentaba una unión anómala entre la cabeza femoral y la base del isquion (Fig. 2A).

Se realizó abordaje morfológico que incluyó disección para observar la musculatura apendicular del miembro extra y luego diafanización y doble tinción (Taylor y Van Dyke, 1985) para observar y medir sus huesos y cartílagos tanto del miembro extra como del miembro posterior izquierdo. Para las mediciones se utilizó una lupa Zeiss Stemi SV11 equipada con grilla para medición (precisión 0,1 mm). El ejemplar con sus partes diafanizadas se encuentra alojado en la colección Herpetológica del Museo de La Plata (A5913).

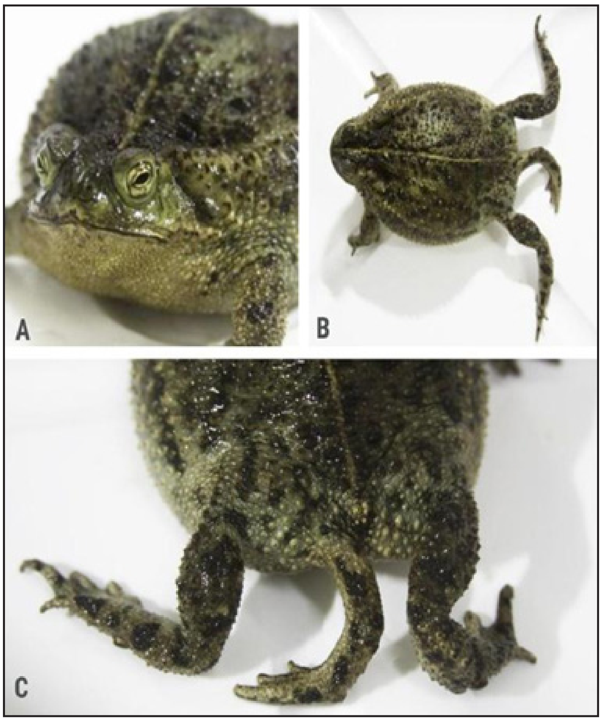

Figura 1. Vista general de la cabeza de la hembra de *Rhinella dorbignyi* estudiada (A), vistas dorsal de todo el ejemplar (B) y posterodorsal en detalle de los miembros posteriores incluyendo el ectópico (C).

En el proceso de disección se observó que todos los órganos eran de apariencia normal y detectamos una gran masa de oocitos maduros en la cavidad.

Del análisis realizado podemos decir que, al estar el miembro supernumerario localizado sobre la zona media posterior y encontrarse leventemente lateralizado, sería una duplicación del miembro izquierdo. Ingresa al cuerpo entre la cara interna del muslo izquierdo y la porción izquierda de la cintura pélvica, entre los músculos *piriformis*, *semimebranoso* y *gracilis minor*, sin establecer contacto óseo entre la cabeza del fémur y el acetábulo mediante una unión flotante en la que intervienen un músculo piriforme propio más un ligamento corto (Fig. 2C-D). La cabeza proximal del fémur forma una epífisis articular medial marcada (Fig. 2B), del tipo de las conocidas para otros grupos de marcha bípeda (aves, primates,

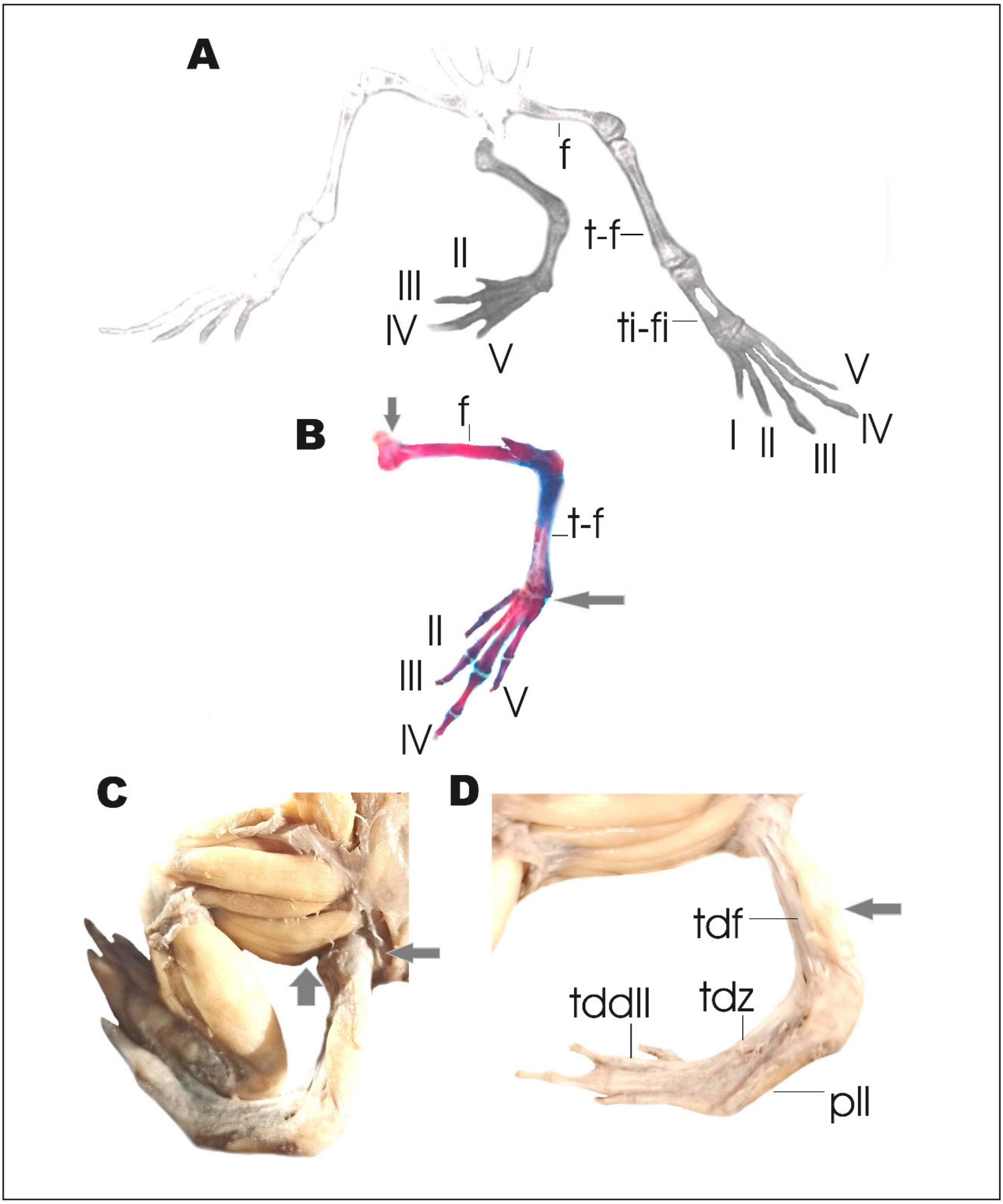

Figura 2. Detalle en vista dorsal de la región posterior del cuerpo del ejemplar estudiado de *Rhinella dorbignyi*: (A) Radiografía, (B) miembro extra teñido y diafanizado, (C) Músculos del miembro posterior izquierdo y de su versión duplicada ectópica, y (D) detalle de la musculatura asociada al miembro ectópico. En (B) la flecha vertical indica la forma marcada de la cabeza articular del fémur y la flecha horizontal denota la zona del basipodio con ausencia casi total de elementos. En (C) las flechas resaltan la diferencia en volumen muscular entre el miembro posterior izquierdo normal y el miembro ectópico. En (D) la flecha indica la masa muscular indiferenciada de la cara posterior del fémur. Referencias: I-V, dedos I a V, f: fémur, pll: músculo *plantaris longus*, tddII: tendón dorsal del dedo II, tdf: tendón dorsal del fémur, tdz: tendón dorsal del zeugopodio, t-f: tibia-fíbula, ti-fi: tibial-fibular.

etc.), lo que resulta una condición anómala para anuros.

El fémur extra carece de músculos salvo por una masa muscular innominada en su cara posterior y una *fascia* de conectivo que reviste el conjunto, más un grueso tendón dorsal (ver más abajo; Fig. 2C-D). La tibia y la fíbula están completamente fusionadas formando una tibia-fíbula donde solo se verifican, con escasa definición entre sí, los músculos *plantaris longus*, *tibialis anticus longus* y *tibialis anticus brevis*. El análisis osteológico del material diafanizado confirma que el autopodio carece de dedo I (formula de falanges miembro duplicado: 0-2-2-3-2; formula normal: 2-2-3-4-3), de tibial-fibular, prehallux (en el miembro normal representado por elemento basal únicamente) y elemento Y. Los únicos tarsales presentes son el tarsal 1 y el tarsal 2-3. La cara ventral del autopodio presenta aponeurosis *plantaris* normal junto con los músculos *tarsalis posticus* (inserto en la tibia por ausencia de tibial) y *flexor digitorum brevis superficialis*. En la cara dorsal del metapodio solo se distingue un tendón, grueso como los dígitos, que corre desde el extremo de la última falange del dedo II hasta el fémur, siendo el único elemento en el dorso del zeugopodio. A nivel de los dígitos, el dedo II tiene un único músculo, el *transversus metatarsi* II. El dedo III presenta solo m. *flexor teres* III mientras que el dedo IV tiene m. *flexor teres* IV y todos los mm. *lumbricalis* (*brevis*, *longus* y *longissimus*). Finalmente, el dedo V presenta sólo al m. *transversus metatarsi* IV.

Por último, las mediciones realizadas sobre el miembro posterior izquierdo y el miembro extra asociado evidencian que todos los componentes óseos de este último resultaron de longitud menor a los elementos correspondientes del miembro normal. Esto resultó particularmente notorio para el autopodio, tanto en valores absolutos como relativos, ya que el miembro extra carece de tibial-fibular (Tabla 1).

Los casos de polimelia reciben distinta designación según el área del cuerpo donde ocurren: cefalomelia (región de la cabeza), notomelia (región dorsal), toracomelia (región toráxica) y pigomelia (región pélvica) (Verma *et al.*, 2013), siendo esta última la que se ajusta al caso reportado en la presente nota.

Tabla 1. Medidas tomadas a los elementos esqueléticos del miembro ectópico y del miembro posterior izquierdo normal de la hembra estudiada de *Rhinella dorbignyi*: Longitud total del miembro (LT) producto de la suma de cada elemento por separado, longitud del fémur (LF), longitud de la tibia-fíbula (LTF), y longitud del autopodio (LA). El asterisco (*) denota que 1,3 cm corresponden al elemento tibial-fibular (ausente en el miembro extra).

	Miembro extra (cm)	Miembro normal izq. (cm)
LT	4,25	7,8
LF	1,65	2,5
LTF	1,1	1,85
LA	1,5	3,45*

Para determinar de manera fehaciente las causas de polimelia que ocurren en la naturaleza se debe realizar un estudio poblacional a partir de marcadores de contexto ambiental (presencia de contaminantes: ver Ouellet *et al.*, 1997; Gurushankara *et al.*, 2007; Pollo *et al.*, 2019) o de signos asociados a la malformación (parásitos como por ejemplo trematodos: ver Stopper *et al.*, 2002) que puedan existir en los individuos. En nuestro caso, relevamientos previos y posteriores realizados en el sitio de estudio en el marco de otras investigaciones no permitieron el hallazgo de otros ejemplares de anfibios con malformaciones, motivo por el cual suponemos que nos encontramos ante un suceso aislado al igual que la mayoría de los casos de polimelia reportados en bufónidos para la región. Su baja frecuencia sugiere que esta malformación es una rareza en poblaciones silvestres.

Finalmente, las malformaciones anatómicas, como el caso de la polimelia, suelen traducirse en menores tasas de supervivencia de animales silvestres (Johnson *et al.*, 1999; Johnson *et al.*, 2001b; Hoppe, 2005), de allí el presente hallazgo resulta relevante, ya que el ejemplar de *R. dorbignyi* aquí reportado alcanzó la adultez, carecía de marcas de depredación sobre su cuerpo y era capaz de producir ovocitos maduros.

Agradecimientos

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Novedad zoogeográfica

Cuad. herpetol. 37 (2): 219-221 (*2023*)

Primeros registros de *Cercosaura parkeri* (Ruibal, 1952) (Sauria, Gymnophthalmidae) en la ecorregión Chaco Seco de Argentina

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Localidades.— República Argentina. Provincia de Formosa, Departamento Bermejo: Reserva Natural Formosa (24°17'59"S; 61°49'5"O), ejemplar colectado el 30/06/1995 por J. Céspedez y M.L. Lions (UNNEC 01338). Provincia de Chaco, Departamento General Güemes: Estancia Doña Irma (25°35'12"S; 61°00'16"O), ejemplar colectado el 19/11/2021 por V. Zaracho, R. Aguirre, J.A. Ruiz García y D. Lencina (UNNEC 13662); Paraje Los Pichis (25°39'25"S; 61°08'46"O), ejemplares colectados el 09/03/2009 y el 24/08/2009 por J.L. Acosta (UNNEC 10456 y 10554 respectivamente) y el 03/05/2009 por V. Zaracho (UNNEC 10619); Paraje Las Delicias (25°09'46"S; 62°10'34"O), ejemplar colectado el 03/04/1995 por R. Aguirre y A. Hernando (UNNEC 01180); Departamento Almirante Brown: Taco Pozo (25°37'2"S; 63°16'7"O), ejemplar colectado el 16/04/2010 por R. Aguirre, E. Etchepare y J.A. Ruiz García (UNNEC 10949); Concepción del Bermejo (26°36'10"S; 60°56'58"O), colectado el 13/07/2007 por R. Aguirre y J.A. Ruiz García (UNNEC 10010). Provincia de Santiago del Estero, Departamento Copo: Seccional El Aybal, Parque Nacional Copo (25°55'12"S; 61°43'6"O), ejemplar colectado el 03/11/2018 por E. Etchepare y D. Aguiar (UNNEC 13429). Provincia de Santa Fe, Departamento 9 de Julio: Tostado (29°13'57"S; 61°46'17"O), ejemplar colectado el 11/03/2001 por M. Morand (UNNEC 07421). Los ejemplares están depositados en la Colección Herpetológica "Lic. Blanca Beatriz Álvarez" de la Universidad Nacional del Nordeste (UNNEC).

Comentarios.— *Cercosaura parkeri* se distribuye en Argentina, Bolivia (que incluye su localidad tipo: Buena Vista, departamento de Santa Cruz), Brasil y Perú (Ruibal, 1952; Viñas y Daneri, 1991). El estado taxonómico de algunas poblaciones se encuentra en revisión y probablemente represente un complejo de varias especies (Ribeiro-Júnior y Amaral, 2017). En Argentina, ha sido citada para las provincias de Catamarca, Jujuy, Salta y Tucumán (Viñas y Daneri, 1991; Abdala *et al*., 2012), particularmente en la ecorregión de selvas de Yungas (Morello *et al*., 2012). Para Santiago del Estero su presencia fue incierta y no se conocían ejemplares de referencia. Sin embargo, fue incluida en un mapa de distribución por Cei (1993) y en una lista de especies de Argentina por Ávila *et al.* (2013), pero no fue considerada por otros autores tales como Abdala *et al.* (2012) y Cabrera *et al.* (2019). En este trabajo se da a conocer la presencia de *C. parkeri* para las provincias de Chaco, Formosa y Santa Fe, y confirmamos su presencia para Santiago del Estero con material de referencia (Fig. 1). Las nuevas localidades amplían el rango de distribución de la especie hacia el Este, en un radio aproximado de 450 km. Además, se destaca la presencia de la especie en ambientes de Argentina con otra morfología geográfica y ecológica, diferente al de Yungas. Las localidades mencionadas se encuentran en la ecorregión del Chaco Seco, subregión del Chaco Semiárido, caracterizado por una vegetación de bosque xerófilo con predominio de *Aspidosperma quebracho-blanco*, *Prosopis kuntzei*, *Prosopis* spp. y

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Trithrinax schizophylla (Morello, 2012).

En algunas localidades del Chaco Seco argentino, *C. parkeri* se encuentra en simpatría con *C. schreibersii* (Fig. 1), aunque algunas diferencias morfológicas entre los ejemplares de *C. schreibersii* analizados durante este trabajo sugieren la existencia de un complejo de especies. En tal sentido, se recomienda una revisión exhaustiva para dilucidar el estado taxonómico de las poblaciones argentinas. La coexistencia en simpatría de *C. parkeri* con otras especies del género no es una novedad, y ha sido previamente señalado por Barreto *et al.* (2012) para los estados de Mato Grosso y Mato Grosso do Sul (Brasil), donde fue registrada junto a *C. ocellata* y otras especies del complejo C. *schreibersii* no determinadas. De acuerdo a la última categorización de lagartijas de Argentina, *C. parkeri* es considerada una especie No Amenazada (Abdala *et al.* 2012).

Información complementaria de los especímenes analizados (sexo, LHC en mm y número de poros femorales). UNNEC 13662: macho, 37,6 y 6/5; UNNEC 10456: hembra, 27,5 y 2/2; UNNEC 10554: macho, 38,3 y 6/6; UNNEC 10619: hembra, 29,0 y 3/3; UNNEC 01180: macho, 32,4 y 5/5; UNNEC 10010: hembra, 35,0 y 2/2; UNNEC 13429: hembra, 37,2 y 2/2; UNNEC 07421: hembra, 29,5

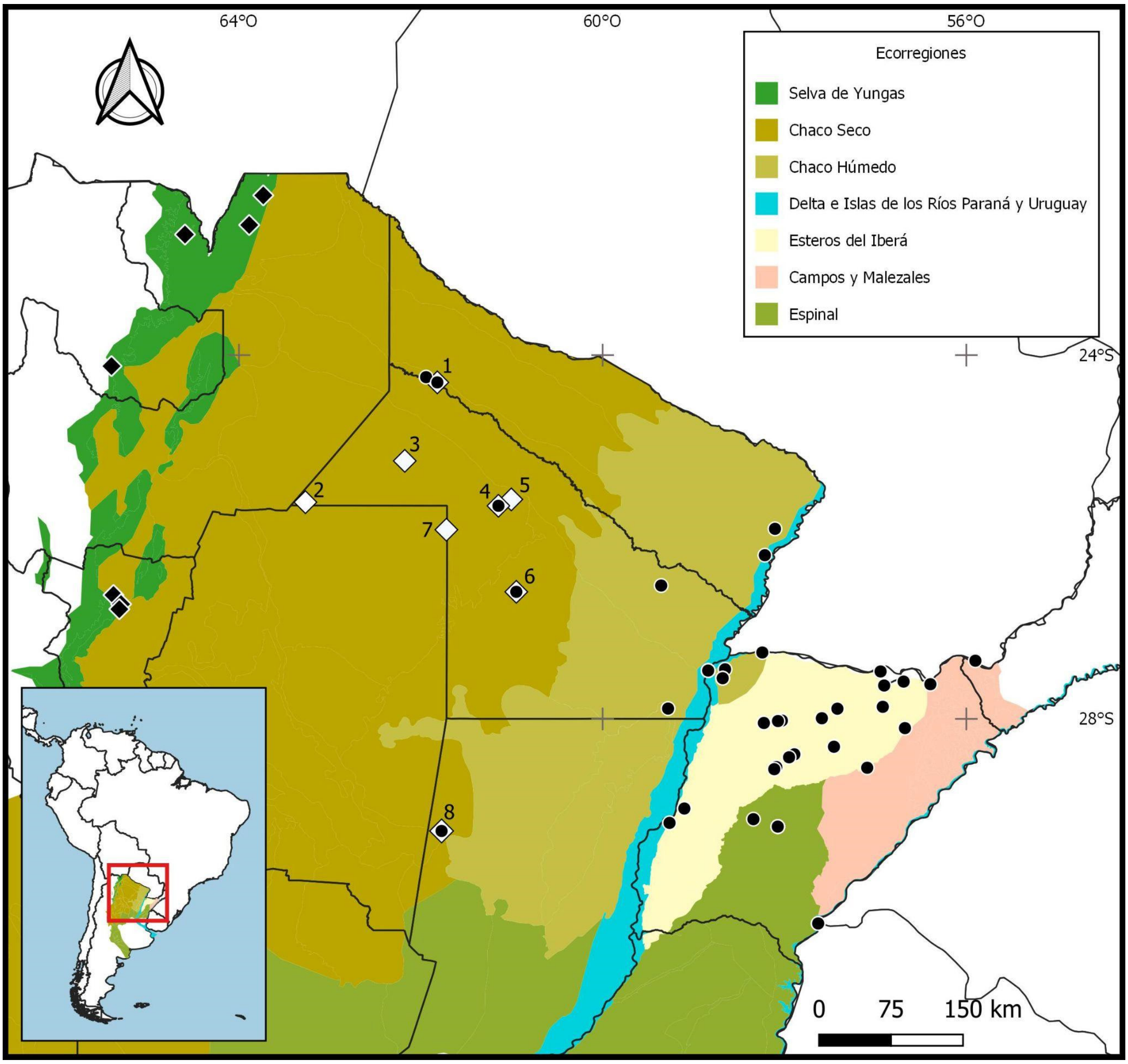

Figura 1. Distribución de *Cercosaura parkeri* en Argentina. Rombos negros: registros bibliográficos (Ruibal, 1952; Viñas y Daneri, 1991). Rombos blancos: nuevos registros. Formosa: 1) Reserva Natural Formosa; Chaco: 2) Taco Pozo, 3) Paraje Las Delicias, 4) Paraje Los Pichis, 5) Estancia Doña Irma, 6) Concepción del Bermejo; Santiago del Estero: 7) Parque Nacional Copo; Santa Fe: 8) Tostado. Círculos negros: *C. schreibersii.*

y 3/3; UNNEC 01338: macho, 36,4 y 5/5; UNNEC 10949: macho, 25,2 y 6/5.

Otros ejemplares revisados. *Cercosaura schreibersii*: UNNEC 00567, 00690, 00752, 00754, 00755, 00757, 00758, 00759, 00879, 01179, 01181, 01242, 01273, 01274, 01299, 01300, 01337, 01773, 01891, 04382, 04833, 04923, 04939, 05002, 05003, 05048, 05049, 05050, 05723, 05724, 05726, 05733, 05734, 05735, 05736, 05737, 05738, 05739, 05794, 06740, 06805, 06825, 06829, 06830, 06836, 06837, 06894, 06896, 07420, 07581, 07582, 07583, 07584, 07585, 07586, 07620, 07632, 07633, 07639, 07640, 07795, 07796, 07797, 07798, 07808, 07809, 07895, 07904, 07905, 08241, 08508, 08781, 08813, 10069, 10101, 10129, 10130, 10451, 10472, 10494, 10520, 10555, 10578, 10579, 11562, 12008, 12009, 12011, 12012, 12015, 12495, 12503, 12517, 12522, 12524, 12526, 13734, 13736, 13737.

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Novedad zoogeográfica

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New records and distribution extension of *Acanthochelys macrocephala* (Rhodin, Mittermeier & McMorris 1984) in midwestern Brazil

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Localities.— We recorded specimens of *A. macrocephala* in five municipalities of Mato Grosso state (Brazil: Fig. 1)

(1) Poconé Municipality, Pantanal ecoregion: I) Malhada Farm (Lat. 16°34'42"S, Long. 57°02'05"W), January 2011, observed by TFD-R. A hatchling (Fig. 2A) was captured in a pitfall trap installed in a seasonally flooded grassland (completely dry),

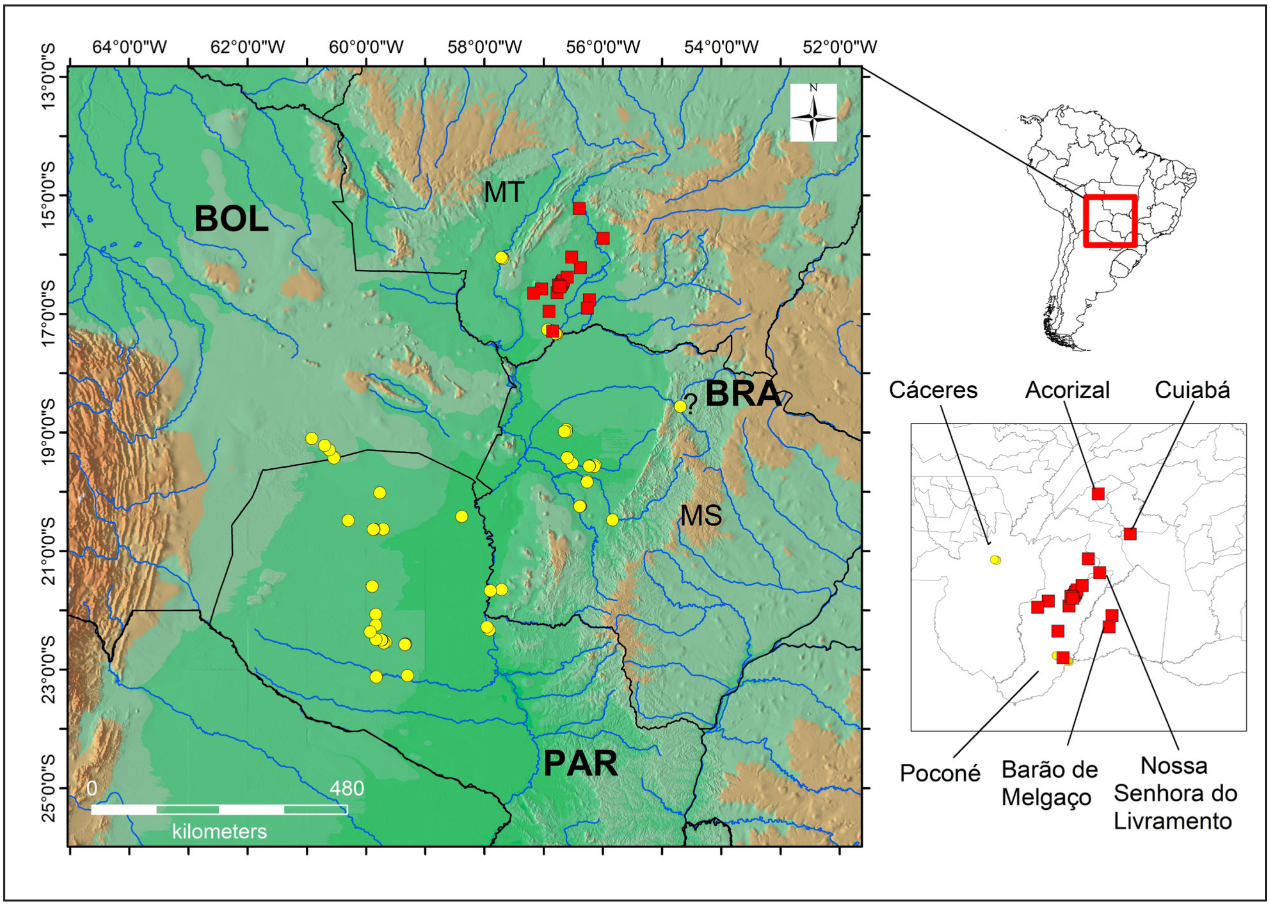

Figure 1. Distribution of *Acanthochelys macrocephala* in South America. BRA = Brazil (MT: Mato Grosso State; MS: Mato Grosso do Sul State). BOL = Bolívia. PAR = Paraguay. Red square = new records reported in this study; yellow dots = previously known records; ? = unconfirmed record in the municipality of Coxim (see Ávila *et al.*, 2006).

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50 m away from the nearest permanently wet area; II) Santa Inês Farm (Lat. 16°39'14"S, Long. 57°10'12"W), May 1991, observed by CS. An unsexed adult was found submerged in shallow water in a seasonally flooded grassland; III) Piuval Farm (Lat. 16°22'58"S, Long. 56°36'08"W), May 2012, observed by MRFC (see Acknowledgements). An unsexed adult was recorded in the edge of a circular forest patch of paleo-fluvial origin distributed along the seasonally flooded grassland matrix of the Pantanal, locally known as *capão*; IV) Pouso Alegre Farm (Lat. 16°31'04"S, Long. 56°44'58"W), January 2011, January and March 2012, observed by ESB and LVSCF. Three individuals were recorded

Figure 2. Individuals of *Acanthochelys macrocephala* recorded in Poconé (a - d), Cuiabá (e), and Acorizal (f), Mato Grosso State, Brazil.

near a *capão* edge and two additional individuals (one female, one unsexed adult) were captured in traps installed in open termite savannas, locally known as *campo de murundu*: the female (Fig. 2B) captured with a funnel trap baited with fresh beef and installed in the center of a small pond, and the unsexed adult captured with a pitfall trap installed in the edge of another small pond ; V) Transpantaneira Park Road (or MT-060), 146 km long, which crosses the Pantanal between the localities of Poconé and Porto Jofre, a small port settlement in the right margin of the Cuiabá River. December 2002 and July 2008, two specimens road-killed collected by CS and deposited in the Herpetological Collection of Universidade Federal de Mato Grosso (UFMT-R 341, UFMT-R 6910); March, April, and July 2011, April 2022, and January 2023, observed by FRT, ESB, CS, MRFC, and RCP. Eight individuals were found alive while crossing the road (Fig. 2C, D);

(2) Barão de Melgaço Municipality, Pantanal: I) São Francisco do Perigara Farm (Lat. 16°54’06”S, Long. 56°15’47”W), April 1989, observed by CS. One unsexed adult was recorded submerged in shallow water; II) RPPN Sesc Pantanal (Lat. 16°45’43”S, Long. 56°13’35”W), January 2021, observed by GS. One unsexed adult was recorded crossing an unpaved road;

(3) Nossa Senhora do Livramento Municipality, Pantanal: Pirizal district (Lat. 16°13’10”S, Long. 56°22’48”W), March 2012, collected by AP and deposited in the Herpetological Collection of Universidade Federal de Mato Grosso (UFMT-R 9893). The individual was captured while crossing a road;

(4) Cuiabá Municipality, Cerrado ecoregion: Peri-urban area (Lat. 15°43’41”S, Long. 55°59’45”W), November 2021, observed by KRS. One unsexed adult (Fig. 2E) was recorded moving towards an artificial pond;

(5) Acorizal Municipality, Cerrado-Pantanal transition zone: MT-10 paved state highway, 70 km long (Lat. 15°13’12”S, Long. 56°24’00”W), February 2010, observed by RMV. One adult (Fig. 2F) was recorded during a wildlife roadkill survey. The monitored stretch of the MT-010 is surrounded by seasonally flooded open environments created by the overflow of the Cuiabá river during the rainy season.

Comments.— The big-headed Pantanal swamp turtle *Acanthochelys macrocephala* (Rhodin, Mittermeier & McMorris, 1984) is a rare and poorly studied medium-sized freshwater turtle (Rhodin *et al.*, 2021), whose individuals can explore a wide variety of habitat types. Although they typically inhabit wetlands and marshes, the species is also occasionally found in slow moving streams, shallow bays, brackish or salty lagoons, natural and or artificial shallow lakes, many of which dry up seasonally (e.g., Mauro *et al.*, 2004; Rhodin *et al.*, 2018). *Acanthochelys macrocephala* has a geographical distribution restricted to central South America, with an estimated extent of occurrence (EOO) of 283,284 km^2 (encompassing localities situated in southern Bolivia, northeastern Paraguay, and midwestern Brazil (Rhodin *et al.*, 2018; 2021, and references therein). In Bolivia and Paraguay, published records correspond to areas of Chaco. In Brazil, records are restricted to the Upper Paraguay river basin, throughout the Pantanal wetlands or nearby Chaco areas. These records were limited until now to four municipalities of Mato Grosso do Sul and two municipalities of Mato Grosso (Rhodin *et al.*, 1984; Cintra and Yamashita, 1989; Kinas *et al.*, 2005; Ávila *et al.*, 2006; Garbin *et al.*, 2016; Métrailler, 2006; Ferronato and Molina, 2009; Rhodin *et al.*, 2009; Souza *et al.*, 2010; Garbin *et al.*, 2016; Brito *et al.*, 2018; Brito *et al.*, 2020). Some of the records we provided here constitute the first occurrence of the species for the municipalities of Barão de Melgaço, Nossa Senhora do Livramento, Cuiabá, and Acorizal, and increase from two to six the number of municipalities where the species is currently known in Mato Grosso (previously known for Poconé and Cáceres - the latter being the species type locality). Our record from Acorizal extends the geographic distribution of the species approximately 170 km northeast from the previous northernmost known record, in the municipality of Cáceres (Rhodin *et al.*, 2021). Moreover, the records from Cuiabá and Acorizal confirm the presence of *A. macrocephala* in habitats situated outside the lowland Pantanal (Ferronato and Molina, 2009; Rhodin *et al.*, 2009; Rhodin *et al.*, 2021), in areas belonging to the plateau of the “Paraná-Guimarães” Cerrado ecoregion (see Sano *et al.*, 2019). The only previous record in a typical Cerrado habitat (in the municipality of Coxim, Mato Grosso do Sul) was based on a turtle skeleton, tentatively identified as *A. macrocephala* by Ávila *et al.* (2006). Overall, we increased the EOO of the species by 10%, expanding it to 314,324 km^2.

Of the total number of records detailed above (n=23), 82.6% took place during the local rainy season, between November and April. However, 48% of the records were concentrated in the Pantanal water drawdown period, between March and May, reinforcing previous observations by Brito *et al.* (2020). During this period, the water level in the flooded grasslands decreases and the remaining ponds are disconnected. In 70% of the records made during the drawdown period, turtles were encountered while moving through dry habitats (crossing a road or on the edge of forested patches). Individuals probably-move more during the water drawdown period, in the search of mates, permanent ponds, and/or suitable reproductive habitats (Brito *et al.*, 2020), thus enhancing the detection of the species.

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