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Una publicación semestral de la Asociación Civil Herpetológica Argentina (Paz Soldán 5100. Piso 1 Dpto 8. Ciudad Autónoma de Buenos Aires, Argentina). Incluye trabajos científicos relacionados con todos los aspectos de la investigación en Anfibios y Reptiles, abarcando tópicos como: sistemática, taxonomía, anatomía, fisiología, embriología, ecología, comportamiento, zoogeografía, etc. Comprende las siguientes secciones: Trabajos, Puntos de Vista, Notas, Novedades Zoogeográficas y Novedades Bibliográficas. Publica en formato digital online y en formato impreso artículos científicos originales asegurando a los autores un proceso de revisión por evaluadores externos sólido y trasparente más una alta visibilidad internacional de sus trabajos. Para los lectores, se garantiza el acceso libre a los artículos. Los idiomas aceptados son castellano, portugués e inglés.

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Trabajo

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Hannemania sp. larvae (Acari: Leeuwenhoekiidae) in *Leptodactylus luctator* (Anura: Leptodactylidae) in the Pampa biome, southern Brazil

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ABSTRACT
The study aims to register *Hannemania* larvae in *Leptodactylus luctator* and their infestation rates in an area of the Pampa biome at the extreme south of Brazil. Forty-six specimens of *L. luctator* (25 females: 21 males) collected in an urban area of the state of Rio Grande do Sul were examined. The prevalence of mites was 93.5%, and 4207 larvae were collected. The mean intensity of infestation was 97.8 mites/host (6-379). There were no significant differences in the prevalence and mean intensity of infestation between male and female hosts. There was no relationship between abundance of larvae and size of anurans. The study increases knowledge of the parasitic fauna and its parasitological indices in anurans from extreme south of Brazil.

Key Words: Amphibians; Chiggers; Intradermic parasite; Infestation rates.

RESUMO
O estudo tem o objetivo de registrar larvas de *Hannemania* sp. em *Leptodactylus luctator* e suas taxas de infestação em uma área do bioma Pampa no extremo sul do Brasil. Foram examinados 46 espécimes de *L. luctator* (25 fêmeas: 21 machos) coletados em uma área urbana do estado do Rio Grande do Sul. A prevalência de ácaros foi de 93,5%, e 4207 larvas foram coletadas. A intensidade média de infestação foi de 97,8 ácaros/hospedeiro (6-379). Não houve diferenças significativas na prevalência e intensidade média de infestação entre hospedeiros machos e fêmeas. Não houve relação entre abundância de larvas e tamanho dos anuros. O estudo amplia o conhecimento da fauna parasitária e seus índices parasitológicos em anuros no extremo sul do Brasil.

Palavras-chave: Anfíbios; Trombiculidae; Parasito intradérmico; Taxa de infestação.

Introduction

Amphibians are potential hosts for several groups of parasites, either endo or ectoparasites. When compared to other vertebrates, amphibians rarely carry ectoparasites, being members of Acari their most common ones (Hatano *et al*., 2007; Luz and Faccini, 2013; Díaz-Páez *et al*., 2016). Mites of the genus *Hannemania* Oudemans, 1911 (Leeuwenhoekiidae) are free-living predators in their adult stage (Hatano *et al*., 2007), while in their larval stage they parasitize dermal tissues of vertebrates. They are present mainly in anurans, and are commonly found in their feet, legs and ventral parts (Quinzio and Goldberg, 2015). Pathologic effects of *Hannemania* larvae can include inflammation, necrosis, dermal abscesses, and consequently thickening of the skin (Quinzio and Goldberg, 2015).

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Leptodactylus luctator (Hudson, 1892) (Leptodactylidae) is a large anuran whose adults that can reach 120-140 mm snout-vent length (Martins *et al.*, 2015). The species occurs in tropical and subtropical South America east to the Andes (Magalhães *et al.*, 2020). It has nocturnal and diurnal habits and occupies many habitats, such as savannas, grasslands, forest borders and riverbanks in humid tropical forests, altered habitats due to anthropic action, and its reproduction depends on medium sized o large ponds or flooded areas (Bernarde, 2012).

Twenty-four species of *Hannemania* known to parasitize anurans in the American continent (Silva-De La Fuente *et al.*, 2016). In Brazil, *Hannemania* larvae were reported in the following hosts and regions: *Leptodactylus latrans* group in São Paulo; *Scinax ruber* (Laurenti 1768) (Hylidae) in Mato Grosso do Sul; *Pristimantis conspicillatus* (Günther, 1858) (Craugastoridae) in Mato Grosso; *Hylodes* sp. from unknown location (Jacinavicius *et al.*, 2018); *Hylodes phyllodes* Heyer & Cocroft, 1986 (Hylodidae) in Rio de Janeiro (Hatano *et al.*, 2007) and *Boana pulchella* (Duméril & Bibron, 1841) (Hylidae) in Rio Grande do Sul (Silveira *et al.*, 2019). *Hannemania* parasitism in anurans has also been reported in other South American countries, such as Bolivia (Wohltmann *et al.*, 2006), Argentina (Attademo *et al.*, 2012) and Chile (Díaz-Paez *et al.*, 2016; Silva-De La Fuente *et al.*, 2016). However, few studies have provided parasitological information associated to the occurrence of larval infestations of *Hannemania* in anurans from the region.

Infestations data from Leptodactylid anurans were provided for Argentina (Attademo *et al.*, 2012), Chile (Díaz-Paez *et al.*, 2016) and northeastern Brazil (Rodrigues *et al.*, 2018). In this study we present the occurrence of *Hannemania* sp. larvae in *L. luctator* from an area located in the Pampa biome of southern Brazil, and provide some parasitological indices.

Materials and methods

Forty-six adult specimens of *Leptodactylus luctator* (25 females: 21 males) were collected in an urban area with wet, dry and flooded fields in the west bank of the Laguna dos Patos, where it meets the São Gonçalo channel, in Pelotas (31°46'30.6"S 52°14'09.3"W), Rio Grande do Sul, Brazil. Anurans were collected in September and December 2017 and in April and August 2018, during active searches since the beginning of twilight. Specimens were placed individually in plastic containers and taken to the Laboratório de Parasitologia de Animais Silvestres of the Universidade Federal de Pelotas (LAPASIL/UFPel), where they were euthanized in agreement with Resolution no. 1000 issued by the Conselho Federal de Medicina Veterinária (CFMV 2012). Mean snout-vent length (SVL) of anurans was 71.36 mm (36.2 - 102 mm ± 14.83).

Mites were preserved in 70º ethanol, clarified in Hoyer medium and identified in agreement with Brennan and Goff (1977). They were photographed under a Olympus BX 41 microscope with an attached digital camera. Vouchers were deposited in the "Coleção de Artrópodes do Laboratório de Parasitologia de Animais Silvestres" (CALAPASIL 558-573) at the Microbiology and Parasitology Department in the Institute of Biology at the Universidade Federal de Pelotas and "Coleção de Acarologia" at the Instituto Butantan (16534-16538 IBSP), Brazil.

Prevalence (P %), mean intensity of infestation (MII) and mean abundance (MA) were estimated in agreement with Bush *et al.* (1997). Prevalence of mites between male and female hosts were compared by the Fisher's Exact test, and mean intensity of infestation with bootstrap test, using of the Quantitative Parasitology (PQweb) (Reiczigel *et al.*, 2019), considering significance at $p \leq 0.05$. Simple regression analysis (RA) and was used to check for associations with host size based on SVL.

Results

Forty-three anurans (93.5 %) were parasitized by *Hannemania* sp. larvae (Fig. 1) and 4207 mites were located in intradermal nodules, mainly in the ventral region of their hindlimbs (Fig. 2). The overall MII was 97.8 mites/host and mean abundance was 91.5 mites (Table 1).

In regression analyses, although the dispersion trends were positive, both the Pearson's (r) and the determination coefficient (r^2) were low. The relationship between both variables (abundance of larvae and host size) was either random or nonexistent (Fig. 3).

Prevalence of mites between females (96 %) and males (90.5 %) did not differ significantly (p = 0.585). Likewise, MII were similar, 109.6 and 88.5 mites/host for males and females, respectively (p = 0.488 (Table 1).

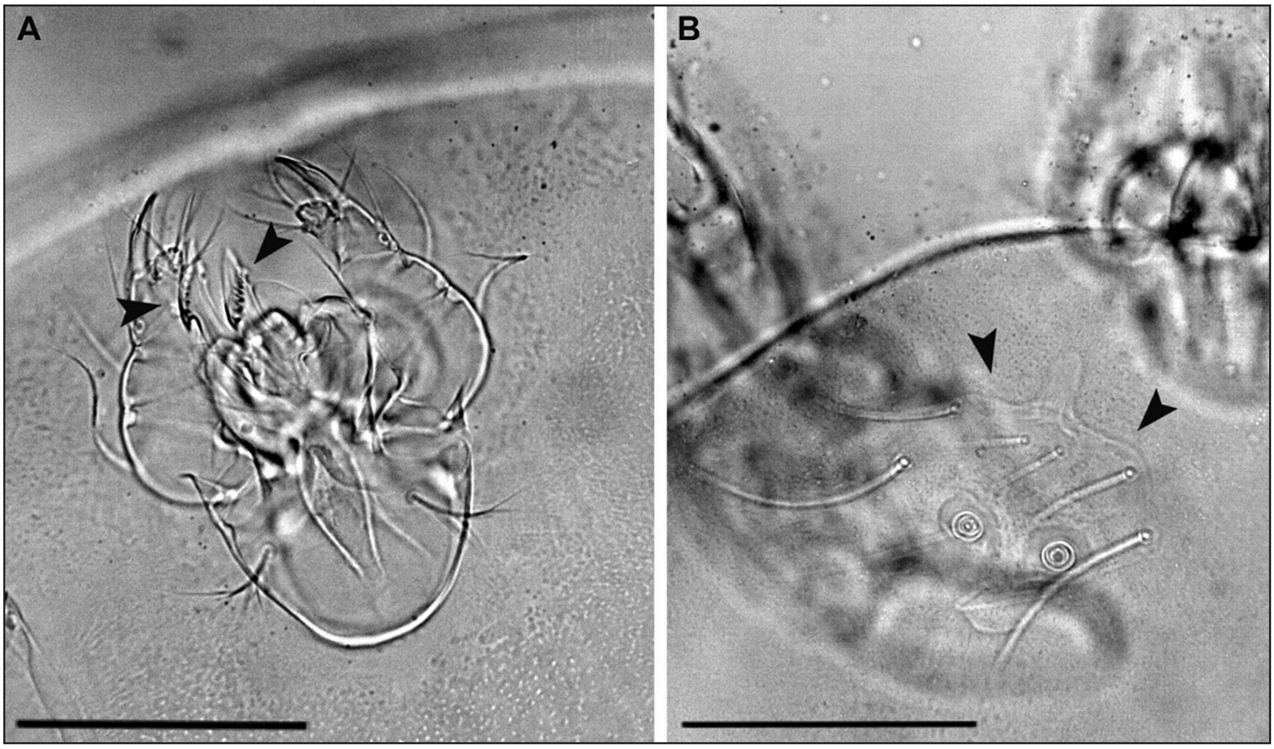

Figure 1. Larva of *Hannemania* sp. (Acari: Leeuwenhoekiidae) parasitizing *Leptodactylus luctator* (Anura: Leptodactylidae), from Pampa biome, southern Brazil. A - Gnathosoma (arrows indicate chelicera) (bar = 57μm). B - Scutum with nasus (arrows) located on the dorsum of the larva (bar = 75μm).

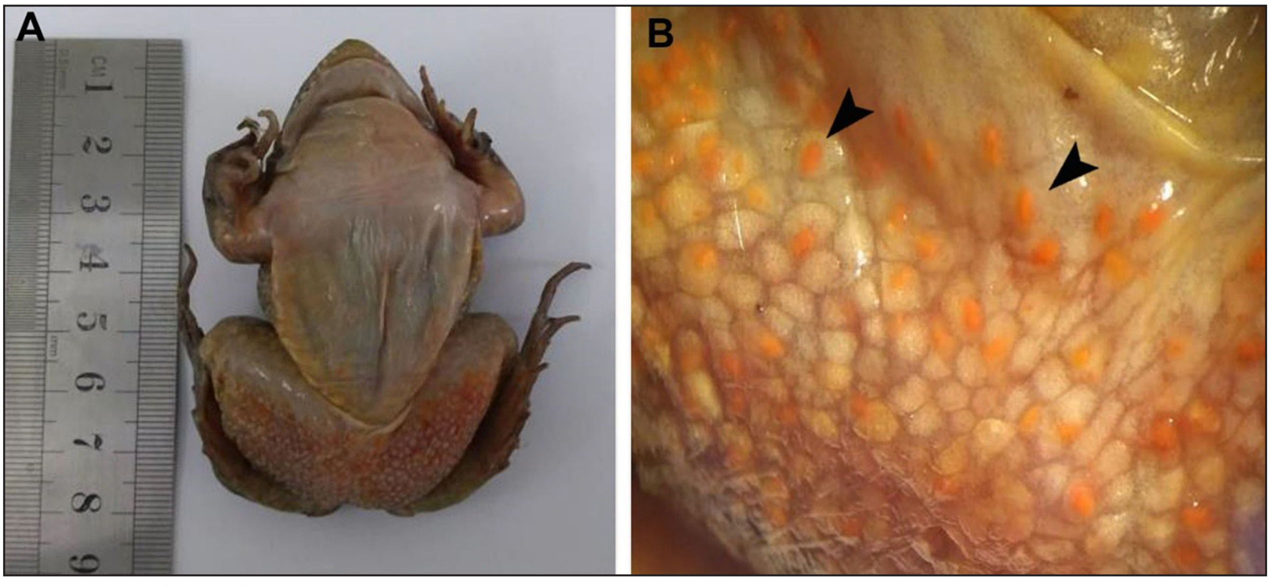

Figure 2. *Leptodactylus luctator* (Anura: Leptodactylidae) parasitized by *Hannemania* sp. larvae in the Pampa biome, southern Brazil. A - Ventral view of the anuran, notice the numerous larvae (orange spots) in the hind limbs. B - Detail with magnification of the intradermic parasitic larvae (arrows).

Discussion

Frogs of the genus *Leptodactylus* are conspicuous components of the anuran fauna in South America, but reports on their ectoparasites are scarce. In Argentina, infestations by *Hannemania* in *Leptodactylus macrosternum* Miranda-Ribeiro, 1926 (n= 40) were observed with higher rates in plantation areas, where prevalence was 100 %, and mean abundance of 194.7 in rice fields and 85.1 mites in soybean crops, as opposed to native forest areas in which prevalen-

Table 1. Parasitological indices of the intradermal parasitic larvae of *Hannemania* sp. (Acari: Leeuwenhoekiidae) in *Leptodactylus luctator* (Anura: Leptodactylidae) from Pampa biome, southern Brazil, discriminated by sex. Abbreviations: prevalence (P%), mean intensity of infestation (MII), mean abundance (MA), range (R), and standard error (SE).

Indices	**Total hosts (N=46)**	**Females (N=25)**	**Males (N=21)**
P%	93.5%	96.0%	90.5%
MII (± SE)	97.8 (± 14.3)	88.5 (± 16.1)	109.6 (± 25.3)
MA (± SE)	91.5 (± 13.8)	85.0 (± 15.9)	99.2 (± 24.0)
R	6-379	6-286	11-379

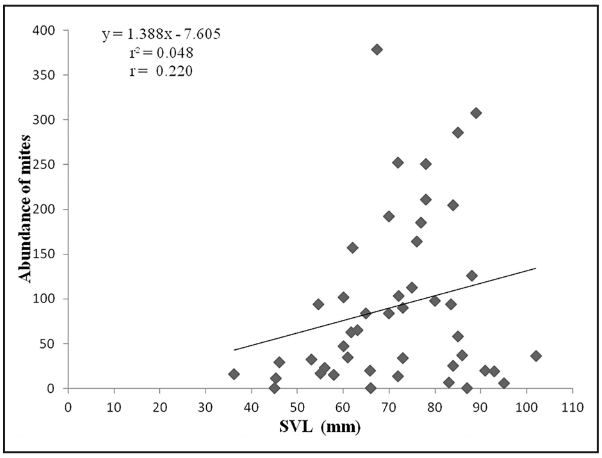

Figure 3. Regression analysis of *Hannemania* sp. larvae (Acari: Leeuwenhoekiidae) abundance in *Leptodactylus luctator* (Anura: Leptodactylidae) from Pampa biome, southern Brazil, in relation to host snout-vent length (SVL, in millimeters).

ces were between 22 and 42 % and a mean abundance between 0.33 and 1.4 (Attademo *et al.*, 2012). The authors commented that the high infestation rates in cultivated areas may be related to the high humidity and exposure to pollutants such as agrochemicals, which may affect the immune system of hosts (Attademo *et al.*, 2012). Although the region of the present study is inserted in an urbanized area, the influence of anthropic alterations on infestations was not analyzed. Already in Brazil, mite parasitic larvae were recorded in *L. macrosternum* (n= 31) with prevalence of 42 % and mean intensity of infestation of 22 mites/host (Rodrigues *et al.*, 2018). In Chile, *Hannemania* sp. larvae were found in *Pleurodema bufoninum* Bell, 1843 (n= 5) and *Pleurodema thaul* (Schneider, 1799) (n= 13) with prevalence of 100 % and mean intensity of 63.2 mites/host (19-137) and 11.6 (4-28) for the first and the latter, respectively (Díaz-Páez *et al.*, 2016).

Hannemania larvae were also reported in associations with Bufonidae, Hylodidae and Hylidae with variable infestation rates in South America. In Chile, a study on *Rhinella spinulosa* (Wiegmann, 1834) (Bufonidae) (n= 15) showed prevalence of 100% and mean intensity of 17.3 mites/host (Díaz-Páez *et al.*, 2016). Studies by Hatano *et al.* (2007) in Brazil observed prevalence of 87.7 % and mean intensity of 48.2 larvae/host in *Hylodes phyllodes* (Hylodidae) (n= 49), while in *Boana pulchella* (Hylidae) (n= 50) Silveira *et al.* (2019) found a prevalence of 18 % and mean intensity of infestation of 1.1 larvae/host.

Variations in infestation rates may be related to the habitat (e.g. terrestrial and arboreal) of the host species and the environmental conditions (e.g. humidity levels) demonstrating the opportunistic habit of the parasite. Jung *et al.* (2001) highlighted that moist soil is important for the survival of adult forms of the free-living mites. Bufonid anurans are preferably terrestrial, the Leptodactylidae is composed of species found in both wet and dry areas (Gallardo 1974), while studied Hylidae present arboreal habits (Bernarde 2012). It seems that species associated with more humid environments may be more likely to be infested by *Hannemania* larvae. Studies with *P. bufoninum*, *P. thaul* (Díaz-Paez *et al.*, 2016), *L. macrosternum* (Attademo *et al.*, 2012; Rodrigues *et al.*, 2018), and species of Ranidae, *Lithobates vaillanti* (Brocchi, 1877), *Lithobates berlandieri* (Baird, 1859) and *Lithobates brownorum* (Sanders, 1973) (Jacinto-Maldonado *et al.*, 2016) found no significant relationships between snout-vent length and abundance of mites, as observed for *L. luctator* in the present study. Like us, Hatano *et al.* (2007) found no significant differences in prevalence of *Hannemania* larvae between males (86.5 %) and females (91.7 %) of *H. phyllodes*.

Further studies are needed to evaluate the infestation rates of *Hannemania* larvae in anurans of different groups, as well as in different anthropized areas to broaden the knowledge about parasite-host relationships. This information that may contribute to the development of ecosystem conservation programs.

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Trabajo

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Herpetofauna of an urban environmental protection area in an Amazon forest remnant of Amapá state, northern Brazil

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ABSTRACT
The herpetofauna of Amazonia biome is one of the richest in the world. However, there is a lack of information on its richness and distribution. Here we provide a list of the herpetofauna from the Environmental Protection Area of Lagoa dos Índios, state of Amapá, Brazil, in the northern Amazon Forest. Sampling effort was conducted by active search in three distinct transects from January to May 2018 (rainy season) and August to December 2019 (drought season). We recorded 46 species composing the local herpetofauna, being 18 amphibians and 28 reptiles. *Scinax ruber*, *Gonatodes humeralis* and *Helicops angulatus* were the most abundant species. Regarding the conservation status, two species are categorized as Date Data deficient, (*Lysapsus bolivianus* and *Eunectes deschauenseei*), and one as Vulnerable (*Podocnemis unifilis) in the* Red List of the International Union for Conservation of Nature. *Podocnemis unifilis* is also considered *Near Threatened* in the Brazilian Red List of Endangered Species. Our results suggest that local biodiversity is still underestimated and, if expanded, could increase the species richness in the area. This study represents preliminary trends and raise further questions concerning the herpetofauna assemblage of Eastern Brazilian Amazonia.

Key words: Amphibians; Herpetological surveys; Reptiles.

RESUMEN
El bioma de la herpetofauna de la Amazonia es uno de los más ricos del mundo. Sin embargo, falta información sobre su riqueza y distribución. Aquí proporcionamos una lista de la herpetofauna del Área de Protección Ambiental de Lagoa dos Índios, estado de Amapá, Brasil, en el norte de la Selva Amazónica. El esfuerzo de muestreo se realizó mediante búsqueda activa en tres transectos distintos de enero a mayo de 2018 (temporada de lluvias) y de agosto a diciembre de 2019 (temporada de sequía). Registramos 46 especies que componen la herpetofauna local, siendo 18 anfibios y 28 reptiles. *Scinax ruber*, *Gonatodes humeralis* y *Helicops angulatus* fueron las especies más abundantes. En cuanto al estado de conservación, dos especies están categorizadas como Datos deficientes en fecha, (*Lysapsus bolivianus* y *Eunectes deschauenseei*), y una como Vulnerable (*Podocnemis unifilis*) en la Lista Roja de la Unión Internacional para la Conservación de la Naturaleza. *Podocnemis unifilis* también se considera Casi Amenazada en la Lista Roja Brasileña de Especies Amenazadas. Nuestros resultados sugieren que la biodiversidad local todavía se subestima y, si se amplía, podría aumentar la riqueza de especies en el área. Este estudio representa tendencias preliminares y plantea más preguntas sobre el conjunto de herpetofauna de la Amazonia brasileña oriental.

Palabras claves: Anfibios; Inventario; Reptiles.

Introduction

The territory of the state of Amapá, in the Brazilian Amazon Forest, is mostly covered by protected areas harboring a mosaic of conservation units and indigenous lands (Mustin *et al.*, 2017). The State is known by harboring a rich biodiversity, and some herpetofauna surveys have corroborated this statement (Benício and Lima, 2017; Lima *et al.*, 2017; Silva e Silva and Costa-Campos, 2018; Costa-Campos and

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Freire, 2019; Pedroso-Santos *et al.*, 2019; Prudente *et al.*, 2020; Costa-Campos *et al.,* 2021).

The herpetofauna of the northern region of Amapá state remained unknown due to the lacking of data on the natural history and geographic distribution of several taxa, which led to a false idea of a low species richness. This scenario has changed, and several inventories were conducted in in order to better describe the herpetofauna, providing new occurrences and describing new species, especially in the eastern Amazon (Fouquet *et al.*, 2016; Ribeiro-Júnior *et al.*, 2016; Costa and Bérnils, 2018; Taucce *et al.,* 2020; Fouquet *et al.,* 2021).

In this study, we present the species of amphibians and reptiles that compose the herpetofauna from the Environmental Protection Area of Lagoa dos Índios, subject to human pressure in the Amazonia biome, northern region of Brazil, and provide the conservation status.

Materials and methods

Study area— Our study was conducted in the Environmental Protection Area (PAs) of Lagoa dos Índios (0.0288°N, 51.0978°W), located on the banks of the Duca Serra Highway, municipality of Macapá, state of Amapá, Brazil (Figure 1). This PAs has an area of 11 km and connects to the Fortaleza Hydrographic Basin, an important tributary to the Amazon River (Tavares-Dias *et al.,* 2013), and it is representative of the "*terra firme*" (upland forest) and flooded forest. The climate is Am according to the Köppen-Geiger system (tropical monsoon, without a dry season) and the mean annual rainfall is nearly 1,885 mm and average is 27 ºC (Peel *et al.*, 2007). The PAs Lagoa dos Índios history presents environmental conflicts arising from urbanization process and high degree of anthropogenic disturbance (Vargas and Bastos, 2013).

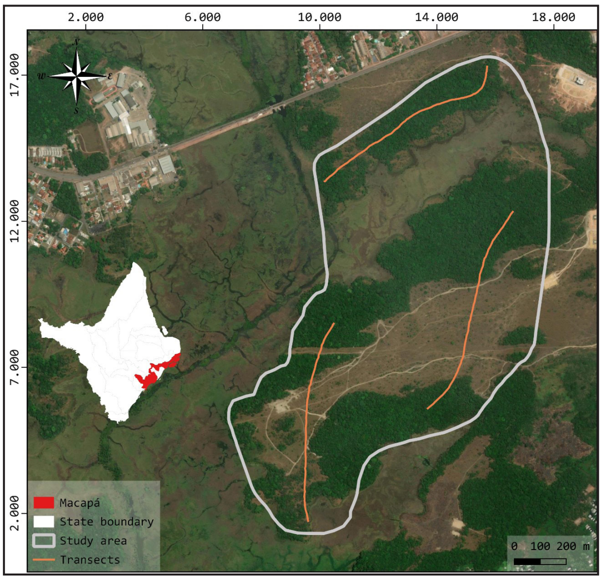

Figure 1. Map showing the geographic location of the Environmental Protection Area of Lagoa dos Índios, in southeast portion of Macapá municipality, Amapá state, northern region of Brazil.

Data collection—We sampled the herpetofauna in two different periods, from January to May 2018 (rainy season) and August to December 2019 (drought season), by using active search (Heyer *et al.*, 1994). Sampling effort was conducted in three distinct transects of 1.5 km distant by at least 1 km by a team made up of four collectors. Our sampling effort comprises about 1.020 hours of observation, of which 720 h during the night (6 pm – 9 pm) and 300 h during the day (8 am – 11 am).

The collected specimens were killed using lidocaine, fixed in 10% formaldehyde, and preserved in 70% ethanol (Pisani, 1973) and housed in the Herpetological Collection of the Universidade Federal do Amapá (CECC, Appendix 1), except for a few species of reptiles (the turtles *Chelonoidis carbonarius* and *Podocnemis unifilis*, the lizards *Iguana iguana iguana*, the snakes *Boa constrictor constrictor*, *Eunectes murinus*, *Hydrodynastes gigas*, and the crocodylian *Caiman crocodilus crocodilus*) that were too large for preservation and storage.

The zoological nomenclature adopted herein follows Segalla *et al.* (2021) for amphibians, and Costa *et al.* (2021) for reptiles. We assessed the conservation status of individuals identified to species level using the International Union for Conservation of Nature red list web interface (IUCN, 2022), and Brazilian Red List of Endangered Species (ICMBio, 2018). Only *Hemidactylus mabouia* was not evaluated since it an exotic species.

Analyzes—We perform a rarefaction curves for amphibians, lizards, snakes and all the herpetofauna combined, based on individuals collected over time (Gotelli and Colwell, 2001), through 1,000 randomizations of an abundance matrix where each column represents a species and each row represents a sample. We used species richness estimator Jacknife1 to determine the expected richness for amphibians, lizards, snakes and all the herpetofauna combined. We did not build chelonian or crocodilian curves because these taxa were only rarely present. Both rarefaction curves and the Jacknife 1 were done performing using the software EstimateS v.9.1.0 (Colwell, 2013). The occurrence of the registered species was evaluated by the constancy index of occurrence (Dajoz, 2005), classified as: abundant (present in > 50% of samples), common (present in 25 to 50% of samples) or rare (present in < 25% of samples).

Results

We recorded a total of 46 species composing the local herpetofauna, being 18 amphibians and 28 reptiles (Table 1; Figure 2). *Scinax ruber*, *Gonatodes humeralis* and *Helicops angulatus* are the most abundant species. According to constancy index of occurrence, the number of abundant species recorded was higher and represented 45.7% of the community (n = 21; >50%), followed by rare species (n = 15; 32.6%; >25% <50%) and common species (n = 10; 21.7%; <25%). Of the 46 species listed, none are considered endemic to the Amazon Forest biome.

According to the Red List of the International Union for Conservation of Nature (IUCN, 2022)

Table 1. List of amphibians and reptiles species, number of specimens, constancy index and the conservation status according to the Red List of the International Union for Conservation of Nature (IUCN) and Brazilian Red List of Endangered Species (ICMBio) recorded from January to May 2018 (rainy season) and August to December 2019 (wet season), in the Environmental Protection Area of Lagoa dos Índios, Macapá municipality, Amapá state, Brazil. NL = not listed; LC = Least Concern; DD = Date Deficient; VU = Vulnerable; NT = Near Threatened.

Family	Species	Number of specimens	Constancy index	IUCN	ICM-Bio
ANURA					
Bufonidae					
	Rhinella major (Muller & Helmich, 1936)	7	Abundant	NL	LC
	Rhinella marina (Linnaeus, 1758)	9	Abundant	LC	LC
Hylidae					
	Boana punctata (Schneider, 1799)	5	Common	LC	LC
	Boana raniceps (Cope, 1862)	9	Abundant	LC	LC
	Dendropsophus leucophyllatus (Beireis, 1783)	7	Abundant	LC	LC
	Dendropsophus walfordi (Bokermann, 1962)	4	Common	LC	LC
	Lysapsus bolivianus Gallardo, 1961	6	Abundant	DD	NL
	Pseudis paradoxa (Linnaeus, 1758)	4	Common	LC	LC
	Scinax ruber (Laurenti, 1768)	10	Abundant	LC	LC
	Sphaenorhynchus lacteus (Daudin, 1800)	7	Abundant	LC	LC
	Trachycephalus typhonius (Linnaeus, 1758)	4	Common	LC	LC
Leptodactylidae					
	Adenomera hylaedactyla (Cope, 1868)	9	Abundant	LC	LC
	Leptodactylus macrosternum Miranda-Ribeiro, 1926	2	Rare	LC	LC
	Leptodactylus pentadactylus (Laurenti, 1768)	4	Common	LC	LC
	Leptodactylus podicipinus (Cope, 1862)	9	Abundant	LC	LC
Microhylidae					
	Elachistocleis helianneae Caramaschi, 2010	3	Rare	LC	LC
Pipidae					
	Pipa pipa (Linnaeus, 1758)	4	Common	LC	LC
GYMNOPHIONA					
Typhlonectidae					
	Typhlonectes compressicauda (Duméril & Bibron, 1841)	2	Rare	LC	LC

TESTUDINES					
Testudinidae					
	Chelonoidis carbonarius (Spix, 1824)	1	Rare	NL	LC
Podocnemididae					
	Podocnemis unifilis Troschel, 1848	4	Common	VU	NT
CROCODYLIA					
Alligatoridae					
	Caiman crocodilus crocodilus (Linnaeus, 1758)	2	Rare	LC	LC
SQUAMATA					
Gekkonidae					
	Hemidactylus mabouia (Moreau de Jonnès, 1818)	8	Abundant	---	---
Sphaerodactylidae					
	Gonatodes humeralis (Guichenot, 1855)	9	Abundant	LC	LC
Mabuyidae					
	Copeoglossum nigropunctatum (Spix, 1825)	6	Abundant	LC	LC
Iguanidae					
	Iguana iguana iguana (Linnaeus, 1758)	6	Abundant	LC	LC
Tropiduridae					
	Tropidurus hispidus (Spix, 1825)	6	Abundant	LC	LC
Gymnophthalmidae					
	Arthrosaura kockii (Lidth de Jeude, 1904)	2	Rare	LC	LC
Teiidae					
	Ameiva ameiva ameiva (Linnaeus, 1758)	7	Abundant	LC	LC
	Cnemidophorus cryptus Cole & Dessauer, 1993	6	Abundant	NL	LC
Aniliidae					
	Anilius scytale (Linnaeus, 1758)	3	Common	LC	LC
Boidae					
	Boa constrictor constrictor Linnaeus, 1758	1	Rare	NL	LC
	Eunectes deschauenseei Dunn & Conant, 1936	3	Common	DD	LC
	Eunectes murinus (Linnaeus, 1758)	6	Abundant	NL	LC
Colubridae					
	Chironius carinatus (Linnaeus, 1758)	1	Rare	LC	LC
Dipsadidae					
	Imantodes cenchoa (Linnaeus, 1758)	1	Rare	LC	LC
	Hydrodynastes gigas (Duméril, Bibron & Duméril, 1854)	1	Rare	NL	LC
	Helicops angulatus (Linnaeus, 1758)	8	Abundant	LC	LC
	Helicops leopardinus (Schlegel, 1837)	6	Abundant	LC	LC
	Helicops trivittatus (Gray, 1849)	7	Abundant	LC	LC

	Pseudoeryx plicatilis plicatilis (Linnaeus, 1758)	1	Rare	LC	LC
	Oxyrhopus melanogenys melanogenys (Tschudi, 1845)	1	Rare	LC	LC
	Thamnodynastes pallidus (Linnaeus, 1758)	5	Common	LC	LC
	Erythrolamprus cobella (Linnaeus, 1758)	1	Rare	LC	LC
	Erythrolamprus reginae semilineatus (Wagler in Spix, 1824)	1	Rare	LC	LC
	Lygophis lineatus (Linnaeus, 1758)	1	Rare	LC	LC
Viperidae					
	Bothrops atrox (Linnaeus, 1758)	7	Abundant	NL	LC

the species from PAs Lagoa dos Índios were classified into four categories: Least Concern (n = 35), Non Evaluated (n = 7), Data Deficient (n = 2) and Vulnerable (n = 1). *Lysapsus bolivianus* (anuran amphibian) and *Eunectes deschauenseei* (snake) are classified Data Deficient and *Podocnemis unifilis (*turtle*)* is considered Vulnerable. According to the Brazilian Red List of Endangered Species (ICMBio, 2018), most of the species found in the Environmental Protection Area of Lagoa dos Índios are Least Concern (n = 43; 93.5%). *Podocnemis unifilis* is considered NT (Near Threatened).

Among the amphibians, the family Hylidae presented the highest species richness (nine species, 52.9%), followed by Leptodactylidae (four species, 23.5%) and Bufonidae (two species, 11.8%). Gymnophiona was represented by one species (Typhlonectidae). Among the reptiles, Dipsadidae (12 species, 48%) and Boidae (three species, 12%) were the richest families among the squamates.

Regarding the sampling effort, the accumulation curve began to reach an asymptote at the end of the study for amphibians and lizards (Figure 3). For snakes and herpetofauna (amphibian and reptile species), the accumulation curve do not reached the asymptote at the end of the sampling effort.

Discussion

The 46 species (18 amphibians and 28 reptiles) recorded in the present study corresponded to 14.5 % of the 124 amphibians species (Lima, 2017) and 17.4 % of the 161 reptiles species (Costa and Bérnils, 2018) known for the country. The herpetofauna richness at Environmental Protection Area of Lagoa dos Índios is relatively low when compared to the other Amazonian sites (23 lizards and amphisbaenians species in Campos *et al.*, 2015; 28 amphibians species in Lima *et al.*, 2017; 50 anurans and reptiles species in Pedroso-Santos *et al.,* 2019; 95 reptiles species in Prudente *et al.*, 2020; and 57 reptiles species in Costa-Campos *et al.,* 2021). Similar results were reported in the Amazonian, where species richness decreased with increasing intensity of human impact (Menin *et al.*, 2019; Peixoto *et al.*, 2019; Almeida-Corrêa *et al.*, 2020). Perhaps habitat loss, urbanization and forest fragmentation comprise primary threats to amphibians and reptiles populations in the Neotropics (Stuart *et al.*, 2004; Verdade *et al.*, 2012).

The high reptile richness supports the hypothesis that these group of animals are not as sensitive to fragmentation as other taxa (e.g. anurans), and that some species can thrive in partially disturbed habitats (Kurz *et al.*, 2013; Bitar *et al.*, 2014). Additionally, we captured a relatively high number of snake species, but few individuals (max three individuals) compared to lizards, which may also account for the steep rarefaction curve.

The snakes' species *Boa constrictor constrictor*, *Chironius carinatus*, *Imantodes cenchoa*, *Hydrodynastes gigas*, *Pseudoeryx plicatilis plicatilis*, *Oxyrhopus melanogenys melanogenys*, *Erythrolamprus cobella*, *E. reginae semilineatus* and *Lygophis lineatus* were recorded only in one sample and according index of constancy of occurrence were considered rare (<25%). Snakes are difficult to sample since they used to appear in low densities (compared to lizards, in this study), most species are of cryptic coloration, and have secretive habitats (Martins and Oliveira, 1998; Santos-Costa *et al.*, 2015), and the detection is even more difficult in tropical forests (Fraga *et al.,* 2014; Frazão *et al.,* 2020).

The prevalence of Hylidae and Leptodactylidae families in relation to the other anuran families is a similar result as in other locations of the Amazon region (Lima *et al.*, 2017; Silva e Silva and Costa-

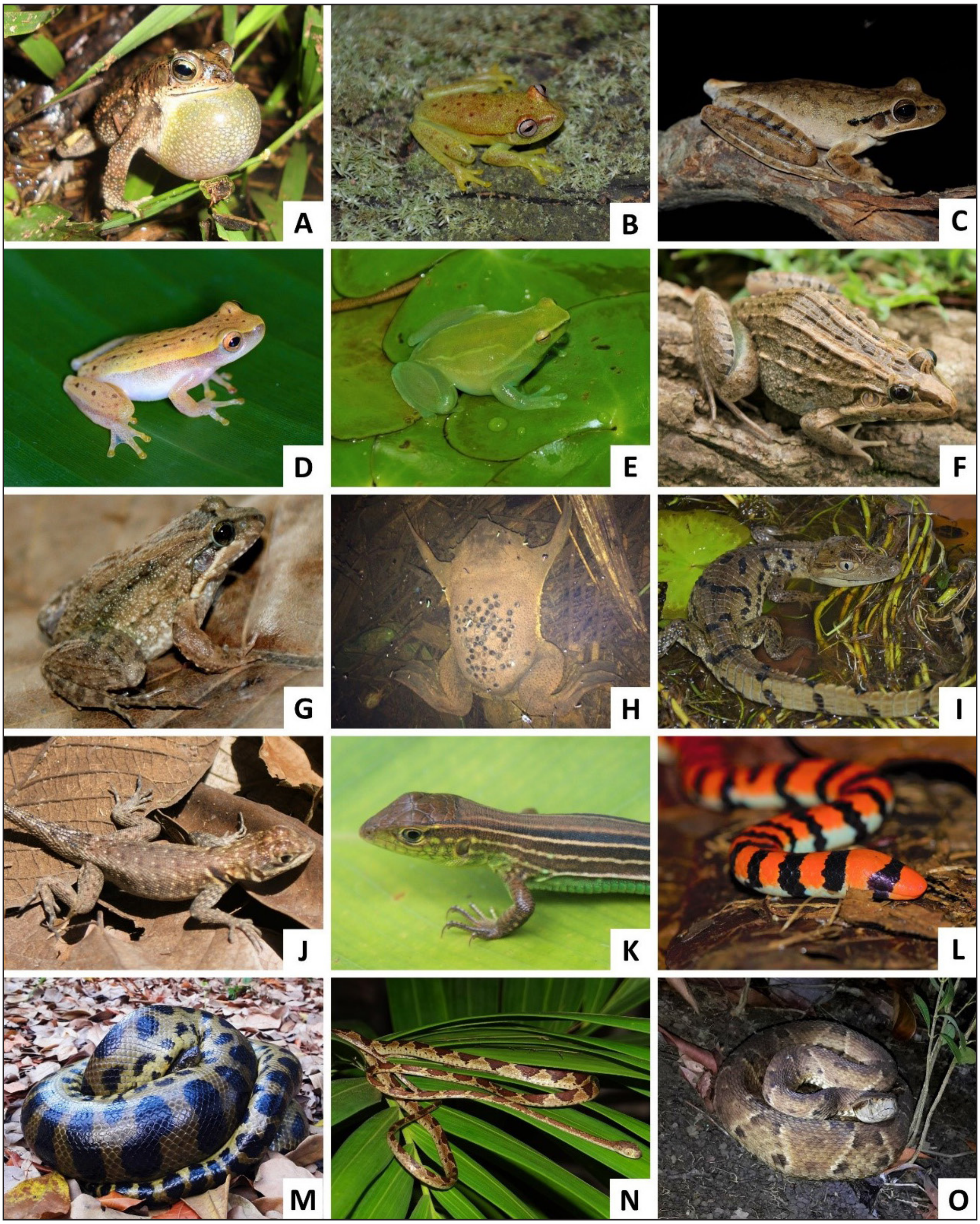

Figure 2. Some species of amphibians and reptiles from the Environmental Protection Area of Lagoa dos Índios, Macapá municipality, Amapá state, northern Brazil. (A) *Rhinella major*; (B) *Boana punctata;* (C) *B. raniceps*; (D) *Dendropsophus walfordi*; (E) *Sphaenorhynchus lacteus*; (F) *Leptodactylus macrosternum*; (G) *L. podicipinus*; (H) *Pipa pipa*; (I) *Caiman crocodilus crocodilus*; (J) *Tropidurus hispidus*; (K) *Cnemidophorus cryptus*; (L) *Anilius scytale*; (M) *Eunectes deschauenseei*; (N) *Imantodes cenchoa*; (O) *Bothrops atrox*.

Campos, 2018; Pedroso-Santos *et al.*, 2019). For lizards, Teiidae family presented greater richness. Species of this family (e.g. *Ameiva ameiva* and *Cnemidophorus cryptus*) seem to benefit from the

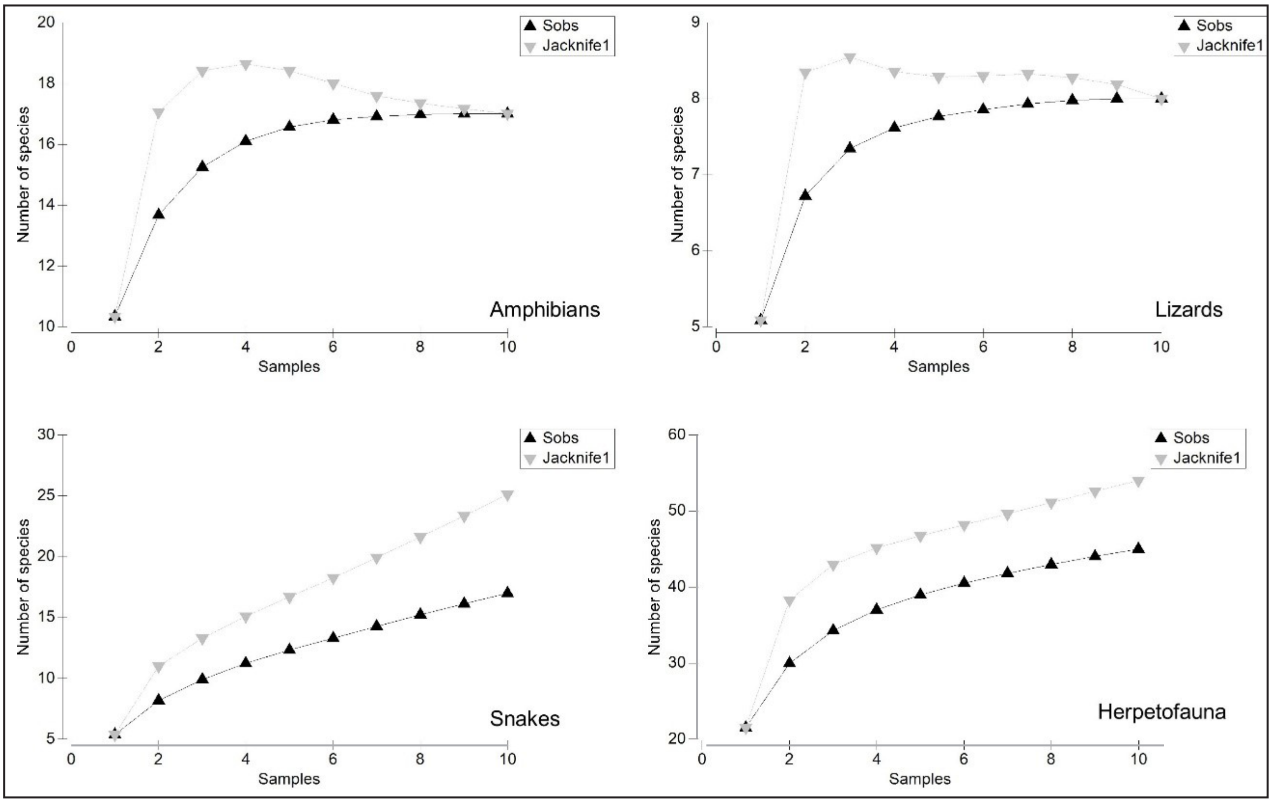

Figure 3. Accumulation and rarefaction curve for (A) amphibians, (B) lizards, (C) snakes and (D) pooled herpetofauna coupled with a species richness estimator Jacknife 1, recorded from January to May 2018 (rainy season) and August to December 2019 (wet season) in the Environmental Protection Area of Lagoa dos Índios, Macapá municipality, Amapá state, northern Brazil.

effects of fragmentation and urbanization (Andrade *et al.*, 2019), responding positively to fragmentation showing a marked increase in abundance (Sartorius *et al.*, 1999). Dipsadidae was the most representative family of snakes, as also reported in other studies (Debien *et al.*, 2019; Frazão *et al.*, 2020), which was expected since this is the richest family of reptile species in Brazil (Costa and Bérnils, 2018; Nogueira *et al.*, 2019).

The herpetofauna of the Environmental Protection Area of Lagoa dos Índios is composed predominantly of species widely distributed in Amazonia biome. Our results represent preliminary trends and raise further questions concerning the assemblage of Eastern Brazilian Amazonia.

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APPENDIX 1

Voucher specimens of amphibians and reptiles from the Environmental Protection Area of Lagoa dos Índios, in southeast portion of Amapá state, Macapá municipality, northern region of Brazil.

AMPHIBIANS: Anura: *Rhinella major* (0030); *Rhinella marina* (0122); *Boana punctata* (0498); *Boana raniceps* (0451); *Dendropsophus leucophyllatus* (0086); *Dendropsophus walfordi* (2345); *Lysapsus bolivianus* (0449); *Pseudis paradoxa* (2757); *Scinax ruber* (2784); *Sphaenorhynchus lacteus* (0113); *Trachycephalus typhonius* (2343); *Adenomera hylaedactyla* (0557); *Leptodactylus macrosternum* (2187); *Leptodactylus pentadactylus* (0119); *Leptodactylus podicipinus* (0521); *Elachistocleis helianneae* (0543); *Pipa pipa* (2391); Gymnophiona: *Typhlonectes compressicauda* (2973). **REPTILES**: Squamata: *Hemidactylus mabouia* (0899); *Gonatodes humeralis* (2700); *Copeoglossum nigropunctatum* (1061); *Tropidurus hispidus* (0872); *Arthrosaura kockii* (1013); *Ameiva ameiva ameiva* (1076); *Cnemidophorus cryptus* (1062); *Anilius scytale* (2974); *Eunectes deschauenseei* (2892); *Chironius carinatus* (2975); *Imantodes cenchoa* (2976); *Helicops angulatus* (1040); *Helicops leopardinus* (1363); *Helicops trivittatus* (1194); *Pseudoeryx plicatilis plicatilis* (2977); *Oxyrhopus melanogenys melanogenys* (2978); *Thamnodynastes pallidus* (2171); *Erythrolamprus cobella* (2979); *Erythrolamprus reginae semilineatus* (2980); *Lygophis lineatus* (2981); *Bothrops atrox* (1434).

Trabajo

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Lipidic cycle, condition factor and reproductive cell maturation in *Gymnodactylus darwinii* Gray, 1845 (Squamata, Phyllodactylidae) from a fragment of Atlantic Forest in northeastern Brazil

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ABSTRACT
In this study, we describe a variation in fat bodies (liposomatic relationship), condition factor (the welfare degree of a population against the environment, K1), and male and female reproductive cells of the gecko *Gymnodactylus darwinii* (Gray, 1845) collected in two contiguous protected areas (Tapacurá and Mata do Camucim forests) in the municipality of São Lourenço da Mata, Pernambuco state, Brazil. We assessed seasonal variation and the influence of biotic (body temperature and K1 condition factor) and abiotic (air temperature and precipitation) factors on the lipidic cycle and reproductive cells' maturation. As typical in geckos, fat bodies in *G. darwinii* decreased markedly during the high temperature and low precipitation periods. A slight variation in K1 suggests similar levels of energetic investment in growth and sexual maturation. Different reproductive cells matured similarly in sexually mature individuals, indicating a continuous, synchronised maturation cycle. Nonetheless, while fat bodies decreased and the number of mature reproductive cells increased in dry months, the opposite pattern was observed in rainy months. Our results indicate that *G. darwinii* does not show reproductive seasonality but instead peaks of reproductive activity (reproductive cell maturation, mating, lay eggs) synchronised in females and males, regulated by body temperature and climatic variables, including air temperature and precipitation.

Key Words: Gender Environmental Changes; Gametogenesis; Gonadal Histology; Lipid Storage; Lizards

Introduction

Studies concerning energetic cycles and reproductive cell maturation provide important insights for testing hypotheses about lizards' life-history evolution strategies (Ramírez-Bautista *et al.*, 2009; Norval *et al.*, 2019; Resendiz, 2020). In these animals, the lipidic cycle, including condition factor (a population degree of well-being against the environment) and gametogenesis, tends to be regulated by the interaction of abiotic (Serrano-Cardozo *et al.*, 2007; Sánchez-Hernández *et al.*, 2013; Lozano *et al.*, 2015)

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and biotics factors (Galdino *et al.*, 2003; Garda *et al.*, 2014). The influence of these factors can be assessed through several parameters, including temperature, precipitation, and photoperiod (Cheng, 1987; Norval *et al.*, 2019; Díaz-Vega *et al.*, 2020), and through correlations between body conditions, such as reproductive cells maturation and the condition factor (K1), body temperature, and variation in energy reserves (Derickson, 1976; Galdino *et al.*, 2003, Salvador, 2011).

The condition factor is measured as the lizards' lipids reserves. It provides valuable insights concerning the individuals' recent nutritional patterns and energetic investment into cyclical, vital activities, including feeding, thermoregulation, and breeding, therefore inferring a population's general well-being (Ballinger, 1977; Cheng, 1987; Autumn y De Nardo, 1995). In addition, identifying morphological characteristics and seasonal changes in cellular structures of reproductive cell maturation in different sexes is key to determining the reproductive strategies employed by the species (Newlin, 1976; Trauth, 1979; Uribe et al., 1995). Therefore, data on energetic cycles and reproductive cell maturation allow inferences on the population reproductive strategies, the prevailing environmental conditions, and the availability of local resources, contributing to the development of more effective conservation and management programs (Uribe et al., 1995; Torki, 2007; Norval et al., 2019).

The reproductive dynamics, body growth patterns, and lipidic cycles of most Neotropical lizards are unknown or scarcely documented so far, as is the case of the naked-toed gecko, *Gymnodactylus darwinii* Gray, 1845. This lizard is a typical ground-dwelling, sit-and-wait forager (Oitaven *et al.*, 2019; Guedes *et al.*, 2020) endemic to the Brazilian Atlantic Forest (Costa y Bérnils, 2018; Oitaven *et al.*, 2019). *Gymnodactylus darwinii* presents a generalist diet (Almeida-Gomes *et al.*, 2012), a continuous reproductive strategy (Guedes *et al.*, 2020), and rupicolous habits, inhabiting mainly rocky outcrops (Huey *et al.*, 2009; Oitaven *et al.*, 2019). Given the great diversity of Neotropical lizards and the environments they inhabit, different reproductive patterns and strategies might be expected to be used by different populations, especially in environments with intense climatic seasonality (Ferreira *et al.*, 2002; Galdino *et al.*, 2003; Ferreira *et al.*, 2009; Migliore *et al.*, 2017).

Several studies have reported that abiotic factors, such as precipitation and air temperature, do not play a clear role in increasing or decreasing lipid storage in individuals' bodies (i.e., in the lipidic cycle). Such factors seem not to influence the investment put by individuals into bodyweight and length gain (i.e., in the growth cycle) (Ramírez-Bautista *et al.*, 2009; Norval *et al.*, 2019). Likewise, the reproductive cells' maturation in lizards also lacks a clear relationship with abiotic factors (Uribe *et al.*, 1995; Lozano *et al.*, 2014; Lozano *et al.*, 2015). However, there is evidence that biotic factors influence the cell maturation process and the lipid cycle (Derickson, 1976; Cooper *et al.*, 1987; Galdino *et al.*, 2003).

In this study, we described the condition factor (K1), the fat body cycle, and the temporal variation in the lizard *G. darwinii* gamete production from an Atlantic Forest remnant in the state of Pernambuco, northeastern Brazil. We use these data to 1) infer how geckos manage their energetic resources over the year, i.e., whether seasonally or not, and 2) evaluate whether and to what extent abiotic and biotic factors influence the reproductive cell maturation process and the lipidic cycle. Specifically, we expected variation in the *G. darwinii* lipidic cycle and gamete production to be driven by biotic factors, including body temperature and K1 condition factor, and abiotic factors, including air temperature and precipitation.

Materials and methods

Study site

We performed this study in two Brazilian Protected Areas (PAs): Mata do Camucim (200 ha; -8.041513S, -35.1923423W, Datum WGS84) and Mata do Tapacurá (100 ha; -8.044166S, -35.201388W, Datum WGS84), which are geographically contiguous and inserted within another protected area, the Tapacurá Ecological Station (Fig. 1). Once both areas constitute interconnected, close-located PAs that do not present significant environmental differences, the collected *Gymnodactylus darwinii* individuals were analysed together. Both PAs comprise seasonal Atlantic Forest remnants surrounded by sugarcane crops in the municipality of São Lourenço da Mata, part of the metropolitan region of Recife city, in the Pernambuco state, northeastern Brazil (Carneiro-Moura *et al.*, 2014; Oitaven *et al.*, 2019). The altitude gradient in the study area ranges from 120 m to 150 m above sea level.

The local climate is tropical, and rainfall is concentrated between March and August, typical of

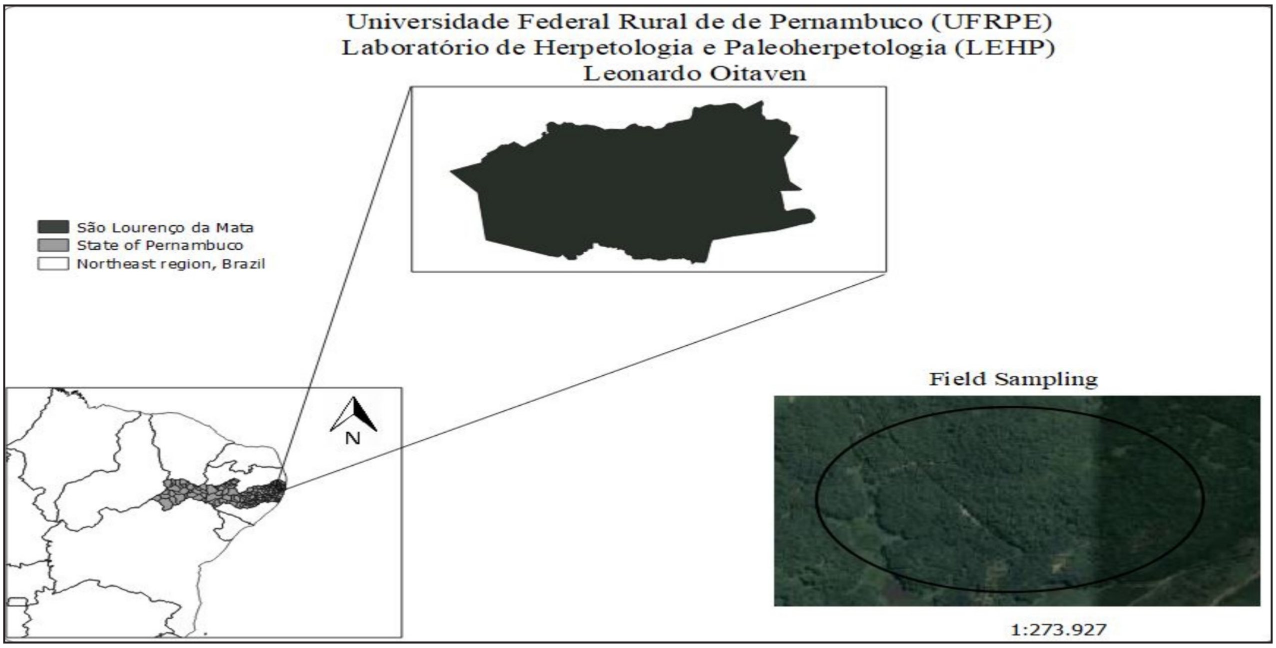

Figure 1. Map of the state of Pernambuco (PE), northeastern Brazil, depicting the municipality of São Lourenço da Mata and the study areas in Mata do Camucim and Mata do Tapacurá, both located within the Tapacurá Ecological Station.

the Brazilian Northeast Rainforest Zone. The annual precipitation is 1900 mm, but precipitation amounts may be less than 100 mm in the driest months (APAC, 2017; Oitaven *et al.*, 2019). Because precipitation and the air temperature vary considerably in the study area, we defined the dry and rainy seasons according to climatic data collected during the study (Fig. 2). The dry season was characterised by low monthly precipitation (0.5–20.7 mm) and relatively high temperatures (30.3–34.9°C). In contrast, we recorded a drastic increase in precipitation levels (13.4–211.4 mm) during the rainy season, although temperatures decreased slightly (32.4–34°C). We obtained data on monthly air temperature (°C) and rainfall (mm) levels from the Pernambuco Water and Climate Agency database (APAC, 2017).

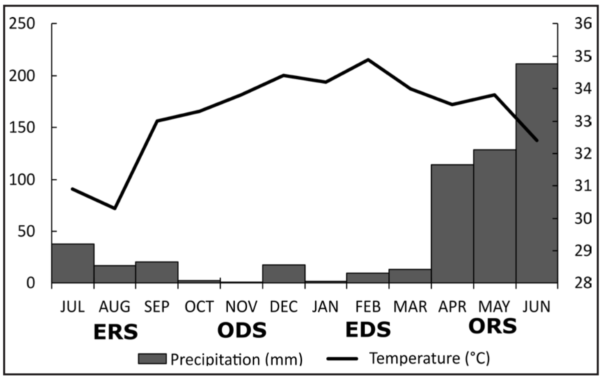

Figure 2. Climatogram from the Tapacurá Ecological Station (Açude de Tapacura, APAC) region, municipality of São Lourenço da Mata, state of Pernambuco, Brazil. The climatogram shows the mean air temperature (°C) and precipitation (mm) monthly between July 2016 and June 2017, according to the onset of dry season (ODS), ending of dry season (EDS), onset of rainy season (ORS), and ending of rainy season (ERS).

Field and laboratory procedures

We collected up to six individuals per month (three males and three females) between July 2016 and June 2017 through active searching, following the recommendation of the collection license available and applying the same effort from previous studies (e.g., Lozano *et al.*, 2015; Hernández-Gallego *et al.*, 2018). Even with a limited number of specimens, the monthly sample size of collected individuals was enough to conduct all analyses in this study.

Immediately after capturing the individuals, we measured the body temperatures using a digital thermohygrometer with precision to the nearest 0.01°C (Colli *et al.*, 2003), weighed the body masses with a digital scale (to the nearest 0.1 g), and then euthanised the specimen with a Ketamine (10 mg/Kg) and Medetomidine (0.1–0.3 mg/Kg) lethal injection. We measured each specimen's snout-vent length (SVL) using a digital calliper (to the nearest 0.01 mm). After the measurements, we removed the specimen's tail and weighed its mass (w) separately on a digital scale (to the nearest 0.001 mm) (Galdino *et al.*, 2003). Because most of the individuals captured presented the entire tail, showing no evidence of earlier autotomy, we also measured the tail length and diameter using a digital calliper (to the nearest 0.01 mm). Then, we calculated their tail volume (π(D/2)2H), where D represents the diameter and H is the extension of the tail. In addition, because abdominal fat bodies were absent in all *G. darwinii* individuals collected, we used tail mass to calculate the lipidic reserves index in the population (Derickson, 1976).

We fixed and preserved the collected specimens in 10% formaldehyde and 70% ethyl alcohol, respectively, and stored them in the UFRPE Herpetological and Paleoherpetological collection (catalogue numbers 4843–4907). The procedures were authorised by the Federal Environment Agency (SISBIO #54374-1) and the UFRPE Ethics Committee on Experimental Use of Animals (CEUA UFRPE 098/2016).

We removed each specimen's gonads through a longitudinal incision from the throat to the cloaca for reproductive cells analysis and conserved the gonads in 70% ethyl alcohol. Then, we fixed the material in Bouin's solution (71% picric acid, 24% formaldehyde, 5% glacial acetic acid) for 24 hours and dehydrated it in an increasing alcohol series for 30 minutes at each of the six stages: 70%, 80%, 90%, 95%, 100%, and 100% (Hopwood, 1990). Finally, we embedded the samples in paraffin to obtain 5 μm thick histological sections, placed them on microscope slides, and stained them with Hematoxylin and Eosin for analysis (Robinson y Gray, 1990).

Reproductive cells and structures analyses and the liposomatic relationship (LR) index and the condition factor (K1) estimation

We analysed the histological slides qualitatively using a conventional, trinocular bench microscope (Olympus AX70, Tokyo, Japan) attached to a digital image acquisition system (ERc 5s camera with Axiovision 6.3, Carl Zeiss, Jena, Germany). Cell types were identified according to Gribbins (2011). The seminiferous tubules were analysed based on a random selection of 27 tubules from each male specimen at a 400x magnification (Mayhew y Wright, 1970; Lozano *et al.*, 2015).

We analysed the male testicle slides stereologically by following Mandarim-de-Lacerda (1995) and Weibel (1979). We calculated the Volume Density (Vv) of the primary and secondary spermatids and the spermatozoa to obtain the reproductive maturation index for each specimen (Torki, 2007). We calculated the Volume Density using Hally's (1964) formula to represent the stereological parameters (Mandarim-de-Lacerda, 1995). We described the morphology of the male reproductive cells following Gribbins (2011) and quantified the density profiles (Qa: primary and secondary spermatids and spermatozoa, which are the best-differentiated structures). We also counted a different number of fields within the Test Area (AT) for each analysed specimen. The results (profiles/mm^2) were obtained from the profiles' average, based on QA = ∑profiles/AT (Mandarim-de-Lacerda, 1995).

We also analysed the female ovaries microscopically. We used the follicles described by Uribe *et al.* (1995) and Santos *et al.* (2020) as references for histological descriptions. Because the thickness of the follicular wall modifies during the previtellogenic and vitellogenic phases, we quantified different cell types of this region using the population density approach, considering a test area of 88 mm2 within which we identified small and intermediate ovarian cells and pyriform cells (Uribe *et al.*, 1995; Santos *et al.*, 2020). We also quantified the number of oviductal eggs in each female over the sampling months to describe temporal variation in mature reproductive structures in the studied population. We measured the length of the oviductal eggs using a digital calliper (to the nearest 0.01 mm) and estimated the eggs' volume using the formula of an ellipsoid (Mesquita *et al.*, 2015).

We calculated the specimens' liposomatic relationship (LR) using the following formula: LR = WT (100)/w, where WT is the tail mass, and w is the total mass. Because abdominal fat bodies were absent in the specimens collected, the LR index was applied to tail measurements, and weight was used to proxy the individuals' lipid reserves (Vazzoler, 1982; Norval *et al.*, 2019). We then used the Allometric Method to calculate the overlap of the individuals' condition factor (K1), based on the equation K1 = W/Lb, where W is the specimen's total mass, L is the specimen's snout-vent-length, and b is a coefficient determined by each specimen mass-length ratio (W = aLb) (Lima-Junior *et al.*, 2002).

Data analysis

To analyse the monthly patterns, we calculated the mean values and the standard deviation of the condition factor (K1), the liposomatic relationship (LR) indices, the number of male reproductive cells (primary and secondary spermatocytes and sperms), and the number of oviductal female eggs in each collecting month. We assessed seasonal variation (regarding the onset and dry and rainy seasons end) in reproductive cell types and K1 and LR indices values using a non-parametric Kruskal-Wallis' analysis of variance (Zar, 1999; Galdino *et al.*, 2003). Due to the limited monthly sample size and the variation in the number of individuals collected throughout the study, only samples with at least four individuals were included.

We modelled linear regressions (R2) using the *Iswr* package, considering conditions (reproductive cell types and LR) as dependent variables and the abiotic (air temperature and precipitation) and biotic factors (body temperature and K1) as independent variables to verify the correlation on reproductive cell types and LR. Finally, we tested the relationship between LR and tail volume using simple linear regression (*r*2) (Zar, 1999). We considered a significance level of $P < 0.05$ For all statistical analyses, and the analyses were performed in the R program v3.6.1 (R Development Core Team, 2019).

Results

Sixty-one adult individuals were used in the present study, corresponding to 81.3% of the total individuals sampled (n = 83). No adults or males were sampled in August and September 2016, respectively. The SVL of gravid females varied from 48.32 mm to 59.23 mm. Based on the ellipsoid formula, the mean egg volume was 63.27 ± 49.5 mm^3 (20.06–185.17 mm^3; n = 14). Eggs were always found in the oviducts, and the ovaries were adhered to the dorsal wall by the mesovarium, showing fixed clutch size (always two eggs).

Follicles in both previtellogenic and vitellogenic phases were observed in the ovaries' histological sections. The previtellogenic phase is characterised by a large number of vitellus granules, with earlier signs of the vitellogenic process and the ooplasm forming the yolk platelets with evident layers (Fig. 3A). The granulosa layer is extremely thick in this phase, presenting a well-developed theca and yolk membrane (Fig. 3B) with small, cuboid, intermediate, and pyriform cells (Fig. 3C). In the vitellogenic phase, the ooplasm is characterised by yolk granules in the cytoplasm (Fig. 3D) and a significant reduction in the granulosa layer, yolk membrane, and theca, with only small cells present (Fig. 3D). Some individuals showed corpus luteum, a hypertrophied theca containing "whorl" cells and lutein mass (Fig. 3E).

The microscopic analysis of testicles' histological sections revealed the presence of germinal cells at all development stages, including spermatogonia, spermatocytes, and sperms, indicating that males are reproductively active throughout the year. The spermatogonia (SPG) mainly occupied the seminiferous tubules' periphery and were ovoid-shaped and dark-coloured (Fig. 4A). The central region of the seminiferous tubules is filled up as the cell maturation process develops (mitotic process), becoming a rod-shaped spermatocyte (SPT), which may be either primary (following meiosis 1) or secondary after meiosis 2 (Fig. 4A). At the final spermatogenesis stage, the cells are fully developed and termed spermatozoa (SPZ) (Fig. 4B), being stored in the epididymis and presenting structures that allow them to move towards and survive in the female's reproductive organ.

Condition factor (K1)

According to the Allometric Method, the body condition factor of the studied *G. darwinii* population had a K1 value of 2.707, based on the positive correlation between the individuals' body mass and body length ($r^2 = 0.81$; $P < 0.001$) (Fig. 5). K1 values in males increased at the beginning of the dry and rainy seasons (Fig. 6), whereas in females, they were higher at the end of the rainy season (Fig. 7). Lower LR values were recorded in males during the end of the dry and rainy seasons (Fig. 6), whilst in females, the lowest value was recorded during the rainy season, even though the highest value was recorded at the beginning of this season (Fig. 7). Mean tail length did not differ between males and females ($t = -1.14$; df = 44.2; $P = 0.25$), nor did tail volume ($t = 1.55$; df = 55.23; $P = 0.12$). Despite the lack of a significant difference between males and females, there was a positive and significant correlation between tail weight and volume (r2 = 0.12; df = 57; p < 0.001), indicating that lipid reserves in tail regions increase according to tail volume.

There was significant seasonal variation in the K1 values obtained for males, with higher K1 values recorded at the beginning of the dry and rainy seasons (Table 1). In contrast, LR values did not vary significantly between seasons for either sex (Table 1). In males, primary (Spt1) and secondary (Spt2) spermatocytes varied significantly between seasons (Kruskal-Wallis: $p < 0.05$), with a peak of Spt1 production at the end of the rainy season and the

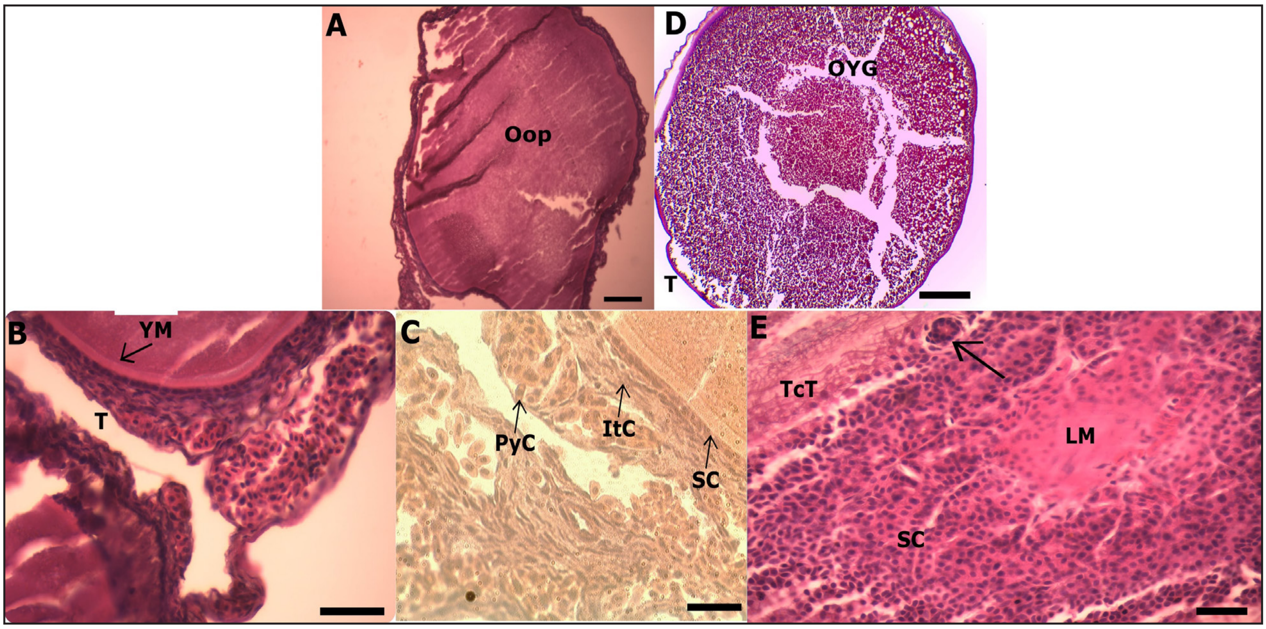

Figure 3. Histological sections of follicles of *G. darwinii* specimens collected in the Tapacurá forest, São Lourenço da Mata, Pernambuco, Brazil. (A) Previtellogenic follicle in early vitellogenic process, with the ooplasm (Oop) forming yolk platelets. H-E Stained. Bar = 150 µm. (B) Ooplasm showing few, small yolk platelets, with yolk membrane (YM) exhibiting a hyaline band and zona radiata. Theca (T) well defined. H-E Stained. Bar = 150 µm. (C) Follicular wall with poly stratified epithelium and cells under apoptosis. Granulosa containing small cells (SC), theca interna with intermediate cells (ItC), and theca externa with pyriform cells (PyC). H-E Stained. Bar = 400 µm. (D) Vitellogenic follicle exhibiting ooplasm with yolk granules (OYG) in their cytoplasm. Thinner theca (T) with blood vessels. H-E Stained. Bar = 500 µm. (E) Hypertrophied theca with lutein mass (LM) within the central cavity of the follicle, connective tissue (TcT), and cell "whorl" (arrow). At the vitellogenic stage, only small cells (SC) are found at theca layer. H-E Stained. Bar = 400 µm.

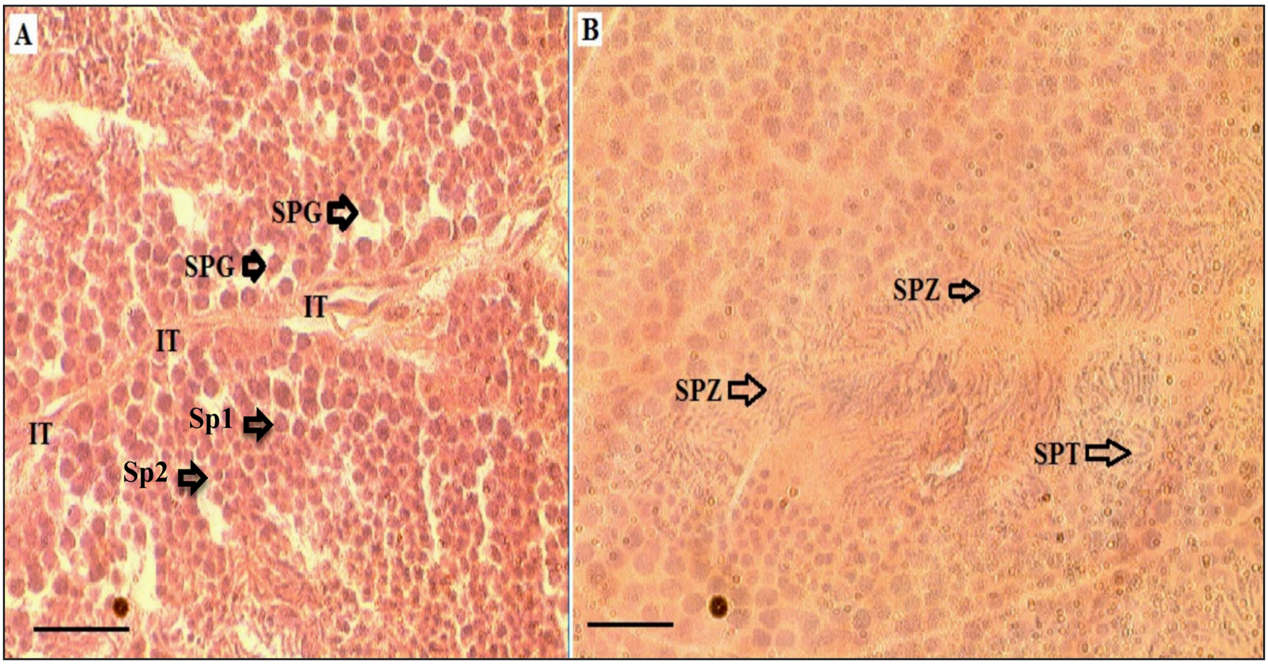

Figure 4. Histological sections of seminiferous tubes of *G. darwinii* specimens collected in October and November 2016 in the Tapacurá forest in São Lourenço da Mata, Pernambuco, Brazil. IT = interstitial tissue; SPG = spermatogonia; SPT = Spermatids; Spt1 = primary spermatocytes; Spt2 = secondary Spermatocytes; SPZ = sperms; (A) October; (B) November. H-E Stained. Bar = 50 µm.

beginning of the dry season, while Spt2 production only peaked at the beginning of the dry season, with no seasonal effects on the stages of cell maturation (Table 1). Females did not present seasonal variation in small cells (Table 1). Males produced reproductive cells year-round, which peaked during the driest

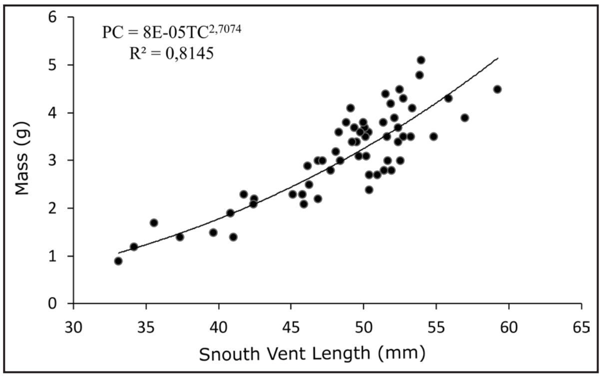

Figure 5. Ratio between body mass (g) and snout-vent length (SVL; mm) of *G. darwinii* specimens collected between July 2016 and June 2017 in the Tapacurá forest in São Lourenço da Mata, Pernambuco, Brazil.

months (October and November), followed by a progressive decrease in the number of spermatozoa during the rainy months (April and May), shifting again and growing during the end of the rainy season (Fig 6). In seven females, fixed clutches size of two eggs were observed both during the dry and the rainy seasons (Fig 7).

The linear regression between the liposomatic relationship (LR), environmental temperatures, and precipitation revealed negative and positive correlations. The number of small cells (SC) in females did not covary with precipitation or air temperature. On the other hand, the number of Spt1 cells in males covaried negatively with both precipitation and air temperature (Table 2). LR covaried positively with K1 and negatively with body temperature, particularly in females. In contrast, small cells in females did not covary with any biotic factor, whereas there was a negative correlation between the number of spermatozoa and body temperature in males (Table 2).

Discussion

Our study showed that reproductive cells of sexually mature individuals of *G. darwinii* were continuously produced over a year, indicating continuous reproductive activity. Energetic reserves, in turn, declined at the end of the dry season and during the rainy season, especially in females, a process associated with precipitation levels. During the rainy season, primary production rates are higher in tropical environments, proportionating a food resource for many lizard populations inhabiting these domains. Those species tend to accumulate energetical reserves constantly, which are used in many phases of the continuous reproductive activity (mating, gestation, and egg-laying) after the investment in growth to reach the sexual maturity, i.e., K1 (Ballinger 1977; Galdino *et al.*, 2003).

Histological changes include the oogenesis in females, with the modifications displayed by the ovary and their structures between previtellogenic and vitellogenic phases, such as the drastic reduction

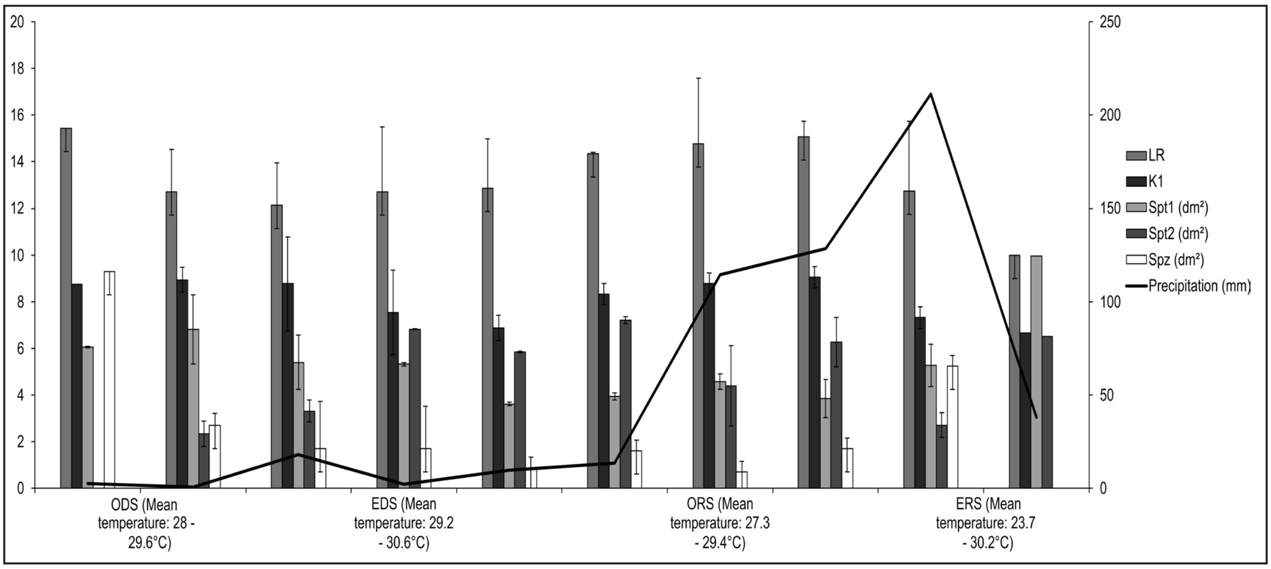

Figure 6. Monthly variation and standard deviation of the condition factor (K1), liposomatic relationship (LR), primary spermatocytes (Spt1), secondary spermatocytes (Spt2), and sperms (SPZ) recorded in males of *G. darwinii*, with the mean air temperature (°C) and precipitation (mm) registered in the same months. Specimens were collected between July 2016 and June 2017 in the Tapacurá forest in São Lourenço da Mata, Pernambuco, Brazil. ODS = onset of dry season (September – November); EDS = ending of dry season (December – February); ORS = onset of rainy season (March – May); ERS = ending of rainy season (June – August).

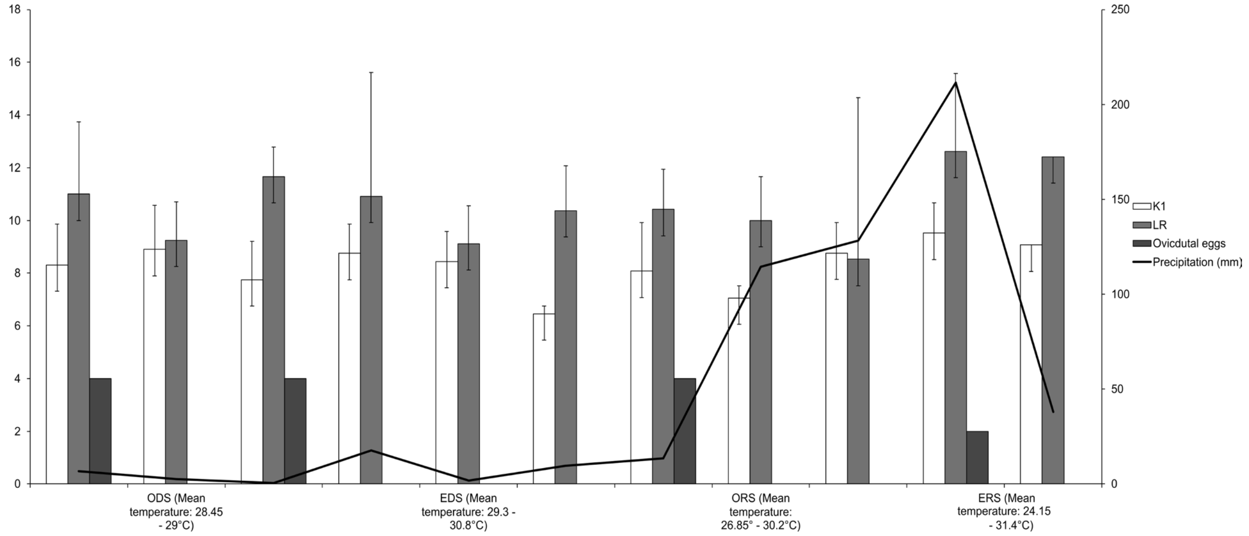

Figure 7. Monthly variation and standard deviation of the condition factor (K1), liposomatic relationship (LR), and number of oviductal eggs recorded in females of *G. darwinii*, with the mean air temperature (°C) and precipitation (mm) registered in the same months, according to seasonality. Specimens were collected between July 2016 and June 2017 in the Tapacurá forest in São Lourenço da Mata, Pernambuco, Brazil. ODS = onset of dry season (September – November); EDS = ending of dry season (December – February); ORS = onset of rainy season (March – May); ERS = ending of rainy season (June – August).

of the Granulosa thickness (Moodley y Van-Wyk, 2007; Santos *et al.*, 2020). Regarding males, these changes include different arrangements of reproductive cells on seminiferous tubes along the maturation stages (Primary and secondary spermatids and sperms). Most reproductive cells at the initial maturation stages (Primary and secondary spermatids) were found in the periphery of the seminiferous epithelium. In contrast, at the final maturation stages (Sperms), most reproductive cells were located in the seminiferous epithelium centre, without reclusion stages on reproductive cell maturation. Histological changes in the shape and arrangement of the reproductive cells in both females and males were similar to patterns documented for other reptiles but displayed some differences compared to other vertebrates (Tumkiratiwong *et al.*, 2012; Sánchez-Ospina *et al.*, 2014; Machado-Santos *et al.* 2015). In other ectothermic vertebrates (amphibians and fishes), male Germ lineage cells forming the seminiferous tubule are grouped into cysts probably due to the strict water dependency for reproduction and the lack of amniotic protection (Sylva y Brender, 1997; Santos y Oliveira, 2008). On the other hand, in endothermic vertebrates (mammals), the Sertoli cells, containing complex folds in their plasmalemma and

Table 1. Results of the Kruskal-Wallis nonparametric analysis of variance applied to the different parameters of the physiology and reproductive biology of the *G. darwinii* specimens (female and male) collected in Tapacurá forest in São Lourenço da Mata, Pernambuco (Brazil), between July 2016 and June 2017. * Significant difference ($p < 0.05$).

Group	H	df	P
Female			
Small Cells	5.50	3	0.13
Liposomatic Relationship	3.98	3	0.26
Condition Factor (K1)	3.70	3	0.29
Male			
Primary Spermatocytes (Spt1)	7.41	3	0.04*
Secondary Spermatocytes (Spt2)	9.28	3	0.02*
Sperms	2.43	3	0.48
Liposomatic Relationship	4.58	3	0.20
Condition Factor (K1)	7.64	3	0.05*

Table 2. Results of the linear regression analyses (r^2) using exogenous (Temperature and Precipitation) and endogenous factors (Body temperature and the K1 Condition Factor) as the independent variables for the comparisons with the following variables: the Liposomatic Relation (LR) and, in the case of the reproductive cells, the Small Cells (SC) in the females, and the Primary (Spt1) and Secondary Spermatocytes (Spt2), and Spermatozoa (SPZ) in the male *G. darwinii* specimens collected in the Tapacurá forest in São Lourenço da Mata, Pernambuco (Brazil), between July 2016 and June 2017. * Significant difference ($p < 0.05$).

	Group analyzed	**Temperature (°C)**		**Precipitation (mm)**		**K1**		**Body Temperature (°C)**	
		r^2	**p**	**r^2**	**p**	**r^2**	**p**	**r^2**	**p**
LR	Female and Male	-0.32	0.01*	0.32	0.01*	0.17	0.02*	-0.17	0.02*
SC	Female	-0.12	0.11	0.09	0.16	-0.03	0.58	-0.03	0.61
Spt1	Male	-0.15	< 0.001*	-0.04	< 0.01*	-0.14	< 0.001*	0.01	0.07
Spt2	Male	0.02	0.88	0.01	0.89	0.01	0.09	0.03	0.64
SPZ	Male	-0.09	0.18	0.01	0.22	0.02	0.75	-0.14	< 0.001*

junctions of occlusion in their lateral membranes, subdivides the seminiferous epithelium into the basal compartment, containing spermatogonia and primary spermatocyte; and the adluminal compartment, containing secondary spermatocytes and sperms (Takashiba *et al*., 2011).

The reproductive cells' maturation in lizards tends to be influenced by extrinsic and intrinsic processes regarding the studied species. (Abu-Zinadah, 2008; Mamou *et al*., 2017). In *G. darwinii*, reproductive cell maturation seems constant, with sexually mature individuals showing mature reproductive cells regardless of the period of the year (Moodley y Van-Wyk, 2007; Torki, 2007). Current studies have reported that environmental conditions, such as appropriate rainfall and optimal temperatures, also contribute to a constant oogenesis and gametogenesis process in neotropical lizards (Salvador, 2011; Migliore *et al*., 2017; Díaz-Vega *et al*., 2020). However, the lack of any significant seasonal variation in the reproductive cells' production in *G. darwinii* suggests that reproductive cell maturation is likely to be firstly determined by the species' biotic rhythm, optimised by suitable environmental conditions. Since *G. darwinii* usually maintain low body temperatures, adopting similar temperatures to the environment (range = 22.4 – 34.6° C), and considering the low-temperature variation in the Atlantic Forest, the resource availability in the environment, such as adequate microhabitats and feeding, seems to display stronger determinant factors for individuals' sexual maturation, also regarding the reproductive cells (Guedes *et al*., 2020; Lara-Resendiz, 2020). Individuals of *G. darwinii* seems to maintain optimised vital activities, including reproduction, growth, and digestion, since it keeps a slightly constant body temperature and display sit-wait behaviour (Autumn and De Nardo, 1995; Lara-Resendiz, 2020).

Spermatozoa were found in both the testes and the males' epididymis, a pattern associated with high testosterone levels in some lizard species (Cooper *et al*., 1987; Galdino *et al*., 2003). This hormone is well known to modulate aggressive, territorial behaviour in males (Galdino *et al*., 2003). Unfortunately, no behavioural data is available for *G. darwinii* so far (Stamps, 1983), which precludes further discussion on whether this species is territorial. On the other hand, the seasonal variation observed in primary and secondary spermatocyte levels seems to be associated with a peak in gonadal activity coinciding with the period in which reproductively active females are more abundant in the population and when copula occurs (Jenssen *et al*., 2001; Widerhecker *et al*., 2002). This scenario also suggests that selective pressures from abiotic environmental factors (such as precipitation and temperature) acting on cell maturation are weak, for both females and males, despite the climatic seasonality observed in the study area (Galdino *et al*., 2003). Nevertheless, our results do not preclude the role of abiotic factors such as air temperature and precipitation on the reproductive biology and gamete production in this species, especially in females (Galdino *et al*., 2003; Norval *et al*., 2019).

It was recorded that females with oviductal eggs during both dry and rainy seasons, despite the importance of precipitation and environmental temperature for the development of reptilian eggs (Ramírez-Bautista *et al*., 2009; Norval *et al*., 2019. Therefore, our results reinforce that *G. darwinii* seems to offset the fixed and/or small clutch size conditions, increasing the frequency of clutches per year (Doughty, 1997, Guedes *et al*., 2020). In addition, due to the rigid-shelled eggs and the low-temperature variance at the study site, eggs seem to be laid at any moment, despite the seasonality and the variation of precipitation levels (Pike et al., 2012; Guedes *et al*., 2020). Due to those conditions and the constant availability of feeding resources in the environment, the energy invested probably did not affect gravid females in different gestation periods (Norval *et al*., 2019; Guedes *et al*., 2020).

Our results indicate that *G. darwinii* increased in weight proportionally to the increase in body length (isometric coefficients of 2.5–4.0), suggesting that individuals invested similarly in accumulating body mass and body length (Vazzoler, 1996; Migliore *et al*., 2017). Tail fat bodies and body size tend to grow at similar rates when lacking previous caudal autotomy (Derickson, 1976). As males and females did not differ in tail length, variation in caudal fat body accumulation in *G. darwinii* is likely related to differences in energetic requirements between the sexes and variation in air temperature and precipitation (Pinilla, 1991). Indeed, caudal fat body accumulation correlated negatively with temperature and positively with precipitation, reflecting the higher temperatures effect during the most favourable period for mating in the study area (i.e., the dry season; Pinilla, 1995; Norval *et al*., 2019). Previous studies have also shown that caudal fat bodies are used in activities other than reproduction, which implies that tail fat loss during the dry season can be regulated by additional factors such as decreasing resource availability and increasing energy-demanding territorial contests (Derickson, 1976; Ramirez-Bautista *et al*., 2009; Norval *et al*., 2019). On the other hand, the tail fat bodies' negative relationship with air temperature and positive relationship with precipitation indicate a high energetic buildup during the rainy season (Ramírez-Bautista *et al*., 2009).

Although there was seasonal variation in the lipid reserves of the studied population, our data indicates that individuals of *G. darwinii* do not accumulate energy reserves for long periods, which suggests that the local availability of food resources is adequate for sustaining other vital individuals' activities (Pinilla, 1991; Serrano-Cardozo *et al*., 2007). As *G. darwinii* is a sit-and-wait forager (Almeida-Gomes *et al*., 2012), little energy is required for foraging (Colli *et al*., 2003; Norval *et al*., 2019). Our results are consistent with other Neotropical lizards, such as *Liolaemus huacahuasicus* Laurent, 1984 (Pinilla, 1991), *Liolaemus bitaeniatus* Laurent, 1984 (Pinilla, 1995) *Iguana iguana* Linneaus, 1785 (Ferreira *et al*., 2002) and *Eurolophosaurus nanuzae* Rodrigues, 1981 (Galdino *et al*., 2003).

Our study presents the first dataset about seasonal variation in energetic and growth cycles and the reproductive cells' maturation of male and female *G. darwinii*. Overall, our findings support that the energetic cycle and reproductive cell maturation of *G. darwinii*'s spermatogenesis, oogenesis, and egg development are influenced firstly by biotic factors and slightly by abiotic factors (Watling *et al*., 2005). The fat body reserves seem to be affected by abiotic factors, probably also sustaining the reproduction during the year, which can be related to the lack of relationship between the gonadal activity and the environment. In conclusion, our study reinforces the continuous gamete production over the year and its optimised production with increased air temperature and precipitation to an acceptable degree. Finally, we suggest that future studies investigate whether the continuous production of reproductive cells in *G. darwinii* modulates other fitness-related behaviours, such as feeding, thermoregulation, mating, and territory defence.

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Trabajo

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Reproductive strategies of the semifossorial snake *Atractus zebrinus* from the Atlantic Forest

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ABSTRACT
Secretive snakes (*e.g.*, fossorial or semifossorial) show morphological adaptations that may constrain potential reproductive output. However, females can store sperm in the reproductive tract, increasing their reproductive potential. Thus, studies on the reproduction of fossorial and semifossorial species are of great interest for a complete understanding of snake reproductive strategies. Here, we investigate the reproductive strategies of the semifossorial snake *Atractus zebrinus* through macroscopic and histological analyzes of the male and female reproductive tracts. Females were larger and had more ventral scales than males, the most common pattern in snakes, and apparently conserved in the genera. The female reproductive cycle was seasonal, with gravid females occurring in spring and summer. Rainfall seems to be the major factor driving female reproductive seasonality, and sperm storage in the infundibular receptacles may ensure fertilization and multiple paternity. The male reproductive cycle was aseasonal. Spermiation, hypertrophy of the sexual segment of the kidney, and sperm storage in the ductus deferentia occurred over several months. This aseasonal reproduction in males may enhance paternity by increasing the species mating chances.

Key words: Female sperm storage; Reproductive cycle; Secretive snakes; Sexual dimorphism; Spermiogenesis.

RESUMO
Las serpientes fosoriales y semifosoriales presentan adaptaciones morfológicas que pueden limitar su reproducción. Sin embargo, las hembras pueden almacenar espermatozoides en el tracto reproductivo aumentando su potencial reproductivo. Por lo tanto, estudios sobre la reproducción de especies fosoriales y semifosoriales son de gran interés para la comprensión de las estrategias reproductivas de las serpientes. En este trabajo, investigamos las estrategias reproductivas de la serpiente semifossorial *Atractus zebrinus* através de análisis macroscópicos y cortes histológicos de los tractos reproductivos de machos y hembras. Como resultado, encontramos que las hembras fueron más grandes y tenían más escamas ventrales que los machos. Por otro lado, el ciclo reproductivo de las hembras fue estacional, con hembras grávidas en primavera y verano. La lluvia parece ser el factor principal que influencia la estacionalidad reproductiva de las hembras, y el almacenamiento de espermatozoide en los receptáculos infundibulares podría asegurar la fertilización y la paternidad múltiple. Además, el ciclo reproductivo de los machos no fue estacional. En varios meses, se encontraron procesos de espermiación, hipertrofia del segmento sexual del riñón y almacenamiento de semen en el conducto deferente. Esta reproducción no estacional en los machos puede aumentar las posibilidades de apareamiento de la especie.

Palavras-chave: Almacenamiento de espermatozoide femenino; Ciclo reprodutivo; Serpientes secretivas; Dimorfismo sexual; Espermiogénesis.

Introduction

Reproductive traits may vary substantially in closely related snake species (Vitt, 1992; Pizzatto *et al.*, 2008). To achieve reproductive success, the timing of mating and gametogenesis can occur in diffe-

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rent periods in congeneric species (Aldridge and Duvall, 2002; Pizzatto and Marques, 2006; Barros *et al.*, 2012). Temperature and rainfall are the main abiotic factors influencing the reproductive cycles in snakes and, therefore, responsible for modulating the seasonality of cycles (Aldridge and Duvall, 2002; Lutterschmidt and Mason, 2009). Thus, reproductive variation between congeneric species is associated with geographical distribution and climate (Aldridge and Duvall, 2002, Pizzatto and Marques, 2006; Barros *et al.*, 2012). Generally, temperate-zone snakes have strictly seasonal reproductive cycles, while tropical snakes have extended reproductive cycles (Saint-Girons, 1982; Bizerra *et al.*, 2005; Mathies, 2011; Silva *et al.*, 2019a).

Most Dipsadidae (*sensu* Zaher *et al.*, 2019) reproduce seasonally, with males presenting spermatogenesis peaks in spring and summer (*e. g.* rainy season; Rojas *et al.*, 2013; Braz *et al.* 2014; Braz *et al.*, 2019), and females showing gravidity/pregnancy and oviposition/parturition mainly in the rainy season (summer) (Pizzatto and Marques, 2002; Pizzatto *et al.*, 2008; Mathies, 2011; Bellini *et al.*, 2013; Braz *et al.* 2014; De Resende and Nascimento, 2015; Loebens et al*.*, 2016; Teixeira *et al.*, 2020). However, relatively few dipsadids have been studied, particularly those with fossorial habits (Braz *et al.*, 2014; Braz *et al.*, 2019; Gualdrón-Durán *et al.*, 2019). This paucity of information probably reflects their secretive nature and the scarcity in zoological collections, even highly biodiverse regions (Martins and Oliveira, 1993; Böhm *et al.*, 2013).

Adaptations to underground life may increase a species' vulnerability to environmental fluctuations and extinction (Cyriac and Kodandaramaiah, 2018; Braz *et al.*, 2019). Evolutionary adaptations to fossorial/semifossorial habitats may require a series of physiological and morphological modifications, such as changes in thermal ecology, head shape, and body shape (Pough, 1980; Navas *et al.*, 2004; Barros *et al.*, 2011; Abegg *et al.*, 2020; Khouri *et al.*, 2020). Consequently, the physiological and morphological constraints imposed by fossoriality may influence a range of life-history traits, such as clutch size and egg size (Marques, 1996; Marques and Puorto, 1998; Balestrin and Di-Bernardo, 2005; Braz *et al.*, 2014; De Resende and Nascimento, 2015; Braz *et al.*, 2019). Thus, studies on the reproduction of fossorial or semifossorial snakes are especially relevant for understand possible reproductive adaptations in species with these habits.

The genus *Atractus* is highly diverse, grouping about 150 species distributed from the eastern region of the Isthmus of Panama to northern Argentina (Fernandes and Puorto, 1993; Giraudo and Scrocchi, 2000; Myers, 2003; Passos *et al.*, 2018). Of these, 39 species occur in Brazil (Costa and Bérnils, 2018). *Atractus zebrinus* (Jan, 1862) inhabits the Atlantic Forest, with some records at high elevations in the Araucaria Forest (Passos *et al.*, 2010a; Barbo *et al.*, 2011; Nogueira *et al.*, 2019). There are few ecological data available for this species in the literature (Marques *et al.*, 2019). Like other congeners, *A. zebrinus* exhibits semifossorial habits, probably nocturnal activity, and females are bigger than males (Fernandes *et al.*, 2000; Marques *et al.*, 2019). However, there is virtually no reproductive data of the species. Herein, we use macroscopic and histological data of male and female reproductive tracts of *A. zebrinus*, to describe the species reproductive cycle. We additionally provide detailed information about the sexual segment of the kidney (SSK), sexual maturity, sexual dimorphism, and clutch size. We also discuss our results for *A. zebrinus* by comparing its reproductive pattern to that reported for another dipsadid species of *Atractus* genus.

Materials and methods

Specimens and area

We examined 38 preserved specimens of *A. zebrinus* housed in museums (Appendix 1). Our sample consisted of 20 females (14 adult and six subadult specimens) and 18 males (13 adults and five subadults) collected in the Atlantic Forest areas of the states of Espírito Santo, Minas Gerais, São Paulo, and Paraná (Southeastern and Southern Brazil; between S 27°22'23", W 51°54'14" and S 22°7' 3", W 45°3'6"; 440-1029 m above sea level). The climate in this region is seasonal, with warmer temperatures occurring from spring (October-December) to summer (January-March) and are associated with higher rainfall, whereas lower temperatures occur from autumn (April-June) to winter (July-September) and are generally associated with lower rainfall (Mendonça and Danni-Oliveira, 2007).

Morphological data

We collected morphological data of adult individuals to test for sexual dimorphism. Before performing the dissections, we measured snout-vent length (SVL) and tail length and counted the number of ventral and subcaudal scales of each specimen following Dowling *et al.*, (1951). Females were considered sexually mature (adult) if they had spermatozoa in the reproductive tract, however indirect evidences were also used, as ovarian follicles bigger than 5 mm length (De Resende and Nascimento, 2015), oviductal eggs, corpora lutea, or folded oviducts (indicative of recent egg-laying; Almeida-Santos *et al.*, 2014; Silva *et al.*, 2020). Males were considered adults if they had spermatozoa in the reproductive tract (Almeida-Santos *et al.*, 2014; Silva *et al.*, 2020). In adult females, we recorded the number of ovarian follicles and oviductal eggs and the diameter of the largest ovarian follicle and egg (Almeida-Santos *et al.*, 2014). In adult males, we recorded the diameter of the right distal ductus deferentia (between the kidney and the cloaca) and the length, width, and thickness of the right testis. The organs were measured using a digital caliper (± 0.1 mm).

Histology

For histological data, only adults were used. For females (N = 14), we excised the posterior portions of the right nonglandular uterus, right posterior infundibulum, and pouch (nomenclature of Siegel *et al.*, 2011) to check for the presence of spermatozoa. We also collected ovarian follicles from each individual to identify highly vacuolated ooplasm (vitellogenic follicle), the last stages that precedes the ovulation (Manes *et al.*, 2007; Vieira *et al.*, 2010).

For males (*N* = 13), we collected the mid-region of the testes, the distal ductus deferentia (the portion between the kidney and the cloaca), and the proximal region of the kidneys (Rojas *et al.*, 2013). Tissue samples were stored in 70% ethanol, dehydrated, and embedded in paraffin. We used a rotary microtome to produce transverse and longitudinal sections (5 μm) and stained the slides with hematoxylin-eosin (Junqueira and Carneiro, 2013). Testis and ductus deferens sections were assessed for sperm production and presence, respectively. Kidney sections were examined to identify SSK hypertrophy. We classified spermatogenesis according to the six cell stages presented by Silva *et al.*, (2019a). However, we only observed the following three stages: spermiogenesis (metamorphosing spermatids), spermiation (mature spermatozoa in the lumen) and early regression (reduced germinal epithelium, with few spermatogonia, spermatocytes, and spermatids). For each specimen, we measured the height of the seminiferous epithelium height, seminiferous tubule diameter, SSK epithelium height, and SSK tubule diameter (Rojas *et al.*, 2013) using ImageJ software v1.46 (Abramoff *et al.*, 2004).

Data analysis

A sexual size dimorphism (SSD) index was calculated as: (mean SVL of females/ mean SVL of males)-1. This index is arbitrarily expressed as positive if females are the larger sex and negative if males are the larger (Lovich and Gibbons, 1992). Morphological sexual dimorphism was tested only in adults. Before the analysis all variable was tested for normality of distribution using the Shapiro-Wilk's test. The only variable that showed deviation for normal distribution were SVL on females ($W = 0.71$, $p < 0.005$), and in this sense we accessed sexual variation in this trait using a Mann-Whitney test. Differences in the number of ventral and subcaudal scales was tested in using a Welch t-test. A previous Analysis of Covariance (ANCOVA) showed that TL does not increase with SVL ($F = 2.68$, $p = 0.11$, $N = 30$), therefore sexual variation in TL were also tested using a Welch t-test. We determined clutch size by counting the number of enlarged ovarian follicle at the beginning of the vitellogenesis process (>5 mm; potential clutch size) or oviductal eggs in preserved specimens. We investigated the relationship between clutch size and maternal SVL using Spearman's rank correlation test. We calculated testis volume (TV) in each season using the ellipsoid formula: $TV = (4/3)\pi(abc/2)$, where a = length, b = width, and c = thickness (Pleguezuelos and Feriche, 1999). Also, a previous test showed that testis volume was not correlated with male SVL ($r = 0.31$, $p = 0.38$, $N = 10$), in this sense we present the raw data of the mean testicular volume. All analyses were performed using R Statistical Software version 4.0.2 (R Core Team, 2021).

Results

Sexual maturity, sexual dimorphism, and clutch size

Our smallest adult female was 423 mm SVL, while the smallest adult male was 315 mm SVL (Table 1). Adult females were larger ($U = 181$, $df = 28$, $p < 0.005$) and had more ventral scales ($t = 9.02$, $df = 24.8$, $p < 0.005$) than adult males (Table 1). However, males had relatively larger tails ($t = -2.86$, $df = 28$, $p = 0.008$) and more subcaudal scales ($t = -5.69$, $df = 19.2$, $p = 1.67e-5$) than females (Table 1). The SSD

Table 1. Variation in snout–vent length (mm), tail length (mm), and number of ventral scales and subcaudal scales in male and female adults of *Atractus zebrinus*. The results are expressed as the mean ± standard deviation (SD) of the total sampling number and range of distribution.

Variable	**Male**			**Female**		
	Mean ± SD	Range	N	Mean ± SD	Range	N
Snout-Vent Length	386 ± 58	315-487	13	463 ± 55	423-621	14
Tail Length	49 ± 9	40-68	13	41 ± 8	31-54	14
Ventral Scales	146 ± 4	140-153	13	159 ± 4	153-166	13
Subcaudal Scales	26 ± 4	21-34	13	19 ± 2	16-23	13

Note: Due to the process of tissue autolysis, there was only one individual for histological measurements.

was 0.20. Clutch size averaged 6 ± 3 eggs/vitellogenic follicles (range = 3-11, N = 8) and was not correlated with female SVL (r = 0.48, p = 0.22, Fig. 1A).

Female reproductive cycle and sperm storage

Females in early vitellogenesis (follicles ranging from 5.0-8.5 mm) were observed from October to March, except in November (Figs. 1B, and Fig. 2A-B). We did not find ovarian follicle in the last stages of follicle maturation before ovulation. Gravid females were observed in October (N = 1), November (N = 2), December (N = 1) and January (N = 1) (Figs. 1B, 3). In July 1992, a clutch of six eggs (ZUEC-REP 1407) was found buried (~10 cm depth) in a pile of pine splinters (*Pinus elliotti*) at Parque Estadual de Campos do Jordão, state of São Paulo (southeastern Brazil). Eggs were collected and fixed and housed in zoological collection. Dissections revealed fully formed *A. zebrinus*, which were close to hatching. One of the individuals was measured (140 mm SVL and 10 mm tail length) and weighed (3 g body mass). Recruitment period (hatchlings with 140-168 mm SVL; N = 5) occurred in October, November, December, and January (Fig. 3).

A female in early vitellogenesis (follicle size = 5 mm) collected in late summer (March) exhibited sperm stored inside the alveolar glands of the posterior infundibulum (Figs. 4A, B). No female had sperm in the nonglandular uterus (Fig. 4C) and the pouch (Fig. 4D). The epithelium lining the posterior infundibulum had ciliated and secretory cells, which were cuboidal to columnar. The outer wall of the nonglandular uterus and the pouch consisted of two layers of smooth muscle: an inner circular layer and an outer longitudinal layer (Fig. 4D). The epithelium lining the nonglandular uterus was pseudostratified, and presented many ciliated cells (Fig. 4C). The pouch had a thick lamina propria with folds, and the epithelium was pseudostratified columnar (Fig. 4D).

Male reproductive cycle

Adult males were collected in February (N = 3), March (N = 2), April (N = 1), May (N = 1), July (N = 1), September (N = 1), and November (N = 4). Males had testes in spermiogenesis (Fig. 5a) or spermiation (Fig. 5b) in all seasons (Fig. 3). In au-

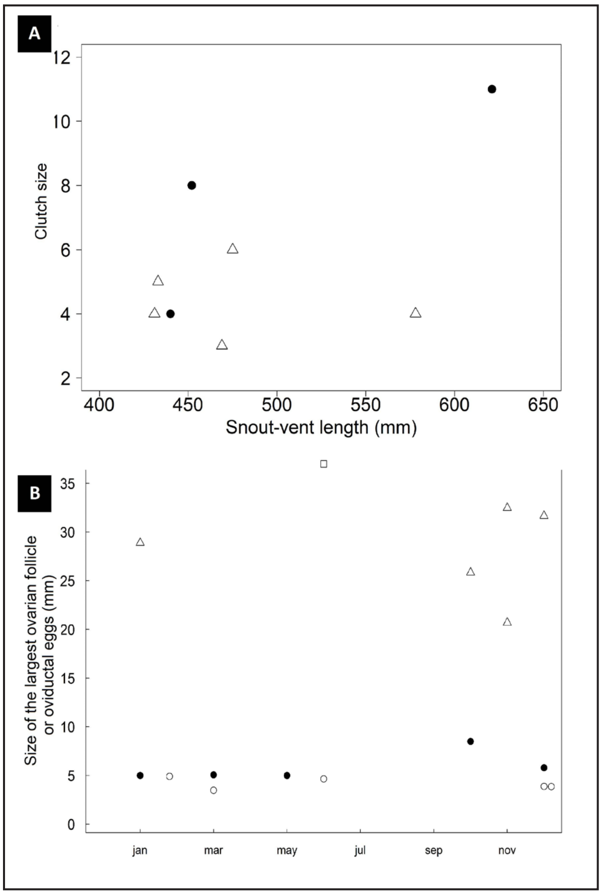

Figure 1. Reproductive traits of female *Atractus zebrinus*. (a) Relationship between maternal snout-vent length and clutch size. (b) Seasonal variation in the size of the largest ovarian follicles/oviductal eggs and timing of eggs found in nature. *Note*: Black circles = follicles in early vitellogenesis; White triangles = oviductal eggs; White circles = previtellogenic follicles; White square = eggs in nature.

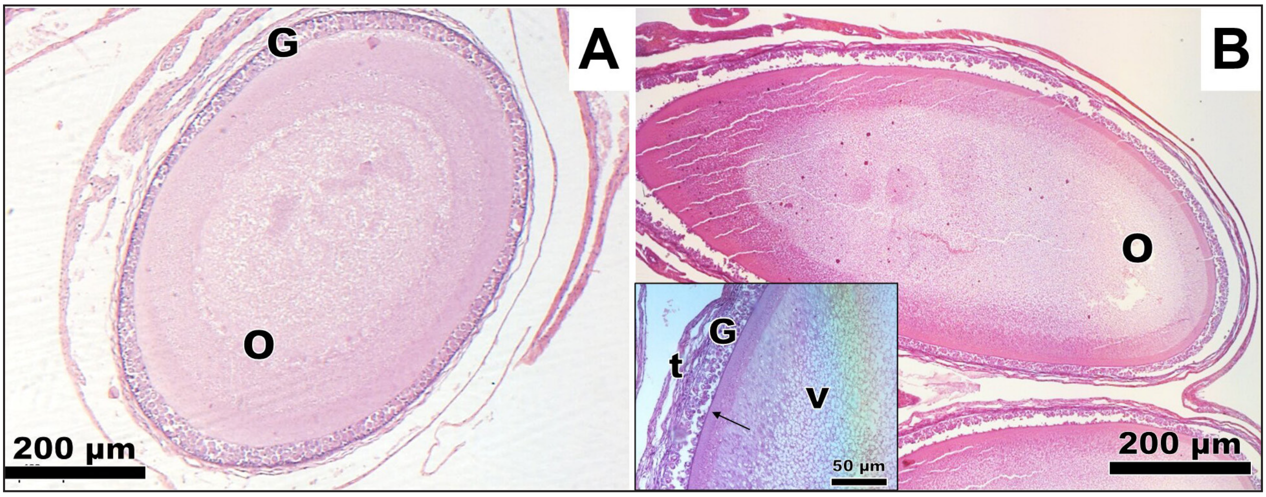

Figure 2. Histology of the ovarian follicles of *Atractus zebrinus* at the onset of vitellogenesis. (a) 5 mm diameter follicle. (b) 8.5 mm diameter follicle. *Note the vacuolated ooplasm:* G = Granulosa layer; V = vesicles; O = oocyte; t = theca. The arrow indicates the zona radiata. Hematoxylin-eosin.

tumn, males had testes in spermiogenesis in April (N = 1) and spermiation in May (N = 1). In late winter (September), the only individual examined had testes in spermiation. In spring (November), males (N = 4) had testes in spermiogenesis or spermiation; only one male had regressed testes, although some spermatids were still present. In summer (February and March), all males (N = 4) had testes in spermiation. All males showed hypertrophied SSKs (Figs. 3, 5C) and ductus deferentia packed with sperm (Figs. 3, 5D). Table 2 shows the variation in testicular volume, seminiferous epithelial height, seminiferous tubule diameter, SSK diameter, and SSK epithelial height. Unfortunately, the sampling per season was too small to test for seasonal differences statistically.

Discussion

Sexual dimorphism, sexual maturity, and clutch size

Our finding of female-biased SSD agrees with previous results reported in this species (Fernandes *et al.*, 2000). Female-biased SSD is consistent with size-related fecundity selection and the absence of male-male combat (Shine, 1994). This SSD pattern and maturity at larger sizes in females are common in *Atractus* (Balestrin and Di-Bernardo, 2005; De Resende and Nascimento, 2015; Zanella and D'Agostini, 2018; Ferreira-Silva *et al.*, 2019; Abegg *et al.*, 2020). In all species studied so far, females are larger than males, and male-male combat is absent, suggesting that the female-biased SSD is phylogenetically conserved. Additionally, female *Atractus* have more ventral scales and fewer subcaudal scales than males (Passos *et al.*, 2010b). In snakes, the number of ventral and subcaudal scales is often correlated with the number of vertebrae and body size (Arnold, 1988; Lindell *et al.*, 1993). Because female snakes commonly have more body vertebrae and fewer caudal vertebrae than males (Shine, 1993), our results on sexual dimorphism in scalation may be a function of sexual variation in the number of vertebrae.

Male *A. zebrinus* show relatively larger tails than females, a pattern similar to that observed in other *Atractus*, except *A. paraguayensis* (Balestrin and Di-Bernardo, 2005; Zanella and D'Agostini, 2018; Ferreira-Silva *et al.*, 2019; Abegg *et al.*, 2020). The morphological constraint hypothesis states that the male tails are longer to accommodate the hemipenis and associated muscles. King, (1989)

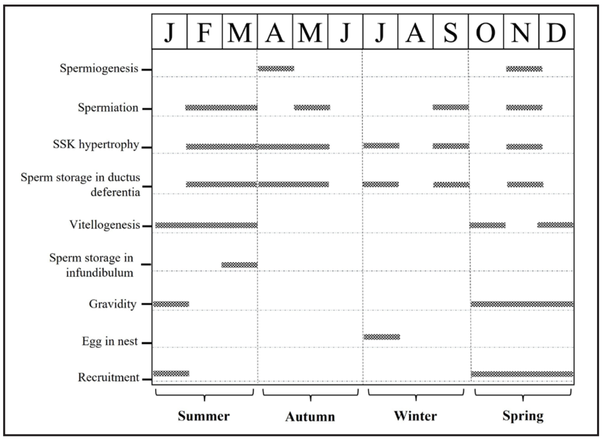

Figure 3. Overview of the reproductive events in male and female *Atractus zebrinus*.

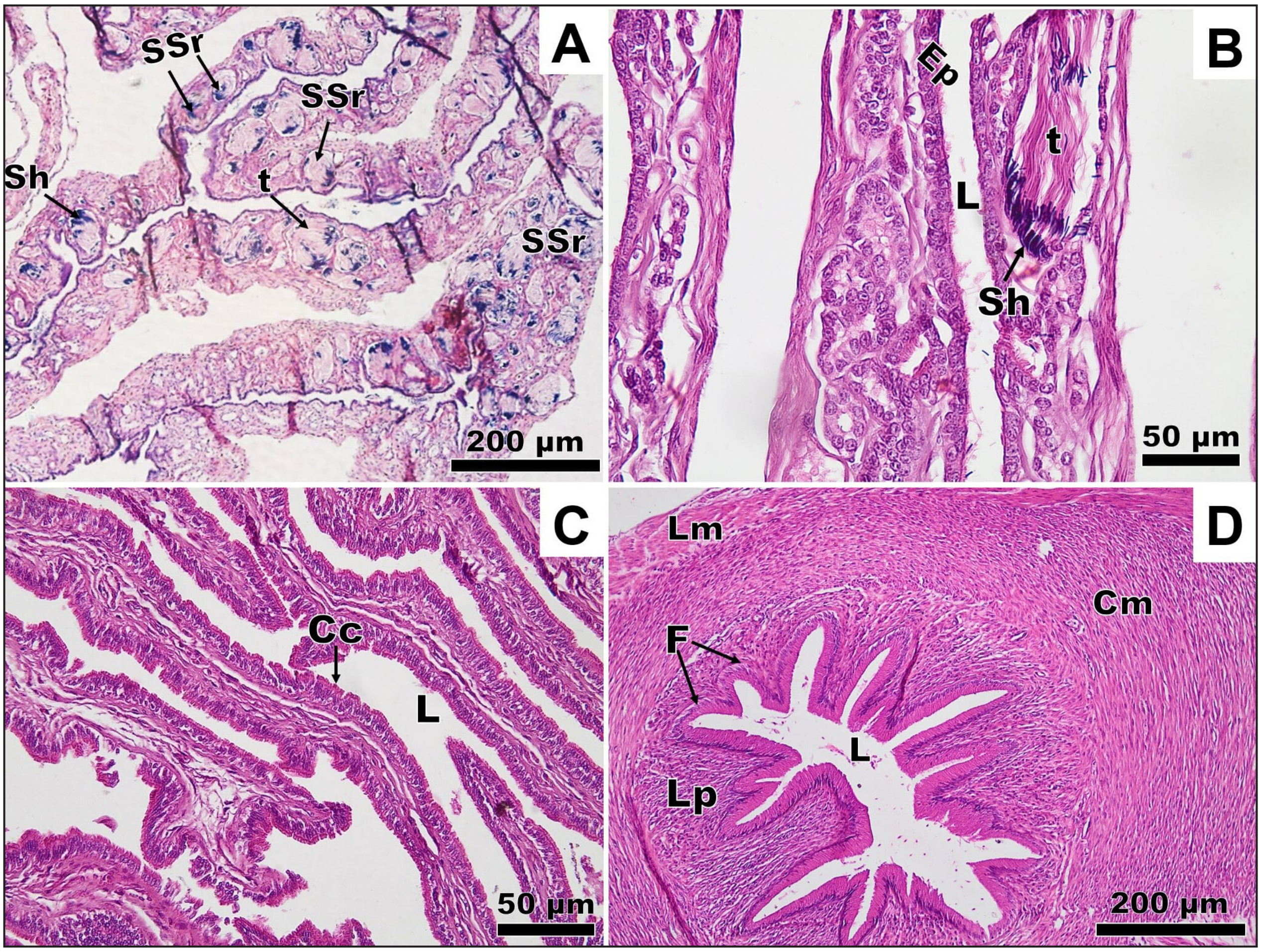

Figure 4. Histology of the reproductive tract of female *Atractus zebrinus.* (a) Posterior infundibulum showing various alveolar glands with sperm. (b) Higher magnification of sperm receptacles. (c) Nonglandular uterus. (d) Pouch showing thick epithelium. *Note:* SSr = Sperm storage receptacles; Sh = sperm head; t = sperm tail; L = lumen; Ep = luminal epithelium; Cc = ciliated cells; F = folds; Lp = lamina propria; Cm = circular muscle; Lm = longitudinal muscle. Hematoxylin-eosin.

found strong support for this hypothesis after testing it using 104 colubrid species. On the other hand, Shine *et al.* (1999) provide evidence that sexual selection may also occur in species that form mating aggregations, as males with intact tails may be until three times more successful in mating. Because mating aggregation has been observed in at least one *Atractus* species (*A. marthae*: Meneses-Pelayo and Passos, 2019), sexual selection could also explain the sexual dimorphism in the tail length of *A. zebrinus*, although, field observations are needed in order to provide evidence for this hypothesis.

Sexual maturation at smaller body sizes in males is also common in snakes (Shine, 1994). Larger females are often more fecund, mainly because a larger celomic cavity allows for larger clutches/litters and offspring (Rivas and Burghardt, 2001). Thus, it is advantageous for females to reach sexual maturity at larger body sizes than males (Shine, 1978). However, the lack of correlation of body size and clutch size suggests that fossorial habits probably constrain clutch size, reducing clutch size variance (Braz *et al.*, 2014; Braz *et al.*, 2019). Alternatively, the small sampling of gravid females may have affected the power of the analysis.

Clutch size of *Atractus* may be more strongly influenced by other factors, such as abiotic/biotic factors (*e.g.*, climate or prey availability, Seigel and Fitch, 1985, Barros *et al.*, 2014), life-history traits (*e.g.,* habitat use), and phylogeny (Zanella and D'Agostini, 2018). Although clutch size increases with maternal body size in some species (*A. pantostictus*: De Resende and Nascimento, 2015), this relationship does not occur in others (*e.g.*, *A. paraguayensis*: Zanella and D'Agostini, 2018; this study). The clutch size of *A. zebrinus* (3-6, considering only eggs) is similar to that of various similar-sized congeners such as *A. pantostictus* (2-4 eggs),

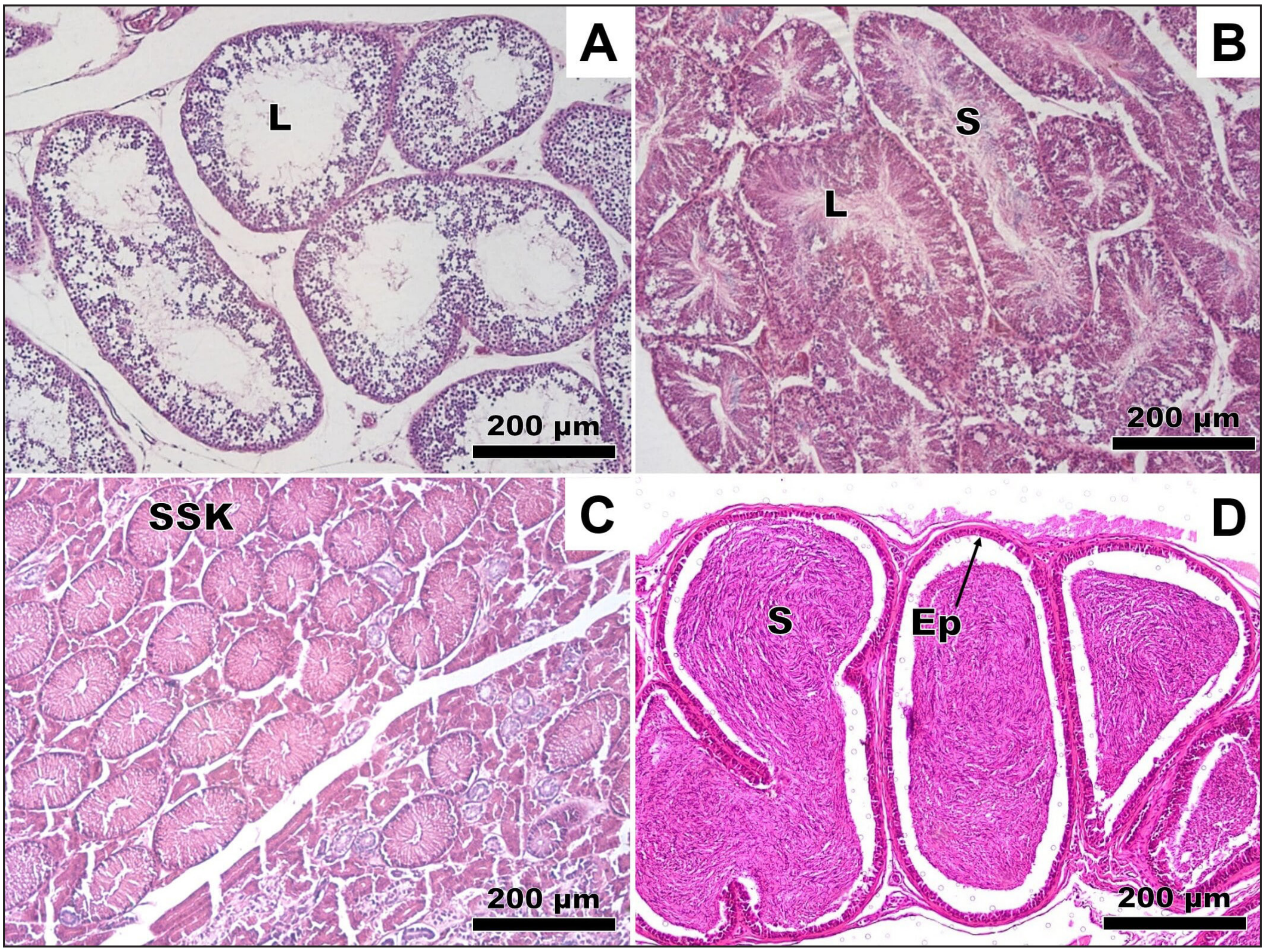

Figure 5. Histology of the reproductive tract of male *Atractus zebrinus*. (a) Seminiferous tubules in spermiogenesis. (b) Seminiferous tubules in spermiation, peak of cell division. (c) Hypertrophy of the sexual segment of the kidney. (d) Ductus deferens with the lumen packed with sperm. *Note:* L = lumen; S = spermatozoa; SSK = sexual segment of the kidney; Ep = epithelium. Hematoxylin-eosin.

A. reticulatus (1-3 eggs), *A. carrioni* (3-5 eggs), *A. paraguayensis* (3-8 eggs), *A. schack* (8 eggs), and *A. francoi* (6 eggs), but it is smaller than the larger ones (*e.g.*, *A.* gigas; 12 eggs) (Martins and Oliveira, 1993; Balestrin and Di-Bernardo, 2005; Passos *et al.*, 2010; Barbo *et al.*, 2011; Passos *et al.*, 2013; De Resende and Nascimento, 2015; Zanella and D'Agostini, 2018; Marques *et al.*, 2019).

Reproductive cycles

The reproductive cycle of female *A. zebrinus* is seasonal, with vitellogenesis, sperm storage, and gravidity occurring in the rainiest seasons (spring and summer). Gravidity is the best metric to classify reproductive cycles, as the completion of reproduction (oviposition) is relatively imminent, and the length of time a female is ovigerous may not be as variable as the time it is vitellogenic, which may vary among individuals due to many factors, such as hormonal levels or body condition (Mathies, 2011). Oviductal eggs, oviposition and recruitment occur mainly in spring and summer in *Atractus carrioni* (Passos *et al.*, 2013), *A. reticulatus* (Fernandes and Puorto, 1993; Balestrin and Di-Bernardo, 2005), *A. paraguayensis* (Zanella and D'Agostini, 2018), *A. pantostictus* (Travaglia-Cardoso and Maia, 2012; De Resende and Nascimento, 2015), and *A. ronnie* (Ferreira-Silva *et al.*, 2019). Therefore, the reproductive cycle of female *Atractus* is completed within the period of high rainfall. However, gravidity appears to occur during the months of lower rainfall in several Amazonian *Atractus* (Martins and Oliveira, 1993). Further studies are needed to understand the environmental influence on the type of reproductive cycle in *Atractus*.

The reproductive cycle of female oviparous snakes is often heavily influenced by rainfall, with oviposition peaking in the months of lower rainfall to ensure hatching success (Brown and Shine, 2006). In contrast, oviposition in *Atractus* occurs during

Table 2. Variation in testicular volume (mm3) and microscopic measurements (µm) of the urogenital structures of *Atractus zebrinus*. The results are expressed as the mean ± standard deviation (SD).

	Summer (N = 5)	**Autumn (N = 2)**	**Winter (N = 2)***	**Spring (N = 4)**
Volume testicular	72 ± 14 (61-87)	126 ± 114 (45-207)	130 ± 32 (107-153)	198 ± 112 (116-326)
Seminiferous tubule diameter	201 ± 22 (187-233)	252 ± 57 (212-292)	115	192 ± 86 (91-267)
Seminiferous epithelial height	58 ± 14 (39-72)	66 ± 3 (64-68)	28	53 ± 16 (28-64)
Sexual segment of kidney (SSK) diameter	142 ± 36 (83-177)	98 ± 3 (97-101)	82	104 ± 51 (65–162)
SSK epithelial height	64 ± 15 (38-76)	41 ± 4 (37-43)	34	43 ± 21 (25-66)

Note: Due to the process of tissue autolysis, there was only one individual for histological measurements.

the rainiest months (December–March; Fernandes and Puorto, 1993; Martins and Oliveira, 1993; Sandoval *et al.*, 2009; Travaglia-Cardoso and Maia, 2012; Ferreira-Silva *et al.*, 2019). For *Atractus zebrinus*, although the finding of eggs in nest (July) and neonates (October-January), it was not possible to identify the oviposition period. This is because, the incubation period of *Atractus* eggs is quite variable (2 to 8 months - Balestrin and Di-Bernardo, 2005; Travaglia-Cardoso and Maia, 2012; Fernandes and Puorto, 1993) and come from captivity, in which they may not reflect the climatic conditions in nature.

In *A. pantostictus*, mating occurs in the rainy season – spring and summer (De Resende and Nascimento, 2015). In *A. marthae*, a mating aggregation was recorded in April, when rainfall peaks (Meneses-Pelayo and Passos, 2019). The mating season of *A. zebrinus* remains unknown, but it probably occurs in the rainy season (spring and summer), when we found receptive vitellogenic females (estrus, DeNardo and Taylor, 2011) and sperm storage (in late summer). Sperm storage until the follicles reach ovulatory size is crucial to ensure fertilization. In addition, estradiol and testosterone levels are elevated in females during vitellogenesis (DeNardo and Taylor, 2011; Taylor *et al.*, 2004). While estradiol stimulates vitellogenesis, testosterone can stimulate receptivity in female (DeNardo and Taylor, 2011).

To ensure fertilization and, in some cases, multiple paternity (Friesen *et al.*, 2020), females of *A. zebrinus* store sperm in the posterior infundibulum in the beginning of the vitellogenesis, a strategy recently reported in the genus (Gualdrón-Durán *et al.*, 2019). Sperm has also been reported in the nonglandular uterus of *Atractus pantostictus* as a result of recent mating (De Resende and Nascimento, 2015). In snakes, female sperm storage occurs mainly in the uterus and infundibulum (Siegel *et al.*, 2011). As in other snakes (Siegel *et al.*, 2011), the spermatozoa aggregated in the alveolar glands are positioned in a parallel alignment, with the acrosomes orientated towards the epithelium of the lamina propria of the posterior infundibulum. This arrangement is often suggested to increase sperm survival (Siegel *et al.*, 2011; Rojas *et al.*, 2015; Rojas *et al.*, 2017).

The morphology of the infundibular glands (sperm receptacles) seems to be phylogenetically conserved among snake families, consisting of alveolar glands in dipsadids (Perkins and Palmer, 1996; Rojas *et al.*, 2015) and tubular glands in viperids (Saint-Girons, 1957; Siegel and Sever, 2008; Silva *et al.*, 2019b). Sperm storage may be advantageous for reproductive success by allowing sperm competition and multiple paternity (Uller and Olsson, 2008; Friesen *et al.*, 2020). In the dipsadid *P. patagoniensis,* gravid and postpartum females remain with sperm stored in the nonglandular uterus, suggesting the ability to produce several clutches from a single mating (Rojas *et al.*, 2015; Loebens *et al.*, 2016). If mating ball (Crews and Garstka, 1982) is a common reproductive strategy in *Atractus*, sperm storage in the female reproductive tract would be extremely advantageous for sperm competition and multiple paternity. However, mating order effects and other mechanisms that might confer cryptic female choice in snakes with long-term sperm storage (up to 6 years) remains unknown (Levine *et al.*, 2021).

Although our sampling of males may be considered small for statistical analyses (N=13), all individuals except one showed seminiferous tubules with epithelium filled with spermatids and/or lumen filled with sperm. These records were registered between the months of February and November, and when considering the seasons, they can be classified as happening during: mid to late summer, early to mid-autumn, late winter, mid-spring. This pattern can be characterized as aseasonal at the population level (Mathies, 2011). The one individual that was not in the spermatogenesis peak showed a reduction in activity in November (mid-spring), which may indicate that the cycles of individual males are continuous cyclical (Mathies, 2011). Thus, our results indicated sperm was produced over a long period, what apparently also occurs in *Atractus marthae* (Gualdrón-Durán *et al.*, 2019).

The reproductive cycle of male *Atractus* has been studied histologically only in *A. marthae* (Gualdrón-Durán *et al.*, 2019). In this species, males have an aseasonal reproductive cycle, with sperm production year-round (Gualdrón-Durán *et al.*, 2019). *Atractus* is taxonomically classified in the subfamily Dipsadinae (Grazziotin *et al.*, 2012), and other studies have already reported dipsadines presenting an aseasonal reproductive cycle for males (Angarita-Sierra and López-Hurtado, 2020; Alves *et al.*, 2005). The arboreal *Dipsas neivai* and *D. catesbyi*, although not confirmed by histological analyses, present constant testes length throughout the year (Alves *et al.*, 2005). Similarly, Angarita-Sierra and López-Hurtado (2020) and Goldberg (2004) suggested that the semifossorial *Ninia atrata* and *Ninia maculata* shows an aseasonal spermatogenesis or a prolonged reproductive cycle. Finally, the aquatic snake (*Helicops pastazae*), males have an aseasonal cycle at the population level (García-Cobos *et al.*, 2021). These findings indicate that aseasonal reproduction is a fairly common strategy among dipsadids.

Among dipsadids with terrestrial habits, the male reproductive cycle has been studied in detail in *Dipsas mikanii* (Rojas *et al.*, 2013), *Philodryas patagoniensis* (Loebens *et al.*, 2017), and *Tomodon dorsatus* (Loebens *et al.*, 2020). In these species, the cycle of individual males is strictly seasonal, with spermatogenesis and SSK hypertrophy peaking in summer (Rojas *et al.*, 2013) or spring and summer (Loebens *et al.*, 2017). Thus, semifossorial snakes such as *A. zebrinus* and *A. marthae* (Gualdrón-Durán *et al.*, 2019) may have more plastic or asynchronous reproductive cycles to ensure paternity, when meeting a receptive female. However, individual males of the fossorial snake *Phalotris lativittatus* exhibit a discontinuous cyclical reproductive cycle, and a spermatogenesis peak in spring (Braz *et al.*, 2014), indicating that abiotic (*e.g.*, climate) or biotic (*e.g.*, diet) factors may modulate the reproductive cycles of dipsadids. While *P. lativittatus* occurs in the Brazilian Cerrado and feeds on amphisbaenids (Braz *et al.*, 2014), *A. zebrinus* occurs in the Atlantic Forest and feeds on annelids (Marques *et al.*, 2019). Biotic factors (*e.g.*, predation risk and food supply) may influence the extension of the reproductive season (Crews and Moore, 1986).

In squamates, SSK hypertrophy has been associated with mating or spermatogenesis (Schuett *et al.*, 1997; Krohmer, 2004; Sever and Hopkins, 2005; DeNardo and Taylor, 2011). The SSK secretory granules are deposited in the female reproductive tract during mating and may contribute to the seminal composition and formation of a copulatory plug (Aldridge *et al.*, 2011; Friesen *et al.*, 2013). In all male *A. zebrinus*, the SSK hypertrophy, spermiation, and the presence of sperm in ductus deferentia were synchronous and occurred in several months of the year. Therefore, male *A. zebrinus* could mate at various times of the year, depending on the signaling of the female (as observed in *A. marthae*, Gualdrón-Durán *et al.*, 2019).

In sum, the reproductive cycle of female *A. zebrinus* is seasonal, and females may potentially enhance their fertility and fitness through oviductal sperm storage and sperm competition. Moreover, male *A. zebrinus* may ensure their paternity due to their prolonged reproductive activity, increasing this way the species mating chances.

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Author Contributions

LHCS – Data collection, analysis and the manuscript writing; JLS - Data collection, analysis and the manuscript writing and KMPS - Data collection, analysis and the manuscript writing.

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Appendix 1 – Voucher specimens of *Atractus zebrinus* analyzed in this study:

Museu Nacional (MNRJ): 18684; 18717. **Coleção Herpetológica Richard Alphonse Hoge, Instituto Butantan (IBSP):** 32367; 44049; 62537; 71747; 71952; 72872; 72904; 72905; 73735; 78563; 81405; 80487; 82070; 83995; 84373; 84374; 84982, 84983; 84984; 84985; 84986; 84987; 84988; 86384; 86462; 87639; 88762; 88763; 88764; 88765; 88766; 88767; 88769; 88768; 89731; 89799; 89896. **Museu de Zoologia da Universidade Estadual de Campinas (ZUEC):** REP 1407. **Museu de História Natural Capão da Imbuia (MNHCI):** 8331.

Trabajo

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Taxonomic notes on the *Phalotris bilineatus* group (Serpentes: Dipsadidae: Elapomorphini), with the description of a new species from northwestern Argentina

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ABSTRACT
The examination of 93 specimens of the *Phalotris bilineatus* group housed in the major Argentinian collections allowed us to recognize three additional species of this group. We propose the resurrection of two names, *Phalotris spegazzinii* and *Phalotris suspectus*, previously placed in the synonymy of *P. bilineatus*, and describe a new species from northwestern Argentina. The new species is recognized based on a unique combination of morphological characters. In addition, we redescribe the resurrected taxa (providing variation in color patterns, meristic and morphometric characters) and present new data on their geographic distributions.

Key Words: *Phalotris*; Species boundaries; Meristic characters; Morphological characters; Color patterns.

RESUMEN
El examen de 93 ejemplares del grupo de *Phalotris bilineatus* depositados en las principales colecciones de la Argentina permitió reconocer tres especies adicionales del complejo. Proponemos la revalidación de dos nombres, *Phalotris spegazzinii* y *Phalotris suspectus*, previamente ubicados en la sinonimia de *Phalotris bilineatus*, y se describe una nueva especie del noroeste de la Argentina. La nueva especie se reconoce con base en una combinación única de caracteres. Adicionalmente, redescribimos los taxones revalidados (brindando información sobre patrones de coloración, caracteres morfológicos y merísticos) y presentamos nuevos datos sobre su distribución geográfica.

Palabras clave: *Phalotris*; Límites de especie; Caracteres merísticos; Caracteres morfológicos; Patrones de coloración.

Introduction

The tribe Elapomorphini includes approximately 50 Neotropical species belonging to the genus *Apostolepis* Cope, 1861; *Coronelaps* Lema & Deiques, 2010; *Elapomorphus* Wiegmann, 1843; and *Phalotris* Cope, 1861 (Entiauspe-Neto *et al.,* 2020). Elapomorphini snakes are characterized by the following suite of characters: reduction of the number of supralabial scales to six; entire nasal plate; frontal bones dorsally included by the anterior-lateral processes of the parietal and almost excluded from the reduced optic foramen; exoccipitals in contact on the dorsal surface of the condyle; second supralabial scale contacting the eye (Zaher *et al*., 2009). The monophyly of the tribe Elapomorphini was corroborated by several recent studies based on molecular phylogenies (Zaher *et al.,* 2009; Grazziotin *et al.,* 2012; Pyron *et al.,* 2013). They are small to moderate-sized fossorial snakes that feed mostly on elongated fossorial vertebrates (e.g., amphisbaenians and snakes) (Lema, 1989; Braz *et al.,* 2014). The complex taxonomy of

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this group is even more intricate due to the scarcity of specimens in herpetological collections of these hard to find fossorial snakes, which hampers morphological studies (Ferrarezzi, 1993a,b).

Ferrarezzi (1993a,b) resurrected the genus *Phalotris* to accommodate Elapomorphini species presenting only one prefrontal. The number of species of the genus is still debated. Uetz *et al.* (2022) mention 14 species, but their list does not include *Phalotris bilineatus*, a species that has been accepted for some time (i.a. Puorto and Ferrarezzi, 1993; Wallach *et al.*, 2014; Williams *et al.*, 2021), they also do not include *Phalotris punctatus*, a species revalidated by Martins and Lema (2017). The recently described *Phalotris shawnella* is also missing from the list (Smith *et al.,* 2022). Therefore, up to now the genus must be considered composed of 17 species, arranged in three species groups: *Phalotris tricolor*, *P. bilineatus*, and *P. nasutus*. The *P. bilineatus* group is characterized by vertebral and dorsal-lateral black longitudinal stripes laterally expanded and black venter (Ferrarezzi, 1993a; Cabral and Cacciali, 2015), comprising five recognized species (see below).

Brief taxonomic history

The taxonomic history of the species of the *Phalotris bilineatus* group is particularly convoluted, with many names with uncertain status demanding careful revision and was in part summarized by Ferrarezzi (1993a) and Puorto and Ferrarezzi (1993). The first species described in this group were *Phalotris bilineatus* (Duméril, Bibron and Duméril, 1854) and *P. lemniscatus* (Duméril, Bibron and Duméril, 1854), followed by *P. reticulatus* (Peters, 1860), *P. iheringi* (Strauch, 1884), *P. melanopleurus* Cope, 1885, *P. trilineatus* (Boulenger, 1889), *P. spegazzinii* (Boulenger 1913), *P. suspectus* (Amaral, 1924), and *P. bollei* (Mertens, 1954). Most of these latter species were described based on a single specimen, and the validity of several assignments generated controversy (Puorto and Ferrarezzi, 1993), until Amaral (1930) and posteriorly, Peters and Orejas-Miranda (1970) considered all these names as synonyms of *P. bilineatus*. Lema (1970, 1979, 1984) proposed the recognition of only one species with several subspecies and later considered two species as follows: (i) *P. lemniscatus* including four subspecies: *P. l. lemniscatus*, *P. l. iheringi*, *P. l. trilineatus*, and *P. l. divittatus* (Lema, 1984); and (ii) *P. spegazzinii*, containing two subspecies: *P. s. spegazzinii* and *P. s. suspectus*. Also, it suggested that the holotype of *Phalotris bilineatus* would be a hybrid or an intergrade specimen between *P. spegazzinii* and *P. suspectus* (Lema, 1979, 1984). Ferrarezzi (1993a) reviewed the taxonomy of Elapomorphini snakes, recognizing three species belonging to the *P. bilineatus* group: *P. bilineatus*, *P. lemniscatus*, and *P. reticulatus*, and described a new species of the group from Brazil, *P. multipunctatus* (Puorto and Ferrarezzi, 1993). This last arrangement was followed by most of the posterior authors that studied the genus (Giraudo, 2001; Giraudo and Scrocchi, 2002). Recently, Cabral and Cacciali (2015) proposed a new species from Paraguay named *P. normanscotti*. In summary, five species are currently recognized in the group, as follows: *Phalotris bilineatus* distributed in central and northern Argentina; *P. lemniscatus* from Uruguay, eastern Argentina and southern Brazil; *P. multipunctatus* in central to southeastern Brazil and eastern Paraguay; *P. reticulatus* that dwells in eastern Argentina and southern Brazil; and *P. normanscotti* present in western Paraguay (Puorto and Ferrarezzi, 1993; Cabral and Cacciali, 2015).

The overlap in pholidosis and meristic characters and the complex variations in color patterns account for the difficulties in precisely identifying many forms of the *Phalotris bilineatus* group. Herein, we discuss the status and validity of the species of the *P. bilineatus* group based on a geographically representative sample. We propose a new taxonomic scheme in which eight species are recognized in the group, including the description of a new species and the recognition of taxa previously placed in the synonymy of *Phalotris bilineatus*.

Materials and methods

We examined 93 specimens from the species included in the *Phalotris bilineatus* group, housed in herpetological collections (Appendix I). Institutional abbreviations are those of Sabaj (2016), except for CZA (Centro de Zoología Aplicada, Universidad Nacional de Córdoba), PNP (Parque Nacional El Palmar, Entre Ríos) and EX-CENAI (CENAI snakes collection now accessed in MACN). The types of *Elapomorphus spegazzinii* (MSNG 30651) and *Elapomorphus suspectus* (USNM 48939) were studied through photographs. Data on the geographic distribution include coordinates, names of localities and respective provinces, and associations with their ecoregions, as defined by Olson *et al.* (2001).

Taxonomic characters. We examined traditional meristic characters. Counts of ventral scales follow Dowling (1951), while for cephalic shields the terminology follows Ferrarezzi (1993a,b) and Puorto and Ferrarezzi (1993). Paired structures are presented as right/left. We took measurements, with the aid of a digital caliper to the nearest 0.1 mm, except for snout-vent length (SVL) and tail length (TL), for which we used a flexible ruler to the nearest 1.0 mm. The head length was measured from the tip of the retroarticular process on the mandible to the tip of the snout. The maximum head width has been taken at the widest point. Cephalic shields measurements follow Peters (1960). The eye was measured on the right side from its anterior to posterior end. We determined sex based on direct observation of the presence/absence of hemipenes. When necessary, we performed a small mesial incision on the base of the tail to verify the presence of hemipenes. We base the description of coloration features mostly on the specimens preserved in ethanol solution 70% after fixation of formalin, increased by examining some photographs of live specimens. Coloration traits follow Ferrarezzi (1993a,b), Puorto and Ferrarezzi (1993) and Harvey (1999). According to Harvey (1999), the nuchal collar length was typically recorded as a range of occupied dorsal scales (i.e., 2–3), and dorsal stripes are described using the dorsal number as a landmark and expressing the width of stripes. We follow Cabral and Cacciali (2015) in using "collar" for structures associated with the neck and "caudal ring" for the black transversal band on the cloacal region, typical of the *Phalotris bilineatus* group.

Morphological Analyses. We compared potential evolutionary lineages considering putative natural barriers (e.g., Paraná River, ecoregions, mountain systems) and geographical proximity of the available sample to define six operational groups (see Passos and Prudente, 2012 for a similar procedure). Based on the cumulative frequencies of character states, we investigated if each group have any geographical basis. Independently, we searched for correspondence between the above groups and the taxa previously described (see taxonomy history account). We performed a discrete analysis of qualitative characters based on the frequency of occurrence of each state throughout the sample. We followed Passos *et al.* (2009) in considering the presence of one or more exclusive, apparently fixed diagnostic characters to distinguish species among the populations of the *Phalotris bilineatus* complex. Our sample size did not allow us to address statistical confidence in qualitative characters from some populations (*sensu* Wiens and Servedio, 2000); therefore, we looked for concordance between the discrete/continuous characters, and the distributional patterns of the analyzed taxa, as our main parameters in sorting out putative evolutionary units.

Results

All the species herein studied from the *Phalotris bilineatus* group shared a unique combination of the following features: prefrontal one; nasal entire; supralabials 6/6 (rarely 5/5), with 2–3rd entering the eye (rarely 3–4th); infralabials 7/7 (rarely 6 or 8), with 4–5 contacting the first chinshield; loreal absent, nasal contacting unique preocular; two postoculars; dorsal scales in 15 rows without reduction; cloacal plate and subcaudals divided.

Our evidence points out the recognition of six species of the *Phalotris bilineatus* group in Argentina. Comparisons with literature data and type specimens allowed us to associate five taxa to available names, whereas the sixth form represents a new species described in the following section.

Species account

***Phalotris illustrator* sp. nov.**
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Figs. 1-2, Table 1
Phalotris bilineatus Scrocchi, Moreta & Kretzschmar, 2006

Holotype. Adult female (FML 23858) collected on October 03, 2010, by M. Paz, 3 km from the cemetery of Nío River (26.438312°S, 64.998233°W; 991 m above sea level, asl hereafter), Departamento Burruyacú, Tucumán, Argentina.

Paratypes. Four specimens, all from Argentina: juvenile of unknown sex (FML 29520) and adult female (FML 29521) collected between 23–28 August 1978 by E. Lavilla, G. Scrocchi and E. Terán at Balderrama (25.489520° S, 65.039234° W; 1337 m asl), Sierra de Metán, Departamento Metán, province of Salta; female (FML 23860, partially damaged with crushed head and cut body) collected on April 24, 2010 by M. Torres-Bugeau at Raco (26.665408° S, 65.420254° W; 1153 m asl), Departamento Tafí Viejo, province of Tucumán; female (FML 12388) collected on May 12, 2001 by G. Coronel at Finca El Milagro (24.858180°S, 65.538322° W; 1321 m asl),

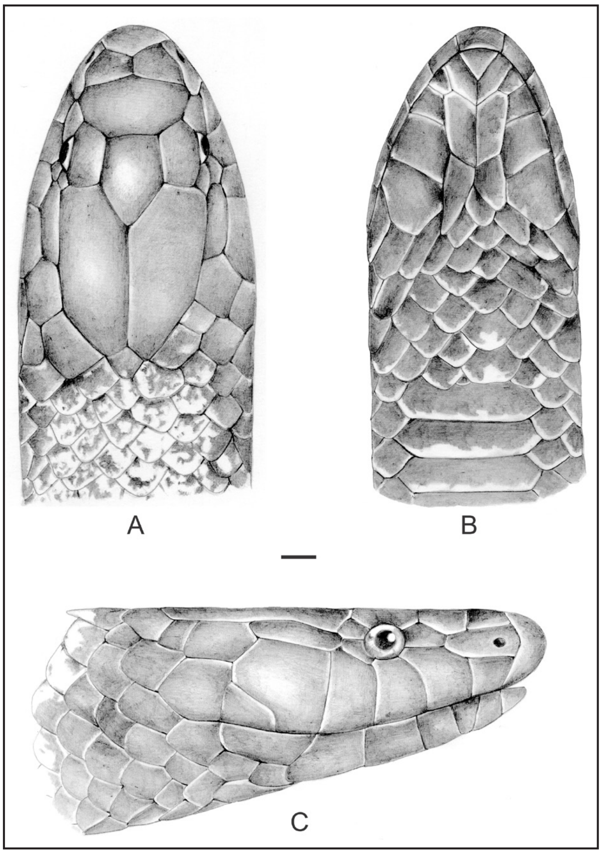

Figure 1. Dorsal (A), ventral (B) and lateral (C) view of the head of the holotype (FML 23858) of *Phalotris illustrator* sp. nov. Scale bar = 1 cm.

La Merced Chica, Departamento Rosario de Lerma, province of Salta.

Diagnosis. *Phalotris illustrator* is distinguished from all congeners in the *P. bilineatus* group (including the two revalidated taxa and *P. bilineatus* redefined in this paper) by a unique combination of the following characters: head entirely black (vs. head with light areas or blotches on rostral and supralabials and head venter almost uniformly white in all other species); nuchal collars absent (vs. present in other species of the group, except for *P. bilineatus* and *P. spegazzinii*); venter almost uniformly black (vs. venter white or with black blotches in all other species); flanks uniformly black to the level of lower half of fifth dorsal scale row (vs. flanks with a narrow black stripe between 4–5th dorsal scale row and 1–3rd dorsal rows white in *P. bilineatus*; white dot in each scale in *P. reticulatus*; or black stripe from 2nd or 3rd to 5th dorsal row, and the first dorsal row (or 1st and 2nd) white in *P. spegazzinii*); cloacal black ring present (vs absent in *P. multipunctatus*); rostral well separated from the prefrontal (vs. rostral contacting the prefrontal or slightly separated in *P. bilineatus*).

Description of the holotype. Total length 554 mm, 512 mm SVL, 42 mm TL; head length 16 mm; maximum head width 8.6 mm; tail 8.2% SVL; head 3.1% SVL; head, not distinct from body; body subcylindrical; eye diameter smaller than one-third length of frontal; rostral protruded, visible from above one-third as long as its distance to frontal; internasals trapezoidal; prefrontal large, single, the wide a half of the long; frontal pentagonal; parietals longer than wide, almost twice as long as frontal; nasal entire; preocular single, contacting nasal; postoculars two, lower postocular contacting third and fourth supralabials; temporals 1+1; supralabials seven, second and third contacting orbit; infralabials seven, first to fourth contacting first pair of chinshields and fourth-fifth contacting second pair of chinshields; chinshields two pairs; dorsal scale rows smooth in 15/15/15; apical pits absent; ventrals 217; subcaudals 27; cloacal plate divided.

Head entirely black in dorsal, lateral and ventral views (Figs. 1-2); dorsum of body between 5–11th

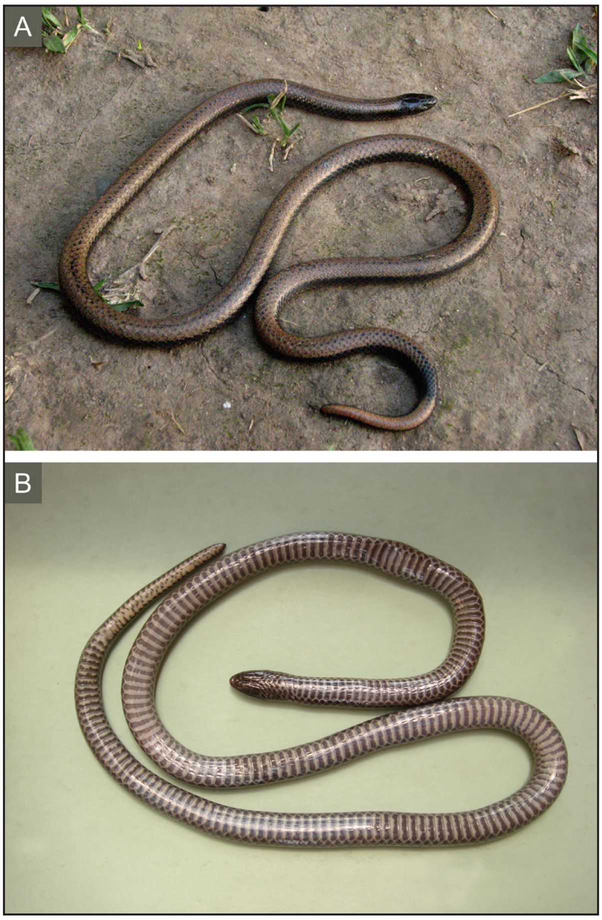

Figure 2. Dorsal (A) and ventral (B) views of the holotype (FML 23858) of *Phalotris illustrator* sp. nov.

Table 1. Meristic and morphometric variation of *Phalotris illustrator* sp. nov. Data on the holotype is marked in boldface. Abbreviations are as follows: SVL= snout-vent length, TL=tail length.

Number	Sex	Supralabials	Ventrals	Subcaudals	Head length	Head width	SVL	TL	% TL/ SVL	Rostral length (mm)	Internasal length (mm)	Prefrontal length (mm)	Frontal length (mm)	Parietal length (mm)
23858	**F**	**7 (2–3)**	**217**	**27+1**	**16**	**8.6**	**512**	**42**	**8.2**	**1.2**	**0.8**	**2**	**3.4**	**4.4**
23860	F	6 (2–3)	–	32+1	8	–	–	23	–	0.4	0.3	1	2	2.9
29521	F	6 (2–3)	202	31+1	10	–	340	31	9.1	0.6	0.5	1.5	2.6	2.9
29520	juv	6–5 (2–3)	215	27+1	8.5	4	172	15	8.7	0.5	0.3	1.2	1.8	2.6
12388	F	6 (2–3)	203	31+1	8	4.4	213	25	11.7	0.5	0.3	1.3	1.7	2.7

scale rows brown, with small black dots irregularly disposed; dots denser on the vertebral row forming an inconspicuous vertebral line; flanks and ventral surface of body black (from the first to lower half of fifth dorsal scale rows), with a thin white border on each dorsal and ventral scales; white border of ventral scales gradually increases toward posterior region of body (Fig. 2B); black caudal ring occupying 5–11th dorsal scale rows; subcaudal predominantly white irregularly marked with black (Fig. 2B).

Variation. The paratypes agree with the holotype in coloration except for the following features: FML 12388, 29520–21 the vertebral line is very faded or absent (coloration in preservative from well-conserved specimens); dorsal color of smaller specimens FML 12388 and 29520 reddish-brown. Table 1 synthesizes the variation of *Phalotris illustrator*.

Etymology. The specific epithet *illustrator* is a noun referring to the secular philosophical movement of the XVII Century, known as "enlightenment" in English and "illustration" in Latin. This philosophy gave rise to both science and Latin American independence movements. Because Argentina celebrates in 2016 the bicentennial of its independence, and several major battles against the Spanish colonizers were fought in northwestern Argentina (Tucumán and Salta), where inhabit the new species; we decided to honor the Argentinian heroes of the independence in Latin America belonging to the "enlightenment" philosophical movement.

Distribution. This species is known from the northern portion of the provinces of Tucumán and Salta. The distribution range of the type-series lies mainly in the Yungas phytogeographic province or southern Andean Yungas and transitional areas between Yungas and the Dry Chaco ecoregion (*sensu* Cabrera, 1976; Olson *et al.*, 2001) (Fig. 3). This region is characterized by several types of forests, ranging from dry to humid rainforests, including some open areas such as high altitude grasslands of the Sub-Andean mountains.

Phalotris bilineatus (Duméril, Bibron & Duméril, 1854)

Fig. 4A

Elapomorphus bilineatus Duméril, Bibron & Duméril, 1854:839.

Holotype MNHN-RA-0.3667. Type Locality "province de Los Corrientes"

Elapomorphus bilineatus, Jan, 1862

Phalotris bilineatus, Cope, 1861.

Elapomorphus bilineatus, Jan & Sordelli, 1865.

P.[*halotris*] *bilineatus*, Cope, 1885.

Elapomorphus bilineatus, Serié, 1936 (in part).

Elapomorphus bollei Mertens, 1954.

Elapomorphus bilineatus, Peters & Orejas-Miranda 1970 (in part).

Elapomorphus bilineatus bilineatus, Lema 1970.

Elapomorphus bilineatus, Ábalos & Mischis, 1975 (in part).

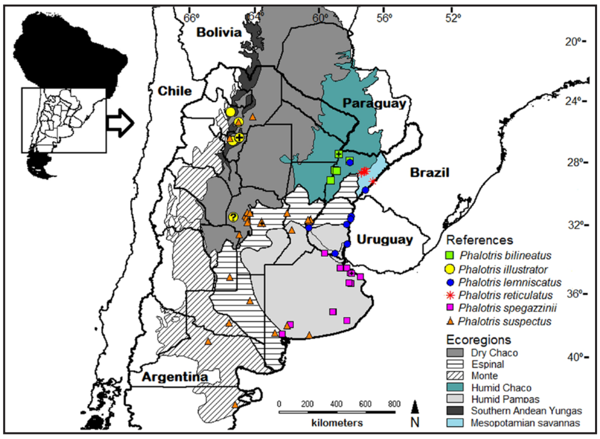

Figure 3. Distribution of the species of *Phalotris bilineatus* group in Argentina. + type localities of *Phalotris illustrator* sp. nov., *P. suspectus*, *P. bilineatus*, *P. bilineatus* and *P. spegazzinii*. The symbol "?" represents doubtful locality based on the confused collection data available.

Figure 4. General view in life of Argentinian members from *Phalotris bilineatus* group, *P. bilineatus* from Estancia La Adelita, Departamento Capital, Corrientes (A), *P. lemniscatus* from Gualeguaychú, Entre Ríos (B), *P. reticulatus* from Colonia Carlos Pellegrini, Corrientes (C), *P. spegazzinii* from City Bell, Partido de La Plata, Buenos Aires (D), and *P. suspectus* from Santa Fe (E). Photos by E. Etchepare (A), S. Nenda (C), L. Giambelluca (D), A. Giraudo (B, E).

Elapomorphus bollei, Ábalos & Mischis, 1975.
Elapomorphus bilineatus bilineatus, Lema 1978b.
Phalotris bilineatus, Ferrarezzi, 1993b (in part).
Phalotris bilineatus, Scrocchi *et al.*, 2000.
Phalotris bilineatus, Giraudo & Scrocchi, 2002.
Phalotris bilineatus, Giraudo, 2004.
Phalotris bilineatus, Giraudo *et al.*, 2012 (in part).
Phalotris spegazzini, Cabral and Cacciali, 2015 (in part).
Phalotris lemniscatus, Cacciali *et al.* 2016 (in part).

Diagnosis. *Phalotris bilineatus* can be distinguished from all other congeners in the *P. bilineatus* group by a unique combination of the following characters:

head dorsally black with rostral and first supralabials lighter; and ventrally almost white (vs. head entirely black in *P. illustrator*); nuchal collars absent (vs. present in other species of the group, with exception of *P. illustrator* and *P. spegazzinii*); belly with ventral scales white, with a half-moon shaped black dot in the anterior portion of each ventral scale; the dots enlarge toward posterior region of body, occupying almost all surface of ventral scales, being white only on the borders in contact with dorsal scale rows (vs. almost entirely black in *P. illustrator*, and *P. spegazzinii*); sides of the body with a narrow black stripe on 4–5th dorsal scale rows and 1–3rd dorsal scales rows white (vs. wider black lateral stripe 3–4 scales wide in *P. illustrator*, *P. reticulatus* and *P. spegazzinii*); cloacal shield with black ring present (vs. absent in *P. multipunctatus*); rostral scales projected backward, reaching or slightly separated from the prefrontal (vs. rostral well separated from the prefrontal in other species of the group).

Coloration (Fig. 4A). Dorsum of head with black cephalic-cap reaching first or second postparietal scales; rostral and three first supralabials lighter, nearly grey; head ventrally black with lighter areas on border of infralabials and chinshields; dorsum of body brown with or without black dots; black dots vary in density and size but never defining a pattern; sometimes dorsal dots arrangement constitute a barely noticeable vertebral stripe on the cloacal region and tail; dorsum often darker on the vertebral area and lightens at upper margins of dorsal-lateral stripes; dorsum of cloacal region with black area, covering 4–7 scales wide; body flanks exhibit dorsal-lateral black stripe (one scale wide) anteriorly connected to cephalic-cap and reaching the tip of tail posteriorly, over lower half of 5th and upper half of 4th dorsal scale rows; dorsal scales below the stripes immaculate white; dorsum of tail similar to body color pattern; belly with ventral scales white, with a half-moon shaped black dot on the anterior portion of each ventral scale; dots enlarges posteriorly, occupying almost all surface of ventral scales, being white only on the borders; ventral surface of tail white with black dots covering the suture region between subcaudals.

Variation. The largest specimen was a female 695 mm SVL, TL 50 mm; TL 9.5% SVL in a single male, 6.1–10.3% in females; ventrals 205 in male, 206–210 in females (mean= 207.5, SD= 1.76, *n*= 6); subcaudals 30 in male, 20–28 in females (mean= 23.8, SD= 3.43, *n*= 6); from ten specimens studied, four have the typical head scales of the genus; four specimens have 5/5 (2nd and 3rd in contact with orbit) supralabials; one has 8/8 infralabials, and one has 1+2+1 temporals in one side of the head.

Distribution. Species known only from Argentina (Ferrarezzi, 1993a, Giraudo and Scrocchi, 2002), our data restricted the distribution of *Phalotris bilineatus* to the northwestern province of Corrientes, eastern from the Paraná River, in the Humid Chaco ecoregion (*sensu* Olson *et al.*, 2001) (Fig. 3). There is only one specimen westwards to the Parana River, from Resistencia, Chaco. This species does not overlap its known distribution with any congeners and probably has a parapatric distribution with *P. lemniscatus*, with the nearest records between these two species separated by a 10 km airline (Manantiales and Mburucuyá National Park).

***Phalotris lemniscatus* (Duméril, Bibron & Duméril, 1854)**

Fig. 4 B

Elapomorphus lemniscatus Duméril, Bibron & Duméril, 1854. Erp. Gen., 7:840.

Holotype. Adult, MNHN-RA-0.3668, from “Amèrique du Sud” (= South America).

Elapomorphus lemniscatus, Jan, 1862.
Elapomorphus lemniscatus, Jan & Sordelli, 1865.
Phalotris lemniscatus, Cope, 1861.
P.[*halotris*] *lemniscatus*, Cope, 1885.
Elapomorphus trilineatus, Boulenger, 1889.
Elapomorphus lemniscatus, Koslowsky, 1898.
Elapomorphus bilineatus, Bertoni, 1914.
Elapomorphus lemniscatus, Bertoni, 1914.
Elapomorphus bilineatus, Schouten, 1931.
Elapomorphus lemniscatus, Schouten, 1931.
Elapomorphus bilineatus, Schouten, 1937.
Elapomorphus lemniscatus, Schouten, 1937.
Elapomorphus bilineatus, Bertoni,1939.
Elapomorphus bilineatus, Gatti, 1955.
Elapomorphus bilineatus, Peters and Orejas-Miranda 1970. In part.
Elapomorphus bilineatus lemniscatus, Lema, 1970
Elapomorphus bilineatus lemniscatus, Lema, 1978b
Elapomorphus lemniscatus lemniscatus, Lema, 1979
Elapomorphus bilineatus, Talbot, 1979.
Elapomorphus (*P*) *lemniscatus lemniscatus*, Lema, 1984
Elapomorphus (*P*) *lemniscatus trilineatus*, Lema, 1984
Elapomorphus lemniscatus lemniscatus, Williams and Francini, 1991.
Elapomorphus (*Phalotris*) *lemniscatus lemniscatus*, Cei 1993.

Phalotris lemniscatus, Ferrarezzi, 1993.
Elapomorphus spegazzinii, Aquino, Scott, and Motte, 1996.
Phalotris lemniscatus, Scrocchi *et al.*, 2000.
Phalotris lemniscatus, Giraudo and Scrocchi, 2002.
Phalotris lemniscatus, Giraudo, 2004.
Phalotris lemniscatus, Carreira, Meneghel, and Achával, 2005.
Phalotris bilineatus, Cacciali, 2009.
Phalotris lemniscatus, Giraudo *et al.*, 2012.
Phalotris lemniscatus, Costa and Bernils, 2014.
Phalotris lemniscatus, Costa and Bernils, 2015.
Phalotris lemniscatus, Cacciali *et al.* 2016 (in part).
Phalotris lemniscatus, Costa and Bernils, 2018.

Diagnosis. *Phalotris lemniscatus* can be distinguished from all congeners in the *P. bilineatus* group by a unique combination of the following characters: head dorsally black, with rostral and first supralabials lighter, and ventrally almost white (vs. head totally black in *P. illustrator* and *P. spegazzinii*); white nuchal collar present, 2–3 dorsal scales width, followed by a narrow black ring, one scale width (vs. nuchal collar absent in *P. bilineatus*, *P. illustrator* and *P. spegazzinii*); ventral scales almost black with the lateral and posterior edge white (vs. venter almost uniformly black in *P. illustrator*); flanks of the body with a black stripe on 3–5th dorsal rows, 1–2nd dorsal rows, white (vs. wider black lateral stripe 3–4 scales wide in *P. illustrator*, *P. reticulatus* and *P. spegazzinii*); cloacal black ring present (absent in *P. multipunctatus*); rostral well separated from the prefrontal (vs. rostral contacting the prefrontal or slightly separated in *P. bilineatus).* Additionally, *P. lemniscatus* have a well-defined vertebral stripe (vs. vertebral stripe absent or faded in other species, except *P. illustrator*, *P. spegazzini* and *P. reticulatus*).

Coloration (Fig. 4B). Dorsum of head dark (black to light brown); snout lighter than other cephalic portion in dorsal view; light area occupies the rostral, internasals and anterior prefrontal; head laterally black with first four supralabials mostly whitish or cream, and last supralabials dark; head ventrally almost white, usually with black to brown spots that run from the mental over the suture between chinshields and all infralabials. Behind the head, there is an immaculate white ring of variable width in the dorsum, occupying from one to three dorsal scales and being commonly two scales wide. In all specimens, the white ring occupies the sides of the body. The white ring is bordered by front and rear by a thin black line. The dorsum of the body, behind the white ring, is reddish-brown. All specimens have three black stripes, one vertebral occupying the vertebral and half of paravertebral scales, and two lateral stripes on the sides of the body that run over the lower half of the fifth to the upper half of the third row of dorsal scales. The dorsal scales below the lateral black stripes to the venter are whitish. The tail coloration continues the pattern of the body. Dorsally, over the cloaca, there is a black ring of 5–7 dorsal scales wide, which extends to the differentiated white anal plates. The ventral scales are almost black, with the lateral and posterior edge white. Ventrally the tail is black, with mostly black subcaudals that have sutures and lateral edges white. The tip of the tail is black.

Variation. The largest specimen was a female 658 mm SVL and 51 mm tail. Tail short, corresponding to 8.5–11.8% of SVL in males and 7.8–9.4% in females. Males with 188–199 ($\bar{x}$= 193.70, SD= 5.51, *n*= 3) ventral scales (180–200 reported for Brazilian and Uruguayan populations by Ferrarezzi, 1993a), females 202–217 ($\bar{x}$= 209.33, SD= 10.60, *n*= 3; 195–217 reported for Brazilian and Uruguayan populations by Ferrarezzi 1993a). Subcaudal scales 29–31 ($\bar{x}$= 30.30, SD= 0.58, *n*= 3) in males (21–36 reported by Ferrarezzi 1993a), and 25–31 in females ($\bar{x}$= 28.00, SD= 2.65, *n*= 3; 19–28 reported by Ferrarezzi, 1993a). The specimens studied have the typical head scales of the genus. The rostral is not projected backwards and is separated from the prefrontal.

Distribution (Fig. 3). Species known to occur in Argentina from Corrientes and Entre Ríos provinces, inhabiting mainly the ecoregion called Southern Cone Mesopotamian Savannas by Olson *et al.* (2001), and marginally in the Espinal and Humid Pampas, mostly associated with the high banks of the Uruguay and Paraná Rivers. All reliable records of *P. lemniscatus* are restricted to the east of Paraná River in the Argentine Mesopotamia. Two specimens labeled as coming from Buenos Aires and Córdoba probably correspond to wrong locations, because the characteristics of the localities are different from all the others known, and they are several hundred km from the closest localities. Our data showed that *P. lemniscatus* has a parapatric distribution with respect to other Mesopotamian species, *P. bilineatus* and *P. reticulatus* (Fig. 3).

***Phalotris reticulatus* (Peters, 1860)**

Fig. 4 C
Elapomorphus reticulatus Peters, 1860

Holotype. Adult ZMB 3811, collected at "Brasilien"

(= Brazil)
Phalotris reticulatus Cope, 1861.
Elapomorphus iheringi Strauch, 1884.
Phalotris melanopleurus Cope, 1885.
Elapomorphus bilineatus, Peters and Orejas-Miranda 1970. In part.
Elapomorphus bilineatus reticulatus, Lema, 1970.
Elapomorphus bilineatus reticulatus, Lema, 1978b.
Elapomorphus lemniscatus reticulatus, Lema, 1979.
Elapomorphus (*P*) *lemniscatus iheringi*, Lema, 1984.
Elapomorphus (*Phalotris*) *lemniscatus divittatus* Lema, 1984.
Elapomorphus lemniscatus iheringi, Williams and Francini, 1991.
Elapomorphus (*Phalotris*) *lemniscatus iheringi*, Cei 1993.
Phalotris reticulatus Ferrarezzi, 1993.
Phalotris reticulatus, Scrocchi *et al.*, 2000.
Phalotris reticulatus, Giraudo, 2001.
Phalotris reticulatus, Giraudo and Scrocchi, 2002.
Phalotris reticulatus, Giraudo *et al.*, 2012.
Phalotris reticulatus, Costa and Bernils, 2014.
Phalotris reticulatus, Costa and Bernils, 2015.
Phalotris reticulatus, Costa and Bernils, 2018.

Diagnosis. *Phalotris reticulatus* can be distinguished from other species of the group by unique combination of the following characters: head dorsally almost totally black, with lighter snout, and a notable white dot on 3rd–4th supralabials (this last character is unique within the *P. bilineatus* group), and ventrally almost white (vs. black in *P. illustrator* and *P. spegazzinii*); white nuchal collar present and reaching ventral scales (2–3 dorsal scales width), followed by a black ring (1–3 scale width) (vs. nuchal collar absent in *P. bilineatus*, *P. illustrator* and *P. spegazzinii*); venter almost totally black, each ventral scale black with thin whitish free posterior border (vs. venter white or with black blotches in all other species); vertebral black stripe can be from very noticeable to almost faded and runs over the vertebral and the inner half of paravertebral dorsal scales. The stripe is formed by irregular dots over each scale and could fade after fixation. On both sides of the vertebral stripe, there is a reddish stripe two dorsal scales wide. The sides of the body, from the reddish stripes to the venter, almost black, where each scale is black with a thin withe posterior border resulting in a reticulated lateral pattern in general view; black in *P. spegazzinii* and *P. illustrator* and a narrow lateral black stripe in others species); cloacal black ring present (vs. absent in *P. multipunctatus*); rostral well separated from the prefrontal (vs. rostral contacting the prefrontal or slightly separated in *P. bilineatus*).

Coloration (Fig. 4C). The head is black, and the coloration extends until to an almost straight line behind the parietals. There is a clear area, with irregular black dots, from the snout to the anterior half of frontal, supraoculars, and first supralabials. There is a noticeable big white spot on the third and fourth supralabials. Ventrally the head is white and presents a sub-triangular to sub-rhombic black spot over the gulars, that extends forward as a line over the chinshields suture. Behind the head, in the dorsum, there is a white nuchal collar of two to three dorsal scales wide; the white collar is posteriorly bordered by a black collar of one to two dorsal scales width, which is formed by the union of the enlarged end of the vertebral stripe, and the color of sides of the body. A vertebral black stripe can be from very noticeable to almost faded and runs over the vertebral and the inner half of paravertebral dorsal scales. The stripe is formed by irregular dots over each scale and could fade after fixation. On both sides of the vertebral stripe, there is a reddish stripe two dorsal scales wide. The sides of the body, from the reddish stripes to the ventral shields, are black, and only the free border of the scales can be clear, forming a reticulated pattern. There is a white line between the first dorsal scale rows and the ventral shields. Ventrally almost black, only a thin whitish border can exist in each ventral shield. Tail ventrally with the same pattern as the belly. Dorsally, in correspondence to the cloacal region, there is a black area of 8–9 dorsal scales wide that extends in a triangular shape over vertebral and paravertebral scales.

Variation. The largest specimen was a female 453 mm SVL and 42 mm tail. Tail short, corresponding to 10.9–12.4% of SVL in males, and 8–9.3 % in females. Males with 188–197 ($\overline{X}$= 192.00, SD= 4.69, *n*= 4) ventral scales (188–198 reported for Brazilian populations by Ferrarezzi, 1993a), females 201–207 ($\overline{X}$= 205.00, SD= 3.46, *n*= 3; 202–211 reported by Ferrarezzi, 1993a). Subcaudal scales 30–33 ($\overline{X}$= 31.30, SD= 1.26, *n*= 4) in males (27–33 reported by Ferrarezzi, 1993a), and 23–27 in females ($\overline{X}$= 24.67, SD= 2.08, *n*= 4; 23–27 reported by Ferrarezzi, 1993a). The specimens studied have the typical head scales of the genus.

Distribution (Fig. 3). In Argentina, only corroborated by voucher specimens from eastern Corrientes Province, inhabiting the Southern Cone Mesopotamian Savannas ecoregion, and cited from the province of Misiones by Lema (1984) without mention of any voucher specimen.

Phalotris spegazzinii (Boulenger, 1913) Status revalidated

Figs. 4 D – 5 A-B
Elapomorphus spegazzinii Boulenger, 1913.
Holotype. Adult, MSNG 30651, collected at "La Plata".
Elapomorphus bilineatus spegazzinii, Lema, 1978b.
Elapomorphus lemniscatus spegazzinii, Lema, 1979.
Elapomorphus (*P.*) *spegazzinii spegazzinii*, Lema, 1984.
Elapomorphus spegazzinii spegazzinii, Williams and Francini, 1991.
Elapomorphus spegazzinii Aquino, Scott, and Motte, 1996.
Elapomorphus (*Phalotris*) *spegazzinii spegazzinii*, Cei, 1993.
Phalotris bilineatus, Cabrera, 2004. In part.
Phalotris bilineatus, Giraudo *et al.*, 2012. In part.
Phalotris spegazzini, Cabral and Cacciali, 2015 (in part).
Phalotris lemniscatus, Cacciali *et al.*, 2016 (in part).

Diagnosis. *Phalotris spegazzinii* can be distinguished from the other species of the group by a unique combination of the following characters: head dorsally black, some specimens with rostral and first supralabials lighter and ventrally almost black (vs. light areas, blotches, or both, present on rostral and supralabials of other species of the group, completely black in *P. illustrator* and ventrally mostly white in other species of the group, but completely black in *P. illustrator*); nuchal collars absent (vs. present in other species of the group, except for *P. bilineatus* and *P. illustrator*) but with two light dots behind parietals (vs. absent in all others species); ventral shields are almost completely black except for the posterior border lighter (vs. venter almost uniformly black in *P. illustrator*, and white or with black blotches in all other species); sides of the body with a black stripe 3–4 scales width in 3–5th or 2–5th dorsal rows (vs. narrow lateral black stripe in others species, except for *P. reticulatus* and *P. illustrator*); cloacal black ring present (vs. absent in *P. multipunctatus*); rostral well separated from the prefrontal (vs. rostral contacting the prefrontal or slightly separated in *P. bilineatus*).

Coloration (Figs. 4D, 5 A–B). Based on photographs of the holotype (MSNG 30651) and live and preserved specimens. Head dorsal, lateral, and ventrally almost black. In a few specimens, the rostral area is somewhat clearer. The body is dorsally light brown with a dark vertebral stripe that continues the coloration of the head and is one dorsal scale wide, occupying the vertebral row of dorsals and the border of paravertebral rows. The scales in the paravertebral rows have black dots, and the posterior border is lighter. Behind the head and between the vertebral stripe and the lateral bands, some specimens (including the holotype) have two spots of two dorsal scales wide, somewhat lighter than the rest of the back and inconspicuous. Dorsally, over the cloaca, there is a black area of 4–9 dorsal scales wide that extends in a triangular shape over vertebral and paravertebral scales. The sides of the body have a black stripe 2–3 dorsal scales wide that runs over the second to fifth dorsal scales rows, from the head to the tip of the tail. In the dorsum, in some specimens appears, between the lateral and vertebral stripes, little black dots not regularly disposed and very inconspicuous. The anterior half of the dorsal scales of the first and second rows are normally black, but in some specimens are immaculate or with a few little dots. In the tail the pattern continues that of the

Figure 5. Dorsal (A) and ventral (B) views of the holotype of *Phalotris spegazzinii* (MSNG 30651) from La Plata, Argentina. Photo by G. Doria.

body. The infralabials and chinshields are normally black, but in some specimens can be lighter. Other scales in the ventral side of the head are black with lighter borders. The ventral shields are almost completely black except for the lighter posterior border. The anal and subcaudals have the same pattern, but the subcaudals can be light with a black suture between them in some specimens.

Variation. The largest specimen was a female 544 mm SVL and 40 mm tail. The tail is short, corresponding to 10.3–11.2% of SVL in males, and 7.1–8.9 % in females. Males with 191–200 ($\overline{X}$= 195.00, SD= 1.15, *n*= 4) ventral scales, females 201–218 ($\overline{X}$= 210.09, SD= 5.01, *n*= 11). Subcaudal scales 29–35 in males ($\overline{X}$= 31.00, SD= 2.71, *n*= 4), and 21–25 in females ($\overline{X}$= 23.09, SD= 1.58, *n*= 11). Almost all the specimens studied (24 of 30) have the typical head scales of the genus, with the follow variation recorded: one specimen with 6(2–3)/7(3–4) supralabials, one with 8/8 infralabials and two with 1+1+1 temporals. The rostral is slightly projected backwards and is separated from the prefrontal.

Distribution (Fig. 3). Known only from eastern and southern Buenos Aires province, Argentina, in the Humid Pampas ecoregion (*sensu* Olson *et al*., 2001).

Phalotris suspectus **(Amaral, 1924) Status revalidated**

Figs. 4 E – 6 A-B

Elapomorphus suspectus Amaral, 1924.

Holotype. Adult male, USNM 48939, collected at "Pilár", near Córdoba, Argentina.

Elapomorphus bilineatus, Serié, 1936. In part.
Elapomorphus bilineatus, Peters and Orejas-Miranda, 1970. In part.
Elapomorphus bilineatus, Ábalos and Mischis, 1975. In part.
Elapomorphus bilineatus suspectus, Lema, 1978a
Elapomorphus lemniscatus suspectus, Lema, 1979
Elapomorphus (P) spegazzinii suspectus, Lema, 1984
Phalotris bilineatus, Ferrarezzi, 1993a. In part.
Elapomorphus (*Phalotris*) *spegazzinii suspectus*, Cei, 1993
Elapomorphus spegazzini (sic) *suspectus*, Reati, 1996.
Phalotris bilineatus, Giraudo and Scrocchi, 2002. In part.
Phalotris bilineatus Cabrera, 2004. In part.
Phalotris bilineatus, Scrocchi *et al.*, 2010.
Phalotris bilineatus, Giraudo *et al.*, 2012. In part.
Phalotris spegazzini, Cabral and Cacciali, 2015 (in part).
Phalotris lemniscatus, Cacciali *et al.,* 2016 (in part).

Diagnosis. *Phalotris suspectus* can be distinguished from all congeners in the *P. bilineatus* group by a unique combination of the following characters: head dorsally black, with rostral and first supralabials lighter; and ventrally almost white (vs. head totally black in *P. illustrator* and *P. spegazzinii*); white nuchal collar present, followed by a narrow black collar (vs. nuchal collar absent in *P. bilineatus*, *P. illustrator* and *P. spegazzinii*); venter whitish with dark, almost black, dots in the anterior portion of the ventral shields, being white at the posterior border (vs. venter almost uniformly black in *P. illustrator*); flanks with a narrow black stripe on 4–5th dorsal scale rows, 1–3rd dorsal scale rows white (vs. wider black lateral stripe 3–4 scales wide in *P. illustrator*, *P. reticulatus* and *P. spegazzinii*); cloacal black ring present (vs. absent in *P. multipunctatus*); rostral well separated from the prefrontal (vs. rostral contacting the prefrontal or slightly separated in *P. bilineatus*).

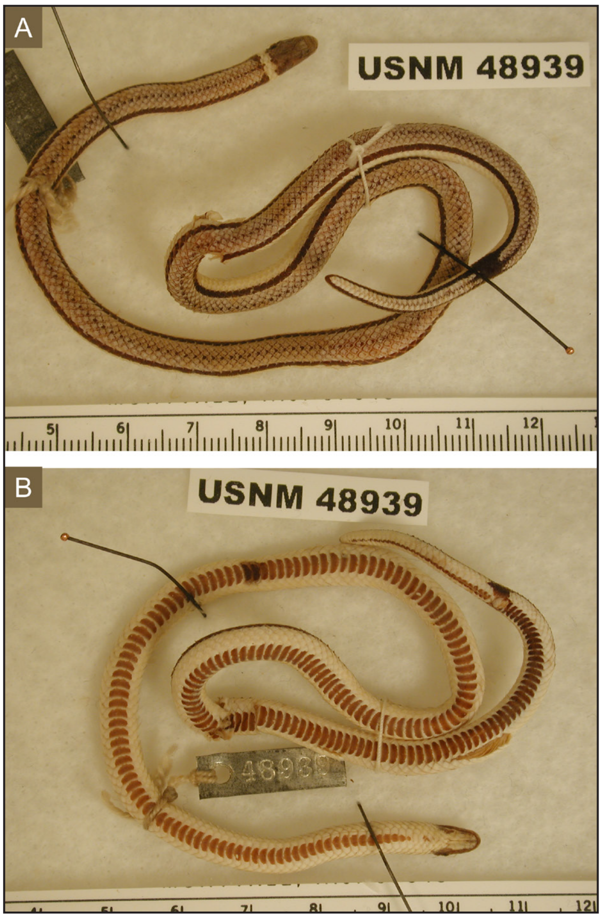

Figure 6. Dorsal (A) and ventral (B) views of the holotype of *Phalotris suspectus* (USNM 48939) from Pilar, Córdoba, Argentina. Photo J.A. Poindexter II.

Coloration (Figs. 4 E, 6 A–B)**.** Based on photographs of the holotype: National Museum of Natural History; Smithsonian Institution, USA. USNM 48939; (additional detailed photographs of the holotype are available at https://www.si.edu/object/nmnhvz_6556633); and live and preserved specimens. The rostral area of the head is clear; the rest of the head dorsally darker (light brown to black). Depending on the specimen, the light area in the head occupies the rostral, internasals, and a big area of prefrontals and supraoculars (MACN 38881, CZA 150); or is extended to parietals (JW 943) or all the head is light brown (MACN 32886, 20629, 11236, 12897). Laterally the pattern is the same of the dorsum, and the last supra and infralabials are dark. Almost all the supralabials are whitish or white, except the last ones that are dark because the black ring of the neck projects over them. Ventrally, the head is almost white or light brown, and almost all the specimens have a dark spot that runs from the mental over the suture between chinshields. Behind the head, there is an immaculate white ring in the dorsum, of variable width, occupying from one to two and a half dorsal scales, being commonly of two scales wide. In almost all specimens, the white ring occupies the sides of the body, but sometimes it is interrupted by the lateral stripes. The white ring is bordered by front and rear by a thin black line. The dorsum of the body posteriorly the white ring is light brown. In most specimens, a diffuse vertebral stripe is formed by the union of small spots on vertebral scales, but it is absent or almost indistinguishable in others. There are black dots irregularly disposed in the paravertebral scales. Dorsally, over the cloaca, there is a black area of 3–7 dorsal scales wide, which extends in a triangular shape over vertebral and paravertebral scales. The body sides have a black stripe that runs over the lower half of the fifth and the upper half of the fourth row of dorsal scales. The dorsal scales below the stripes are whitish, like the venter. In the tail, the coloration continues the pattern of the body, but the vertebral stripe is less conspicuous. The venter is whitish, with dark (almost black) dots in the anterior portion of the ventral shields that are white on their posterior border. The dots progressively increase in size being reduced only to the center of the gular and first ventral shields, while in the rest of the belly occupy almost the entire width of the ventral shield leaving only the lateral ends immaculate. The tail is immaculate white ventrally. Some specimens had little spots or a diffuse black line in the suture of subcaudals. The tip of the tail is black.

Variation. The largest specimen was a female 695 mm SVL and 50 mm tail. The tail is short, corresponding to 9.9–12.3% of SVL in males and 6.8–10.5 % in females. Males with 195–206 ventral scales, females 200–226. Subcaudal scales 28–39 in males and 22–32 in females. Almost all the specimens (22 of 28) studied have the typical head scales of the genus. Two specimens have five supralabials on the left side of head, and in one of them the 3rd and 4th entering the eye; two with 6/6 infralabials; in one specimen the 1–5 infralabials contacting the first chinshield.

Distribution (Fig. 3). It is the species with the largest distribution in Argentina within the *Phalotris bilineatus* group, known from Buenos Aires, Chubut, Córdoba, La Pampa, Río Negro, Salta, San Luis, Santa Fe, and Tucumán Provinces, over at least four ecoregions, the Dry Chaco, Espinal, Monte, Humid Pampas (where it is marginally distributed and partially sympatric with *P. spegazzinii*), and Yungas (where it is sympatric with *P. illustrator*).

Discussion and Conclusion

The taxonomic history of the *Phalotris bilineatus* group is very complex, probably because of the small sample sizes used in previous studies (Ferrarezzi, 1993a,b; Lema, 1970, 1978a,b, 1979, 1984). As a rule, the lack of information regarding geographical variation in the group precluded a more robust assessment of the taxonomic status of most species. Lema (1979, 1984) suggests that the holotype of *Phalotris bilineatus* represents a hybrid specimen or an intergraded phenotype between *Phalotris spegazzinii* and *P. suspectus*. The examined specimens of *P. bilineatus* (including three topotypes) let us conclude that this species can be unambiguously distinguished from *P. spegazzinii* and *P. suspectus* (see species account). On the other hand, the distribution patterns of each of the three species do not support Lema's (1979, 1984) hypothesis because *P. bilineatus* is restricted to the northwest of the Corrientes Province, eastwards from the Paraná River, while *P. suspectus* and *P. spegazzinii* occur to the southwest of this river, with the closest records 300 and 600 km, respectively, from the nearest locality of the first. Moreover, we found no putative hybrids or intergrade phenotypes (i.e., presenting a mosaic of characteristic of different parental taxa) along the sympatric area of *P. suspectus* and *P. spegazzinii* in the south of Buenos Aires Province (Fig. 3). These arguments support our taxonomic decision for the resurrections of *P. suspectus* and *P. spegazzinii*, independently of their parapatric distributions. We agree partly with Ferrarezzi (1993a,b) about the priority of the name *P. bilineatus*, but restricting it to northwestern Corrientes populations, defined by several unique characters that allow its distinction from the other Mesopotamian species, *P. lemniscatus* and *P. reticulatus*.

The *Phalotris bilineatus* group now included eight species; a resume of their principal color characteristics is presented in Table 2. The species of *Phalotris bilineatus* group in Argentina may be characterized by their distribution and principal characters:

Phalotris illustrator occurs in northwestern Argentina (Tucumán and Salta provinces), where it is sympatric with *P. suspectus* (Fig. 3). It is characterized by a head (dorsal and ventral) entirely black, nuchal collar absent, venter and flanks of the body almost black with only a thin white border on the scales.

Phalotris bilineatus is restricted to the northwestern of the province of Corrientes, eastwards from the Paraná River in the Argentine Mesopotamia (Fig. 3), without overlap with any other species analyzed here. It is characterized by rostral scales projected backwards, reaching or slightly separated from the prefrontal (a feature found exclusive of this taxon along with the *P. bilineatus* group), nuchal collars absent, flanks of the body with a narrow black stripe on 4–5th dorsal scale rows and white 1–3rd dorsal scale rows.

Phalotris lemniscatus showed a parapatric distribution with respect to *P. bilineatus* and *P. reticulatus* in the central and southern Argentine Mesopotamia, eastwards from the Paraná River (Fig. 3). It is diagnosed by a white and black nuchal collars, three longitudinal black stripes (one vertebral and two dorsal-lateral) with one scale width, and flanks of the body with 1–2nd dorsal scale rows white pigmented. All Argentinian specimens have similar coloration patterns, but Lema (1984) interpreted some variations of coloration pattern of Brazilian and Uruguayan populations as subspecies (see synonymy)*;* nevertheless, Ferrarezzi (1993a) and Puorto and Ferrarezzi (1993) rejected this posture.

Phalotris reticulatus, as previously mentioned, shows a parapatric distribution with respect to *P. bilineatus* and *P. lemniscatus* in northeastern Argentine Mesopotamia (Fig. 3). It is distinguished by head dorsally almost totally black, with lighter snout, a notable white dot on 3–4th supralabials, white and black nuchal collars and flanks of the body almost black.

Phalotris spegazzinii is distributed in Buenos Aires Province (Pampas ecoregion), with a narrow contact area with *P. suspectus* (Fig. 3). It is diagnosed by the lack of nuchal collars, two light and very inconspicuous dots behind the parietals, flanks of the body with a broad black stripe covering three or four scale rows width (3–5th or 2–5th).

Phalotris suspectus distribution extends from northwestern (where it is sympatric with *P. illustrator*) throughout central Argentina (where it marginally overlaps with *P. spegazzinii*), and up to the south (northern Patagonia), always westwards from the Paraná River (Fig. 3). It is characterized by a head dorsally black, with lighter rostral and first supralabials, a white nuchal collar (three scales width) followed by a narrow black one, and two dorsal-lateral narrow longitudinal black stripes on the flanks.

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Table 2. Principal color characteristics of the species of *Phalotris bilineatus* group.

	Dorsal Head coloration	Ventral Head coloration	Nuchal collars	Vertebralstripe	Sides of the body	Cloacal black ring	Ventral tail coloration
P. illustrator sp. nov. (n = 5)	Totally black(100%).	Totally black(100%).	Absent (100%).	Present (40%), faded (20%) or absent (40%)	Almost black (100%).	Present (100%)	Subcaudals are mostly white with black dots irregularly disposed (100%).
P. bilineatus (n = 10)	Black with rostral and first supralabials lighter (100%).	Almost black (100%).	Absent (100%).	Mostly absent (80%), some exemplars have a faded one (20%)	With a narrow black stripe on 4th–5th dorsal rows, 1st to 3rd dorsal rows white (100%).	Present (100%)	Almost white with the medial suture with black dots (100%).
P. lemniscatus (n = 9)	Black with rostral and first supralabials lighter (100%).	Almost White (100%).	A white collar, reaching the ventral scales, and 2–3 dorsal scales width, followed by a narrow black collar, 1 scale width (100%).	Present (100%)	With a black stripe on 3th–5th dorsal rows, 1st to 2nd dorsal rows white (100%).	Present (100%)	Mostly black (90%) or almost white with the medial suture with black dots (10%).
P. multipunctatus (n = 2)	Black with white contrastant dots and bigger dots in the supralabials (100%).	Almost white, with some scales with black borders (100%).	A white collar reaching the ventral scales, and 3–4 dorsal scales width, followed by a narrow black collar, 1 scale width (100%).	Absent (100%)	The first 5 dorsal scale rows are black with a white dot in each scale. The dots are bigger in the first row and decreases to the fifth (100%).	Absent (100%)	Reticulate in black, with a white dot in each subcaudal (100%).
P. normanscotti (n = 3)	Completely black (100%).	Almost white (100%).	A white nuchal collar on tip of parietals 4–6 dorsal scales width, followed by a black collar 4–6 dorsal scales width (100%).	Absent (100%)	With a narrow black stripe on 4th–5th dorsal rows, 1st to 3rd dorsal rows white (100%).	Present (100%)	White (100%)
P. reticulatus (n = 8)	Almost totally black, with lighter snout (100%). A white notable dot on 3rd–4th supralabials (100%).	Almost white (100%).	A white collar, reaching the ventral scales, and 2–3 dorsal scales width, followed by a black collar, 1–3 scale width (100%)	Present (80%) to faded (20%)	Almost black (100%).	Present (100%)	Almost black (100%).
P. spegazzinii (n = 17)	Almost black, with rostral and first supralabials lighter (96%).Almost black (4%).	Almost black (100%).	Absent (100%). In most exemplars (95%) there are two light dots posterior to parietals.	Present (100%)	A black stripe 3 or 4 scales width (3–5 or 2–5 dorsal rows). The first dorsal rows (or 1st and 2nd) are white with or without black dots (100%).	Present (100%)	Black with white posterior border (90%). Mostly White (10%).
P. suspectus (n = 26)	Black with rostral and first supralabials lighter (96%).Almost black (4%).	Almost white (88%).Almost black (12%).	A white collar, reaching the ventral scales, 3 dorsal scales width (100%), usually followed by a narrow black collar, 1 scale width (62%).	Mostly absent (52%), some exemplars have a faded one (48%).	With a narrow black stripe on 4th–5th dorsal rows, 1st to 3rd dorsal rows white (100%).	Present (100%).	Almost white with the medial suture with black dots or line (56%). Mostly white (44%).

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Appendix

***Phalotris illustrator* (*n*=1, not included in the type series).** ARGENTINA**: Córdoba: Departamento Pocho**: Pocho river (-31.4166 -65.3666), near Las Palmas: MACN 33132.

***Phalotris bilineatus* (*n*=10).** ARGENTINA: **Chaco**: **Departamento San Fernando**: Resistencia (-27.45° -58.9833°): FML 07501; **Corrientes**: **Departamento Capital**: Corrientes Capital (-27.482780° -58.851772°): UNNEC 068, 10667; Estancia La Adelita (-27.4808° -58.7416°): UNNEC 13063; Laguna Pampín, Corrientes (-27.483675° -58.745306°): MACN 49165; **Departamento Bella Vista**: Estación Hidrobiológica Bella Vista (-28.453526° -58.984808°): JW 04, 0525; **Departamento Goya:** Goya (-29.133333° -59.25°): MACN 44593–94 (former CENAI 1478 a–b); **Departamento Mburucuyá:** Manantiales (-27.921341° -58.098208°): MACN 14692.

***Phalotris lemniscatus* (*n*=11).** ARGENTINA: **Córdoba**: **Departamento Calamuchita:** Río de los Sauces (-32.53° -64.58°): CZA 112; **Corrientes**: **Departamento Mburucuyá:** Mburucuyá (-28.046251297° -58.2251396179°): UNNEC 05776; **Departamento Paso de los Libres:** Paso de los Libres (-29.716666º -57.083333º): CENAI 3184; **Entre Ríos**: **Departamento Colón:** Parque Nacional El Palmar, Jardín de la Portada (-31.853055º -58.3225º): PNP 26; **Departamento Concordia:** INTA El Alambrado, near Concordia (-31.381408º -58.115306º): INALI 1116; Puerto Yeruá (-31.5314º -58.0153º): MACN 44597 (former CENAI 3739); **Departamento Diamante:** Diamante (-32.066666º -60.65º): INALI 4042, 5508; **Departamento Gualeguaychú:** Ñandubaysal beach (-33.066229º -58.389108º): INALI 3986; **Departamento Islas del Ibicuy:** Villa Paranacito (-33.7º -58.683333º): MACN 44590 (former CENAI 2145); **Departamento Paraná:** Paraná (-31.744444º -60.5175º): MACN 7822.

***Phalotris reticulatus* (*n*=8).** ARGENTINA: **Corrientes**: **Departamento Mercedes:** Rincón del Socorro (-28.55º -57.2166º): UNNEC 10553; **Departamento San Martín**: Colonia Carlos Pellegrini (-28.5333º -57.1666º): UNNEC 10487–88, MLP.R. 5757, MACN 36085, 37824, 38882; La Cruz (-29.1666º -56.6333º): UNNEC 10164.

***Phalotris spegazzinii* (*n*=30).** ARGENTINA: **Buenos Aires**: without specific locality: JW 683, 937; **Ciudad Autónoma de Buenos Aires:** Palermo (-34.575789º -58.424704): MACN 18; **Partido de Bahía Blanca:** Bahía Blanca (-38.7166º -62.2833º): MACN 24794; **Partido de Balcarce:** Sierra La Barrosa (-37.8833º -58.2666º): MACN 30820; **Partido de Chascomús:** Chascomús F.C.S. (-35.570706º -58.015807º): MACN 7063–64, Laguna Vitel (-35.529546º -58.115744º): MACN 5924–25; **Partido de La Plata:** City Bell (-34.8666º -58.05º): JW.0539; La Plata (-34.9313º -57.9488º): MLP 58A, 1886, 3106, JW 938, 953, 1682; **Partido de Magdalena:** 15 km E from Magdalena (-35.145742º -57.414751º): MACN 24795; **Partido de San Miguel:** Bella Vista (-34.576207º -58.698456º): MACN 9485, 10998; **Partido de San Pedro:** San Pedro (-33.674218º -59.661570º): MACN 4560; **Partido de Tandil**: Sierras de Tandil: MACN 11092, 11357–59; **Partido de Tornquist:** Sierra de la Ventana (-38.134288º -61.792563º): MACN 44592 (former CENAI 2935); Tandil (-37.318889º -59.134722º): MACN 12193–96, 24838.

***Phalotris suspectus* (*n*=28).** ARGENTINA**:** without specific locality: JW 943; **Buenos Aires: Partido de Coronel Dorrego:** Oriente (-38.7333º -60.6166º): MACN 38881; **Partido de Tornquist:** Parque Provincial Tornquist, Sierra de la Ventana (-38.15º -61.9833º): MACN 32886; **Partido de Villarino:** Salinas “Las Barrancas” Salinas Chicas (-38.604115º -62.769800º): MACN 12897; **Chubut: Departamento Rawson:** Ruta Nacional 3, 17 Km N from Trelew (-43.093326° -65.233343º): FML 9324; **Córdoba: Departamento Alta Gracia** (-31.6666º -64.4333º): MACN 20629; **Departamento Colón**: Salsipuedes (-31.15º, -64.3166º): CZA not catalogued; Unquillo (-31.23º -64.32º): CZA 31; **Departamento Punilla:** La Falda (-31.083333º -64.5º): INALI 4021; Tanti (-31.3333º -64.6º): CZA 150–51; **Departamento Santa María:** La Serranita (-31.754125º -64.462009º): MACN 36279; Ave María: CZA not catalogued; Villa Carlos Paz (-31.4357º -64.4991º): CZA 163; **La Pampa**: without specific locality: FML 2093 (1–2); **Departamento Capital:** Santa Rosa (-36.620277º -64.290555º): FML 26170; **Departamento Lihué Calel:** Santa María Lihué Calel (-37.995219º -65.610087º): MACN 11236; **Río Negro: Departamento El Cuy:**15 Km SE from Chichinales, Valle Azul (-39.162009º -66.772884º): FML 09382; **Salta**: **Departamento Metán:** Balderrama, Sierra de Metán (-25.489520º -65.039234º): FML 1044-1,3,5; **San Luis**: **Departamento Chacabuco:** Villa Elena, in front of Cortaderas, western slope of Comechingones (-32.507075º -64.968790º): MACN 43034; **Departamento Gobernador Dupuy:** Bagual (F.C.N.S.), Escuela N°51 (-35.139145º -65.568143º): MACN 15261; **Santa Fe: Departamento La Capital**: Leyes Stream: INALI 5444; S from Colastiné (-31.698294º -60.606804º): MACN 28781; **Departamento San Martín:** El Trébol (-32.1892º -61.7261º): MACN 44596 (former CENAI 2902); **Tucumán**: **Departamento Trancas:** Rodeo Grande (-26.484170º -65.549086º): FML 1523.

Nota

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Preliminary inventory of the anuran fauna of the Mata Estrela Private Natural Heritage Reserve, in the Atlantic Forest of the State of Rio Grande do Norte, Brazil

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ABSTRACT
Tropical regions harbor the highest amphibian biodiversity, nevertheless, information on species composition is lacking in most areas yet these baseline data are essential to guide conservation strategies. The goal of the present study was to survey the anurans of a Private Natural Heritage Reserve named Mata Estrela, a fragment of about 26 km^2 in the Northern Atlantic Forest, Brazil. The study was carried out as an active census in June 2015. We found 27 anuran species assigned to five families: Bufonidae (2 spp.), Craugastoridae (1 sp.), Hylidae (11 spp.), Leptodactylidae (12 spp.) and Phyllomedusidae (1 sp.). All species are widespread in the Atlantic forest as well as in other South American ecoregions. Descriptions of local diversity such as this one are a fundamental baseline for conservation work and are especially needed in the northern limits of the Atlantic forest, for which there is still a huge lack of biological knowledge.

Key Words: Amphibians, Anurofauna, Species Richness, Diversity.

The Neotropical region harbours the highest amphibian diversity in the world (Baillie *et al.*, 2010). For example, South America hosts about three times more amphibian species than Africa (Wells, 2007), and Brazil leads in amphibian diversity worldwide with ca. 14% of the over 8 000 current amphibian species (AmphibiaWeb, 2021). More than half of the Brazilian amphibian diversity is found in the threatened Atlantic Forest, characterized by a high degree of endemism with over 80% of anuran species

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restricted to this ecoregion (Rossa-Feres *et al.*, 2017).

Originally the Atlantic Forest extended for ca. 1300 km^2 in the east coast of Brazil. Currently, however, only 11% of its original vegetation remains in very fragmented pieces (Ribeiro *et al.*, 2009; Tabarelli *et al.*, 2010). The northern Atlantic Forest is currently in its worst state of conservation, with most fragments covering less than 100 km^2, and almost half of the remaining forest is less than 100 m from the forest edges (Ribeiro *et al.*, 2009). The northern Atlantic Forest is presently in its most dire state of conservation (Ribeiro *et al.*, 2009). This scenario reinforces the need for amphibian surveys in the northen fragments as the rapid and ongoing decline of amphibians worldwide is mainly related to anthropogenic actions (Stuart *et al.*, 2004; Lips *et al.*, 2005; Verdade *et al.*, 2012). Recent efforts have been directed to uncover amphibian diversity in the northern Atlantic Forest (e.g. Santana *et al.*, 2008; Morato *et al.*, 2011; Pazinato *et al.*, 2013; Palmeira and Gonçalves 2015; Pereira *et al.*, 2016; Barbosa *et al.*, 2017; Leite-Filho *et al.*, 2017; Roberto *et al.*, 2017; Dubeux *et al.*, 2020), but some gaps still need to be filled. Understanding which species occur in a certain region is the first step for conservation management (Vilela *et al.*, 2018).

In this work, our objective was to carry out a rapid inventory of the anuran fauna in the private reserve of the Mata Estrela natural heritage, a fragment of Atlantic Forest located in the municipality of Baía Formosa, State of Rio Grande do Norte, Brazil.

The anuran survey was carried out in the Private Natural Heritage Reserve Mata Estrela (RPPN Mata Estrela) (06°22'53"South, 35°01'06"West; WGS 84; 34 m a.s.l.; Fig. 1), municipality of Baía Formosa, Rio Grande do Norte State, Brazil. This protected area is 20,35 km^2 and mainly covered by semideciduous forest (18,88 km^2), sand dunes (0,82 km^2) and lagoons (0,65 km^2) (Govindin and Miller, 2015). The climate is classified as tropical with dry summers (according with Köppen criteria, Alvares *et al.*, 2013) and annual temperature and rainfall of 25.8 °C and 1504 mm, respectively (EMPARN, 2019). The vegetation is composed mainly by ombrophilous forest (Dorado *et al.*, 2006).

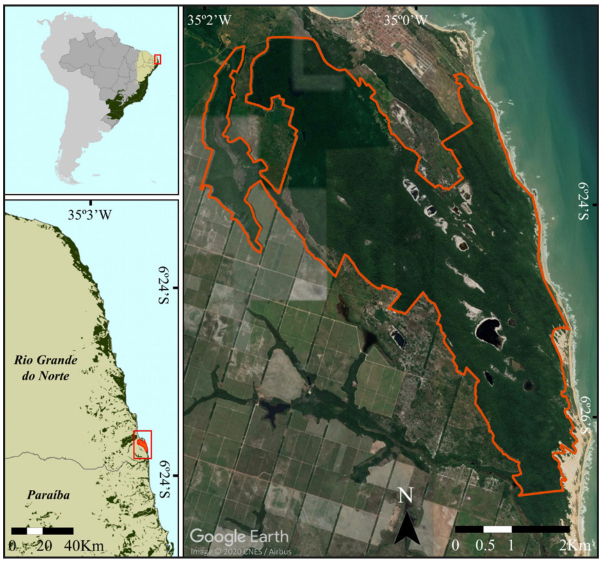

Figure 1. Satelite image of the Private Natural Heritage Reserve Mata Estrela (with boundaries shown in orange). Inset map is South America with rectangle showing the study area.

We sampled the study area for two nights (June 3 and 4, 2015). Individuals were located by active search (visual and acoustic encounter searches) in nocturnal transects in the middle of the forest lasting at least one hour each and in reproductive places such as puddles in roads, lakes and weirs. Two transects were carried out in the forest, one on the road at the edge of the forest and three around lakes and weirs. During each transect (between 17:00 – 23:00 h), 12 hours/collector were invested, where six observers walked slowly with headlights in search of frogs under the leaves or branches of bushes, moving litter and felling trunks and branches in all microhabitats suitable for the presence of frogs (Heyer *et al.*, 1994) (Fig. 2).

All observed individuals were registered, and at least five individuals of each species was collected (Collection authorization ICMBio/SISBIO 48770) and deposited in the *Coleção Herpetológica do Museu de História Natural* of *Universidade Federal de Alagoas* (MHN-UFAL) following the standard protocol of euthanasia and fixation (Resolução CFMV N°

Figure 2. Survey sites of Private Natural Heritage Reserve Mata Estrela, municipality of Baía Formosa, Rio Grande do Norte state, Brazil. A – C: lagoons; D: temporary pool.

1000 de 11 de maio de 2012). Morphological identification was performed using available literature, consulting specialists, and the taxonomy followed Frost (2021).

The conservation status of each species was based on the Red List of the International Union for the Conservation of Nature and Natural Resources (IUCN, 2022) and the *Livro Vermelho da Fauna Brasileira Ameaçada de Extinção* (ICMBio, 2018). The geographic distribution of the observed anurans was obtained from Haddad *et al.* (2013) and Frost (2021).

In total, 1122 individuals of anurans of 27 species, eleven genera and five families were recorded visually (68.7%) and/or vocally (31.3%). One-hundred twenty one specimens were collected, to compose the witness material. Leptodactylidae and Hylidae were the most diverse families with twelve and eleven species, respectively, followed by Bufonidae (two species), Craugastoridae and Phyllomedusidae (one species each; Table 1; Figs. 3 – 5). The most abundant species was *Scinax nebulosus* (Spix, 1824) (119) and together with *Pseudopaludicola mystacalis* (Cope, 1887) (112), *Dendropshohus branneri* (Cochran, 1948) (89), *Pleurodema diplolister* (Peters, 1870) (82), *Scinax x-signatus* (Spix, 1824) (76), *Leptodactylus fuscus* (Schneider, 1799) (61), *Pristimantis ramagii* (Boulenger, 1888) (56), *Leptodactylus troglodytes* Lutz, 1926 (52), *Dendropsophus oliveirai* (Bokermann, 1963) (51), *D. nanus* (Boulenger, 1889) (45), *Leptodactylus macrosternum* Miranda-Ribeiro, 1926 (45), and *Pseudopaludicola pocoto* Magalhães, Loebmann, Kokubum, Haddad, and Garda, 2014 (45) were responsible for 74% of the records (n = 833) (Fig. 6). All species were classified as least concern according to ICMBio (2018) and IUCN (2022), except *Pithecopus gonzagai* Andrade, Haga, Ferreira, Recco-Pimentel, Toledo and Bruschi, 2020", which was not evaluated so far.

Only three anuran surveys are available for the Atlantic Forest fragments located less than 100 km from the RPPN Mata Estrela. The Guaribas Biological Reserve (REBio Guaribas, Paraíba) is 33 km south of the RPPN and has an area of 43,21 km^2, formed by several formations with a predominantly flat landscape, with fragments of savannah, Atlantic Forest, Semideciduous Seasonal Forest and tableland vegetation, where thirty-four species of frogs were recorded using drop traps and 9720 hours/person of active search (Mesquita *et al.*, 2018). Another survey in Paraíba was carried out in the Benjamim Maranhão Botanical Garden (Mata do Buraquinho), 88 km south of the Mata Estrela RPPN, an area of 5,15 km^2, comprising typical Atlantic Forest vegetation. Sixteen species of anurans were recorded in 60 days of sampling effort distributed over a year (August 2011 to July 2012, Leite-Filho *et al.*, 2017). At the Escola Agrícola de Jundiaí, state of Rio Grande do Norte, 70 km north of the Mata Estrela RPPN, it is characterized by direct contact between Caatinga and Atlantic Forest, 34 species of anurans were recorded during 11 months of active survey and is characterized by vegetation of Caatinga and Atlantic Forest (Magalhães *et al.*, 2013). Each of these locations have peculiarities that can influence their compositions (Moraes *et al.*, 2007; Gambale *et al.*, 2014). For example, the Mata do Buraquinho is isolated in a densely populated region, under great pressure from the surrounding communities (Santana *et al.*, 2008). A different situation from Mata Estrela and REBio Guaribas, which are in sugarcane plantation areas, despite having areas in contact with urbanized stretches, do not suffer to the same degree (Govindin and Miller, 2015; Mesquita *et al.*, 2018). On the other hand, the Escola Agrícola de Jundiaí, despite being outside the sugarcane plantation areas, is located in an important food production hub for the region (Marinho *et al.*, 2021). Hylidae and Leptodactylidae were the most diverse families in the RPPN Mata Estrela. This result was similar to other surveys in the northern Atlantic Forest, for example (Santana *et al.*, 2008; Palmeira and Gonçalves 2015; Leite-Filho *et al.*, 2017 and Melo *et al.*, 2018; Mesquita *et al.*, 2018; Dubeux *et al.*, 2020). Hylids have many morphological adaptations to several types of environments (the presence of adhesive discs in their fingers, for example) that allow them to explore a greater diversity of surfaces (Cardoso *et al.*, 1989). Leptodactylids are ecologically generalists that are resistant to open areas, with most species depending on the presence of water bodies for their reproduction (Coelho and Oliveira, 2010).

An investment of more days in the field, respecting seasonality, combined with other complementary methods for sampling anurans (such as drop traps), as well as in other available environments, not sampled in this study, may reveal greater species richness. In fact, nocturnal searching, especially in water bodies has been shown to be more efficient to record large numbers of anuran species because they are water dependent for their reproduction (Brassaloti *et al.*, 2010). Nevertheless some authors have recommended the use of more than one

Table 1. Species of anuran amphibians collected on 3 and 4 June 2015 a Private Natural Heritage Reserve Mata Estrela, Baía Formosa, Rio Grande do Norte state, Brazil.

Species	Conservation status	Enviroments		Geographic distribution
		Forest	*Open areas*	
BUFONIDAE				
Rhinella granulosa (Spix, 1824)	Least Concern (LC)	Sandy soil	Sandy soil and clayey	
Rhinella diptycha (Cope, 1862)	Data Deficient (DD)	Sandy soil and clayey	Sandy soil and clayey, streams, temporary and permanent ponds and weirs edge	In the Brazil, Pará, Maranhão to Piauí, Ceará, and Alagoas, south to Rio Grande do Sul and Espíritu Santo. Inland Paraguay to Amazonian and eastern Bolivia; and southwest to northern and central Argentina and northern Uruguay.
CRAUGASTORIDAE				
Pristimantis ramagii (Boulenger, 1888)	Least Concern (LC)	Tree branch, edge vegetation of streams and vegetation of temporary ponds	Edge vegetation of temporary ponds and weirs	From RN to BA.
HYLIDAE				
Boana albomarginata (Spix, 1824)	Least Concern (LC)	–	Vegetation in temporary and permanent ponds and vegetation in dams	In Brazil from RN to SC. Apart from Colombia to Guianas.
Boana crepitans (Wied-Neuwied, 1824)	Least Concern (LC)	Tree branches	Vegetation in temporary and permanent ponds and vegetation in dams	GO, MG, RJ, BA, SE, AL, PE and PB.
Boana raniceps (Cope, 1862)	Least Concern (LC)	–	Vegetation in temporary and permanent ponds and vegetation in dams	Colombia, Venezuela, French Guiana, Paraguay, Argentina and Brazil in AL, AM, BA, CE, MG, MS, MT, MA, PA, PB, PE, PI, PR, RJ, RN, SE and SP.
Boana sp.	–	Tree branches	–	
Dendropsophus branneri (Cochran, 1948)	Least Concern (LC)	–	Vegetation in temporary and permanent ponds	PE, AL, SE, BA, MG, ES, RJ, RN and PB.
Dendropsophus minutus (Peters, 1872)	Least Concern (LC)	–	Vegetation in temporary and permanent ponds	Colombia, Venezuela, Guianas, Trindade, Ecuador, Peru, Bolivia, Paraguay, Uruguay and Argentina. In all of Brazil.
Dendropsophus nanus (Boulenger, 1889)	Least Concern (LC)	–	Vegetation in temporary and permanent ponds.	Northeast to the extreme South of Brazil. Suriname, French Guiana, Paraguay, Argentina, Bolivia and Uruguay.
Dendropsophus oliveirai (Bokermann, 1963)	Least Concern (LC)	–	Vegetation in temporary and permanent ponds	From RJ to RN.
Scinax fuscomarginatus (A. Lutz, 1925)	Least Concern (LC)	–	Vegetation in temporary and permanent ponds	South, Central and Eastern of Brazil (PI, CE and AM), Bolivia, Paraguay, Argentina, Venezuela, Guiana and Suriname.

Scinax nebulosus (Spix, 1824)	Least Concern (LC)	–	Vegetation in temporary and permanent ponds and vegetation in dams	Venezuela, Guianas and Bolivia. In Brazil, from low amazon river to Alagoas.
Scinax x-signatus (Spix, 1824)	Least Concern (LC)	–	Vegetation in temporary ponds	Colombia, Venezuela andd Suriname. Eastern, south and southeast of Brazil.
LEPTODACTYLIDAE				
Adenomera hylaedactyla (Cope, 1868)	Least Concern (LC)	–	–	Colombia, Venezuela, Guianas, Ecuador, Peru and Bolivia. From south to north, eastern and central of Brazil.
Leptodactylus fuscus (Schneider, 1799)	Least Concern (LC)	–	Herbaceous vegetation with soggy soil and edge of temporary ponds	Panama, Andes, Bolivia, Paraguay and Argentina; south to southeast of Brazil.
Leptodactylus macrosternum Miranda-Ribeiro, 1926	Least Concern (LC)	Sandy soil near temporary ponds	Sandy soil near temporary ponds and edge of dams	Colombia, Venezuela, Guianas, Bolivia, Trindade and Brazil.
Leptodactylus mystaceus (Spix, 1824)	Least Concern (LC)	Edge of temporary ponds	Herbaceous vegetation with soggy soil	Amazon to the southern limit of Brazil for Bolivia, Peru, Ecuador, Colombia, Venezuela and Guianas. Isolated records from Northeastern Brazil to the south of PN
Leptodactylus natalensis A. Lutz, 1930	Least Concern (LC)	–	Herbaceous vegetation with soggy soil and temporary ponds	Coastal region of Brazil: MA, RN to RJ
Leptodactylus podicipinus (Cope, 1862)	Least Concern (LC)	Herbaceous vegetation with soggy soil	Herbaceous vegetation with soggy soil and temporary ponds	Paraguay, Argentina, Bolivia and Uruguay. In Brazil, central part extending along the Madeira River and the Amazon River in the Amazon Basin.
Leptodactylus troglodytes A. Lutz, 1926	Least Concern (LC)	Herbaceous vegetation with soggy soil	Herbaceous vegetation with soggy soil and temporary ponds	Northeast Brazil to MG.
Leptodactylus vastus (lutz 1930)	Least Concern (LC)	–	Edge of temporary ponds	Northeast Brazil
Physalaemus cuvieri Fitzinger, 1826	Least Concern (LC)	–	Herbaceous vegetation with soggy soil	Northeast, central and southern Brazil; Argentina, Paraguay, Bolivia and Venezuela.
Pleurodema diplolister (Peters, 1870)	Least Concern (LC)	Sandy soil near temporary pools	Sandy soil edge near temporary ponds and edge of dams	Northeast Brazil in the Caatinga region of MA, PB, TO, BA, GO and MG.
Pseudopaludicola mystacalis (Cope, 1887)	Least Concern (LC)	–	Herbaceous vegetation with soaked soil, edge of dams, edge of streams and temporary ponds.	South and east of Brazil. Bolivia, Paraguay, Argentina and Uruguay.
Pseudopaludicola pocoto Magalhães, Loebmann, Kokubum, Haddad & Garda, 2014	–	–	Herbaceous vegetation with soaked soil, edge of dams, edge of streams and temporary ponds.	Northeast Brazil in caatinga regions in the CE, PI, RN, PB and PE. An isolated log in the east of MG.
PHYLLOMEDUSIDAE				
Pithecopus gonzagai Andrade, Haga, Ferreira, Recco-Pimentel, Toledo, and Bruschi, 2020.	Not Evaluated (NE)	–	Vegetation in temporary and permanet ponds and vegetation of dams	RN, PB, PE, AL, CE, PI and MA.

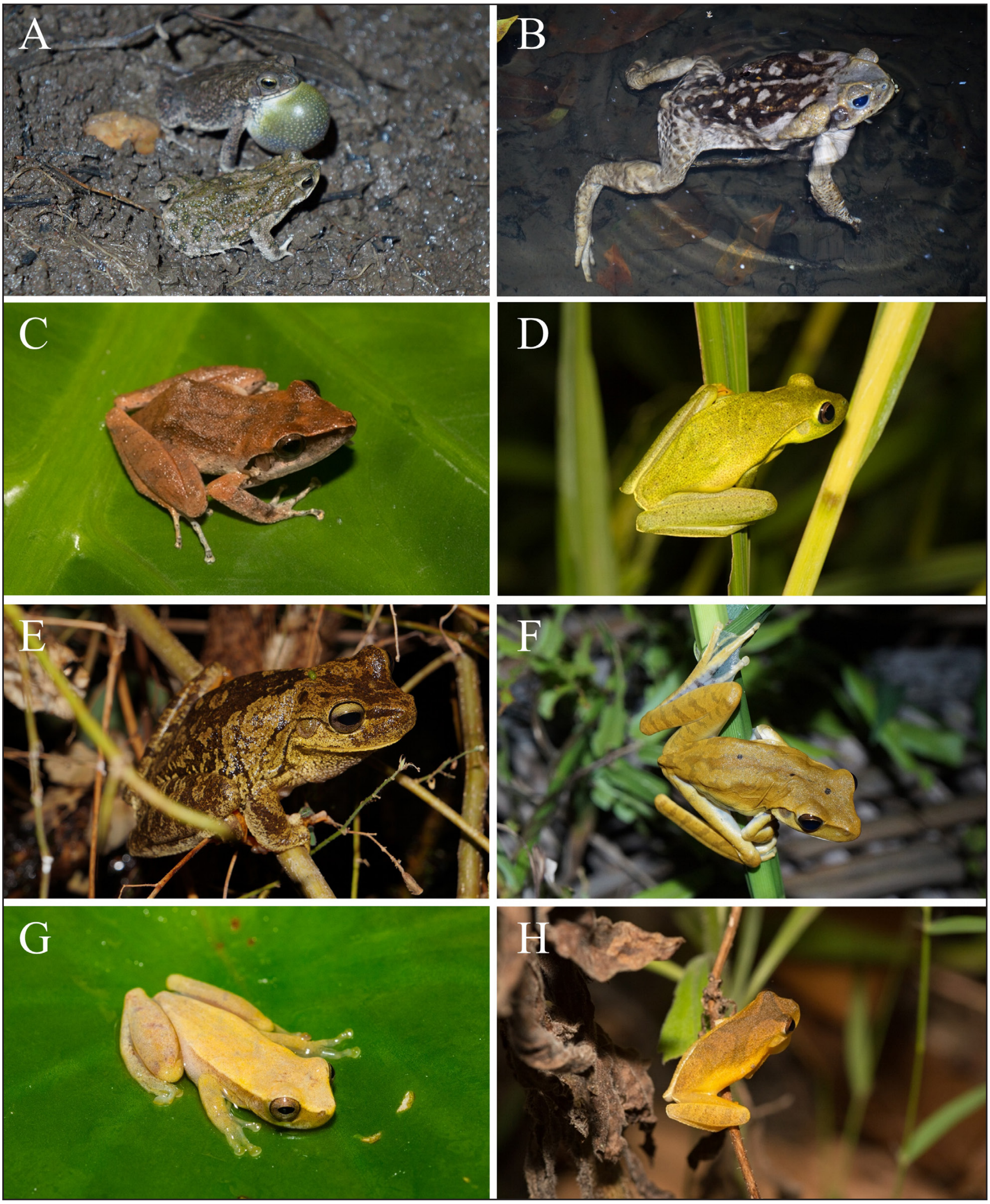

Figure 3. Anurans of the Private Natural Heritage Reserve Mata Estrela, municipality of Baía Formosa, Rio Grande do Norte State, Brazil. **A.** *Rhinella granulosa* (Spix, 1824). **B.** *R. diptycha* (Cope, 1862). **C.** *Pristimantis ramagii* (Boulenger, 1888). **D.** *Boana albomarginata* (Spix, 1824). **E.** *B. crepitans* (Wied-Neuwied, 1824). **F.** *B. raniceps* (Cope, 1862). **G.** *Dendropsophus branneri* (Cochran, 1948). **H.** *D. minutus* (Peters, 1872).

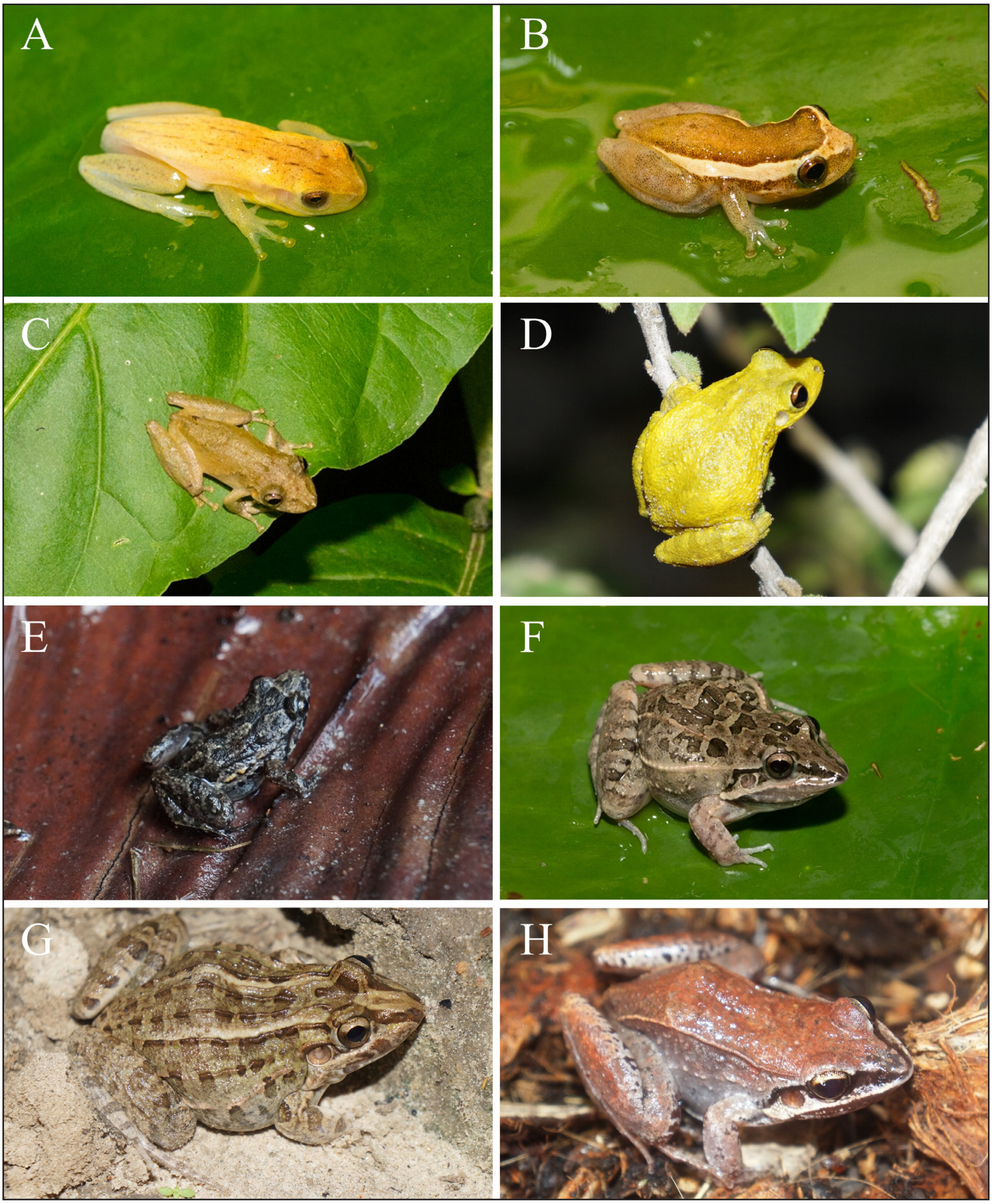

Figure 4. Anurans of the Private Natural Heritage Reserve Mata Estrela, municipality of Baía Formosa, Rio Grande do Norte State, Brazil. **A.** *Dendropsophus nanus* (Boulenger, 1889). **B.** *D. oliveirai* (Bokermann, 1963). **C.** *Scinax nebulosus* (Spix, 1824). **D.** *S. fuscovarius* (Lutz, 1925). **E.** *Adenomera hylaedactyla* (Cope, 1868). **F.** *Leptodactylus fuscus* (Schneider, 1799). **G.** *L. macrosternum* Miranda-Ribeiro, 1926. **H.** *L. mystaceus* (Spix, 1824).

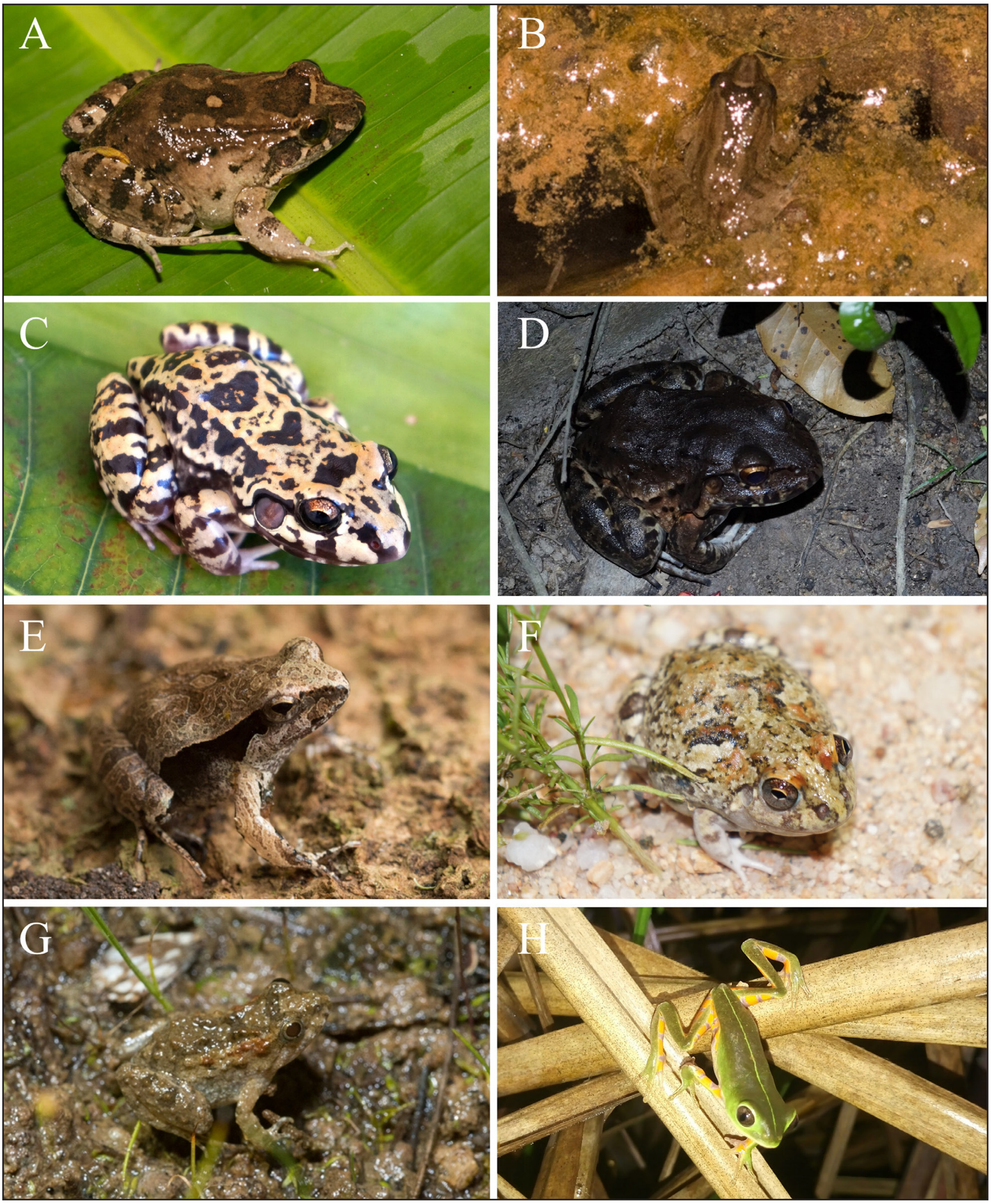

Figure 5. Anurans of the Private Natural Heritage Reserve Mata Estrela, municipality of Baía Formosa, Rio Grande do Norte State, Brazil. **A.** *Leptodactylus natalensis* Lutz, 1930. **B.** *L. podicipinus* (Cope, 1862). **C.** *L. troglodytes* Lutz, 1926. **D.** *L. vastus* Lutz, 1930. **E.** *Physalaemus cuvieri* Fitzinger, 1826. **F.** *Pleurodema diplolister* (Peters, 1870). **G.** *Pseudopaludicola mystacalis* (Cope, 1887). **H.** *Pithecopus gonzagai* Andrade, Haga, Ferreira, Recco-Pimentel, Toledo, and Bruschi, 2020.

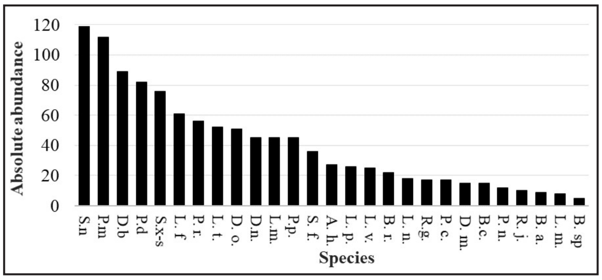

Figure 6. Total number of anurans recorded in the Private Natural Heritage Reserve Mata Estrela, municipality of Baía Formosa, Rio Grande do Norte State, Brazil between June 03 – 04, 2015 (*N* = 1122). *Scinax nebulosus*, *Pseudopaludicola mystacalis*, *Dendropsophus branneri*, *Pleurodema diplolister*, *Scinax x-signatus*, *Leptodactylus fuscus*, *Pristimantis ramagii*, *Leptodactylus troglodytes*, *Dendropsophus oliveirai*, *Dendropsophus nanus*, *Leptodactylus macrosternum*, *Pseudopaludicola pocoto*, *Scinax fuscomarginatus*, *Adenomera hylaedactyla*, *Leptodactylus podicipinus*, *Leptodactylus vastus*, *Boana raniceps*, *Leptodactylus natalensis*, *Rhinella granulosa*, *Physalaemus cuvieri*, *Dendropsophus. minutus*, *Boana crepitans*, *Pithecopus gonzagai*, *Rhinella diptycha* (Cope, 1862), *Boana albomarginata*, *Leptodactylus mystaceus*, *Boana* sp.

sampling method to optimize the area and increase the number of species recorded (Ribeiro-Júnior *et al.*, 2008; Condez *et al.*, 2009; Fonseca *et al.*, 2019).

Environmental impacts caused by anthropogenic actions comprise the majority of the responsibility for biodiversity loss. Private protected areas such as the RPPN Mata Estrela preserve essential environmental conditions to sustain populations of amphibians and other vertebrates dependent on forested habitats (e.g. *Alouatta belzebul* (Linnaeus, 1766), the Guariba monkey). Thus, the Mata Estrela RPPN can be considered an important area of the Atlantic Forest in its northern portion, with a good level of conservation.

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Nota

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O canto de soltura de *Rhinella crucifer* (Wied-Neuwied, 1821), no Estado de Pernambuco, Nordeste do Brasil

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ABSTRACT
In this work, we describe for the first time the release call of *Rhinella crucifer* in addition to comparing its advertisement call with other species of your group. The records were made in an area of Atlantic Forest, named Refúgio de Vida Silvestre Matas de Sirij, in the municipality of São Vicente Férrer, state of Pernambuco, in the Northeast Region of Brazil. At 18 June 2019, 24 advertisement vocalizations of four males were recorded, of which three emit 31 release calls. The results showed a structural difference between the two types of vocalizations, such as the presence of pulses defined, harmonics and greater modulation in the release call. Compared to other *Rhinella* species of the *crucifer* group, the release call of *R. crucifer* had a greater resemblance to *R. ornata* in the duration and interval of notes and number and interval of pulses. While the proximity of the dynamic parameters of the advertisement call with *R. casconi*, the highlight was the compatibility of the dominant frequency with *R. inopina*. This work adds new information on the vocal repertoire of the species, which contributes to information about your natural history.

Key Words: Bioacoustics; Anura; Vocalization; Reproduction; Courtship

RESUMO
Neste trabalho descrevemos pela primeira vez o canto de soltura de *Rhinella crucifer* além de comparar o seu canto de anúncio com outras espécies de seu grupo. Os registros foram feitos em uma área de Floresta Atlântica denominada Refúgio de Vida Silvestres Matas de Sirij, no município de São Vicente Férrer, Pernambuco, na Região nordeste do Brasil. No dia 18 de junho de 2019 foram registradas 24 vocalizações de anúncio de quatro machos, dos quais três reproduziram 31 chamadas de soltura. Os resultados mostraram que houve diferença estrutural entre os dois tipos de cantos, como a presença de pulsos definidos, harmônicos e maior modulação no canto de soltura. Quando comparado com outras espécies de *Rhinella* do grupo *crucifer*, o canto de soltura do *R. crucifer* teve maior semelhança com *R. ornata* na duração e intervalo de notas e número e intervalo de pulsos. Apesar da proximidade dos parâmetros dinâmicos do canto de anuncio com *R. casconi*, o destaque foi a similaridade da frequência dominante com *R. inopina*. Com este trabalho adicionamos mais informações do repertório vocal da espécie, o que contribui com informações sobre sua história natural.

Palavras-chave: Bioacústica; Anura; Vocalização; Reprodução; Corte.

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O canto mais comum em anfíbios anuros é o de anúncio (Toledo *et al.*, 2014; Köhler *et al.*, 2017). Esta chamada pode demonstrar a qualidade do vocalizador (Gerhardt, 1991), sendo usada pelos machos na atração de fêmeas coespecíficas para o acasalamento, indução de outros machos a se juntarem a coros locais e no estabelecimento do espaço interindividual dos sítios vocais (Toledo *et al.*, 2014). No repertório vocal de um anuro podem ocorrer diferentes tipos de cantos, sendo um deles o canto de soltura, que é caracterizado por ter uma curta duração e poucas notas (Grenat e Martino, 2013; Guerra *et al.*, 2020; Mângia *et al.*, 2017). De acordo com alguns autores (e.g., Martof e Thompson 1958; Toledo *et al.*, 2014), estes cantos são emitidos quando o indivíduo é amplexado ou tocado no dorso, lados e/ou região axilar por outros indivíduos machos.

De maneira geral, tanto as fêmeas, quanto os machos de anfíbios anuros podem apresentar canto de soltura quando são alvos de amplexo intra ou interespecífico (McClelland e Wilczynski, 1989; Köhler *et al.*, 2017), sendo através deste tipo de comportamento, uma maneira dos indivíduos pouparem energia em um amplexo enganoso (Batista *et al.*, 2017). As chamadas de soltura podem variar de acordo com as espécies, sendo estas chamadas diferenciadas até mesmo em espécies filogeneticamente próximas (Castellano *et al.*, 2002).

No gênero *Rhinella* já existem registros de cantos de soltura (Guerra *et al.*, 2011; Guerra *et al.*, 2020), que consistem em poucas notas e curta duração, além da baixa frequência (Ver Tabela 1 em Guerra *et al.*, 2020). Todavia, esses registros são ocasionais (Vieira *et al.*, 2014) e, provavelmente essa ausência ocorre pelo fato das poucas informações comportamentais disponíveis na literatura, como o contato pré e pós- oviposição (Toledo *et al.*, 2014).

Rhinella crucifer é uma espécie de grande porte (Baldissera *et al.*, 2004) que possui ampla distribuição no domínio da Mata Atlântica do Brasil e, apesar de ser encontrado no estado de Minas Gerais, possui maior ocorrência em estados litorâneos como Paraíba, Pernambuco, Sergipe, Bahia, Espírito Santo e Rio de Janeiro (Frost, 2020). Indivíduos desta espécie costumam viver no centro ou borda de mata, sobre a serrapilheira e também em regiões antrópicas (Pereira *et al.*, 2016). Em relação ao repertório vocal da espécie, recentemente, apenas o canto de anúncio foi descrito com indivíduos provenientes do sul da Bahia (Oliveira *et al.*, 2014), sendo definido o canto como não-harmônico, composto por notas pulsadas e com o aumento gradual de intensidade. De acordo com Pereyra *et al.* (2021), essa espécie pertence ao grupo *R. crucifer* dentro do clado *R. marina*, que atualmente é composto por mais quatro espécies: *R. casconi* (Roberto *et al.*, 2014); *R. henseli* (Lutz, 1934); *R. inopina* (Vaz-Silva *et al.*, 2012); e *R. ornata* (Spix, 1824). Também existe nesse grupo a presença de uma possível hibridização natural entre *R. crucifer* e *R. ornata* representada por espécimes anteriormente considerados como *R. pombali* (Baldissera *et al.*, 2004) (Thomé *et al.*, 2010, 2012; Pereyra *et al.*, 2021). Dentre esses táxons apenas *R. ornata* possui descrição de seu canto de soltura (Batista *et al.*, 2017; Costa *et al.*, 2020). Diante disto, o objetivo deste trabalho foi descrever os cantos de soltura e anúncio de R. crucifer, comparando com outros cantos de mesma função em outras espécies de seu grupo.

Os cantos foram registrados com um gravador portátil Tascam DR-40 acoplado a um microfone unidirecional Sennheiser ME 66, configurado a 44 KHz em 16 bits. Todos os cantos foram gravados a uma distância de 0,5 m dos indivíduos. Para averiguar dados abióticos (temperatura e umidade relativa do ar) utilizamos um termohigrômetro digital marca Incoterm® e para os dados bióticos (comprimento rostro-cloacal-CRC e massa dos indivíduos) utilizamos um paquímetro digital (0.1 mm) e balança pesola (0.1 g). Na análise dos parâmetros acústicos, utilizou-se o programa Raven Pro 1.5 (Bioacoustics Research Program, 2020), com as seguintes configurações: DFT = 256, tipo = Hann; brilho e contraste= 50, tamanho de janela de espectrograma= 2500, valores padrão). As características físicas de cada chamada analisada foram: duração do canto, (s), intervalo entre canto (s), pico energético (dB), número de notas, duração de notas, intervalo entre notas (s), número de pulsos por nota, duração de pulsos, intervalo entre pulsos, frequência dominante (Hz), frequência mínima (Hz) e frequência máxima (Hz). Após a coleta, todas as gravações foram depositadas na Sonoteca do Laboratório Interdisciplinar de Anfíbios e Répteis na Universidade Federal Rural de Pernambuco (UFRPE-SLIAR) e no Laboratório de Herpetologia da Universidade Federal de Campina Grande (LHUFCG-MNCK). Após as gravações, os indivíduos foram soltos nos mesmos locais de captura.

O registro ocorreu durante atividades do projeto de pesquisas Herpetofauna do Refúgio de Vida Silvestres Matas de Sirij, no município de São Vicente Férrer, Pernambuco, na Região nordeste

Tabela 1. Parâmetros acústicos espectrais e temporais do canto de soltura de três machos coletados e valores atribuídos ao canto de anúncio gravado de quatro animais na Unidade de Conservação Refúgio de Vida Silvestre Matas de Siriji, São Vicente Férrer, Pernambuco, Brasil. Gravações de 18 de junho de 2019. Umidade relativa do ar a 95% em uma temperatura de 22.1 °C. Formato dos valores em: Média + desvio padrão (mínimo – máximo) (número amostral).

	Canto de soltura				**Canto de anúncio**
Parâmetros	**Macho 1**	**Macho 2**	**Macho 3**	**Média/Dp**	**Média/Dp**
Duração de canto (s)	0.604±0.347 (0.21-1.337) (n=15)	0.895±0.343 (0.499-1.311) (n=8)	0.683±0.2844 (0.34-1.056) (n=8)	0.699±0.342 (0.21-1.337) (n=31)	3.882±0.859 (2.744-6.869) (n=24)
Intervalo entre cantos (s)	0.233±0.1778 (0.108-0.761) (n=14)	0.466±0.371 (0.162-1.188) (n=7)	0.187±0.140 (0.085-0.5) (n=7)	0.274±0.243 (0.085-1.188) (n=28)	9.175±9.551 (2.665-47.791) (n=21)
Pico energético(dB)	82.473±1.990 (78.9-85.7) (n=15)	82.25±3.605 (75.9-87.1) (n=8)	82.512±4.130 (75.5-88.6) (n=8)	82.425±2.979 (75.5-88.6) (n=31)	98.316±3.531 (87.4-104.7) (n=24).
Número de notas	3.133±2.166 (1-8) (n=15)	5.375±2.669 (2-9) (n=8)	3.375±2.326 (1-7) (n=8)	3.774±2.459 (1-9) (n=31)	77.166 ± 11.157 (58-100) (n=24)
Duração de notas	0.114±0.116 (0.014-0.429) (n=47)	0.074±0.086 (0.029-0.429) (n=43)	0.126±0.142 (0.025-0.49) (n=27)	0.102±0.114 (0.014-0.49) (n=117)	0.023±0.004 (0.006-0.041) (n=1851)
Intervalo entre notas (s)	0.115±0.103 (0.055-0.657) (n=32)	0.113±0.037 (0.027-0.22) (n=35)	0.107±0.028 (0.081-0.194) (n=19	0.112±0.068 (0.027-0.657) (n=86)	0.025±0.004 (0.009-0.057) (n=1826)
Número de pulsos/Nota	11.234±10.891 (1-45) (n=47)	7.093±7.318 (3-39) (n=43)	12.518±14.213 (3-60) (n=27)	10.008±10.813 (1-60) (n=117)	2.921±0.715 (1-4) (n=1851)
Duração de pulsos	0.0059±0.0016 (0.003-0.017) (n=528)	0.0057±0.002 (0.002-0.0260) (n=305)	0.0055±0.0014 (0.003-0.019) (n=339)	0.0057±0.0017 (0.002-0.026) (n=1172)	0.007±0.0022 (0.003-0.057) (n=5487)
Intervalo entre pulsos	0.0042±0.0029 (0.001-0.037) (n=481)	0.0043±0.0035 (0.001-0.028) (n=262)	0.0047±0.0023 (0.001-0.014) (n=312)	0.0044±0.0029 (0.001-0.037) (n=1055)	-
Frequência Dominante (Hz)	844.08±35.648 (775.2-861.3) (n=15)	882.862±60.927 (775.2-947.5) (n=8)	839.775±39.856 (775.2-861.3) (n=8)	852.977±46.412 (775.2-947.5) (n=31)	1065.9±61.241 (1033.6-1205.9) (n=24)
Frequência Mínima (Hz)	666.113±39.457 (602.9-689.1) (n=15)	678.312±55.217 (602.9-775.2) (n=8)	678.325±30.476 (602.9-689.1) (n=8)	672.412±41.147 (602.9-775.2) (n=31).	789.5 ± 32.777 (775.2-861.3) (n=24)
Frequência Máxima (Hz)	1498.72±315.306 (1033.6-2497.9) (n=15)	1894.925±419.439 (1464.3-2670.1) (n=8)	1399.675±157.827 (1033.6-1550.4) (n=8)	1575.406±362.846 (1033.6-2670.1) (n=31)	1209.475 ± 47.382 (1119.7-1292) (n=24)
Comprimento (mm)	74.3	87.2	77	-	-
Peso (g)	35.5	35.7	35.2	-	-

do Brasil (07° 36’ 56.6” S, 35° 30’ 21.3” W). No dia 18 de junho de 2019, às 21 horas foram observados sete indivíduos de *Rhinella crucifer* emitindo cantos de anúncio nas margens de um reservatório d’água. Após gravarmos seus cantos de anúncio, coletamos os animais e os manipulamos na região lateral do corpo simulando um amplexo. Em resposta, os indivíduos passaram a emitir o canto de soltura, inflando tanto a região abdominal quanto a região gular, realizando movimentos de tremulação do peito e abdômen. Ao todo foram obtidas 24 vocalizações de anúncio de quatro machos (UFRPE-SLIAR 295–298), onde os três últimos machos gravados emitiram 31 chamadas de soltura (UFRPE-SLIAR 299–301). Umidade relativa do ar a 95% em uma temperatura de 22.1 °C.

O canto de soltura de *Rhinella crucifer* foi multipulsionado e harmônico (Tabela 1, Fig. 1. C-D).

Apresentou duração de 0.699 ± 0.342 s (0.21–1.337 s; n = 31), intercalados com intervalos de 0.274 ± 0.243 s (0.085–1.188 s; n = 28) e com um pico energético de 82.425 ± 2.979 dB (75.5–88.6 dB, n = 31). As chamadas foram compostas por 3.774 ± 2.459 notas (1–9 notas; n = 31) que duraram 0.102 ± 0.114 s (0.014–0.49 s; n = 117), com intervalos de 0.112 ± 0.068 s (0.027–0.657 s; n = 86). O número de pulsos/nota ficou em 10.008 ± 10.813 pulsos (1–60 pulsos; n = 117), sendo estes pulsos com uma duração média de 0.0057 ± 0.0017 s (0.002–0.026 s; n = 1172), intercalados em períodos de 0.0044 ± 0.0029 s (0.001–0.037 s; n = 1055). A frequência dominante foi de 852.977 ± 46.412 Hz (775.2–947.5 Hz, n = 31), a mínima ficou em 672.412 ± 41.147 Hz (602.9–775.2 Hz, n = 31) e a frequência máxima

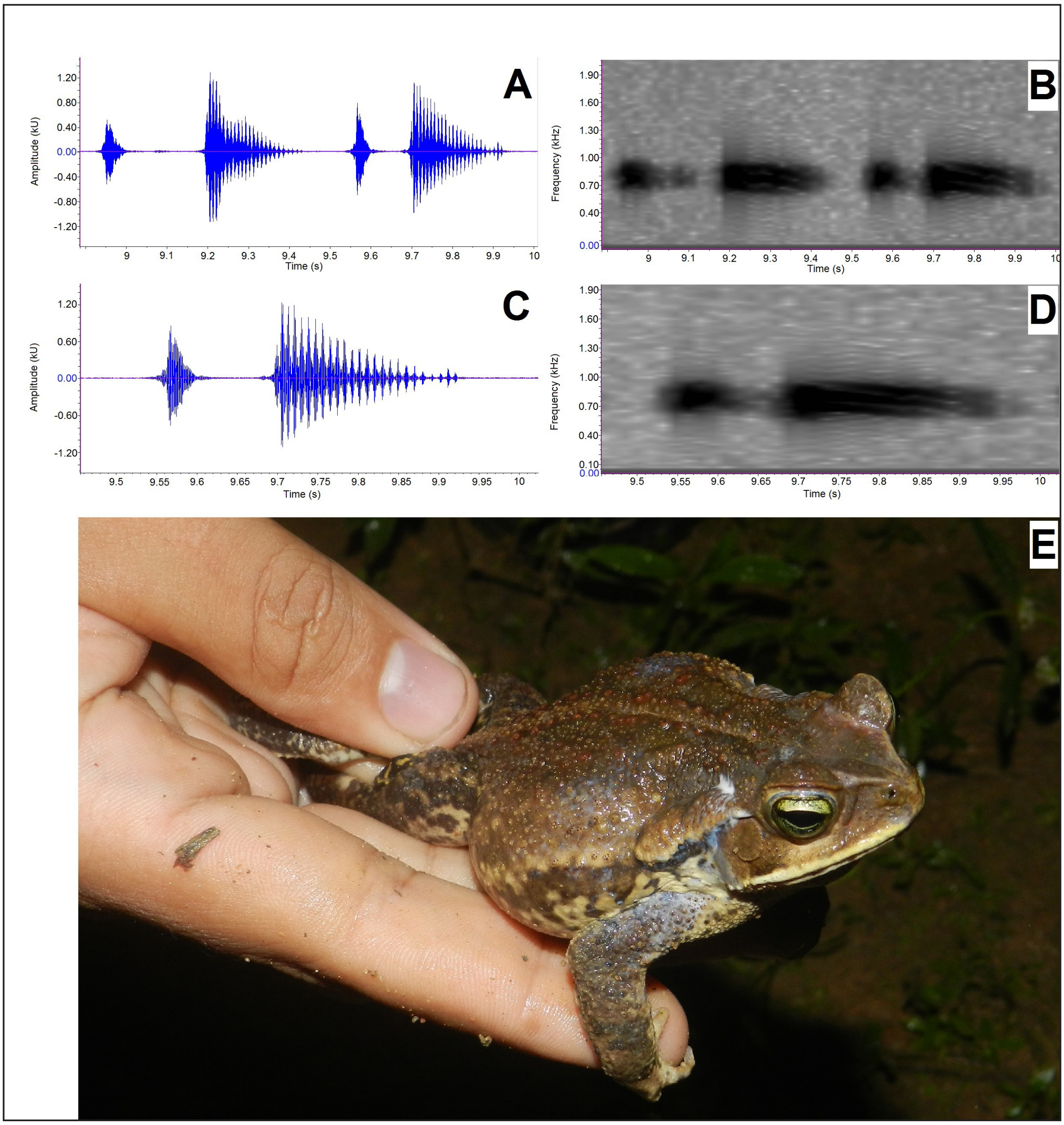

Figura 1. Canto de soltura de um macho adulto de *Rhinella crucifer* (UFRPE-SLIAR 299). **A)** Oscilograma de duas vocalizações consecutivas. **B)** Espectrograma (kHz) demonstrando a presença de harmônicos nas ultimas notas do canto. **C)** Oscilograma de canto único, vê-se a curta nota inicial seguida de uma segunda mais duradoura com pulsos finais separados. **D)** Espectrograma de canto único. E) Macho alvo das gravações.

atingiu 1575.406 ± 362.846 Hz (1033.6–2670.1 Hz, n = 31). As chamadas de soltura tinham entre 1 e 9 notas iniciais com duração curta de 0.0563 ± 0.1284 s (0.014–1.236 s, n = 87) separadas por 0.102 ± 0.028 (0.027–0.22; n = 63) atingindo frequência dominante de 864.293 ± 74.831 Hz (775.2–1205.9 Hz, n = 87). O canto terminou em uma nota mais longa, que durava cerca de 0.283 ± 0.092 s (0.106–0.49 s; n = 29), com intervalo de 0.141 ± 0.120 s (0.055–0.657 s, n = 23) e manteve a média de 837.551 ± 45.432 Hz (775.2–947.5 Hz, n = 29) de frequência dominante.

O canto de anúncio de *Rhinella crucifer* demonstrou ser multipulsionado e sem harmônicos (Tabela 1, Fig. 2. A-D), com duração média de 3.882 ± 0.859 s (2.744–6.869 s; n = 24) e intervalos de 9.175 ± 9.551 s (2.665–47.791 s; n = 21) atingindo o pico energético médio de 98.316 ± 3.531 dB (87.4–104.7 dB, n = 24). O número médio de notas por canto foi de 77.166 ± 11.157 notas (58–100 notas, n = 24), com duração média de 0.023 ± 0.004 s (0.006–0.041 s, n = 1851) e intervalos de 0.025 ± 0.004 s (0.009–0.057 s, n = 1826). Cada nota apresentou 2.921 ± 0.715 pulsos (1–4 pulsos; n = 1851), parcialmente fusionados, com duração de 0.007 ± 0.0022 s (0.003–0.057 s, n = 5487) (Fig. 2.B). A frequência dominante ficou em 1065.9 ± 61.241 Hz (1033.6–1205.9 Hz; n = 24) e, as frequências mínima e máxima foram 789.5 ± 32.777 Hz (775.2–861.3 Hz, n = 24) e 1209.475 ± 47.382 Hz (1119.7–1292 Hz, n = 24).

No grupo *Rhinella crucifer*, poucos cantos de soltura foram descritos (Tabela 2) e, comparando com os estudos encontrados, nossas gravações mostraram um perfil de canto mais curto, de notas rápidas e de baixa frequência (Tabela 2; Fig. 1.A). Entre os espécimes postulados, os mais próximos acusticamente de *Rhinella crucifer* foram indivíduos de *Rhinela ornata* gravados por Costa *et al.* (2020), onde as convergências se deram principalmente em aspectos dinâmicos do canto, como: a duração e intervalo entre notas (0.099 ± 0.148 s e 0.186 ± 0.086 s, respectivamente); número de pulsos (11.4 ± 15.1 pulsos) e intervalo entre pulsos (0.004 ± 0.003 s).

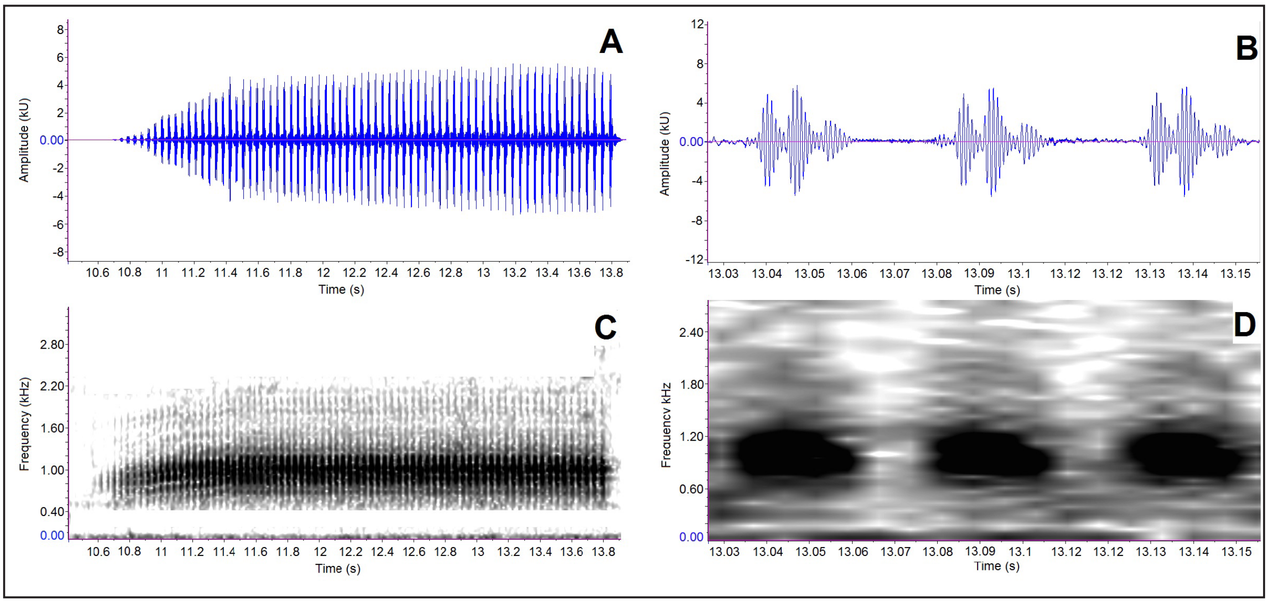

Figura 2. Canto de anúncio de um macho adulto de *Rhinella crucifer* (UFRPE-SLIAR 297). **A)** Canto composto por notas curtas e rápidas. **B)** Conjunto de três notas com três pulsos parcialmente fusionados. **C)** Oscilograma do canto de anúncio. **D)** Conjunto de três notas com ausência de harmônicos.

O canto de soltura, quando comparado ao de anúncio, evidenciou tanto diferenças acústicas temporais quanto espectrais. No canto de soltura, além da presença de pulsos definidos, principalmente no fim das notas (ver Fig. 1.C) e presença de harmônicos (Fig. 1.B), também se mostrou curto, com poucas notas e mais modulado (comparar fig. 1.B com fig. 2.C). Em relação as frequências dos cantos, a chamada de soltura apresentou valor inferior na frequência mínima e dominante enquanto a frequência máxima apresentou valores maiores, identificando uma maior faixa de frequência por parte deste canto (Tabela 1). Os dois cantos não corresponderam ao padrão geral de semelhança estrutural, mas isso pode ser uma resposta a limitações morfológicas do aparato vocal da espécie (Köhler *et al.*, 2017), sendo

Tabela 2. Parâmetros acústicos temporais e espectrais de cantos de soltura e anúncio em espécies do grupo *Rhinella crucifer* deste estudo e compilados da literatura. Autores: (*) Presente estudo; 1 - Costa *et al.*, 2020; 2 - Batista *et al.*, 2017; 3 - Lingnau, 2009; 4 - Lourenço *et al.*, 2010; 5 - Heyer *et al.*, 1990; 6 - Roberto *et al.*, 2014; 7 - Andrade *et al.*, 2015; 8 - Batista *et al.*, 2016. Formato dos valores (quando disponível) em: Média + desvio padrão (mínimo – máximo) (número amostral). dos valores em: Média + desvio padrão (mínimo – máximo) (número amostral).

	Espécie	**Duração de canto (s)**	**Intervalo entre canto (s)**	**Nº de notas**	**Duração de notas**	**Intervalo entre notas(s)**	**Nº pulso/ Nota**	**Duração de pulsos**	**Inter. entre pulsos**	**Frequência Dominante (Hz)**	**Frequência Mínima (Hz)**	**Frequência Máxima (Hz)**
	*Rhinella crucifer**	0.699±0.342 (0.21–1.337) (n=31)	0.274±0.243 (0.085–1.188) (n=28)	3.774±2.459 (1–9) (n=31)	0.102±0.114 (0.014–0.49) (n=117)	0.112±0.068 (0.027–0.657) (n=86)	10.008±10.813 (1–60) (n=117)	0.006±0.0017 (0.002–0.026) (n=1172)	0.004±0.0029 (0.001–0.037) (n=1055)	852.977±46.412 (775.2–947.5) (n=31)	672.412±41.147 (602.9–775.2) (n=31)	1575.406±362.846 (1033.6–2670.1) (n=31)
Canto de soltura	*R. ornata*[1]	2.011±1.903 (n=58)	–	7.5 ±7.5 (n=58)	0.099±0.148	0.186±0.086	11.4±15.1	0.004±0.001	0.004±0.003	960–1750	–	–
	R. ornata (como *R. abei*)[2]	1.62±0.679 (0.585–2.906) (n=10)	–	Média de 2 (2–8) (n=10)	0.206±0.366 (0.058–0.161) (n=42)	0.215±0.056 (0.142–0.395) (n=32)	Média de 5 (4–71) (n=42)	0.007±0.002 (0.003–0.013) (n=42)	0.006±0.004 (0.001–0.021) (n=131)	1214.1±37.12 (125.9–1378.1) (n=42)	824.40±775.17 (344.50–1033.60) (n=42)	1533.98±189.52 (1378.10–1894.90) (n=42)
	*R. crucifer**	3.882±0.859 (2.744–6.869) (n=24)	9.175±9.551 (2.665–47.791) (n=21)	77.166 ± 11.157 (58–1) (n=24)	0.023±0.004 (0.006–0.041) (n=1851)	0.025±0.004 (0.009–0.057) (n=1826)	2.921±0.715 (1–4) (n=1851)	0.007±0.0022 (0.003–0.057) (n=5487)	–	1065.9±61.241 (1033.6–1205.9) (n=24)	789.5 ± 32.777 (775.2–861.3) (n=24)	1209.475 ± 47.382 (1119.7–129) (n=24)
	R. henseli[3]	0.25 ± 0.04 (0.21 – 0.31) (n = 7)	–	–	–	–	–	–	–	980±20 (950–1010)(n=7)	–	–
Canto de anúncio Canto de anúncio	*R. crucifer x R. ornata* (como *R. pombali*)[4]	4.28±0.65 (3.43–4.93) (n=7)	12.82±2.94 (7.79–15.29) (n=6)	66±9.92 (51–76) (n=7)	0.031±0.003 (0.025–0.039) (n=35)	0.034±0.004 (0.026–0.041) (n=28)	–	–	–	959±90 (861–1033) (n=7)	–	4692 ± 622 (4218–5943 (n=7)
	Rhinella ornata[5]	4–7	–	60–150	0.03–0.06		–	–	–	–	960–1750	
	Rhinella casconi[6]	2.9±0.4 (2.2–3.8) (n=12)	8.6±4.3 (4.4–17)(n=8)	63.7±2.4 (61–67) (n=6)	0.02±0.004 (0.009–0.04) (n=382)	0.02 ± 0.006 (0.002–0.07) (n=376)	2.8±0.4 (2–3) (n=233)	–	–	911.6±77.6 (861.3–1119.7) (n=12)	493.7±63.4 (392.3–587.7)	1550.5±497.8 (1134.9–2275.6) (n=12)
Canto de anúncio	*Rhinella inopina*[7]	3.40 ± 0.87 (1.31–4.8) (n=39)	6.12±3.76 (0.21–15.88) (n=34).	62±18 (23–89)	0.019 ± 0.003 (0.013–0.025) (n=1562)	0.036 ± 0.003 (0.033–0.043) (n=1482)	1–3		–	1070±30 (940–1100) (n=38)	–	–
	Rhinella ornata (como *Rhinella abei*)[8]	6.839±2.109 (2.621–12.71) (n= 30)	28.712±30.669 (2.569–134.633) (n=30)	85±26 (32–152) (n=30)	0.038 ± 0.003 (0.007–0.055) (n= 300)	0.038 ± 0.004 (0.008–0.073) (n= 300)	1–6			1418.8±94.5 (1312.5–1500) (n=30)	1131 ± 34.2 (1125–1313) (n= 30)	1606 ± 94.5 (1500–1688) (n=30)

escassos trabalhos que abordem tal correlação (ver Leary, 2001 e Castellano *et al.*, 2002).

Na vocalização de anúncio, o canto se assemelha em parâmetros específicos com *Rhinella casconi* (Roberto *et al.*, 2014) e *R. inopina* (Andrade *et al.*, 2015) (Tabela 2). Em *R. casconi*, além do número de pulsos por nota (2.8 ± 0.4 pulsos [2–3 pulsos; n = 233]), a semelhança se deu principalmente para dados temporais, como o intervalo de canto (8.6 ± 4.3 s [4.4–17 s; n = 8]) e duração e intervalo de notas (0.02 ± 0.004 s [0.009–0.04 s; n = 382] e 0.02 ± 0.006 s [0.002–0.07 s; n = 376], respectivamente). Já *R. inopina*, além de dados dinâmicos, como a duração da chamada (3.40 ± 0.87 s [1.31–4.80 s; n = 39]) e duração de notas (0.019 ± 0.003 s [0.013–0.025 s; n = 1562]), também se aproximou com a média de sua frequência dominante (1070 ± 30 Hz [940–1100 Hz; n = 38]). Entre todos táxons observados, o mais divergente da espécie em estudo foi *R. ornata* (nomeado como *R. abei* em Batista *et al.* 2016), que superou outros congêneres do grupo em todos parâmetros acústicos, com exceção da frequência máxima observada em espécimes de "*R. pombali*" (4692 ± 622 Hz [4218–5943 Hz; n = 7]) (Lourenço *et al.*, 2010; Pereyra *et al.*, 2021).

O registro de canto de anúncio de *R. crucifer* foi realizado por Oliveira *et al* (2014), em uma população presente no município de Igrapiúna, estado da Bahia (Brasil). Semelhante aos nossos registros, os machos apresentaram cantos sem harmônicos, multipulsionados, com duração de chamadas (3.54 ± 0.85 s [1.47–5.53 s; n = 33]) e duração de notas (notas centrais de 0.023 ± 0.013 s [0.011–0.15 s; n = 104]) muito próximas as nossas. O canto analisado neste estudo apresentou maiores médias de número de notas (72 ± 16 notas [31–104 notas; n= 33]) e frequência dominante (855 ± 91 Hz [750–937.5 Hz; n = 32).

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Nota

Cuad. herpetol. 36 (1): 85-88 (*2022*)

Breeding activity of *Dendropsophus counani* (Anura: Hylidae) in eastern Brazilian Amazonia

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ABSTRACT
The current knowledge on the natural history are still scarce for most species of Amazonian anurans. Among these, the hylid *Dendropsophus counani* has incipient data on its breeding biology. Herein, we studied the breeding biology of *D. counani* in the breeding seasons at Cancão Municipal Natural Park, municipality of Serra do Navio, Amapá state. Males of *D. counani* often start calling in the late afternoon (17:45 pm) and continue at night. All clutches had been deposited on tree leaves (n = 8) and branches (n = 9) above the water, and after a few days the eggs hatching into exotrophic tadpoles that dropped from the gelatinous clutch in lentic waters. The number of eggs per egg clutch ranged from 40 to 153 eggs. Our study adds necessary data about the natural history of this species, in addition to increasing the knowledge of the breeding biology of *Dendropsophus* species.

Key Words: Clutches; eggs; reproductive mode.

In the genus *Dendropsophus* Fitzinger, 1843, the reproductive mode with aquatic eggs and tadpoles is present in approximately 80% of the species (Touchon and Warkentin, 2008). Species with terrestrial eggs and others that exhibit reproductive plasticity (i.g., aquatic and terrestrial spawns) have also been registered for some species (Hartmann *et al*., 2010; Mageski *et al*., 2014; Silva *et al*., 2019; Orrico *et al*., 2021). However, the current knowledge on the natural history of anurans from the eastern Amazon are still scarce. As with many other species of *Dendropsophus* in Amazonia, there is little information on the natural history of *D. counani* Fouquet, Orrico, Ernst, Blanc, Martinez, Vacher, Rodrigues, Ouboter, Jairam & Ron, 2015. Indeed, some information has already been published on the vocalization, morphology of adults and juveniles, tadpoles and spawning for this species, however, its breeding activity remains incipient (Fouquet *et al*., 2015). Thus, the aim of this study was to provide data on breeding biology of *D. counani* from a primary forest in the state of Amapá, Eastern Amazon, Brazil.

Field observations were conducted in breeding sites in the temporary ponds in River Amapari trail (0°54'02.9"N, 52°00'48.2"W, 146 m a.s.l.) in the Cancão Municipal Natural Park, during two field trips to the study area in 2018 (19–21 January; 10–12 February). This Protected area is located about 2.5 km NW of the village of Pedra Preta, municipality of Serra do Navio, Amapá state, Brazil.

Forty-nine adult specimens were collected (37 males and 12 females), measured the snout-vent-length (SVL) using a digital caliper (0.5 mm precision, Mitutoyo) and weighed using a digital balance (0.1 g precision, Pesola). We counted the total number of males and females in the temporary pond

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and recorded the variation in calling site height, amplexus, clutches and the reproductive mode. All specimens were deposited in the Herpetological Collection of Universidade Federal do Amapá, municipality of Macapá, Amapá, Brazil (Voucher specimens 1603–1652).

Eggs were obtained from clutches deposited by pairs, placed in plastic bags and preserved in 5% formalin after spawning. The diameter of 10 eggs per clutch was measured using the stereomicroscope, with a micrometric ocular (Hartmann *et al.*, 2010). Spearman correlation analyses were performed to test the relationship between size of female (snout-vent length SVL) and size of clutch (number of eggs counted in each female), and size of clutch and egg diameter. The operational sex ratio (OSR) was obtained by dividing the number of males by the number of females present at the breeding sites (Emlen and Oring, 1977). The Chi-squared was used to evaluate possible differences in sex ratio (Zar, 1999).

Males of *D. counani* often start calling in the late afternoon (ca. 17:45 pm) and continue at night. We recorded males of *D. counani* on tree branches and leaves after heavy rains at 0.60 and 1.70 m above the ground using overhanging vegetation as calling sites. We found 17 clutches of *D. counani* in natural environment. All clutches were deposited on tree leaves (n = 8) and branches (n = 9) above the water (Fig. 1), and after a few days the eggs hatching into exotrophic tadpoles that dropped from the gelatinous clutch in lentic waters (reproductive mode 24; *sensu* Haddad and Prado, 2005). We also observed other anuran species (e.g., *D. leucophyllatus* and *Phyllomedusa bicolor*) using leaves as perches to vocalize.

The number of eggs per egg clutch ranged from 40 to 153 eggs (91.1 ± 42.9 eggs, n = 6 clutches), and egg diameter ranged from 1.42–1.51 mm (1.47 ± 0.04 mm, n = 60 eggs from 6 clutches). We did not find significant correlations between female body size (SVL) and clutch size (rs = - 0.3714, $p = 0.47$), body mass and clutch size (rs = - 0.6667, $p = 0.15$), and body mass and egg diameter (rs = - 0.1029, $p = 0.85$) as well as egg diameter and SVL (rs = 0.1449, $p = 0.49$) (Fig. 2). The operational sex ratio was 4.2:1 males/female (average = 3.2 ± 1.4) and a Chi-squared value greater than expected ($X^2 = 62.77$, $p = 0.07$).

The clutches of *D. counani* follow the same pattern found in other species of the genus. *Dendropsophus berthalutzae* (Hartmann *et al.*, 2010), *D. ebraccatus* (Touchon and Warkentin, 2008), *D. haddadi* (Toledo *et al.*, 2012; Mageski *et al.*, 2014; Silva *et al.*, 2019), *D. leucophyllatus* (Crump, 1974), and *D. microcephalus* (Duellmann, 1970) present the same reproductive mode (terrestrial eggs on leaves above lentic environments and exotrophic tadpoles that complete development in the water).

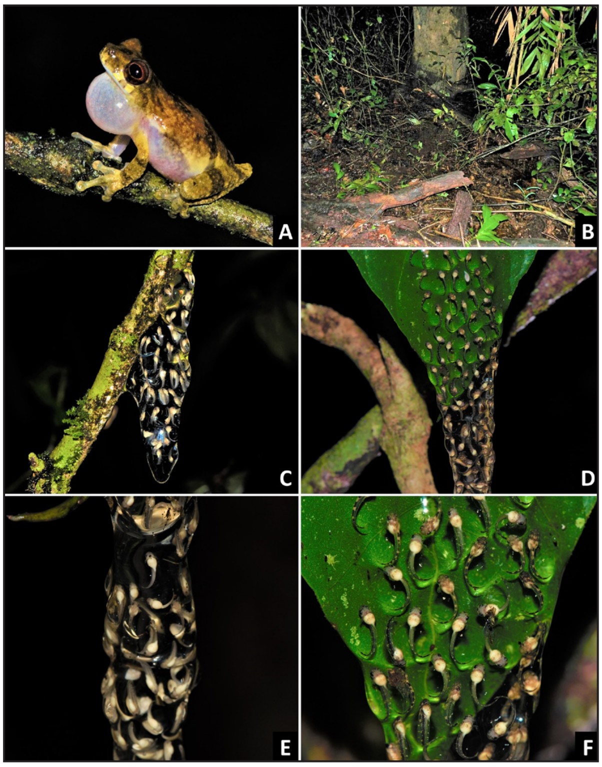

Figure 1. *Dendropsophus counani*: (A) calling male; (B) reproductive habitat located in municipality of Serra do Navio, Amapá state, Brazil, where frogs were sampled; (C), (D), (E) and (F) mature clutch on the leaves and tree branches hanging above water.

According to Fouquet *et al.* (2015), *Dendropsophus counani* is considered as an explosive breeding (*sensu* Wells, 1977). The males calling usually after heavy rains and male density at some choruses was high, resulting in some males calling a few centimeters away from each other. Amplexus is axillary and egg deposition occurred at the same tree branches and leaves where choruses were calling. Amplectant pairs and individuals moving actively out of their reproductive period were also observed during nighttime.

The number of eggs found in our study (153 eggs; 40–153) is higher than that found by Fouquet *et al.* (2015), (50–79 eggs). Clutch size in *D. counani* was not positively correlated with body size (SVL) and body mass of females. Crump (1974) found an inverse relationship between the reproductive investment and body size. This negative trend indi-

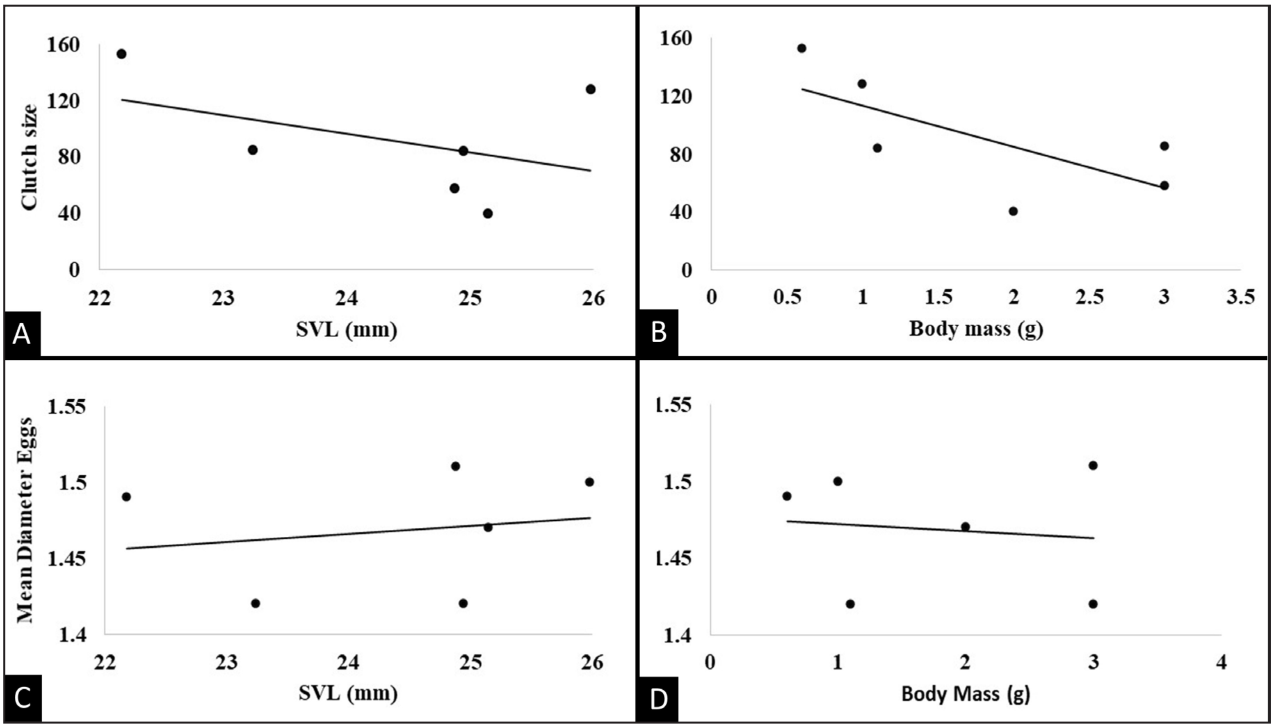

Figure 2. Relationship between (A) clutch size and SVL; (B) clutch size and body mass; (C) mean diameter eggs and SVL, and (D) mean diameter eggs and body mass in *Dendropsophus counani* females from a primary forest in eastern Amazon.

cates that as body size increases or less is invested in gonads. The females, although present in all samples, were often in fewer numbers. During the shorter reproduction period explosive breeding, the occurrence of reproductively active males is concentrated at a breeding site for only a few days. This could lead to relatively high daily proportions of males and a decline in operational sex ratio (A. Fouquet pers. comm.)

Information about species' ecology and behavior provides elements to take conservation and management decisions regarding an endangered or poorly known species (Sanabria *et al*., 2005; Loyola *et al*., 2008). In this way, our study adds necessary data on the natural history of a species that is still poorly known in the Amazon region. We expect that our data can improve knowledge about reproductive biology in *Dendropsophus* species.

Acknowledgments

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Nota

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Tail-break frequency inferences on *Lygodactylus klugei* (Smith, Martin & Swain, 1977) (Squamata: Gekkonidae) in northeastern Brazil

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ABSTRACT
We investigated the sexual, interpopulational, and microhabitat use effects on the autotomy frequencies in the Kluge's Dwarf Gecko *Lygodactylus klugei* in 12 populations inserted in the Caatinga ecoregion, in northeast Brazil. We observed the frequency of autotomized tails in each population by analyzing sexual differences and the characteristics of the trees used, such as the number of branches, presence of thorns, and trunk texture (smooth or rough). The autotomy frequencies ranged from 28.5% to 75% among these populations. We did not find differences in population, sex or on the presence of thorns and number of branches available in the trees used on autotomy rates. On the other hand, lizards found on smooth trunks had a higher rate of autotomized tails compared to those found on rough trunks. We suggest that the presence of crevices in trees with rough trunks is an important factor responsible for protecting lizards against predator attacks.

Key Words: Antipredator behavior; Caatinga; Lizards; Predation; Tail autotomy.

Caudal autotomy in lizards is very common, occurring in many families (Gordeev *et al.*, 2020), and it serves to benefit the organism in two distinct ways: i) the initial detachment of the tail from the body enables the lizard to escape away from a predator's grasp even after having been captured (Clause and Capaldi, 2006), and ii) once severed from the rest of the body, the shed tail is frequently capable of vigorous, violent movements on its own, distracting the predator from any further attack and providing the animal with time to flee to safety (Higham *et al.*, 2013). However, the use of this strategy can cause social, energetic, locomotor, as well as in the life history losses of the species (see review in Bateman and Fleming, 2009).

In many studies, tail-break frequency is used as indirect measure of predatory pressure, as the presence of autotomized tails may be the result of unsuccessful predation attempts (Bateman and Fleming, 2011; Passos *et al.*, 2013; Lin *et al.*, 2017). Other interpretations also refer the frequency of autotomies to intraspecific competition, in cases of lizards competing for territory or for reproduction (Donihue *et al.*, 2016; Itescu *et al.*, 2017; BeVier *et al.*, 2021; for more interpretations, see Bateman and Fleming, 2009).

Among the causes known for affecting caudal autotomy, there are factors such as: temperature, sex, age, and body shape (Bateman and Fleming, 2009; Fleming *et al.*, 2013). In addition, research has also shown that the autotomy frequencies can also vary in response to habitat (Duckett and Stow, 2011; Sousa *et al.*, 2016). Therefore, investigating differences in the presence of lizards with autotomized

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tails between different conditions expressed by the microhabitat can provide information on how these locations affect the risk of predation (Smith and Ballinger, 2001). That being said, this study aimed to investigate the populational, sexual, and mainly effects on the microhabitat use on the frequency of tail break of the Kluge's Dwarf Gecko *Lygodactylus klugei* (Smith, Martin & Swain, 1977), considering this parameter as indirect measure of susceptibility to predation.

Data collection was conducted during 13 months (from July 2018 to July 2019) in 12 municipalities, along five states in northeastern Brazil: Aiuaba (6°36'03"S, 40°07'24"W), Crateús (5°08'14"S, 40°51'58"W), Pentecoste (3°49'06"S, 39°20'20"W), Potiretama (5°45'35"S, 38°08 '22"W) and Quixadá (5°07'49"S, 39°03'59"W), in the state of Ceará; Caetés (8°46'32"S, 36°43'06"W), Carnaubeira da Penha (Type locality, 8°30'09"S, 38°39'42"W) and Petrolina (9°15'15"S, 40°23'57"W), in the state of Pernambuco; João Câmara (5°33'37"S, 35°54'12"W) and Mossoró (5°11'48"S, 37°20'02"W), in the state of Rio Grande do Norte; Patos (7°03'35"S, 37°16'30"W) in the state of Paraíba; and Coronel José Dias (8°50'34"S, 42°33'36"W) in the state of Piauí. We performed active searches during daytime (0700 – 1800) collecting about 20 individuals per locality. After the capture, we made notes on the characteristics of the perches used, such as: trunk texture (smooth or rough, see Fig. 1), presence of thorns, and the number of branches available at chest level (approximately 1.50 m). During the collection processing, we observed the presence of autotomized tails. After taking notes, the lizards were anesthetized with the use of 2% lidocaine gel orally, then sacrificed with an overdose of 2% liquid lidocaine via intracoelomic (following the Conselho Federal de Biologia, Portaria 148/2012), and housed in the Coleção Herpetológica da Universidade Federal of Ceará – CHUFC (Appendix). We used chi-square goodness-of-fit test to verify whether the proportion of captured males and females deviated from the expected 1:1 ratio (Chapple and Swain, 2004). We investigated possible interpopulational, sexual and perch differences in the characteristics of the perch used (trunk texture, presence of thorns, and number of branches available) in the frequencies of intact and autotomized tails using G-test (Sokal and Rohlf, 1995). We conducted all tests using the R v.4.0.4 software (R Development Core Team, 2021) with significance levels of 0.05.

In total, we captured 249 individuals of *L. kluguei* among which 134 males, 105 females, and 10 juveniles. One male who performed multiple autotomies during the capture was excluded from the analysis, as it was not possible to determine previous autotomies. Eight individuals found in non-arboreal habitats were excluded from the analyzes that addressed perch characteristics. The proportion of males (133) and females (105) did not deviate from the expected pattern of 1:1 (X-squared = 3.2941, df = 1, P = 0.06). The percentage of individuals with autotomized tails among the populations ranged from 28.5% to 75% (Table 1). There were no differences in the autotomiy frequencies between locations (G-test = 13,186, df = 11, P = 0.281). Considering all records, males (54.13%; n = 133) and females (46.66%; n = 105) did not differ in the presence of autotomized tails (G-test = 1.3106, df = 1, P = 0.252; Fig. 2A). There were also no differences between the classes of branches available at chest level (G-test = 2.3507, df = 3, P = 0.502; Fig. 2B), or in lizards that used thorn trees (G-test = 3.0557, df = 1, P = 0.08; Fig. 2C). However, we found differences for the trunk texture used (G-test = 4.4036, df = 1, P = 0.03; Fig. 2D), with individuals found in smooth trunks (55.71%; n = 140) presenting higher frequency of autotomized tails in comparison to individuals found on rough trunks (42%; n = 100).

Previous studies with *L. klugei* performed in Exú, Pernambuco, Vitt and Ballinger (1982) found a lower autotomy frequencies (38.5%) than those recorded in most of the localities investigated here (Table 1). Generally, geckos (Gekkota) have relatively high autotomy rates, above 50% (Vitt, 1983; Daniels, 1985; Vitt and Zani, 1997; Vitt *et al*., 2007; Duckett and Stow, 2011; Recoder *et al*., 2012; Itescu *et al*., 2017), ranging from 40.9 to 59% for the African congener, *L. capensis* (Pianka and Huey, 1978; Medger *et al*., 2008; Fleming and Bateman, 2012). This pattern remained similar for most of our observations. Although there are reports of intraspecific clashes for the species (Costa, 2014) we consider it unlikely that these encounters are violent enough to result in tail loss by individuals. Except for a single report of predation by *Felis catus* (Costa, 2014), nothing is known about natural predation on *L. klugei*. However, due to their small size, these lizards can have a great diversity of potential predators, such as: arthropods, other lizards, snakes, birds and small mammals, which can contribute to high autotomy rates (Sousa *et al*., 2016; Savvides *et*

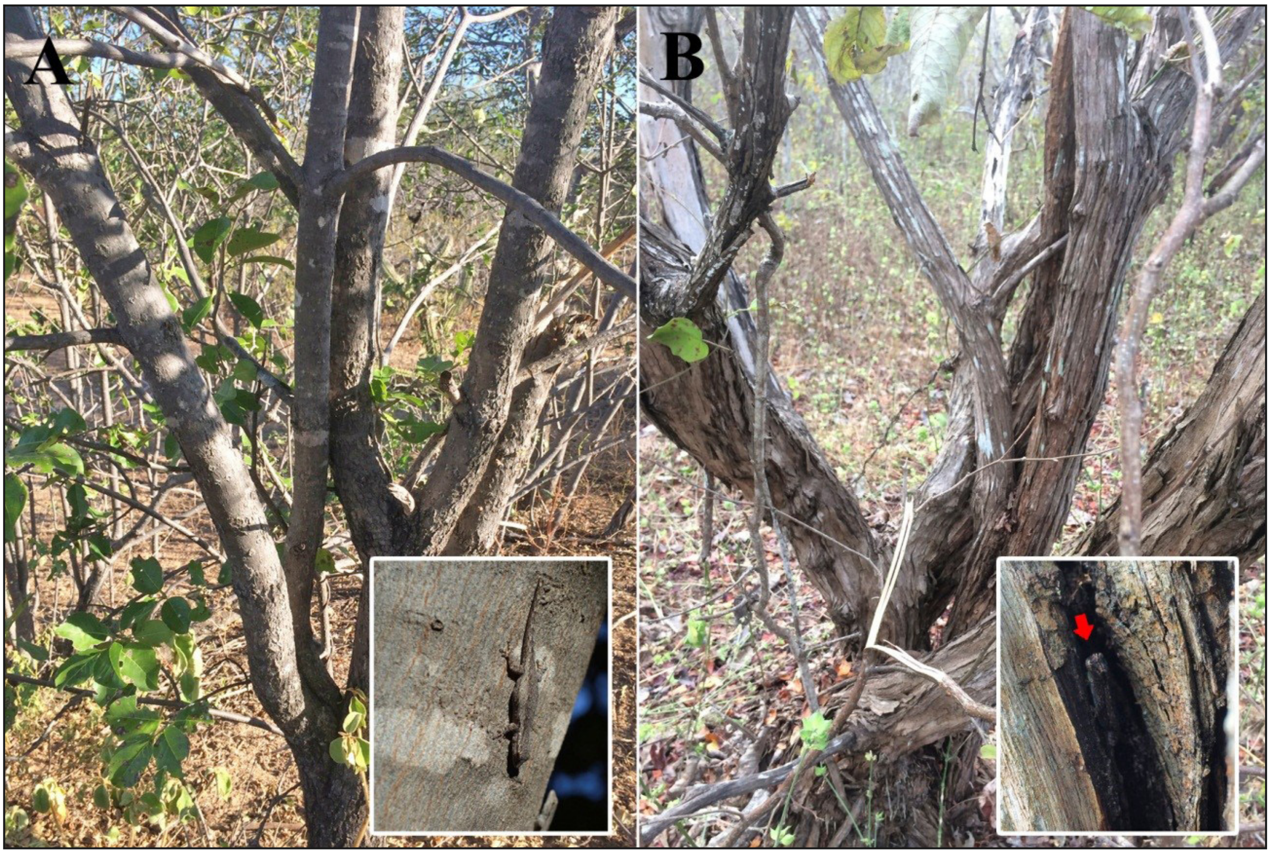

Figure 1. Specimens of trees with smooth (A) and rough (B) trunks with details for the use of the respective trunk types by *L. klugei*.

al., 2018). Although the use of this strategy can cause locomotor impairments (Fleming *et al.*, 2009), the associated costs seem to be minimized by rapid limb regeneration in the species (Vitt and Ballinger, 1982).

Vegetation cover and habitat complexity are some of the known factors that can influence the autotomy frequencies among different populations, with individuals living in more open environments which make them more susceptible to being preyed upon (Tanner and Perry, 2007; Duckett and Stow, 2011; Galdino *et al.*, 2017). Although our surveys were conducted in places that have different phytophysiognomies, including places inserted in urban environments (municipality of Patos), this difference was not significantly evidenced in our results; however, a large amplitude in the frequencies observed among populations (28.5% to 75%) may be an indication for further investigation.

Since they have different social roles, male and female lizards may show differences in the patterns in which they perform autotomy (Itescu *et al.*, 2017). However, most studies investigating sexual bias in tail loss not found these differences (Nunes *et al.*, 2012; Recoder *et al.*, 2012; Passos *et al.*, 2013; Sousa *et al.*, 2016; Pafilis *et al.*, 2017; and others). The absence of these differences in *L. klugei* may represent behavioral similarities, suggesting that both sexes may be similarly exposed to predatory pressure (Passos *et al.*, 2013). Our results are in line with those obtained by Vitt and Ballinger (1982), where sexual differences were observed with female bias, which was attributed to possible behavioral differences in individuals from that population.

The antipredatory behavior exhibited by lizards can vary in response to the physical environment in which they are inserted (Grolle *et al.*, 2014; Salido and Vicente, 2019). Differences in autotomy frequencies between trunk texture observed in our results may reflect the cryptic quality provided by these sites, with lizards being more or less exposed to visually oriented predators (Cuadrado *et al.*, 2001; Bateman and Fleming, 2011; Galdino *et al.*, 2017). Furthermore, even thoughtrees with rough trunks may have uneven surfaces, which can be detrimental to the escape velocity (Vanhooydonck *et al.*, 2005), proximity to places that function as shelters, represented in this case by the crevices that this type of microhabitat provides, can be an advantage during

Table 1. Tail-break frequency by population of *Lygodactylus klugei* of the Caatinga ecoregion, in northeast Brazil.

Localities	% (n)
Aiuaba	50.0 (20)
Caetés	45.0 (20)
Carnaubeira da Penha	42.8 (21)
Coronel José Dias	60.0 (20)
Crateús	45.0 (20)
João Câmara	75.0 (20)
Mossoró	28.5 (21)
Patos	52.1 (23)
Pentecoste	57.14 (21)
Petrolina	47.6 (21)
Potiretama	52.38 (21)
Quixadá	35.0 (20)
All	49.19 (248)

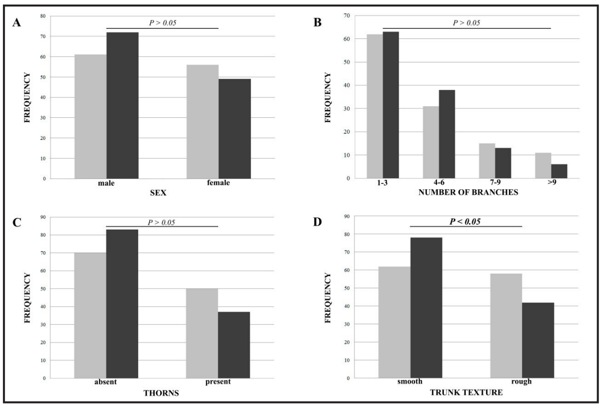

Figure 2. Frequency of lizards with tails intact (light gray bars) and autotomized (dark gray bars) in relation to sex (A), number of branches available at chest level (B), thorns (C) and trunk texture (D) with the respective significance values of the G-test.

the escape (Cooper and Whiting, 2007; Salido and Vicente, 2019). Even though *L. klugei* frequently performs short jumps between branches to get around (Vitt and Ballinger, 1982) and escape capture (pers. obs.), the number of branches available in the tree may not be a relevant element for escape. Likewise, the presence of thorns on the trees used may not provide mechanical protection against the lizard natural predators. Our studies provide new perspectives to be investigated in arboreal lizards, helping to understand how the microhabitat structure can affect the predation risk for these animals.

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Appendix. Material examined from Coleção Herpetológica da Universidade Federal of Ceará (CHUFC): Aiuaba (CHUFC L7420-7423, 7425-7432, 7434-7438 and 7440); Caetés (CHUFC L7508-7509 and 7511-7526); Carnaubeira da Penha (CHUFC L7527-7530, 7532-7534, 7536-7537 and 7539-7547); Coronel José Dias (CHUFC L7461-7471, 7473-7477 and 7479-7480); Crateús (CHUFC L7441-7450 and 7452-7460); João Câmara (CHUFC L7355-7357 and 7359-7373); Mossoró (CHUFC L7375-7377, 7379-7383, 7385-7388, 7391-7392 and 7394-7397); Patos (CHUFC L7312-7314, 7316, 7318-7323 and 7325-7335); Pentecoste (CHUFC L7251-7271); Petrolina (CHUFC L7483-7487, 7489-7495 and 7497-7500); Potiretama (CHUFC L7293-7296, 7298-7300, 7302-7304 and 7307-7312); and, Quixadá (CHUFC L7272-7287 and 7289-7290).

Nota

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First observations on courtship and nesting behavior of *Kinosternon vogti* (Testudines: Kinosternidae)

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ABSTRACT

Kinosternon vogti is one of the most narrowly distributed kinosternid turtles in Mexico; it is apparently restricted to small streams and ponds in the urban area of Puerto Vallarta, Jalisco and Bahía de Banderas, Nayarit. There is little information on the biology of this recently described and endangered species, particularly regarding reproductive biology. We report reproductive behaviors on two mating pairs of *K. vogti* in captivity. Males exhibited combat for copulating with female. During courtship, the male blocks the female movements, the female bites male´s rostrum scute, the male displays titillation posture, and finally the male moves to position himself above the female. The reproductive season begins with courtship in August-September and ends with egg-laying in November-December. Six and two eggs per clutch (non-viable eggs; average size, n = 5: 28.83 ± 1.19 x 15.95 ± 0.55 mm). Minimal reproductive size of females and males were CL (carapace length) = 88.37 mm and CL = 78.7 mm, respectively.

Key Words: Clutch Size; Laying Eggs Period; Mating; Mature Size; Vallarta Mud Turtle.

Kinosternon is one of the most diverse genera of turtles in Mexico (Legler and Vogt, 2013), and two new species were recently discovered. The Vallarta Mud Turtle (*Kinosternon vogti* López-Luna *et al.,* 2018) was described from the city of Puerto Vallarta, Jalisco, and the Cora Mud Turtle (*K. cora* Loc-Barragán *et al*., 2020) was described from the coastal plains in the state of Nayarit, both exhibit a restricted distribution (López-Luna *et al*., 2018; Loc-Barragán *et al*., 2020). The distribution of the Vallarta Mud Turtle is limited to the urban area of Puerto Vallarta and Bahía de Banderas, Nayarit, which are located on the western-central coast of Mexico, where its known to exist in small streams and ponds at low densities (López-Luna *et al*., 2018; Rosales-Martínez *et al*., 2021).

Kinosternon vogti was recently cataloged as Endangered by Mexican law (Secretaría de Medio Ambiente y Recursos Naturales, 2019) based on low population size, as only 30 individuals are known to science. Moreover, the 30 individuals that have been reported were observed within urban areas and human-made habitat (F.G.C-M. pers. obs.).

To date, the life history of this species is limited to anecdotal observations of diet (Ramírez-Ramírez *et al*., 2019), morphology (López-Luna *et al*., 2018) and reproduction (Montaño-Ruvalcaba *et al*., 2020). For example, Montaño-Ruvalcaba *et al.* (2020) provided the only information on reproduction of this species by dissecting an adult female in early November and observed four oviductal eggs (one crush egg, and the size of the oviductal eggs was

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26 x 15 mm, 28 x 15 mm, and 28 x 16 mm), and approximately 18 enlarged follicles.

Herein, we provide additional data on the reproductive biology of *K. vogti* based on a video of 97 s, recorded on 23 October 2017 at the Laboratory of Biodiversity and Ecosystem Services in the Centro Universitario de la Costa of the Universidad de Guadalajara. From this video, four photos were extracted that exhibit the courtship and mating behaviors (Fig. 1). During these recording turtles were being held in a 40 l glass aquarium (water depth < 11 cm) that measured 26 cm wide x 50 cm long x 35 cm tall. The individuals in the video were the paratypes of the original description of *K. vogti* (López-Luna *et al.*, 2018): female #1 Carapace length (CL) = 88.37 mm, male #1 CL = 80.0, and male #2 CL = 83.5 mm.

In addition, we captured three individuals on a dirt road within Puerto Vallarta City; a female in October 2019 (female #2: CL = 95.18 mm), and one female (female #3: CL = 90.71 mm) and one male #3: (CL = 78.7) mm in August 2020. These individuals were placed in a terrarium that consisted of a dry area (43 cm wide x 30 cm long x10 cm tall), and a freshwater area (75.5 cm wide x 97.5 cm long x 10 cm tall). On 18 August 2020 courtship and mating behavior was observed in these two individuals and captured in a 96 s recording (https://www.youtube.com/watch?v=3CvuPf8pAzE; cellular phone camera iPhone 7). In both videos the observations were made underwater.

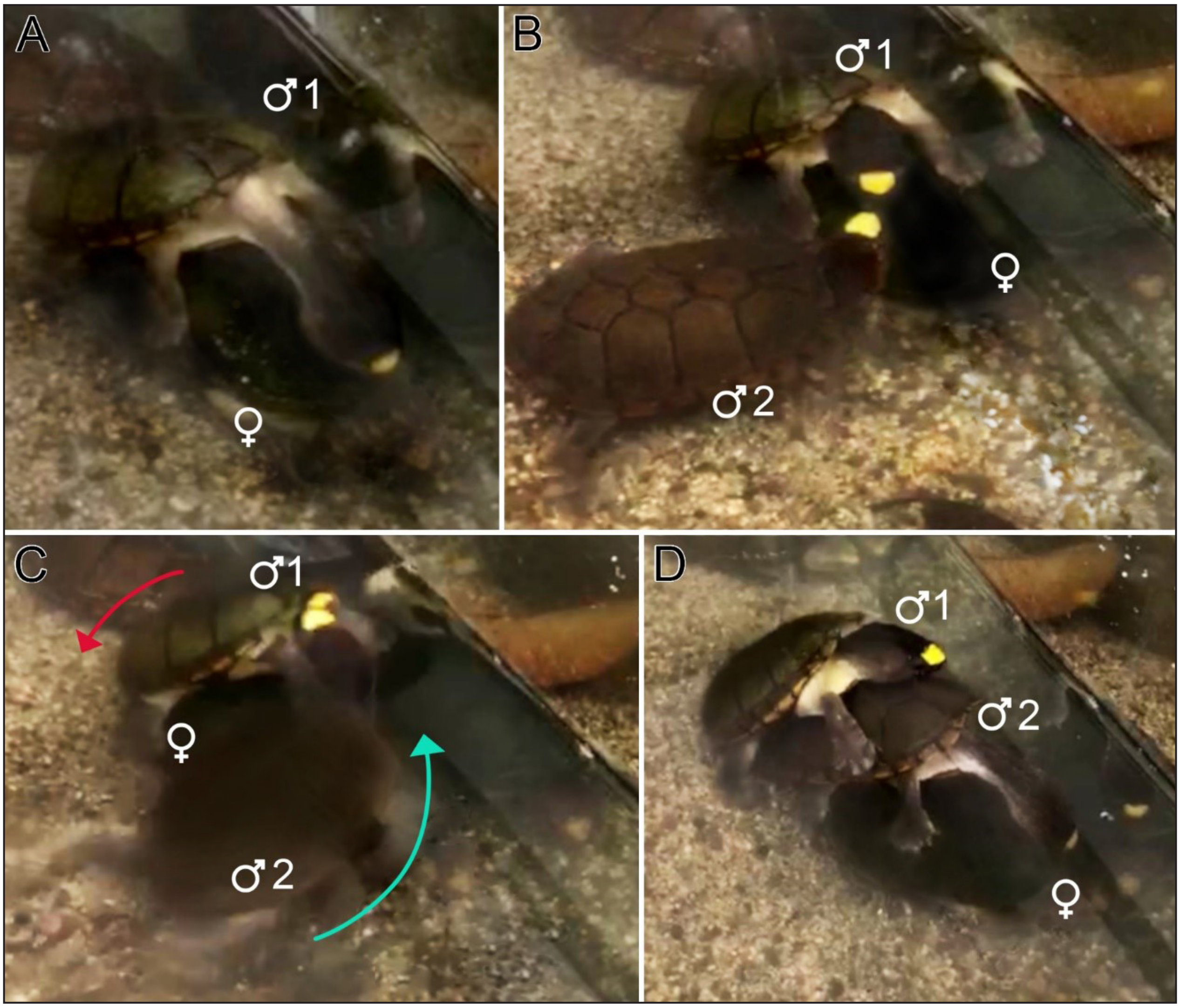

Figure 1. Reproductive combat behavior sequence between two captive *Kinosternon vogti* males. A) Male #1 in mount of female #1. B) Confrontation between males. C) Slam of male #2 (the direction is indicated by the green arrow) on male #1 to knock him off the mount of female #1 (the red arrow indicates the direction of his movement). D) Male #2 mounts female #1 and this in turn is mounted by male #1.

In the images of the first video, male #1 is seen mounting female #1 (Fig. 1A), and at the same time male #2 approaches male #1 and faces him head-to-head (Fig. 1 B). Male #2 then propels himself with his head and body, knocking male #1 out of the mount (Fig. 1C). Male #2 then assumed the mount of female # 1, then male #1 attempted to mount male # 2 (Fig. 1D).

Competition between males (male-male competition) to mate with females like we observed in *K. vogti* is an uncommon behavior in other kinosternid species (previously observed in *K. hirtipes*, a sister species of *K. vogti*), as they are primarily known to mate via forced insemination (Berry and Shine, 1980). Moreover, mating behavior between males, like we observed when male #1 mounted male #2, has been previously documented and it is speculated that this occurs due to a low capacity for sexual recognition (mainly by smell) or by intrasexual behavior with unknown function (Mahmoud, 1967).

In the second video, male #3 was observed mating with female #2 underwater, which is a common behavior in other kinosternid species (Legler and Vogt, 2013). In this video, male #3 extended his neck and placed himself in front of the female #2, impeding her ability to advance by making lateral movements left and right (Fig. 2A). Female #2 responded by biting the yellow rostral scute male # 3 a total of eleven times (Fig. 2B).

When facing female # 2, male # 3 walked forward on the bottom of the terrarium. Also, during this process, six movements that involved one or both front legs were observed with variable duration. Our first impression of these movements was that the male was making swimming type propulsion and changes of direction. However, on two occasions the male executed a pair of horizontal movements with his front legs in front of female # 2 (Fig. 2C). We interpret that this movement pattern of the forelegs was used to attract the attention of the female and facilitate mounting, as has also been suggested by Liu *et al.* (2013) in other freshwater turtles.

The swimming behavior observed in male #3, where the male executes a series of stereotyped movements with his forelimbs has been described as "titillation" and has been observed in other species of freshwater turtles (Liu *et al.*, 2013). This behavior is a type of visual signal, in which the male places their front legs parallel to the female's head in order to vibrate and drum the claws against the female's eyes and interocular region (Kramer and Burghadt, 2010; Liu *et al.*, 2013). In the case of *K. vogti*, male # 3 performed the "titillation" several centimeters away from female # 2 (Fig. 2C), which could be a variant of titillation or a different courtship behavior that has not been described.

The mating behavior occurred after a final stroke of male # 3 and a bite of female # 2 on the male's rostral yellow patch. Subsequently, female # 2 turned, positioning the posterior end of her carapace towards male # 2, and then the male approached the right posterior side of the female and proceeded to mount her (Fig. 2D). Once mounted, with his plastron in contact with the female's carapace, he tried to grasp her posterior margin of the carapace by grabbing her with his hind legs, and in order not to fall from the mount he placed both his front and hind legs on the lateral scutes, as well as the head and neck extended in contact on the middle and anterior part of the carapace (Fig. 2E). This postural pattern of mounting falls within one of the two postures used by freshwater turtles during mounting and couple (Liu *et al.*, 2013). The couple was not consummated.

The courtship observed in *K. vogti* in the two videos from this study is consistent with behavior that has been observed in other *Kinosternon* species (Legler and Vogt, 2013). At least three behaviors have been described in *Kinosternon*: tactile (male approaching another turtle from behind to smell it and apparently determine its sex; which was not observed in this work), mounting and intromission, and biting and rubbing (Mahmoud, 1967). We did not observe the couple, but after 13 weeks, on 7 November 2020, female #2 laid six eggs, and on 3 December 2020 female #3 laid three eggs (for this last female, the courtship occurs on 16 September 2020, but not recorded in video). We don't have evidence to define the exact date of fertilization of both females # 2 and # 3, therefore it was not possible to establish egg retention period. In addition, no courtship or other spawning was observed in months before or after those documented in this note.

Egg laying by females took place in dug holes in the groundwood pulp substrate of the terrarium. No eggs exhibited band formation in the shell that would have indicated normal embryo development (Ewert, 1985), thus the incubation period was unknown. We did not control the incubation temperature and humidity, which could have affected normal embryo development (Packard *et al.*, 1987). From female #2 and female #3, two and three eggs (the remaining

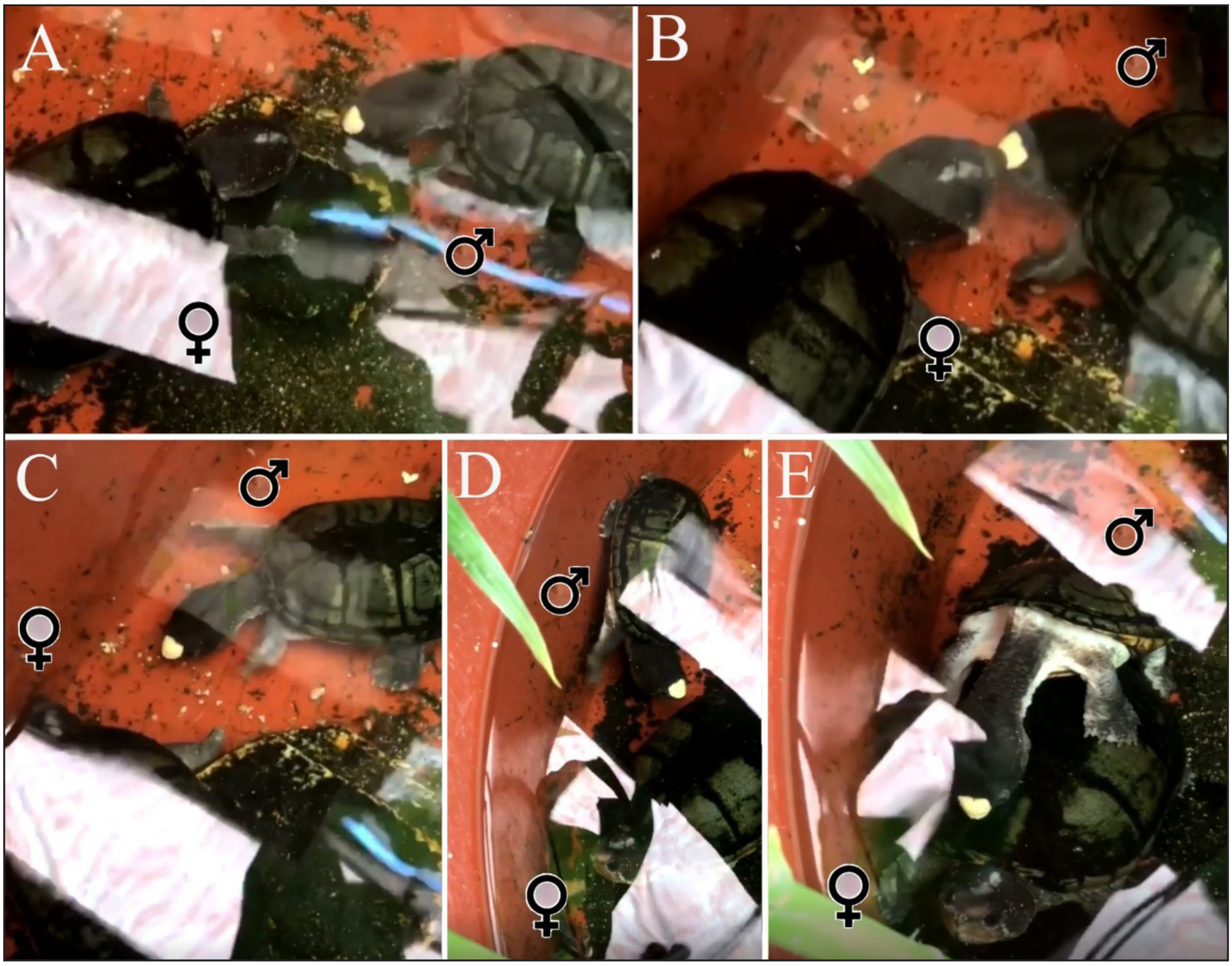

Figure 2. *Kinosternon vogti* female #2 and male #3, courtship and mount. A) Male block the advancement of the female. B) Female bites the male's rostral scale. C) Male in titillation posture. D) Approximation of the male to the back of the female. E) Male in mount on the female. Images from video available at https://www.youtube.com/watch?v=3CvuPf8pAzE. ♀ = female #2; ♂ = male #3.

four eggs were broken) were measured respectively. On their major and minor axes eggs measured: 26.88 x 15.17 mm, and 27.23 x 15.30 mm (Fig. 3); 28.49 x 16.14 mm, 29.38 x 16.71 mm, and 29.70 x15.88 mm. The average size for the five eggs was 28.83 ± 1.19 x 15.95 ± 0.55 mm.

Based on the observations presented in this work, female #1 (CL = 88.37 mm) and male #3 (CL = 78.7 mm) represent the lowest record of sexual maturity size for each sex in this species. The reproductive body-size, clutch size, and the egg traits are within the ranges observed for other species of the genus *Kinosternon* (Legler and Vogt, 2013) and even for this same species (similar clutch size and egg straits observed in Montaño-Ruvalcaba *et al.*, 2020). *Kinosternon acutum* is similar to *K. vogti* in that it has a small body size (CL = 116 mm), and the minimum CL of sexual maturity females is between 77 mm and 97 mm, and as small as 74 mm in males (Legler and Vogt, 2013). Clutch size (one to three) and egg size (33 x 17 mm) in *K. acutum* are also similar to *K. vogti*, but *K. acutum* are known to lay two to four clutches per year (Legler and Vog,t 2013).

Observations on the reproductive biology of *K. vogti* in captivity is an important first step to identify the reproductive season for this species. Based on our observations and the anecdotal observations published previously, the reproductive season in *K. vogti* seems to be from mid-August to early December (end of summer and beginning of winter). Thus, with seasonal reproductive activity, *K. vogti* can make use of trophic and spatial resources for courtship provided by the streams and temporary ponds it inhabits, formed during the summer rains (García-Oliva *et al.*, 2002), as do other species of the genus in Mexico (Iverson, 1996).

Figure 3. *Kinosternon vogti* female #2 with two of its unviable eggs laid in captivity.

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Nota

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Defensive behaviors of *Leptodactylus rhodomystax* (Anura: Leptodactylidae) from northern Brazil

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ABSTRACT
The study of defensive behaviors in anurans can lead to new hypotheses for a better understanding of prey-predator interactions. Herein, based on field observations, we described for the first time defensive behaviors of *Leptodactylus rhodomystax*. The studied individual displayed stretching limbs posture and evidenced hidden aposematism. Hidden aposematism was occasionally described to occur with stretching limbs posture. Our observations suggest that synergistic defense mechanisms in anurans may be more common than currently known.

Key Words: Amphibians; Defense; Predators; Leaf-litter.

Anurans are a common prey for several vertebrate and invertebrate taxa (Duellman and Trueb 1994; Rubbo *et al.*, 2003). Predator-prey interactions have been important in the evolution of many anti-predator mechanisms in these animals, such as toxic and distasteful skin secretions, cryptic and aposematic colouration, and a variety of postures (Dreher *et al.*, 2015; Rebouças *et al.*, 2019; Ferreira *et al.*, 2019). These anti-predator mechanisms act in different life history stages and phases of predation, avoiding or interrupting a predation attempt. On the other hand, predators may evolve strategies to overcome some anuran defences (Brodie and Brodie 1999; Greenbaum, 2004).

Recently, the anti-predatory mechanisms of anurans were arranged in a classification system (Ferreira *et al.*, 2019), showing geographic, taxonomic, and evolutionary patterns. Some of these behaviors have been recognized in previous studies as being effective in reducing predator attacks (Johnson and Brodie 1975; Dreher *et al.*, 2015; Gally *et al.*, 2014). Among them, death feigning and escape behaviors are the most commonly reported (Toledo *et al.*, 2010; 2011). However, there are some defensive postures in anurans that are still poorly understood in terms of their influence on predator-prey interactions. One of these is the stretching limbs posture, which is characterized by full or partial extension of the fore or hind limbs (Ferreira *et al.*, 2019). Several authors have suggested that this behavior is displayed only by forest-floor species in order to mimic fallen leaves, and thus confuse visual predators (Sazima, 1978; Bertoluci *et al.*, 2007; Toledo *et al.*, 2011; Mângia and Santana 2013; Mira-Mendes *et al.*, 2016; Ferreira *et al.*, 2019). With the aim to increase the knowledge of anuran defensive mechanisms, we report here the occurrence of stretching limbs posture with hidden aposematism in *Leptodactylus rhodomystax* Boulenger, 1884.

The Leptodactylidae is one of the most widely distributed anuran families in the Americas, comprising species of large, medium, and small-sized frogs that occupy a wide range of environments, including lowland dense rainforests and open habitats (de Sá *et al.*, 2014; Barrio-Amorós *et al.*, 2019). *Leptodactylus rhodomystax* is a nocturnal terrestrial

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frog of moderate size (SVL 58.5–91.4 mm in adult females and 59.0–89.6 mm in adult males), and a member of the *L. pentadactylus* group; it dwells in Amazonian Bolivia, Brazil, Colombia, Ecuador, French Guiana, Peru, and Suriname (de Sá *et al.*, 2014; Barrio-Amorós *et al.*, 2019).

On 8 July 2019, during faunal inventories and monitoring at Reserva de Desenvolvimento Sustentável do Rio Iratapuru (0.5627°S, 52.5775°W; datum: WGS84; 66 m a.s.l), located in the municipality of Laranjal do Jari, state of Amapá, Brazil, one of us (VAMBF) observed a male of *L. rhodomystax* on the leaf-litter, at about 19:30 h, accommodated it in a plastic bag, and transported it to the nearby laboratory to obtain measurements and photographs. The specimen could be confidently identified by its morphological traits, as described by de Sá *et al.* (2014). During the photographic session, we observed that the individual (male, SVL = 62.5 mm) displayed extension of the hindlimbs when touched, while exposing a contrasting conspicuously colored area of the thighs and post-femoral regions (Fig. 1). It maintained this posture for about 5 min. After these observations, the individual was released at the original place of encounter. According to Ferreira *et al.* (2019), the behavior displayed by the individual in our study could be classified as stretching limbs posture with hidden aposematism. These authors indicate that the stretching limbs posture was recorded in 39 species belonging to seven different families. However, this is likely an underestimation. Our record corresponds to the second occurrence of this behavior in the genus *Leptodactylus*, and the first formal description of a defensive behavior in *L. rhodomystax*. We think that in *L. rhodomystax*, the stretching limbs posture may mimic fallen leaves or a dead frog, in addition to the synergism by hidden aposematism. This species inhabits both leaf-litter areas (e.g., in forest floor and swamps within tropical rainforest), but also non-forested habitats (de Sá *et al.*, 2014), in which mimicking a dead frog with scarce leaf-litter around would be alternatively advantageous. Hind limbs of dead frogs may become stiff for a time under rigor mortis similarly to the stretching limb posture (Borteiro *et al.*, 2018). Therefore, both defensive mechanisms would act as warning signals (Ferreira *et al.*, 2019). Hidden aposematism is common in some leptodactylids and phyllomedusids, and its exhibition is commonly related to the postures of rear elevation, unken reflex, death feigning, and escape behavior; usually, the hidden aposematism occurs at the axila, underside of the body, tongue, thighs, or post-femoral region (Ferreira *et al.*, 2019).

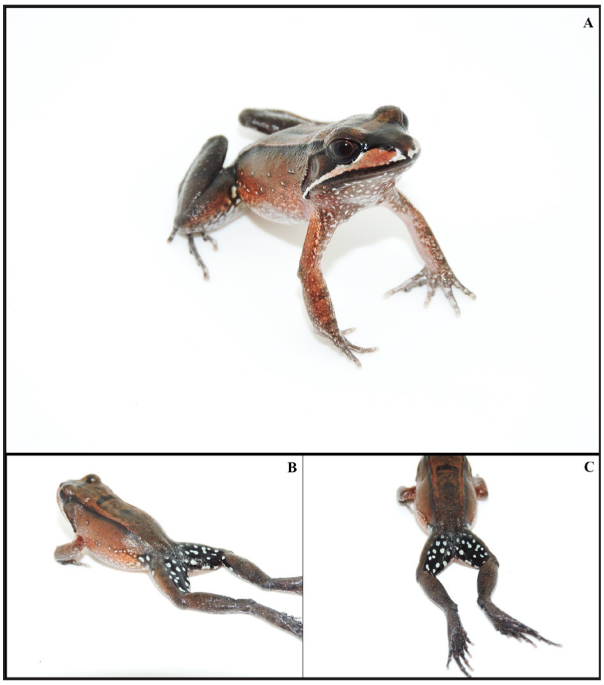

Figure 1. (A) Usual posture of *Leptodactylus rhodomystax*. (B) Postero-lateral and (C) posterior view of stretching limbs posture evidencing hidden aposematism.

As pointed by Sazima (1978), stretching limbs posture may have convergently evolved among unrelated leaf-litter anuran groups. However, this defensive posture can also occur in forest-dwelling and arboreal species, which are distributed in open and flooded areas with scarce leaf-litter (e.g., Mângia and Santana 2013, Rocha and López-Baucells, 2014; Figueiredo-de-Andrade and Silveira 2018; Ferrante *et al.*, 2020). In addition, this behavior has also been described in open grasslands (referred to as "death feigning", Kolenc *et al.*, 2009). As suggested by Mângia and Santana (2013), phylogenetic mapping of this behavior would help to understand its appearance in Neobatrachia during recent anuran diversification. In this sense we encourage field researchers to further record defensive behaviors in still poorly known anurans.

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Diet composition of two hylid species (*Dendropsophus haraldschultzi* and *D. minutus*) in anthropogenic environments in eastern Amazonia

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ABSTRACT
The diet composition of hylids ranges from generalist to specialist, depending on the relative proportions of different types of prey found in their gastrointestinal tracts and trophic niche. Here, we report the diet composition and compare the niche breadth of two syntopic hylid species in eastern Amazonia, *Dendropsophus haraldschultzi* and *D. minutus*, which use anthropogenic environments during the rainy season. We collected 32 individuals of *D. haraldschultzi* and 30 individuals of *D. minutus*. The most important preys found in the diet of *D. haraldschultzi* were Hemiptera (34.6%), whereas Lepidoptera larvae (63.8%) were predominant in the diet of *D. minutus*. Both *Dendropsophus* species had a similar niche breadth with generalist characteristics. The consumption of mobile and slow-moving prey, and hard-bodied and soft-bodied arthropods, indicated a combined use of both "sit-and-wait" and "active search" foraging strategies.

Key Words: Trophic ecology; generalist; anurans; natural history; urbanization.

Anurans are opportunistic and generalist predators, feeding on any potential prey available in the environment (Ceron *et al.,* 2018; Michelin *et al.,* 2020; Moroti *et al.,* 2021), and their diet composition is closely related to foraging strategies (Toft, 1980; 1981; Huey and Pianka, 1981; Taigen and Pough, 1983). Active foragers are effective in capturing small, slow-moving prey that sting or those generally considered distasteful to predators (e.g., ants and termites) and have specialized feeding habits (Toft, 1981). In contrast, sit-and-wait foragers are effective in capturing actively moving prey (e.g., coleopterans and orthopterans) and have generalized feeding habits (Toft, 1981). However, most species can also adapt their feeding strategy according to food availability (Menin *et al.,* 2005; Petrozzi *et al.,* 2021).

The diet composition of hylids ranges from generalist (Leivas *et al.,* 2018; Moser *et al.,* 2019; Silva *et al.,* 2021) to specialist (Parmelee, 1999; Castro *et al.,* 2016), depending on the relative proportions of different types of prey found in their gastrointestinal tracts and trophic niche (López *et al.,* 2009). Among the Neotropical hylids, *Dendropsophus haraldschultzi* (Bokermann 1962) and *D. minutus* (Peters, 1872) are two small-sized, nocturnal, arboreal species. *Dendropsophus haraldschultzi* can be found perched on leafy branches or aquatic vegetation in floating meadows associated with ponds and large (permanent) rivers that discharge into the Amazon River (Hödl, 1977; Böning *et al.,* 2017; Menin *et al.,* 2020). *Dendropsophus minutus* inhabits a variety of open and forest habitats and can be found in both natural and anthropogenic environments (Abegg *et al.,* 2014; Leivas *et al.,* 2018).

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The diet of *D. haraldschultzi* is unknown. The few aspects of its biology and natural history already studied include (i) a brief call description of a population from the Amazon basin (Hödl, 1977), (ii) a possible association with bromeliads and an Amazonian plant species in northern Brazil (Sanches *et al.,* 2019; Figueiredo *et al.,* 2021), and (iii) the tadpole description (Menin *et al.,* 2020). In contrast, previous studies on the diet composition of *D. minutus* were based on populations from northern (Van Sluys and Rocha, 1998), northeastern (Santos *et al.,* 2004), and southern (Leivas *et al.,* 2018) Brazil. Despite this, little is known about the diet of these two species in anthropogenic environments in Amazonia. Here, we report the diet composition and compare the niche breadth of these two hylid species during the rainy season.

We collected the two hylid species during the rainy season, from February to June 2019, using the nocturnal visual search method (Crump and Scott Jr., 1994). Both species were collected in Amapá state, eastern Amazonia, northern Brazil. *Dedropsophus haraldschultzi* was sampled in a seasonally flooded area (regionally referred to as the "ressaca" area) surrounding human settlements in the municipality of Santana (0.0365ºS, 51.1626ºW). *Dendropsophus minutus* was sampled in an abandoned temporary pool in the urban perimeter of the municipality of Serra do Navio (0.9066°N, 52.0073°W). Specimens were collected under ICMBio/RAN, Institutes of Ministry of Environment, Government of Brazil, permit number 48102-2. This permit was subject to the approval of all procedures for collecting and euthanizing organisms. Hylids were euthanized because this work was part of a research project on anuran-parasite networks, whose methodology requires animal euthanasia. Both species are classified as "Least Concern" according to the International Union for the Conservation of Nature (IUCN, 2022). All individuals are housed at Coleção Herpetológica of the Universidade Federal do Amapá, in Macapá, Brazil with the acronym CECC.

We euthanized the specimens with a topical anesthetic (lidocaine), fixed them in formaldehyde 10%, and preserved them in ethanol 70%. We dissected the preserved specimens to remove the gastrointestinal tract (stomach and intestine). For the diet analysis, we determined the prey items to the taxonomic level or Order by analyzing the material under a stereomicroscope, following the taxonomic key developed by Rafael *et al.* (2012). We measured the width (w) and length (l) of all prey items to estimate the ellipsoid volume per prey using Griffiths and Mylotte's (1987) formula: $V = (4\pi/3)(l/2)(w/2)^2$. To determine the importance of each prey item, we used the Pinkas *et al.* (1971) importance index through the occurrence percentage (F%), numeric percentage (N%), and volumetric percentage as follows: IRI = F% (N% + V%).

We calculated the amplitude of the trophic niche through the Levins' Trophic Niche Amplitude Index (B) (Krebs, 2004), defined by: $B = 1 / \Sigma pi^2$, in which p is the proportion of individuals of a given resource i (taxon) found in the diet. To facilitate comparisons between the two species, we calculated the standardized Levins' index (Bsta), which limits the index to a scale from 0 to 1 according to the following equation: Bsta = (B-1) / (n-1), where n is the number of resources (prey categories) recorded. Values near zero are assigned to a specialized diet, whereas those closer to 1 are to a generalist diet.

To estimate the richness of prey categories in the diet of each species, we made rarefaction curves based on samples using Estimates 9 (Colwell, 2013), with 1,000 random permutations with no reposition. This analysis treated stomachs as samples and prey types as richness categories (curves were made for each species).

We collected 62 individuals, 32 individuals of *D. haraldschultzi* and 30 of *D. minutus*. Only ten individuals of *D. haraldschultzi* (31.3%) and 14 of *D. minutus* (46.7%) analyzed had gastrointestinal contents. For *D. haraldschultzi*, four prey categories were identified: Coleoptera, Coleoptera larvae, Hemiptera, and Isoptera (Table 1). Hemipterans were the most important prey category (34.6%). *Dendropsophus minutus* exhibited a lower richness of prey categories: Araneae, Diptera, and Lepidoptera larvae (Table 1). Lepidoptera larvae were the most important prey category (63.8%). *Dendropsophus haraldschultzi* (Bsta = 0.82) and *D. minutus* (Bsta = 0.73) displayed a similar niche breadth.

The number of prey categories consumed by *D. haraldschultzi* and *D. minutus* was lower than that reported for congeners (*D. branneri*, Castro *et al.,* 2016; *D. counani*, Sanches *et al.,* 2021; *D. microcephalus*, Fonseca-Pérez *et al.,* 2017; *D. minutus*, Van Sluys and Rocha, 1998; Santos *et al.*, 2004; Leivas *et al.,* 2018; *D. nanus*, and *D. sanborni*, Menin *et al.,* 2005). The lower number of prey categories in the diet of *D. haraldschultzi* and *D. minutus* in anthropogenic areas are consistent with findings of

Table 1. Prey categories found in the gastrointestinal contents of *Dendropsophus haraldschultzi* and *D. minutus* in anthropogenic environments in eastern Amazonia. N = number of individuals, F = frequency of occurrence of prey categories, V = total volume (in mm3) occupied by prey categories, IRI = Index of Relative Importance, (%) = percentage values over the total number of prey items.

Prey categories	***Dendropsophus haraldschultzi* (N = 32)**				***Dendropsophus minutus* (N = 30)**			
	N (%)	F (%)	V (%)	IRI	N (%)	F (%)	V (%)	IRI
Araneae	-	-	-	-	5 (35.7)	4 (50.0)	23.1 (45.7)	43.8
Coleoptera	4 (18.2)	2 (20.0)	1.9 (13.4)	17.2	-	-	-	-
Coleoptera larvae	3 (13.6)	1 (10.0)	1.5 (10.3)	11.3	-	-	-	-
Diptera	-	-	-	-	2 (14.3)	2 (25.0)	5.2 (10.3)	16.5
Hemiptera	3 (13.6)	3 (30.0)	8.4 (60.0)	34.6	-	-	-	-
Isoptera	10 (45.5)	2 (20.0)	2.3 (16.3)	27.2	-	-	-	-
Lepidoptera larvae	-	-	-	-	7 (50.0)	2 (25.0)	58.8 (116.4)	63.8

previous studies (Menin *et al.,* 2015; Santana *et al.,* 2019; Sanches et al., 2019) and indicate that human alteration of natural areas likely reflects the diet composition of anurans.

Although *D. haraldschultzi* and *D. minutus* exhibited a similar trophic niche breadth with generalist characteristics, the prey rarefaction curve in relation to the number of stomachs analyzed reached the asymptote, indicating that the number of individuals sampled was sufficient to estimate the richness of prey items (Fig. 1).

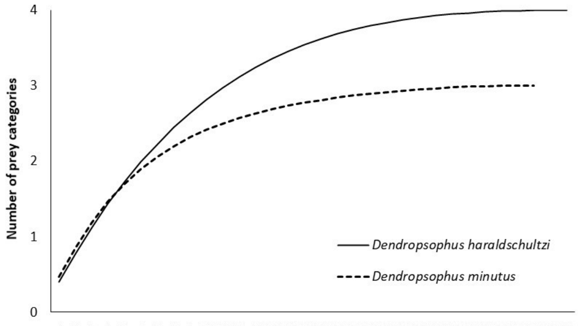

Figure 1. Accumulation curve showing the number of prey categories relative to the number of stomachs containing prey items analyzed in the diet of *Dendropsophus haraldschultzi* and *D. minutus* in anthropogenic environments in eastern Amazonia.

Both *Dendropsophus* species consumed ephemeral resources, such as larvae (coleopterans and lepidopterans) and termites, reinforcing the idea that anurans are opportunistic predators (Toft, 1981). Although the two species were sampled in anthropogenic environments, *D. haraldschultzi* had a higher numerical frequency of prey due to the greater availability of arthropods associated with aquatic macrophytes where this species was sampled (see Menin *et al.,* 2020). On the other hand, the low numerical frequency of prey found in *D. minutus* when compared to natural environments, such as the ombrophilous forest in Amazonia (N = 6 preys; Van Sluys and Rocha, 1998); semi-deciduous rainforest in northeastern Brazil (N = 6 prey; Santos *et al.,* 2004), and ombrophilous mixed forest in southern Brazil (N = 10 prey; Leivas *et al.,* 2018) should be related to the sampled environment, swimming temporary rain pool in an urban area.

The consumption of mobile (e.g., Araneae, Coleoptera, Diptera, and Hemiptera) and small and slow-moving prey (e.g., larvae and termites) and two types of prey in different proportions in their diet: hard-bodied (e.g., Coleoptera and Hemiptera) and soft-bodied arthropods (e.g., Araneae, larvae, Diptera, and Isoptera), indicated a combined use of both “sit-and-wait” and “active search” foraging strategies (Toft, 1980; Huey and Pianka, 1981).

The fact that the two studied hylid species were collected during the rainy season limited the interpretation of our results. Future research focusing on increased sampling during the rainy and dry seasons and assessing prey availability in non-urbanized environments will produce relevant information about the diet composition, foraging strategies, and how species act in trophic webs. Nevertheless, our findings provide a basic description of the diet of *D. haraldschultzi* and *D. minutus* in Amazonia, which can help us understand more aspects of the natural history of these anuran species in urban landscapes.

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Novedad zoogeográfica

Cuad. herpetol. 36 (1): 111-113 (*2022*)

Leiosaurus jaguaris Laspiur, Acosta & Abdala, 2007 (Squamata: Leiosauridae): Primer registro para el Parque Nacional Talampaya, provincia de La Rioja, Argentina

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Localidad.— Argentina, provincia de La Rioja, Departamentos de General Felipe Varela e Independencia, Parque Nacional Talampaya (29°47'38.5"S, 67°59'05.8"W). Fecha: 4 de marzo de 2020. Avistado y fotografiado por Lucía Martínez Retta, Victor M. Pardo y Ana C. Ochoa. Fotografías depositadas en la Colección Herpetológica de la Fundación Miguel Lillo (FML 30834).

Comentarios.— El género *Leiosaurus* se encuentra representado por cuatro especies que ocupan una variedad de ambientes en las regiones fitogeográficas de Monte, Chaco y Estepa patagónica. *L. jaguaris* Laspiur, Acosta & Abdala, 2007, presenta una distribución restringida, encontrándose únicamente en las provincias de La Rioja y San Juan, en zonas de gran altitud donde predomina la estepa con baja cobertura vegetal (Laspiur *et al.*, 2007). En la última evaluación del estado de conservación de las lagartijas de Argentina, *Leiosaurus jaguaris* fue categorizada como una especie no amenazada (Abdala *et al.*, 2012).

En el presente trabajo se da a conocer el hallazgo de *Leiosaurus jaguaris* dentro del Parque Nacional Talampaya (PNT), que conserva la ecorregión de Monte de Sierras y Bolsones, la cual se caracteriza por presentar una estepa arbustiva xerófita o halófita, con bosques marginales de algarrobos (*Prosopis* sp.). Tiene un clima desértico, caracterizado por variaciones extremas de temperatura, que oscilan entre -7°C y 40°C, con precipitaciones menores a 200 mm anuales (APN, 2019). El ejemplar de *L. jaguaris* fue registrado de manera ocasional durante la realización de un muestreo de vegetación (DRC-383; Fig. 1), el día 4 de marzo de 2020 a las 10.30 am., bajo una planta de zampa (*Atriplex* sp.) en una zona caracterizada por la presencia de médanos, con parches de vegetación conformada principalmente por jume (*Allenrolfea vaginata*), jarilla (*Larrea cuneifolia, L. divaricata) y* zampa (*Atriplex* sp.). Como no

Figura 1. Fotografías del ejemplar de *Leiosaurus jaguaris* registrado en el Parque Nacional Talampaya, provincia de la Rioja, Argentina (en 2020). (A) Vista dorsal general. (B) Vista dorso-lateral de la parte anterior del cuerpo.

Autor para correspondencia: maxivictor.arce@gmail.com se contaba con los permisos correspondientes para captura y manipulación de ejemplares, únicamente se tomaron fotografías, que se depositaron en la Colección Herpetológica de la Fundación Miguel Lillo (FML 30834).

L. jaguaris es un lagarto de mediano a gran tamaño (hocico-cloaca: 90 mm aproximadamente; largo cola: 110 mm aproximadamente), sin dimorfismo sexual. Esta especie presenta un patrón particular de coloración. El dorso de *L. jaguaris* es de color marrón o gris claro. Presenta entre cinco y siete marcas dorsales en la línea vertebral en forma de círculos o semicírculos con bordes difusos, de color gris claro en el centro y varios puntos negros en el borde exterior. En los laterales se distinguen numerosas manchas de diferente tamaño, marrón oscuro o claro distribuidas irregularmente. Cola con el mismo patrón que el cuerpo. Región dorsal de la cabeza con numerosas manchas y puntos marrones oscuros distribuidos irregularmente y un dibujo de "cola de ballena" blanco o gris claro rodeado por un borde negro o marrón oscuro. Dos franjas paralelas marrón oscuro o negro se distinguen en la cabeza lateralmente (Laspiur *et al.*, 2007). El individuo registrado en PNT se identificó siguiendo los caracteres de coloración diagnósticos de la especie. Además, se solicitó colaboración a A. Laspiur, especialista en el taxón, quien corroboró la identificación mediante fotografías (Laspiur, com. pers.).

Este nuevo registro extiende la distribución de la especie aproximadamente 55 kilómetros hacia el este (Fig. 2), siendo también el primer registro de la especie en la ecorregión de Monte de Sierras y Bolsones, ya que los registros anteriores han sido en zonas de ecotono entre Monte y Puna, por lo que se creía que la especie estaba asociada a zonas de transición (Laspiur & Acosta, 2015). Teniendo en cuenta la escasa información sobre la distribución del género y sobre las especies que lo conforman (Laspiur *et al.*, 2007), este primer registro de *L. jaguaris* en un área protegida, contribuye a fomentar proyectos de investigación que aporten a profundizar los conocimientos sobre este taxón y su hábitat. El Parque Nacional Talampaya es de especial interés por su herpetofauna, habiéndose registrado 29 especies de reptiles anteriormente (Kass *et al.*, 2018), en este trabajo se extiende el listado a 30 especies en total, enfatizando la importancia del parque para la conservación de las especies de reptiles del semiárido argentino. Además, Kass *et al.* (2018) reportan haber encontrado la especie *L. catamarcensis* en la misma unidad de hábitat (psamófilo, dominado por dunas de arenas), quedando planteada la incógnita acerca de la posible simpatría de estas dos especies en el área protegida.

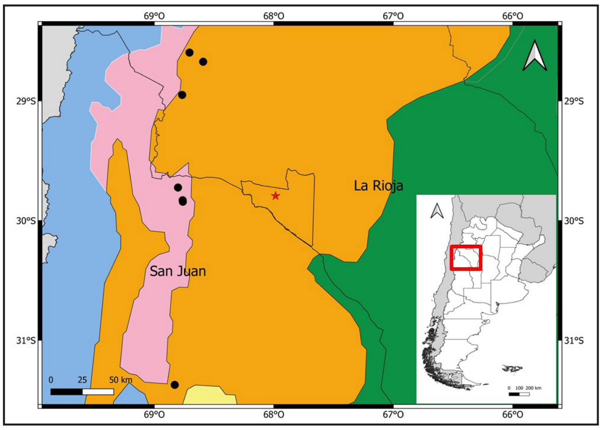

Figura 2. Mapa donde se indican los sitios donde se ha registrado a *L. jaguaris*. Los puntos negros representan los registros anteriores; la estrella roja representa el registro de este trabajo. En colores se representan las ecorregiones según Burkart *et al.* (1999): Chaco seco (Verde), Monte de Sierras y Bolsones (Naranja), Puna (Rosa), Altos Andes (Celeste).

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A A. Laspiur por su colaboración en la identificación de la especie, al personal del Parque Nacional Talampaya por su predisposición y a la Colección Herpetológica de la Fundación Miguel Lillo por recibir las fotografías del registro. A N. Manzanos por sus aportes en el campo. A los revisores anónimos por sus valiosas contribuciones. Al Programa de Pesquisas en Biodiversidad (PPBio-Argentina), por el marco de trabajo colaborativo. Fuentes de financiamiento PROICO 02-2818, Secretaría de Ciencia y Técnica, FQByF, de la Universidad Nacional de San Luis. Rufford Foundation.

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Novedad zoogeográfica

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New record and updated distribution map for *Drymoluber brazili* (Gomes, 1918) (Serpentes: Colubridae)

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Locality.— Brazil, state of Piauí, municipality of Pedro II, Grota rural village (4°29’56” S; 41°28’59” W, datum WGS84, ca. 580 m above sea level). Collected by Felipe Paixão de Sena on April 21, 2021, around 11:00 am. Deposited in the Biological Collection of the Instituto Federal de Educação, Ciências e Tecnologias do Piauí-IFPI Campus Pedro II (CBPII 189).

Comments.— The genus *Drymoluber* Amaral, 1930 currently comprises three species of aglyphous snakes with wide distribution in South America: *Drymoluber apurimacensis* Lehr, Carrillo and Hocking, 2004 is restricted to Apurímac Valley in Peru (Lehr *et al.*, 2004); *Drymoluber brazili (*Gomes, 1918) mainly known for Brazil with a single record for Paraguay (Costa *et al.*, 2013); and *Drymoluber dichrous (*Peters, 1863) with distribution to Peru, Ecuador, Bolivia, Colombia, Venezuela, Brazil, Guyana, Suriname, and French Guiana (Costa *et al.*, 2013). *Drymoluber brazili* is a diurnal and terrestrial snake that feeds on small vertebrates (Guedes *et al.*, 2014) and presents evidence of pseudoautotomy (non-spontaneous separation from the tail) (Ribeiro and Mesquita, 2014). It is a typical species from open areas of the Brazilian Cerrado, being also found in savanna enclaves in the Caatinga, and in anthropized areas of Atlantic Forest, between 334–1,100 m elevation (Costa *et al.*, 2013; Guedes *et al.*, 2014).

Despite the wide distribution in the central region of Brazil, there are few records of this species in the states of the Northeastern region of the country (see Guedes *et al.*, 2014). Herein, we present a new record of *Drymoluber brazili* for the state of Piauí, increasing its distribution and providing an updated distribution map of the species. A juvenile of *Drymoluber brazili* [Snout-Vent Length (SVL): 327 mm, Caudal Length (CL): 142 mm; Fig. 1] was accidentally found in a rural residence of the municipality of Pedro II, state of Piauí. Pedro II is located on the Serra dos Matões, a mountain range inserted in the Serra da Ibiapaba Environmental Protection Area, a transition area between Cerrado and Caatinga ecoregions, with predominance of Cerrado floral elements (IBGE, 2012; Santos *et al.*, 2019). The specimen, killed by a domestic animal, was immediately colleted and sent to the laboratory for fixation. The species identification was verified by Henrique Costa.

Drymoluber brazili can be distinguished from its congeners by having 17/17/15 smooth dorsal scales rows with two apical pits, 182–202 ventral scales, and 109–127 subcaudal scales, without secondary sexual dimorphism in segmental counts (Lerh *et al.*, 2004; Costa *et al.*, 2013). In general, it presents a dorsal coloration ranging from olive-green to grayish-brown on the anterior part of the body, becoming reddish-brown posteriorly, with a yellowish-white belly (Gomes, 1918; Costa *et al.*, 2013). When young, individuals have a red-orange head, with broad dark transverse bands separated by narrower light bands on the back and a yellowish-cream belly (Costa *et al.*, 2013).

Previous records of *Drymoluber brazili* are known only from two localities in the southern region of the state of Piauí (Fig. 2), for municipality of São Gonçalo do Gurguéia (Freitas *et al.*, 2012) and Serra das Confusões National Park (Dal Vechio *et al.*, 2016). This new record extends the known geographical distribution of *Drymoluber brazili* in about 536 km airline northeastern of the Serra das

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Figure 1. Juvenile of *Drymoluber brazili* (CBPII 189; SVL 327 mm, CL 142 mm) from the municipality of Pedro II, state of Piauí, Brazil.

Confusões National Park and about 744 km airline northeastern of the São Gonçalo do Gurguéia. In addition, we increased about 500 km northwestern of the municipality of Milagres, state of Ceará, the closest known record of *Drymoluber brazili* (Fig. 2).

Despite presenting a significant increase in its geographic distribution, *Drymoluler brazili* is still underrepresented in zoological collections (França *et al.*, 2006; Costa *et al.*, 2013), mainly after the fire at the Butantan Institute, where a large part of preserved specimens was destroyed (Costa *et al.*, 2013). This new occurrence within a Caatinga-Cerrado transition area in the northern region of the state of Piauí reinforces the lack of herpetological research and the need for systematic surveys to increasing knowledge about the distribution of this and other species in the state of Piauí.

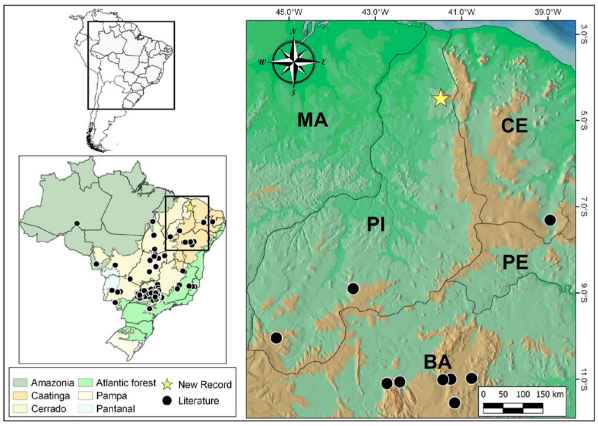

Figure 2. Geographic distribution of *Drymoluber brazili* based on literature records (Nogueira, 2001; Lehr *et al.*, 2004; Moreira *et al.*, 2009; Freitas *et al.*, 2012; Ribeiro *et al.*, 2012; Costa *et al.*, 2013; França and Braz, 2013; Costa *et al.*, 2014; Dal Vechio *et al.*, 2016; Nogueira *et al.*, 2019), and new record to the municipality of Pedro II (yellow star), state of Piauí, Brazil.

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Novedad zoogeográfica

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New distribution record of endangered and endemic frog *Pristimantis quantus* (Lynch, 1998) in western Andes of Colombia

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Locality— Colombia, western Andes, Antioquia department, Jardín municipality, vereda Macanas, Natural Reserve El Centello, (5°30’11.48”N; 75°51’6.05”O, WGS84, 2461 m elevation). The 17 July 2018 at 20:15 h. during a field trip Beatriz Rendón-Valencia and her assistant Divier Idárraga found an adult of *Pristimantis quantus* (MHUA-A 11308, Fig. 1). The specimen was found on leaves of *Anthurium* sp. at 1,20 m above the ground, in riparian habitat with herbaceous vegetation (mainly

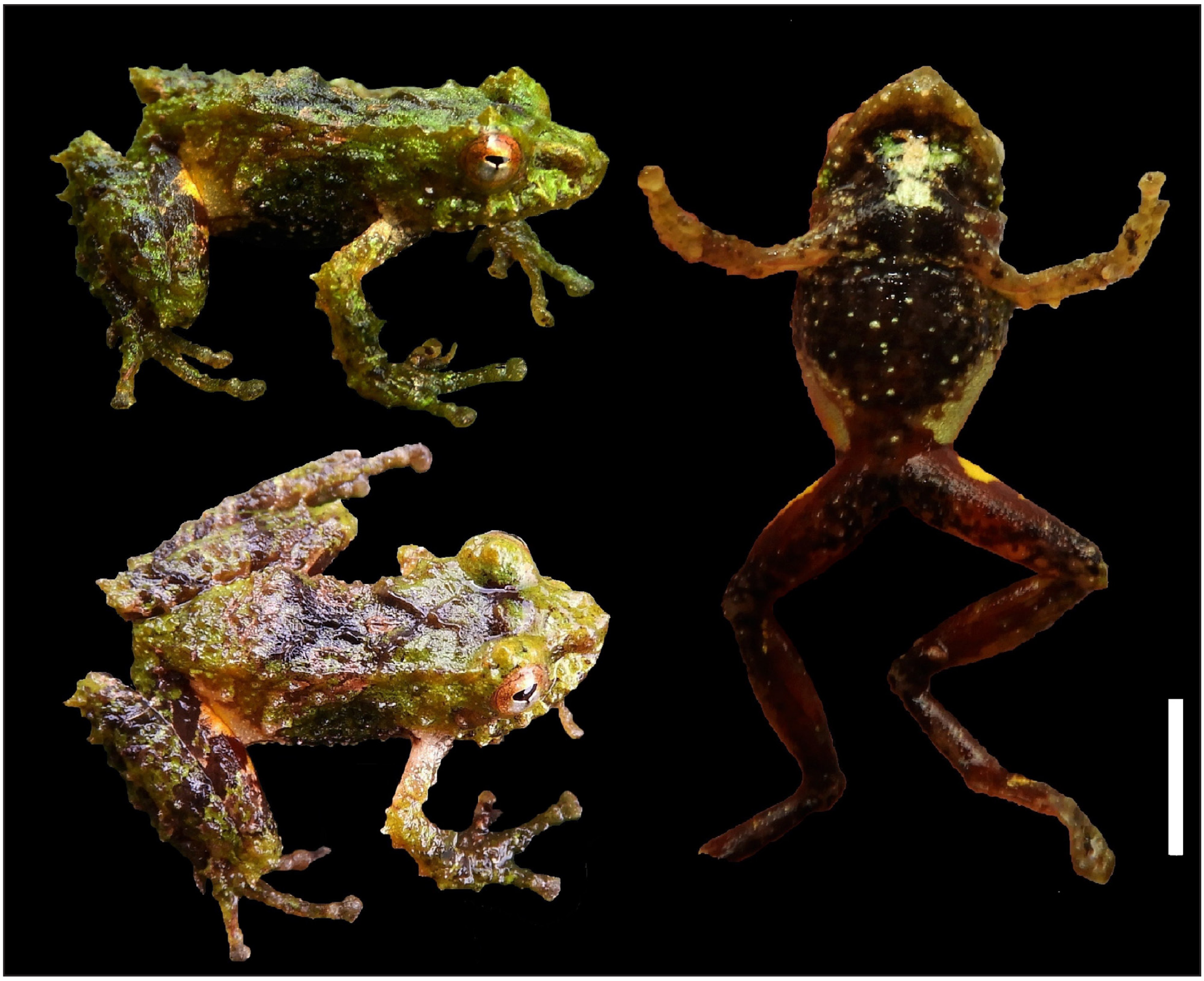

Figure 1. Female of *Pristimantis quantus* (MHUA-A 11308), from Natural Reserve El Centello, north of the western Andes, Jardín municipality, Antioquia departament, Colombia. Scale bar 0,5 mm.

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Araceas) in the edge of a stream located in the Montane Humid Forest (*sensu* Holdrige, 1967). The specimen was collected with the authorization of Autoridad Nacional de Licencias Ambientales (ANLA), resolution number 01711, 30 December 2016. The specimen was deposited in the Museo de Herpetología Universidad de Antioquia (MHUA-A 11308).

Comments.— The genus *Pristimantis* is recognized for their reproductive mode with direct development (Lynch and Duellman, 1997), and for their incredible diversity of species becoming the highest among all amphibians, with most species distributed in the Andean region (Hedges *et al.,* 2008; Frost, 2021). Currently, Colombia has the greatest richness of the genus *Pristimantis* with 232 species (Frost, 2021; Rivera-Correa *et al.,* 2021), with more than half of these endemics (146 species), and with 80 species included in some threat category (i.e., CR: 4, EN: 38, VU: 38; Armesto and Señaris, 2017; Frost, 2021; IUCN, 2021).

Pristimantis quantus (Lynch, 1998) is a species categorized as Endangered (EN) by the IUCN Red List (IUCN, 2021) and as Vulnerable (VU) by the Red Book of Amphibians of Valle del Cauca (Castro-Herrera and Bolivar-García, 2010). *Pristimantis quantus* was assigned to *Pristimantis unistrigatus* species group by Hedges *et al.,* (2008), although recently placed to *Pristimantis boulengeri* species group by Gonzalez-Durán *et al.* (2017).

Pristimantis quantus is endemic to Colombia and is distributed on the frontier between Chocó and Valle del Cauca departments, in western Andes (Lynch, 1988; Acosta-Gálvis, 2021; Cuellar-Valencia *et al.,* 2021). Since its description in 1988, *P. quantus* is known only from two localities on the Serrania de los Paraguas: "Alto de Galápagos" and "El Boquerón", both sites in the El Cairo municipality, Vereda Las Amarillas from 2,100-2,250 m asl (Lynch, 1988; Castro-Herrera and Bolivar-García, 2010). The estimated extent of occurrence (EOO) of *P. quantus* is approximately 65 km^2 and coincides with the protected area Reserva Natural Comunitaria Cerro El Ingles, owned by the Serraniagua foundation (Castro-Herrera and Bolivar-García, 2010; IUCN, 2021). Despite the above, major threats to the species include habitat loss and degradation due to logging, agriculture activities, opening of rural roads, cattle grazing, and the presence of *Batrachochytrium dendrobatidis* within its range (IUCN, 2021), but there is no information on whether chytridiomycosis affects the population.

Pristimantis quantus is a little-known species and information is only available from the original description (Lynch, 1998), and the Red Book of Valle del Cauca Amphibians (Castro-Herrera and Bolivar-García, 2010). Additionally, *P. quantus* is mentioned in checklists that compile regional or local species richness and threat (e.g., Castro-Herrera and Vargas-Salinas, 2008; Stuart *et al.,* 2008; Cardona-Botero *et al.,* 2012; García-R *et al.,* 2014; Armesto and Señaris, 2017; Cuellar-Valencia *et al.,* 2021). There is no ecological information on the species, but it is known to inhabit primary and secondary cloud forest and is found in the understory. *Pristimantis quantus* occurs in sympatry with congeneric species such as *P. achatinus*, *P. angustilineatus, P. brevifrons*, *P. calcaratus*, *P. erythropleura*, *P. kelephus, P. molybrignus*, *P. myops, P. palmeri*, *P. phalarus, P. ptochus*, *P. restrepoi*, *P. silverstonei, P. xylochobates* and the recently described *P. alius* and *P. ingles* (Lynch, 1998; Castro-Herrera and Bolivar-García, 2010; IUCN, 2021; Cuellar-Valencia *et al.*, 2021).

The specimen referenced in the present study agree with the characters proposed for the species *Pristimantis quantus* by Lynch (1988) and Gonzalez-Durán *et al.* (2017): 1) snout subacuminate in dorsal view, with papilla at the tip; 2) conical tubercle on upper eyelid and between eyes; iris pale gray with gray flecks; are bellow pupil is nearly white; 3) skin of dorsum smooth with scattered subconical tubercles; dorsum green and brown above with bright green interorbital bar 4) ventral skin coarsely areolate; chin bears yellow in males with some green tint in females; venter black to brown with cream flecks 5) nuptial pads present on finger I; ulnar tubercles conical; small subconical tubercles on heel and tarsus; two metatarsal tubercles; small double distal subarticular tubercle on Finger III; double distal subarticular tubercle present on finger IV; finger I shorter than finger II; digital discs expanded and round, except in finger I and toe I; toe V much longer than toe III; anterior surface of thigh red with yellow spot; 6) adults minute males 11.6-14.5 mm (n = 4); female 14.4-16-7 mm SVL (n = 4) (Fig. 1).

This record represents the northernmost occurrence of the species and the first evidence of distribution outside the Serranía de los Paraguas. Therefore, the distribution range of the species is extended approximately 100 kilometers from its type locality in the Valle del Cauca department (Serranía de los Paraguas), to the southwest of Antioquia departament in the Natural Reserve El Centello owned by Fundación Jardín Botánico de Medellín Joaquín Antonio Uribe (Fig. 2). It is probable that the *P. quantus* occurs in several sites between the two previously referenced localities with a broader distribution, as occurs with other species of the genus *Pristimantis* in the cloud forest in the north of the Western Cordillera (Lynch, 1998; Cuellar-Valencia *et al.*, 2021).

Protected areas are shielded against different anthropic pressures that can negatively affect threatened amphibian populations (p.e. pollution, fragmentation, urbanization) (Lannoo, 2005; Acevedo *et al.*, 2016; Aguilar-López *et al.*, 2020). The presence of the *P. quantus* in the Natural Reserve El Centello could be an indication of an established population. Additionally, it was possible to register a complex assembly of nine congeneric species that occur in sympatry with *P. quantus*, such as *P. achatinus*, *P. calcaratus, P. zophus, P. erythropleura, P. angustilineatus, P.* cf. *urani*, and two more undetermined species of *Pristimantis.*

The present finding of a new locality for *P. quantus* together with endemic and threatened congeners (*P. zophus*, *P. calcaratus*) and the possibility of two new candidate species and in the Natural Reserve El Centello, highlights the importance of implementing samplings in unknown areas to fill information gaps in sensitive groups such as Andean amphibians. It is also necessary to develop monitoring programs focused on determining the population trends (stability, increase or decrease) of threatened amphibian species, in order to promote effective conservation strategies in which protected areas play an indispensable role.

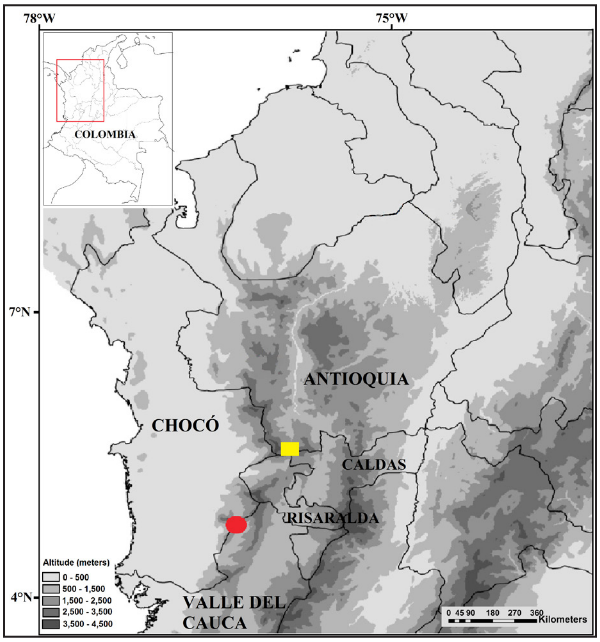

Figure 2. Distribution map of *Pristimantis quantus*. Yellow square correspond to the new locality reported by the present study, and the red circle to the type locality.

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Novedad zoogeográfica

Cuad. herpetol. 36 (1): 123-124 (*2022*)

Primera cita de *Hemidactylus mabouia* (Moreau de Jonnès, 1818) en la provincia de Jujuy, Argentina

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Localidad.— Argentina. Provincia de Jujuy, Departamento Ledesma, Libertador Gral. San Martín (23°48´S - 64°47´W; 482 m s.n.m) (Fig.1). 29 de octubre de 2019. Lucas Flores. Colectado en el interior de una vivienda. Colección de Herpetología de la Fundación Miguel Lillo FML 31080.

Comentarios.— El género *Hemidactylus*, con más de 173 especies reconocidas, es uno de los grupos con mayor número de especies y ampliamente distribuidos a nivel mundial (Cyriac y Umesh, 2021) y contribuyó a la reducción y extinción de lagartijas nativas en diferentes regiones del mundo (Anjos *et al*., 2008). Varias especies de este género son comunes en viviendas humanas y han colonizado con éxito nuevas áreas (Vences *et al*., 2004). *Hemidactylus mabouia* es oportunista y generalista, lo que le confiere ventajas respecto a su posibilidad de colonizar nuevas áreas (Álvarez-Romero *et al*., 2008) y por ello es conocida por su gran capacidad de dispersión tanto en áreas antrópicas como naturales, y en muchos casos, resulta favorecida por el tráfico de mercaderías (Vanzolini, 1978; Baldo *et al*., 2008). Sus poblaciones originales radican en África, pero en la actualidad está ampliamente distribuida en la Región Neotropical (Vanzolini, 1968; Kluge, 1969; Cei, 1993; Carreira *et al*., 2005) y se la encuentra en Sudamérica desde hace casi 500 años (Carranza y Arnold, 2006).

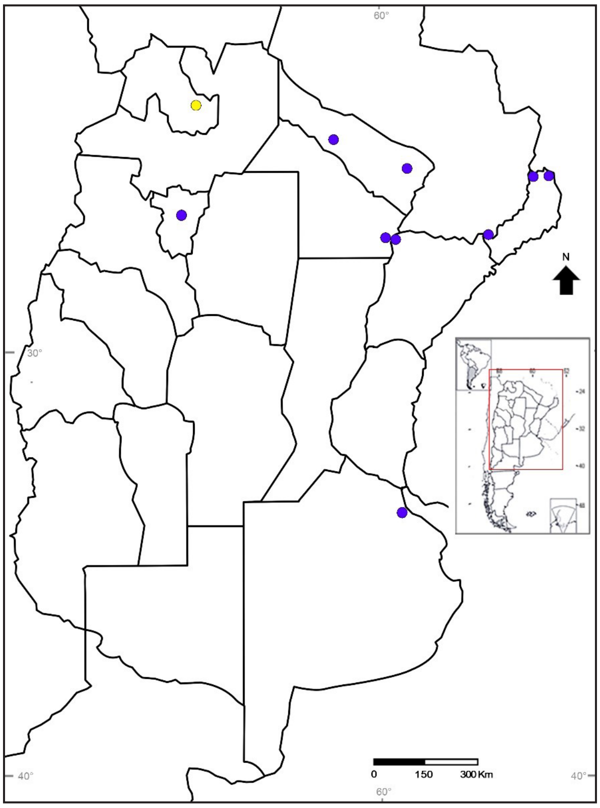

Figura 1. Registros de distribución de *Hemidactylus mabouia* (puntos azules) en la Argentina. El punto amarillo representa el primer registro de la especie para la provincia de Jujuy.

El primer registro del género en Argentina fue realizado por Williams (1988), quien la menciona como *Hemidactylus turcicus*, en Parque Chacabuco, un área urbana de la ciudad de Buenos Aires, con base en un ejemplar juvenil (Museo de La Plata, (MLP S.1092), sin embargo, un examen posterior de dicho ejemplar reveló que pertenece a *H. mabouia* (J.D. Williams de acuerdo a Baldo *et al.,* 2008). Luego, diferentes autores la citaron en Misiones (Genise y Montanelli, 1991; Baldo *et al*., 2008), Chaco (Federico y Cacivio, 2000), Corrientes (Álvarez *et al*., 2002), Formosa (Álvarez *et al*., 2009; Torres *et al*., 2018) y Tucumán (Scrocchi *et al*., 2019).

El ejemplar objeto de esta novedad mide 45,42 mm de LHC (longitud hocico-cloaca) y no se pudo determinar el sexo debido al estado del espécimen, pero sí se pudieron observar las características de la especie: dedos con almohadillas digitales ensanchadas y divididas, que en el IV dedo de la pata trasera no se extienden hasta la base del dedo (Fig.

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Figura 2. Pata trasera de *Hemidactylus mabouia* donde se observa que las almohadillas del IV dedo no llegan a la base del dedo.

2); subcaudales mediales agrandadas y dispuestas en series, pupila vertical y párpados ausentes (Krysko y Daniels, 2005). Este es el primer registro en la provincia de Jujuy, extendiendo la distribución conocida en Argentina 340 km al N del registro más cercano reportado previamente (Tucumán, Scrocchi *et al.*, 2019). Estos dos trabajos representan las primeras citas de la especie para la ecorregión de Selvas de Yungas.

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