

ISSN 1852 - 5768 (en línea)

CUADERNOS *de* HERPETOLOGÍA

VOLUMEN 36 - NUMERO 1 - MAYO 2022

ojs.aha.org.ar - aha.org.ar



Revista de la
Asociación Herpetológica Argentina

Asociación Herpetológica Argentina

Presidenta: María Laura Ponssa

Vicepresidenta: Vanesa Arzamendia

Secretaria: Marta Duré

Prosecretaria: Laura Nicoli

Tesorera: Ana Duport

Vocales Titulares: Gabriela Gallardo, Cristian Abdala

Vocal Suplente: Julián Faivovich

Junta Revisora de Cuentas: Darío Cardozo, Diego Barrasso

CUADERNOS de HERPETOLOGÍA

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 transparente 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.

Comité Científico

Virginia Abdala

Instituto de Biología Neotropical (CONICET-UNT), Tucumán, Argentina.

Gabriela Perotti

Instituto de Investigaciones en Biodiversidad y Medioambiente (CONICET-UNComa), San Carlos de Bariloche, Rio Negro, Argentina.

Taran Grant

Departamento de Zoología, Instituto de Biociências, Universidade de São Paulo (USP), São Paulo, Brasil.

Vanesa Arzamendia

Instituto Nacional de Limnología (CONICET-UNL), Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral, Santa Fe, Argentina.

Juliana Sterli

Museo Paleontológico Egidio Feruglio (CONICET), Trelew, Chubut, Argentina.

James A. Schulte II

Department of Biology, 212 Science Center, Clarkson University, Potsdam, NY, EE.UU.

María Laura Ponssa

Unidad Ejecutora Lillo (CONICET-FML), Tucumán, Argentina.

Lee Fitzgerald

Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas, EE.UU.

Esteban O. Lavilla

Unidad Ejecutora Lillo (CONICET-FML), Tucumán, Argentina.

María Florencia Vera Candiotti

Unidad Ejecutora Lillo (CONICET-FML), Tucumán, Argentina.

Darrel Frost

Division of Vertebrate Zoology, Herpetology, American Museum of Natural History, New York, EE.UU.

Gustavo Scrocchi

Unidad Ejecutora Lillo (UEL, CONICET-FML), Tucumán, Argentina.

Margarita Chiaraviglio

Instituto de Diversidad y Ecología Animal (CONICET-UNC), Córdoba, Argentina.

Célio F. B. Haddad

Departamento de Zoologia, Instituto de Biociências, UNESP, Rio Claro, São Paulo, Brasil.

Directores / Editores

Javier Goldberg / Diego Baldo

Instituto de Diversidad y Ecología Animal, (IDEA, CONICET-UNC), Córdoba, Argentina / Laboratorio de Genética Evolutiva, Instituto de Biología Subtropical (CONICET – UNaM), Facultad de Ciencias Exactas Químicas y Naturales, Universidad Nacional de Misiones, Argentina

Editores asociados

María Gabriela Agostini

Instituto de Ecología, Genética y Evolución de Buenos Aires (CONICET-UBA), Ciudad Autónoma de Buenos Aires, Argentina.

Thais Barreto Guedes

Universidade Estadual do Maranhão (UEMA), Caxias, Maranhão, Brasil.

Paulo Passos

Dpto de Vertebrados, Setor de Herpetologia, Museu Nacional, Universidade Federal do Rio de Janeiro, Brasil.

Luciana Bolsoni Lourenço

Departamento de Biología Estructural e Funcional, Instituto de Biología, Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brasil.

Nora Ruth Iburgüengoytia

Instituto de Investigaciones en Biodiversidad y Medioambiente (CONICET-UNComa), San Carlos de Bariloche, Argentina.

Paola Peltzer

Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

Claudio Borteiro

Sección Herpetología, Museo Nacional de Historia Natural de Montevideo, Montevideo, Uruguay.

Adriana Jerez

Departamento de Biología, Universidad Nacional de Colombia, Bogotá, Colombia.

Sebastián Quinteros

Instituto de Bio y Geociencias del NOA (IBIGEO, CONICET-UNSa), Salta, Argentina.

David Buckley

Dpto de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, Madrid, España.

Claudia Koch

Alexander Koenig Research Museum, Bonn, Alemania (ZFMK).

Silvia Quinzio

Instituto de Diversidad y Ecología Animal, (IDEA, CONICET-UNC), Córdoba, Argentina.

Mario R. Cabrera

Departamento Diversidad Biológica y Ecología, FCEfyN, Universidad Nacional de Córdoba, Córdoba, Argentina.

Julián N. Lescano

Instituto de Diversidad y Ecología Animal, (IDEA, CONICET-UNC), Córdoba, Argentina.

Alex Richter-Boix

Evolutionary Biology Centre, Uppsala Universitet, Norbyvägen, Uppsala, Suecia.

Clarissa Canedo

Departamento de Zoologia, IBRAG, UERJ, Maracanã, Rio de Janeiro, Brasil.

Antonieta Labra

1. ONG Vida Nativa, Chile. 2. Centre for Ecological and Evolutionary Synthesis, University of Oslo, Noruega.

Miguel Tejedo

Departamento de Ecología Evolutiva Estación Biológica de Doñana (EBD-CSIC), Sevilla, España.

Santiago Castroviejo-Fisher

Laboratorio de Sistemática de Vertebrados, Pontificia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Brasil.

Carlos A. Navas

Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo (USP), São Paulo, Brasil.

Marcos Vaira

Instituto de Ecorregiones Andinas (CONICET-UN-Ju), San Salvador de Jujuy, Argentina.

Ana Lucia da Costa Prudente

Coordenação de Zoologia, Museu Paraense Emílio Goeldi, Belém, Estado do Pará, Brasil.

Daniel E. Naya

Dpto de Ecología y Evolución, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

Soledad Valdecantos

Instituto de Bio y Geociencias del NOA (IBIGEO, CONICET-UNSa), Salta, Argentina.

Julián Faivovich

Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Ciudad Autónoma de Buenos Aires, Argentina.

Laura Nicoli

Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Ciudad Autónoma de Buenos Aires, Argentina.

Volumen 36 - Numero 1 - Mayo 2022

CUADERNOS *de* HERPETOLOGÍA

Revista de la Asociación Herpetológica Argentina

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

Juliana H. Wolter¹, Carolina S. Mascarenhas¹, Bruna M. Chaviel¹, Ana Beatriz D. Henzel¹, Ricardo R. C. Silva², Frank Lira³, Gertrud Müller¹

¹ Laboratório de Parasitologia de Animais Silvestres (LAPASIL), Instituto de Biologia, Universidade Federal de Pelotas, Campus universitário, caixa postal 354, Pelotas, 96010-900, RS, Brazil.

² Universidade Federal de Pelotas, Campus universitário, caixa postal 354, Pelotas, 96010-900, RS, Brazil.

³ Grupo especial de proteção do ambiente aquático do Rio Grande do Sul (GEEPAA -RS), Pelotas, 96010-900, RS, Brazil.

Recibido: 28 Enero 2021

Revisado: 23 Mayo 2021

Aceptado: 14 Noviembre 2021

Editor Asociado: C. Borteiro

doi: 10.31017/CdH.2021.(2021-004)

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).

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 or 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 \pm 14.83).

Mites were preserved in 70° 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).

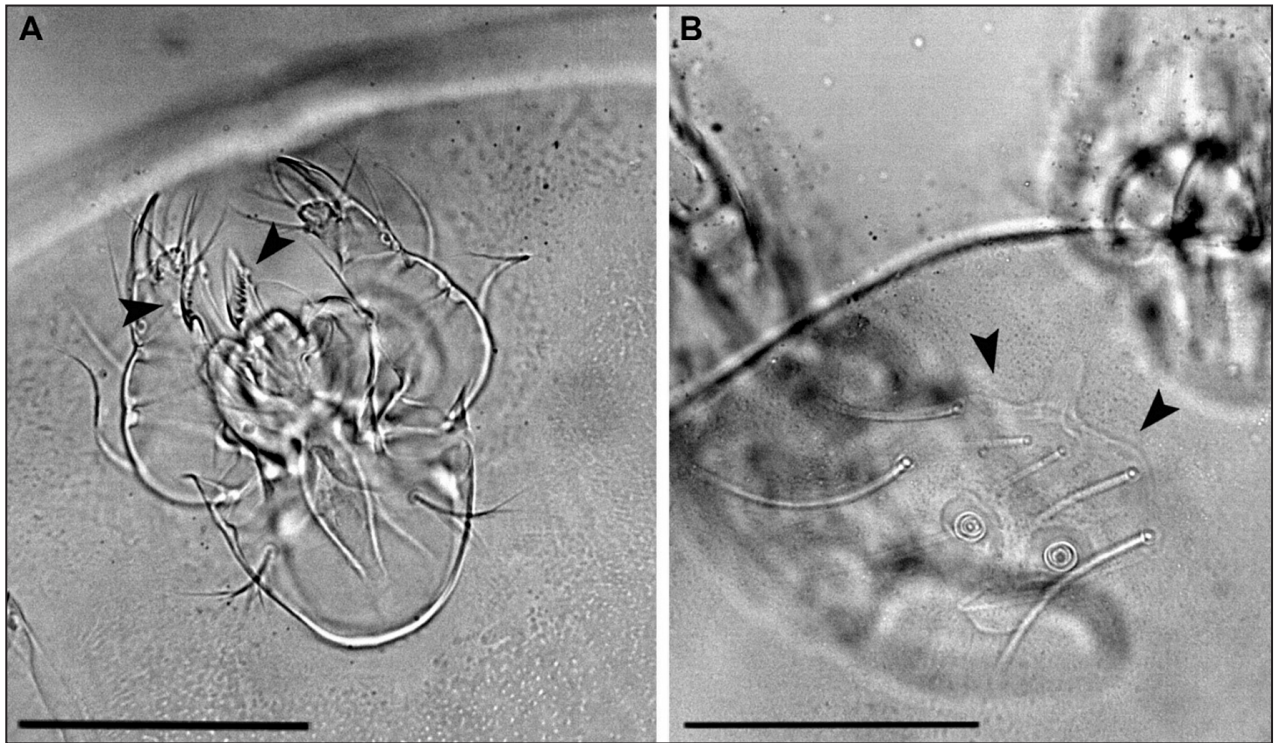


Figure 1. Larva of *Hannemania* sp. (Acari: Leeuwenhoekiidae) parasitizing *Leptodactylus luctator* (Anura: Leptodactylidae), from Pampa biome, southern Brazil. A - Gnathosoma (arrows indicate chelicera) (bar = 57 μ m). B - Scutum with nasus (arrows) located on the dorsum of the larva (bar = 75 μ m).

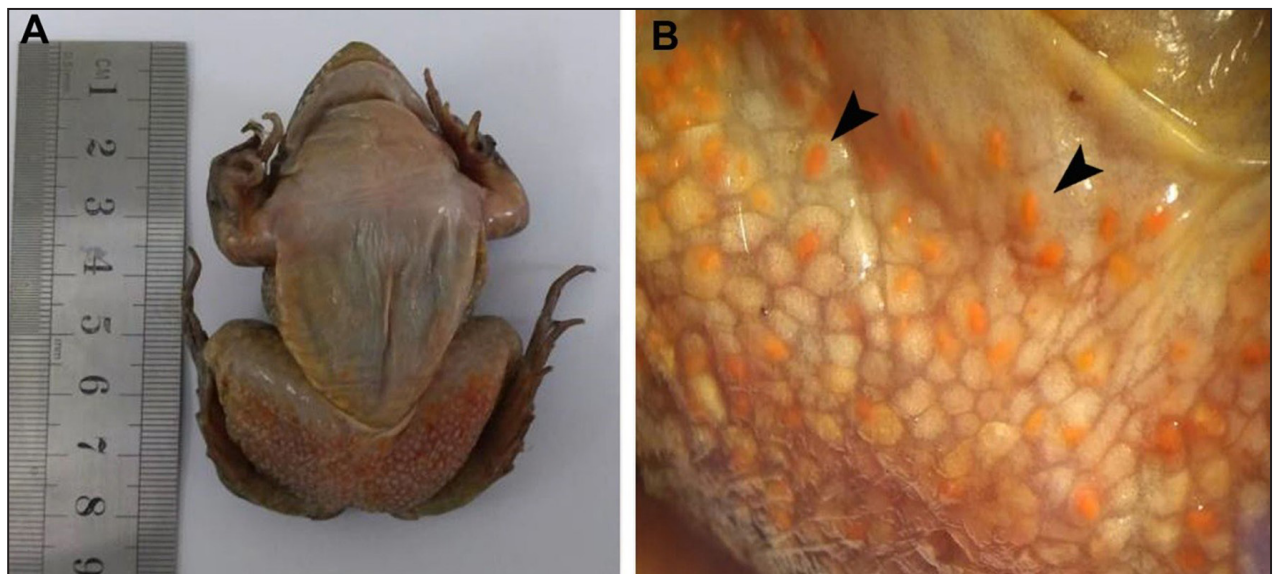


Figure 2. *Leptodactylus luctator* (Anura: Leptodactylidae) parasitized by *Hannemania* sp. larvae in the Pampa biome, southern Brazil. A - Ventral view of the anuran, notice the numerous larvae (orange spots) in the hind limbs. B - Detail with magnification of the intradermic parasitic larvae (arrows).

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

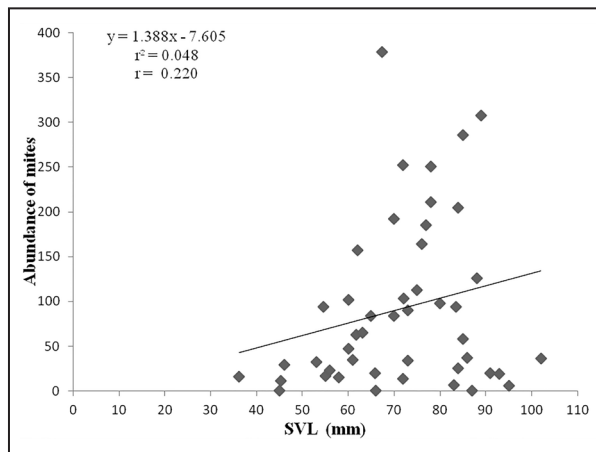


Figure 3. Regression analysis of *Hannemania* sp. larvae (Acari: Leeuwenhoekiidae) abundance in *Leptodactylus luctator* (Anura: Leptodactylidae) from Pampa biome, southern Brazil, in relation to host snout-vent length (SVL, in millimeters).

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 asso-

ciations 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-Páez *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.

Acknowledgments

Special thanks to Julia Veiga Pereira, Emily Costa Silveira and Marco Antônio Afonso Coimbra for their assistance. Authors are grateful to journal reviewers for the suggestions to improve the manuscript. This study was funded by CAPES (Coordenação de Aperfeiçoamento do Pessoal de Nível

Superior, process no. 32/2010), licensed by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio no. 47397-5), and approved by the Ethics Committee on Animal Experimentation (CEEA/UFPEL no.1859/2015).

Literature cited

- Attademo, A.M.; Peltzer, P.M.; Lajmanovich, R.C.; Junges, C.; Bassó, A. & Cabagna-Zenkhusen, M. 2012. Trumbiculid mites (*Hannemania* sp.) in *Leptodactylus chaquensis* (Amphibia: Anura) inhabiting selected soybean and rice agroecosystems of Argentina. *Journal of Zoo and Wildlife Medicine* 43: 579-584.
- Bernarde, P.S. 2012. Anfíbios e Répteis Introdução ao Estudo da Herpetofauna Brasileira. Curitiba, Brasil. Anolisbooks. Curitiba.
- Brennan, J.M. & Goff, M.L. 1977. Keys to the genera of chiggers of the western hemisphere (Acarina: Trombiculidae). *The Journal of Parasitology* 63: 554-566.
- Bush, A.; Lafferty, K.; Lotz, J. & Shostak, A. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* 83: 575-583.
- CFMV (Conselho Federal de Medicina Veterinária). 2012. Retrieved on 25 September 2017. from: <http://portal.cfmv.gov.br/>
- Díaz-Páez, H.; Cortez, E.; De La Fuente, C.S. & Moreno Salas, L. 2016. Body distribution of *Hannemania* sp. (Acari: Leeuwenhoekiidae) in *Rhinella spinulosa*, *Pleurodema bufonina*, and *Pleurodema thaul* from Chile. *Journal of Zoo and Wildlife Medicine* 47: 594-600.
- Gallardo, J.M. 1974. Anfíbios de los alrededores de Buenos Aires. Buenos Aires, Argentina. Editorial Universitaria de Buenos Aires. Buenos Aires.
- Hatano, F.H.; Gettinger, D.; Van Sluys, M. & Rocha, C.F.D. 2007. Parasitism of *Hylodes phyllodes* (Anura: Cycloramphidae) by *Hannemania* sp. (Acari: Trombiculidae) in an area of atlantic forest, Ilha Grande, southeastern Brazil. *Parasite* 14: 107-112.
- Jacinavicius, C.F.; Bassani-Silva, R.; Mendoza-Roldan, A.J.; Pepato, R.A.; Ochoa, R., Welbourn, C. & Barros-Battesti, M.D. 2018. A checklist of chiggers from Brazil, including new records (Acari: Trombidiformes: Trombiculidae and Leeuwenhoekiidae). *ZooKeys* 743: 1-41.
- Jacinto-Maldonado, M.; León-Paredes, R.; Salgado-Maldonado, G.; García, A. & Suzán, G. 2016. New records of amphibians parasitized by chiggers in Los Tuxtlas Biosphere Reserve, Mexico, and taxonomic notes on *Hannemania mexicana* (Acariformes: Prostigmata: Leeuwenhoekiidae). *Systematic & Applied Acarology* 21: 13-20.
- Jung, R.E.; Claeson, S.; Wallace, J. & Welbourn, W.C. 2001. Mite infestation. *Herpetological Review* 32: 33-34.
- Luz, H.B. & Faccini, J.L.H. 2013. Parasitismo por carrapatos em anuros no Brasil. Revisão. *Veterinária e Zootecnia* 20: 100-111.
- Magalhães, F. de M.; Lyra, M.L.; de Carvalho, T.R.; Baldo, D.; Brusquetti, F.; Burella, P.; Colli, G.R.; Gehara, M.C.; Giaretta, A.A.; Haddad, C.F.B.; Langone, J.A.; López, J.A.; Napoli, M.F.; Santana, D.J.; de Sá, R.O. & Garda, A.A. 2020. Taxonomic review of South American Butter frogs: phylogeny, geographic patterns, and species delimitation in the *Leptodactylus latrans* species group (Anura: Leptodactylidae). *Herpetological Monographs* 34: 131-177.
- Martins, L.A.; Kwet, A. & Lema, T. 2015. Anfíbios do Rio Grande do Sul: Lista sistemática comentada, com distribuição e iconografia das espécies de anfíbios ocorrentes no Rio Grande do Sul, Brasil, registradas até 2013. Porto Alegre, Brasil. EdUPUCRS. Porto Alegre.
- Quinzio, S. & Goldberg, J. 2015. Intra-dermal infections by chigger mites (*Hannemania* sp.) in the Andean frog *Telmatobius atacamensis* (Anura, Telmatobidae). *Salamandra* 51: 263-268.
- Reiczigel, J.; Marozzi, M.; Fábíán, I. & Rózsa, L. 2019. Biostatistics for parasitologists – a primer to Quantitative for Parasitology. *Trends in Parasitology* 35: 277-281.
- Rodrigues, J.K.; Tales, D.A.; Filho, J.A.A.; Teixeira, A.A.M.; Mesquita, D.O. & Ribeiro, S.C. 2018. Infestation by Chiggers (*Hannemania* sp.) of Miranda's White-lipped Frog (*Leptodactylus macrosternum*) from a Semiarid, Neotropical Region of Brazil. *Journal of Wildlife Diseases* 54: 397-399.
- Silva-De La Fuente, M.C.; Moreno-Salas, L. & Castro-Carrasco, C. 2016. Review of the genus *Hannemania* (Acari: Leeuwenhoekiidae) with description the two new species in amphibians from Chile. *Zootaxa* 4200: 580-590.
- Silveira, E.C.; Mascarenhas, C.S. & Loebmann, D. 2019. Occurrence of *Hannemania* sp. (Acariformes: Leeuwenhoekiidae) larvae in males of *Boana pulchella* (Anura: Hylidae) from southern Brazil. *Revista Mexicana de Biodiversidad* 90: 1-5.
- Wohltmann, A.; Köhler, J. & Martin, P. 2006. Endoparasitic mite infections of anuran amphibians from Bolivian montane rain forests, with descriptions of two new *Hannemania* species (Acari: Parasitengona: Trombiculidae: Leeuwenhoekiinae). *Organisms, Diversity & Evolution* 6: 141-150.

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

Rodrigo Tavares-Pinheiro, Vinícius A. M. B. de Figueiredo, Fernanda S. Melo, Aline E. Oliveira-Souza, Abdiel Pinheiro-Freitas, Carlos E. Costa-Campos

Universidade Federal do Amapá, Departamento de Ciências Biológicas e da Saúde, Laboratório de Herpetologia, 68903-419, Macapá, Amapá, Brazil

Recibido: 15 Marzo 2021

Revisado: 04 Agosto 2021

Aceptado: 05 Octubre 2021

Editor Asociado: T. Guedes

doi: 10.31017/CdH.2022.(2021-016)

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 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

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.

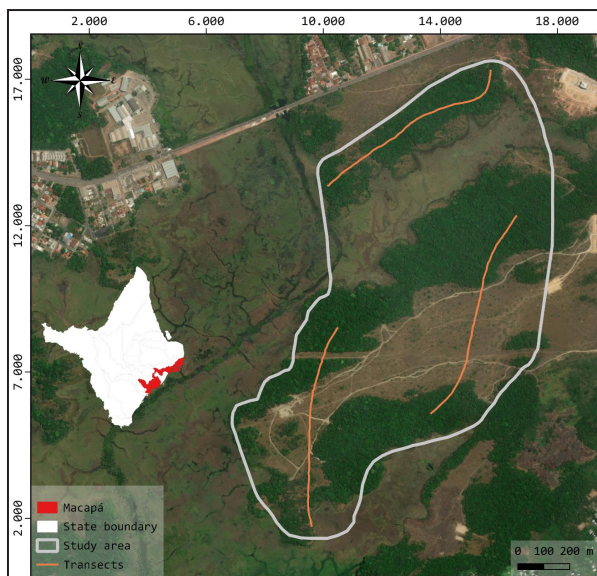


Figure 1. Map showing the geographic location of the Environmental Protection Area of Lagoa dos Índios, in southeast portion of Macapá municipality, Amapá state, northern region of Brazil.

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 °C (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).

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 Jackknife1 to determine the expected richness for amphibians, lizards, snakes and all the herpetofauna combined. We did not build chelonian or crocodylian curves because these taxa were only rarely present. Both rarefaction curves and the Jackknife 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	ICMBio
ANURA					
Bufonidae					
	<i>Rhinella major</i> (Muller & Helmich, 1936)	7	Abundant	NL	LC
	<i>Rhinella marina</i> (Linnaeus, 1758)	9	Abundant	LC	LC
Hylidae					
	<i>Boana punctata</i> (Schneider, 1799)	5	Common	LC	LC
	<i>Boana raniceps</i> (Cope, 1862)	9	Abundant	LC	LC
	<i>Dendropsophus leucophyllatus</i> (Beireis, 1783)	7	Abundant	LC	LC
	<i>Dendropsophus walfordi</i> (Bokermann, 1962)	4	Common	LC	LC
	<i>Lysapsus bolivianus</i> Gallardo, 1961	6	Abundant	DD	NL
	<i>Pseudis paradoxa</i> (Linnaeus, 1758)	4	Common	LC	LC
	<i>Scinax ruber</i> (Laurenti, 1768)	10	Abundant	LC	LC
	<i>Sphaenorhynchus lacteus</i> (Daudin, 1800)	7	Abundant	LC	LC
	<i>Trachycephalus typhonius</i> (Linnaeus, 1758)	4	Common	LC	LC
Leptodactylidae					
	<i>Adenomera hylaedactyla</i> (Cope, 1868)	9	Abundant	LC	LC
	<i>Leptodactylus macrosternum</i> Miranda-Ribeiro, 1926	2	Rare	LC	LC
	<i>Leptodactylus pentadactylus</i> (Laurenti, 1768)	4	Common	LC	LC
	<i>Leptodactylus podicipinus</i> (Cope, 1862)	9	Abundant	LC	LC
Microhylidae					
	<i>Elachistocleis helianneae</i> Caramaschi, 2010	3	Rare	LC	LC
Pipidae					
	<i>Pipa pipa</i> (Linnaeus, 1758)	4	Common	LC	LC
GYMNOPHIONA					
Typhlonectidae					
	<i>Typhlonectes compressicauda</i> (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 Jonnés, 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

<i>Pseudoeryx plicatilis plicatilis</i> (Linnaeus, 1758)	1	Rare	LC	LC
<i>Oxyrhopus melanogenys melanogenys</i> (Tschudi, 1845)	1	Rare	LC	LC
<i>Thamnodynastes pallidus</i> (Linnaeus, 1758)	5	Common	LC	LC
<i>Erythrolamprus cobella</i> (Linnaeus, 1758)	1	Rare	LC	LC
<i>Erythrolamprus reginae semilineatus</i> (Wagler in Spix, 1824)	1	Rare	LC	LC
<i>Lygophis lineatus</i> (Linnaeus, 1758)	1	Rare	LC	LC
Viperidae				
<i>Bothrops atrox</i> (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 Bérnills, 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-

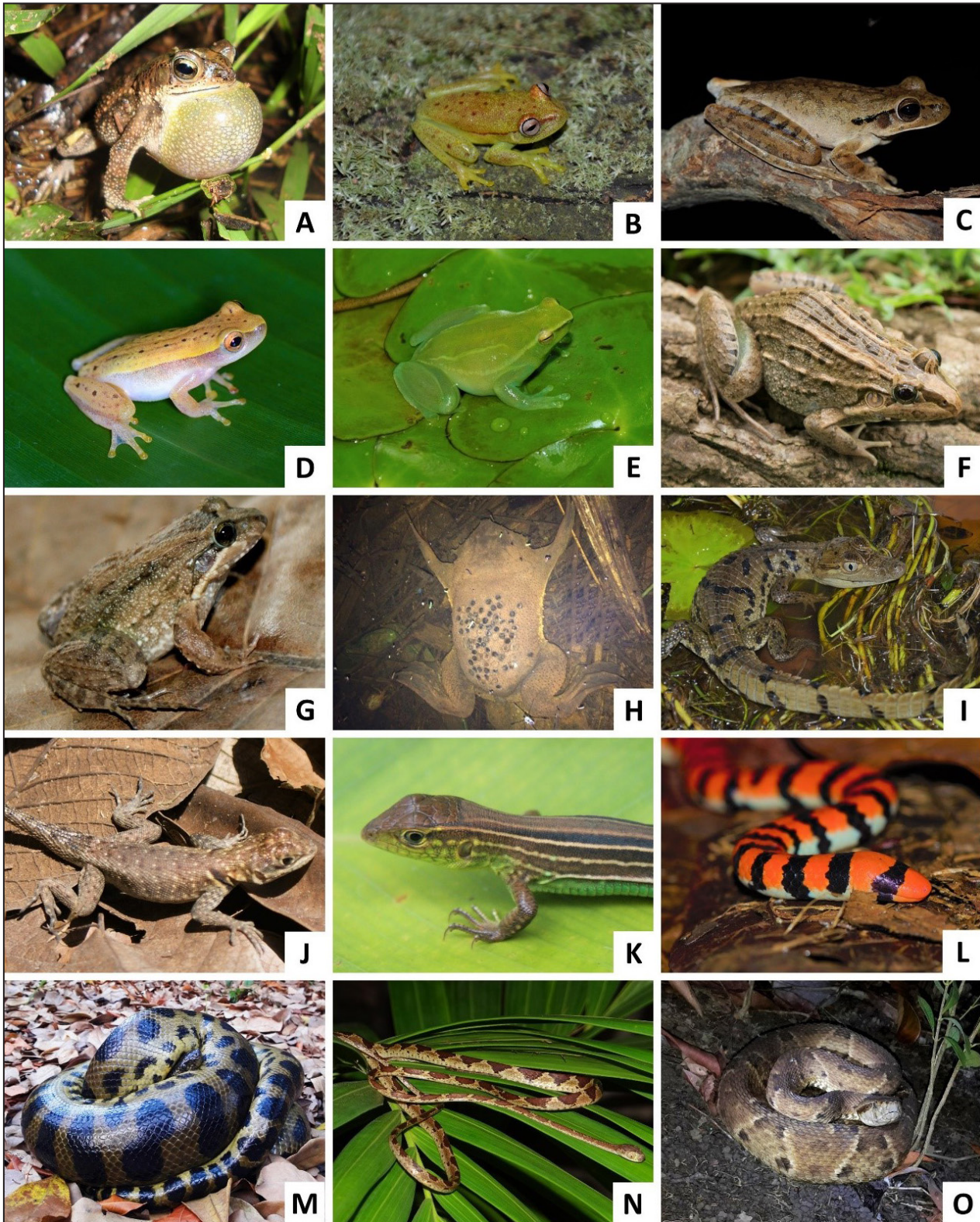


Figure 2. Some species of amphibians and reptiles from the Environmental Protection Area of Lagoa dos Índios, Macapá municipality, Amapá state, northern Brazil. (A) *Rhinella major*; (B) *Boana punctata*; (C) *B. raniceps*; (D) *Dendropsophus walfordi*; (E) *Sphaenorhynchus lacteus*; (F) *Leptodactylus 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

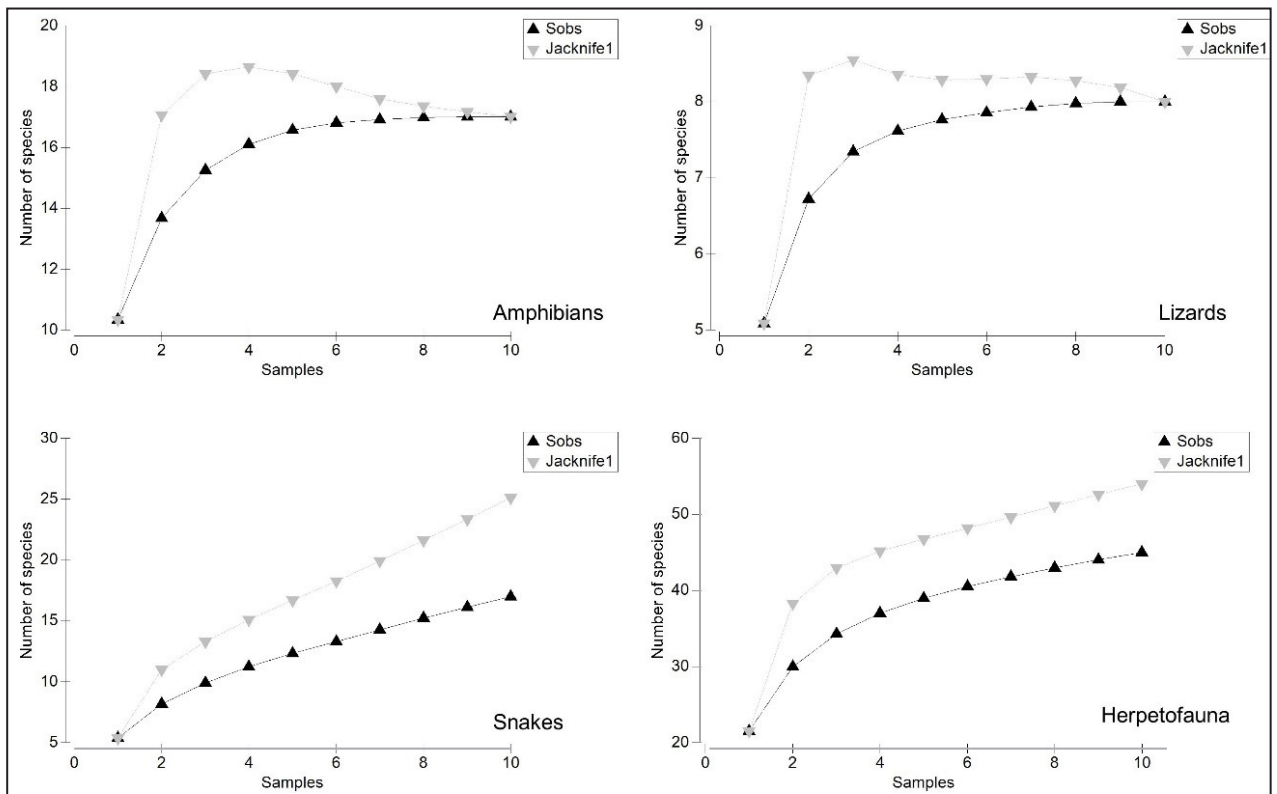


Figure 3. Accumulation and rarefaction curve for (A) amphibians, (B) lizards, (C) snakes and (D) pooled herpetofauna coupled with a species richness estimator Jackknife 1, recorded from January to May 2018 (rainy season) and August to December 2019 (wet season) in the Environmental Protection Area of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

Acknowledgments

We are grateful to Patrick Sanches, Fillipe Santos, Jéssica Anaissi, Madalena Santana, Aline Souza and Tamylls Reis of the Laboratório de Herpetologia of Universidade Federal do Amapá for the assistance during fieldwork. Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for providing permits

(ICMBio/SISBIO license # 48102-2) for the collection of zoological specimens. Finally, we thank to the associate editor Thaís Guedes, that along with anonymous reviewers, provided helpful comments on our manuscript.

Literature cited

- Almeida-Corrêa, T.; Frazão, L.; Costa, D.M.; Menin, M. & Kaefer, I.L. 2020. Effect of environmental parameters on squamate reptiles in an urban forest fragment in central Amazonia. *Acta Amazonica* 50: 239-245.
- Andrade, A.C.; Franzini, L.D. & Mesquita, D.O. 2019. Assessing the effect of urbanization on tropical forest dwelling teiid lizards. *Ecological Indicators* 99: 225-229.
- Araújo, A.S. & Costa-Campos, C.E. 2014. Anurans of the Reserva Biológica do Parazinho, Municipality of Macapá, state of Amapá, eastern Amazon. *Check List* 10: 1414-1419.
- Benício, R.A. & Lima, J.D. 2017. Anurans of Amapá National Forest, Eastern Amazonia, Brazil. *Herpetology Notes* 10: 627-633.
- Bitar, Y.O.C.; Juen, L.; Pinheiro, L.C. & Santos-Costa, M.C. 2014. Anuran Beta Diversity in a Mosaic Anthropogenic Landscape in Transitional Amazon. *Journal of Herpetology* 49: 75-82.
- Campos, C.E.C.; Lima, J.D. & Lima, J.R.F. 2015. Riqueza e composição de répteis Squamata (lagartos e anfisbenas) da Área de Proteção Ambiental da Fazendinha, Amapá, Brasil. *Biota Amazônia* 5: 84-90.

R. Tavares-Pinheiro *et al.* — Herpetofauna an urban area in the Amazon Forest of Amapá

- Colwell, R.K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9.1.0. Available from: <<http://viceroy.eeb.uconn.edu/estimates/>>. Last access: 8 April 2020.
- Costa, H.C.; Guedes, T.B. & Bérnils, R.S. 2021. Lista de répteis do Brasil: padrões e tendências. *Herpetologia Brasileira* 10: 110-279.
- Costa-Campos, C.E. & Freire, E.M.X. 2019. Richness and composition of anuran assemblages from an Amazonian savana. *ZooKeys* 843: 149-169.
- Costa-Campos, C.E.; Sanches, P.R.; Pedroso-Santos, F.; Figueiredo, V.A.M.B.; Tavares-Pinheiro, R. & Almeida-Santos, W. 2021. Checklist of the reptiles from the Cancão Municipal Natural Park, state of Amapá, eastern Amazon, Brazil. *Herpetology Notes* 14: 539-550.
- Dajoz, R. 2005. Princípios de Ecologia. Artmed. Brazil.
- Debien, I.V.; Waldez, F. & Menin, M. 2019. Diversity of reptiles in flooded and unflooded forests of the Amanã Sustainable Development Reserve, central Amazonia. *Herpetology Notes* 12: 1051-1065.
- Fouquet, A.; Martinez, Q.; Zeidler, L.; Courtois, E.A.; Gaucher, P.; Blanc, M.; Lima, J.D.; Souza, S.M.; Rodrigues, M.T. & Kok, P.J. 2016. Cryptic diversity in the *Hypsiboas semilineatus* species group (Amphibia, Anura) with the description of a new species from the eastern Guiana Shield. *Zootaxa* 4084: 79-104.
- Fouquet, A.; Marinho, P.; Réjaud, A.; de Carvalho, T.R.; Caminer, M.A.; Jansen, M.; Rainha, R.N.; Rodrigues, M.T.; Werneck, F.P.; Lima, A.P.; Hrbek, T.; Giaretta, A.A.; & Venegas, P.J.; Chávez, G. & Ron, S. 2021. Systematics and biogeography of the *Boana albopunctata* species group (Anura, Hylidae), with the description of two new species from Amazonia. *Systematics and Biodiversity* 0(0): 1-38.
- Fraga, R.; Stow, A.J.; Magnusson, W.E. & Lima, A.P. 2014. The costs of evaluating species densities and composition of snakes to assess development Impacts in Amazonia. *Plos One* 9(8): 1-9.
- Frazão, L.; Oliveira, M.E.; Menin, M.; Campos, J.; Almeida, A.; Kaefer, I.L. & Hrbek, T. 2020. Species richness and composition of snake assemblages in poorly accessible areas in the Brazilian Amazonia. *Biota Neotropica* 20(1): e20180661.
- Gotelli, N.J. & Colwell, R.K. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* 4: 379-391.
- Heyer, W.R.; Donnelly, M.A.; McDiarmid, R.W.; Hayek, L.C. & Foster, M.S. 1994. Measuring and monitoring biological diversity: Standard methods for amphibians. Smithsonian Institution Press. Washington.
- ICMBio. 2018. Instituto Chico Mendes de Conservação da Biodiversidade. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. Ministério do Meio Ambiente. Brasília.
- IUCN. 2022. The IUCN Red List of Threatened Species. Version 2020-3. Available from: <<https://www.iucnredlist.org>>. Last access: 21 January 2022.
- Kurz, D.J.; Nowakowski, J.A.; Tingley, M.W.; Donnelly, M.A. & Wilcove, D.S. 2013. Forest-land use complementarity modifies community structure of a tropical herpetofauna. *Biological Conservation* 170: 246-255.
- Lima, J.D. 2017. Anfíbios e répteis do estado do Amapá: contribuições para a conservação da herpetofauna Amazônica. Tese (Doutorado). Universidade Federal do Amapá. Programa de Pós-Graduação em Biodiversidade e Biotecnologia. Macapá, Amapá.
- Lima, J.R.F.; Lima, J.D.; Lima, S.D.; Silva, R.B.L. & Andrade, G.V. 2017. Amphibians found in the Amazonian Savanna of the Rio Curiaú Environmental Protection Area in Amapá, Brazil. *Biota Neotropica* 17: 1-10.
- Martins, M. & Oliveira, M.E. 1998. Natural history of snakes in forests of the Manaus region Central Amazonia Brazil. *Herpetological Natural History* 6: 78-150.
- Menin, M.; Ferreira, R.F.B.; Melo, I.B.; Gordo, M.; Hattori, G.Y. & Sant'anna, B.S. 2019. Anuran diversity in urban and rural zones of the Itacoatiara municipality, central Amazonia, Brazil. *Acta Amazonica* 49: 122-130.
- Mustin, K.; Carvalho, W.D.C.; Hilário, R.R.; Costa-Neto, S.V.; Silva, C.; Vasconcelos, I.M.; Castro, I.J.; Eilers, V.; Kauano, E.E.; Mendes-Júnior, R.N.G.; Funi, C.; Fearnside, P.M.; Silva, J.M.C.; Euler, A.M.C. & Toledo, J.J. 2017. Biodiversity, threats and conservation challenges in the Cerrado of Amapá and Amazonian savanna. *Nature Conservation* 22: 107-127.
- Nogueira, C.C.; Argôlo, A.J.S.; Arzamendia, V.; Azevedo, J.A.; Barbo, F.E.; Bérnils, R.S.; Bolochio, B.E.; Borges-Martins, M.; Brasil-Godinho, M.; Braz, H.; Buononato, M.A.; Cisneros-Heredia, D.F.; Colli, G.R.; Costa, H.C.; Franco, F.L.; Giraud, A.; Gonzalez, R.C.; Guedes, T.; Hoogmoed, M.S.; Marques, O.A.V.; Montingelli, G.G.; Passos, P.; Prudente, A.L.C.; Rivas, G.A.; Sanchez, P.M.; Serrano, F.C.; Silva, N.J.; Strüssmann, C.; Vieira-Alencar, J.P.S.; Hussam, Z.; Sawaya, R.J. & Martins, M. 2019. Atlas of Brazilian Snakes: Verified Point-Localities Maps to Mitigate the Wallacean Shortfall in a Megadiverse Snake Fauna. *South American Journal of Herpetology* 14: 1-274.
- Pedroso-Santos, F.; Sanches, P.R.; Costa-Campos, C.E. 2019. Anurans and reptiles of the Reserva Extrativista Beija-Flor Brilho de Fogo, Amapá state, eastern Amazon. *Herpetology Notes* 12: 799-807.
- Peel, M.C.; Finlayson, B.L. & McMahon, T.A. 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences Discussions* 4: 439-473.
- Peixoto, G.; Leitão, P.; Kaefer, I.L. & Lima, A.P. 2019. The lizards along the road BR-319 in the Purus-Madeira interfluvium, Brazilian Amazonia (Squamata, Lacertilia). *Herpetology Notes* 12: 689-697.
- Pisani, G.R. 1973. A guide to preservation techniques for amphibians and reptiles. *Herpetological Circulars* 1: 1-22.
- Prudente, A.L.C.; Sarmento, J.F.M.; Costa, K.K.C.; Dourado, A.C.M.; Santos, M.M.; Lima, J.R.F.; Lima, J.D. & Galatti, U. 2020. Serra do Navio, Guiana Shield lowland area, Brazil: a region with high diversity of Squamata. *Cuadernos de Herpetología* 34: 145-162.
- Ribeiro-Júnior, M.A.; Silva, M.B. & Lima, J.D. 2016. A New Species of *Bachia* Gray 1845 (Squamata: Gymnophthalmidae) from the Eastern Guiana Shield. *Herpetologica* 72: 148-156.
- Santos-Costa, M.C.; Maschio, G.F. & Prudente, A.L.C. 2015. Natural history of snakes from Floresta Nacional de Caxiuanã, eastern Amazonia, Brazil. *Herpetology Notes* 8: 69-98.
- Sartorius, S.S.; Vitt, L.J. & Colli, G.R. 1999. Use of naturally and anthropogenically disturbed habitats in Amazonian rainforest by the teiid lizard *Ameiva ameiva*. *Biological Conservation* 90: 91-101.
- Segalla, M.V.; Berneck, B.; Canedo, C.; Caramaschi, U.; Cruz,

- C.A.G.; Garcia, P.C.A.; Grant, T.; Haddad, C.F.B.; Lourenço, A.C.C.; Mângia, S.; Mott, T.; Nascimento, L.B.; Toledo, L.F.; Werneck, F.P. & Langone, J.A. 2021. List of Brazilian Amphibians. *Herpetologia Brasileira* 10: 121-216.
- Silva e Silva, Y.B. & Costa-Campos, C.E. 2018. Anuran species composition of Cancão Municipal Natural Park, Municipality of Serra do Navio, Amapá state, Brazil. *ZooKeys* 762: 131-148.
- Stuart, S.; Chanson, J.S.; Cox, N.A.; Young, B.E.; Rodrigues, A.S.L.; Fischman, D.L. & Waller, R.W. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783-1786.
- Taucce, P.P.G.; Costa-Campos, C.E.; Haddad, C.F.B. & de Carvalho, T.R. 2020. A New Amazonian Species of the Diminutive Frog Genus *Adelophryne* (Anura: Brachycephaloidea: Eleutherodactylidae) from the State of Amapá, Northern Brazil. *Copeia* 108: 746-757.
- Tavares-Dias, M.; Neves, L.R.; Pinheiro, D.A.; Oliveira, M.S.B. & Marinho, R.G.B. 2013. Parasites in *Curimata cyprinoides* (Characiformes: Curimatidae) from eastern Amazon. Brazil. *Acta Scientiarum Biological Sciences* 35: 595-601.
- Vargas, G.M. & Bastos, C.M.C.B. 2013. Urban environmental conflicts and urbanization processes at Ressaca Lagoa dos Índios, Macapá/AP. *Cadernos Metr pole* 15: 265-288.
- Verdade, V.K.; Valdujo, P.H.; Carnaval, A.C.; Schiesari, L.; Toledo, L.F.; Mott, T.; Andrade, G.V.; Eterovick, P.C.; Menin, M.; Pimenta, B.V.S.; Nogueira, C.; Lisboa, C.S.; Paula, C.D. & Silvano, D.L. 2012. A leap further: the Brazilian Amphibian Conservation Action Plan. *Alytes* 29: 27-42.

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).

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

Leonardo P. C. Oitaven^{1,3}, Márcio F. Chaves², José L. de Araújo², Felipe S. Ribeiro³, Geraldo J.B. de Moura³

¹ Laboratório de Estudos Herpetológicos e Paleoherpetológicos, Departamento de Biologia, Universidade Federal Rural de Pernambuco – UFRPE. Graduate Program in Animal Bioscience, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP 52171-900, Recife, PE, Brasil.

² Laboratório de Biologia de Anuros, Centro de Saúde e Educação, Universidade Federal de Campina Grande, Rua Olho D'Água da Bica s/n, CEP 58175-000, Cuité, PB, Brasil.

³ Laboratório de Estudos Herpetológicos e Paleoherpetológicos, Departamento de Biologia, Universidade Federal Rural de Pernambuco – UFRPE, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP 52171-900, Recife, PE. Graduate Program in Animal Bioscience-UFRPE. Graduate Program in Biodiversity-UFRPE. Graduate Program in Environmental Sciences-UFRPE, Brasil.

Recibido: 23 Junio 2021
Revisado: 05 Agosto 2021
Aceptado: 02 Mayo 2022
Editor Asociado: N. Ibargüengoytia

doi: 10.31017/CdH.2022.(2021-049)

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)

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; Toriki, 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

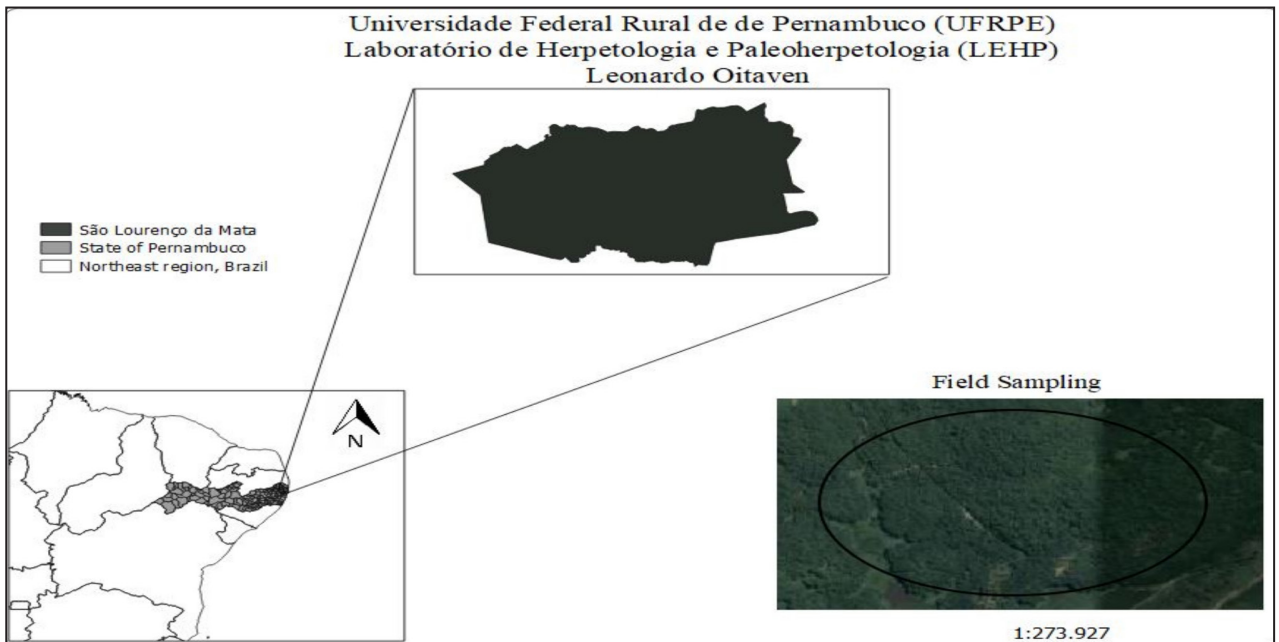


Figure 1. Map of the state of Pernambuco (PE), northeastern Brazil, depicting the municipality of São Lourenço da Mata and the study areas in Mata do Camucim and Mata do Tapacurá, both located within the Tapacurá Ecological Station.

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).

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

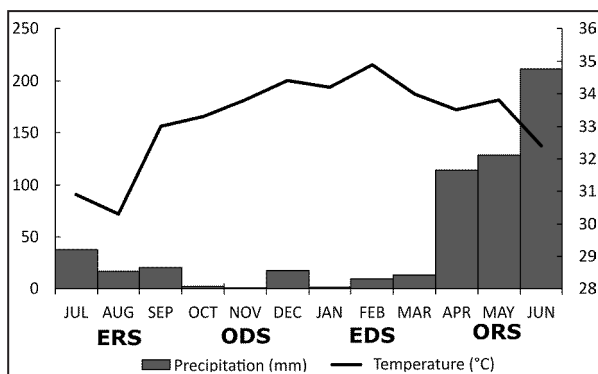


Figure 2. Climatogram from the Tapacurá Ecological Station (Açude de Tapacura, APAC) region, municipality of São Lourenço da Mata, state of Pernambuco, Brazil. The climatogram shows the mean air temperature (°C) and precipitation (mm) monthly between July 2016 and June 2017, according to the onset of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

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 ($\pi(D/2)2H$), 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 μ m 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 (V_v) 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 $QA = \Sigma \text{profiles} / AT$ (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 mm^2 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 $63.27 \pm 49.5 \text{ mm}^3$ (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. darwini* 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 ($r^2 = 0.12$; $df = 57$; $p < 0.001$), 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

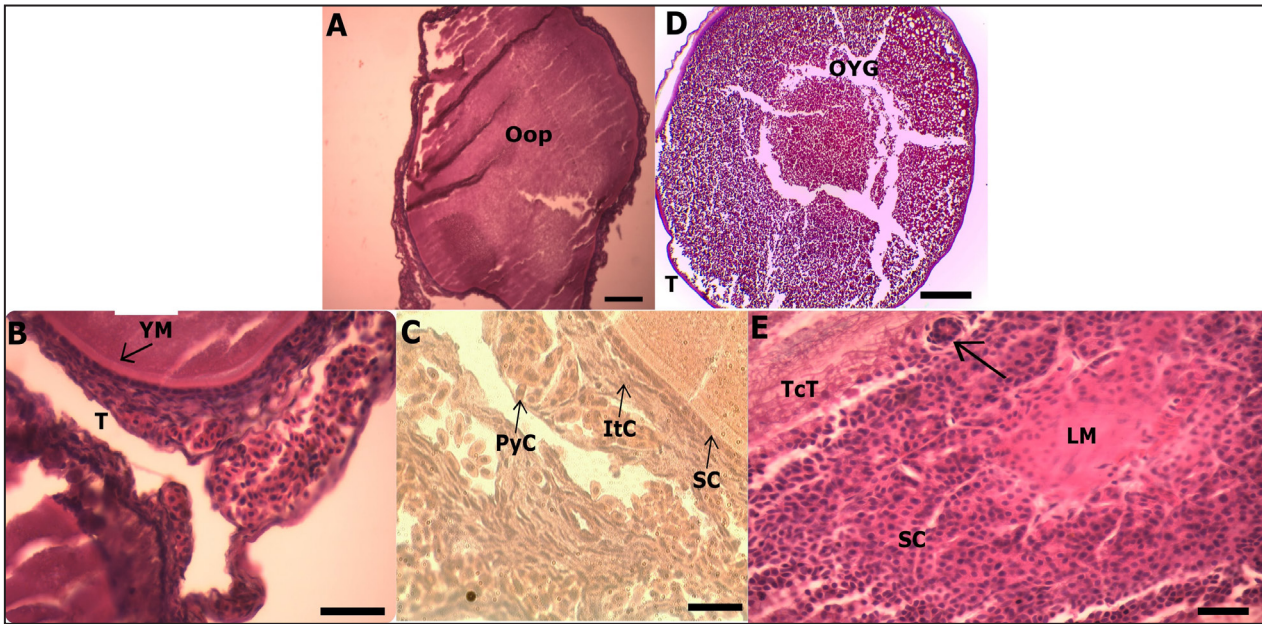


Figure 3. Histological sections of follicles of *G. darwinii* specimens collected in the Tapacurá forest, São Lourenço da Mata, Pernambuco, Brazil. (A) Previtellogenic follicle in early vitellogenic process, with the ooplasm (Oop) forming yolk platelets. H-E Stained. Bar = 150 μ m. (B) Ooplasm showing few, small yolk platelets, with yolk membrane (YM) exhibiting a hyaline band and zona radiata. Theca (T) well defined. H-E Stained. Bar = 150 μ m. (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 μ m. (D) Vitellogenic follicle exhibiting ooplasm with yolk granules (OYG) in their cytoplasm. Thinner theca (T) with blood vessels. H-E Stained. Bar = 500 μ m. (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 μ m.

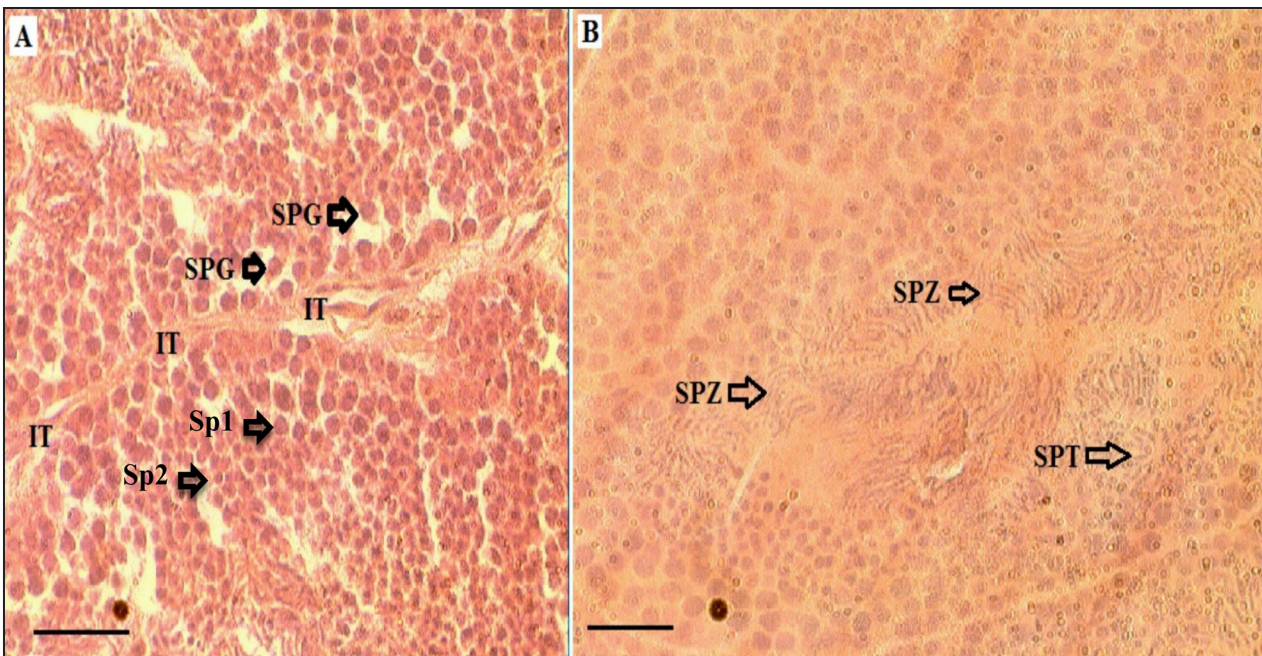


Figure 4. Histological sections of seminiferous tubes of *G. darwinii* specimens collected in October and November 2016 in the Tapacurá forest in São Lourenço da Mata, Pernambuco, Brazil. IT = interstitial tissue; SPG = spermatogonia; SPT = Spermatids; Sp1 = primary spermatocytes; Sp2 = secondary Spermatocytes; SPZ = sperms; (A) October; (B) November. H-E Stained. Bar = 50 μ m.

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

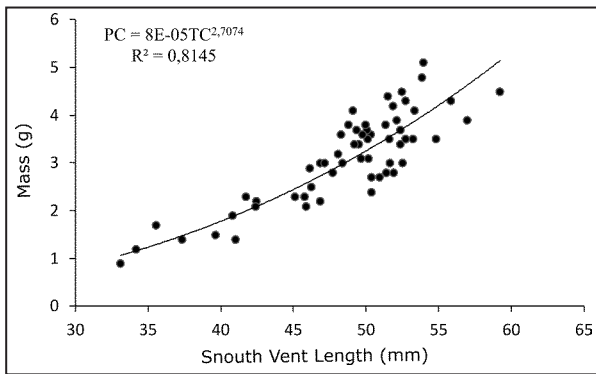


Figure 5. Ratio between body mass (g) and snout-vent length (SVL; mm) of *G. darwinii* specimens collected between July 2016 and June 2017 in the Tapacurá forest in São Lourenço da Mata, Pernambuco, Brazil.

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

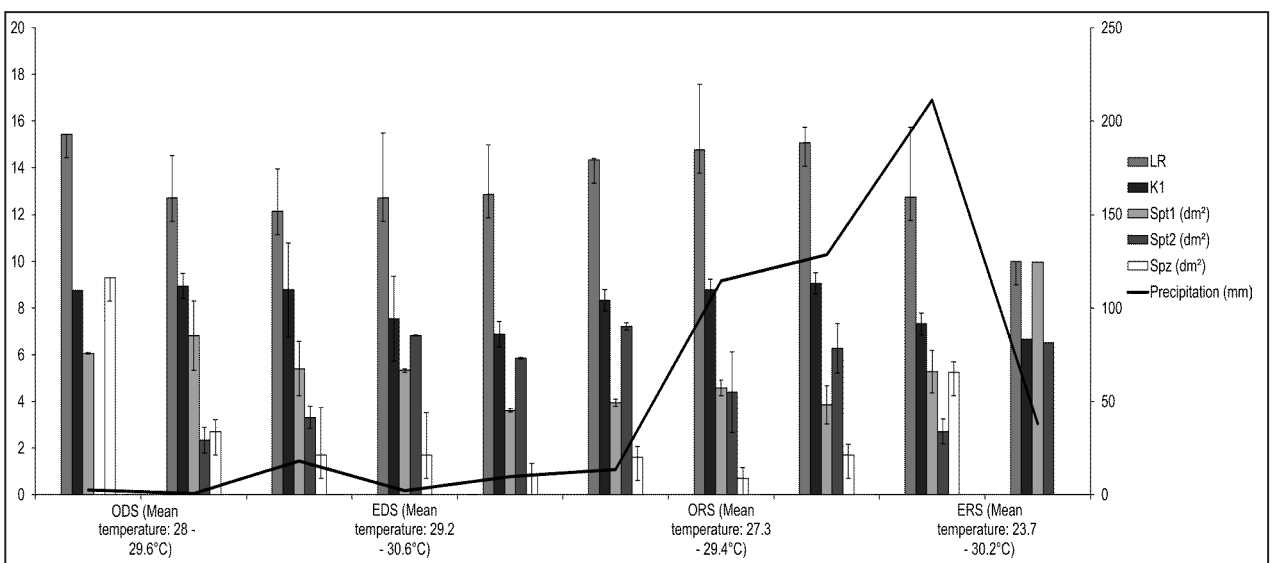


Figure 6. Monthly variation and standard deviation of the condition factor (K1), liposomatic relationship (LR), primary spermatocytes (Spt1), secondary spermatocytes (Spt2), and sperms (SPZ) recorded in males of *G. darwinii*, with the mean air temperature (°C) and precipitation (mm) registered in the 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).

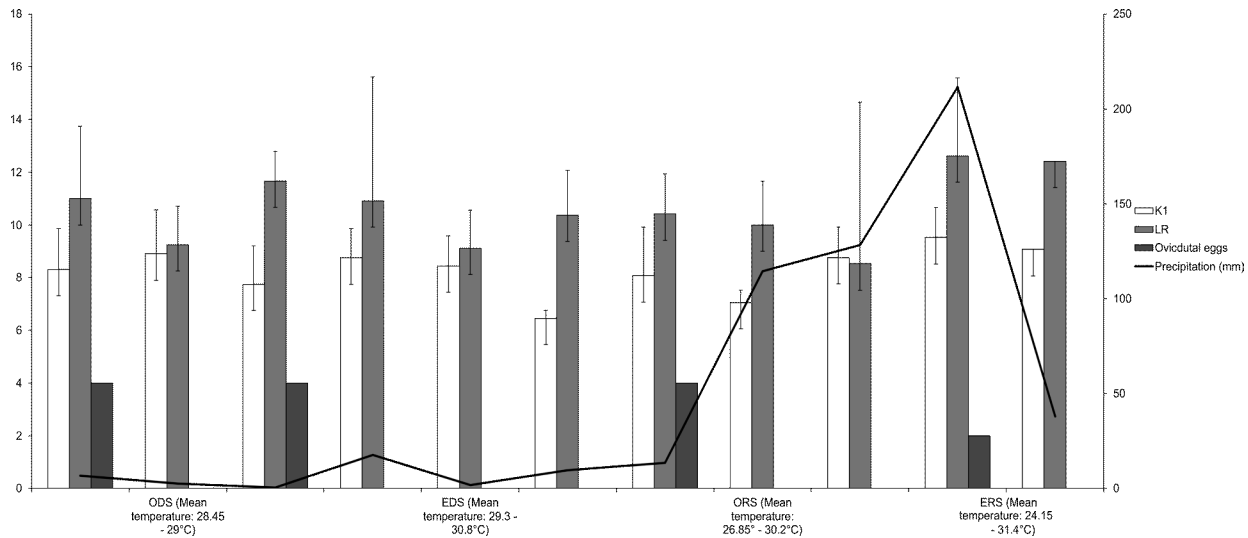


Figure 7. Monthly variation and standard deviation of the condition factor (K1), liposomatic relationship (LR), and number of oviductal eggs recorded in females of *G. darwini*, with the mean air temperature (°C) and precipitation (mm) registered in the same months, according to seasonality. Specimens 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. darwini* 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; Toriki, 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* Linnaeus, 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.

Acknowledgments

We are grateful to Alexandre Marques for helping in statistical analyses. We also thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for financial support to this study (0287-2.05114), and the federal environmental agency, Instituto Chico Mendes (ICMBio), and the Comitê de Ética e Uso de Animais (CEUA-UFRPE), for providing the study licenses and authorizations. To Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq, for the research productivity grant awarded to G.J.B.Moura (1D).

Literature cited

- Abu-Zinadah, O.A. 2008. Variation in testicular histology of the spiny tailed lizard *Uromastix aegyptius microlepis* during hibernation and active periods. *Pakistan Journal of Biological Sciences* 11: 1615 – 1619.
- APAC - Agência Pernambucana de Águas e Clima. 2017. Boletins Pluviométricos. Available in: <https://www.apac.pe.gov.br/>. Last access: 20/08/2017.
- Almeida-gomes, M.; Vrcibradic, D. Maia-Carneiro, T. & Rocha, C.F.D. 2012. Diet and endoparasites of the lizard *Gymnodactylus darwinii* (Gekkota, Phyllodactylidae) from an Atlantic Rainforest area in southeastern Brazil. *Biotemas* 25: 203 – 206.
- Autumn, K. & De Nardo D.F. 1995. Behavioral thermoregulation

- increases growth rate in a nocturnal lizard. *J Herpetol* 29: 157 – 162.
- Ballinger, R.E. 1977. Reproductive strategies: food availability as a source of proximal variation in lizard. *Ecology* 58: 628–635.
- Carneiro-Moura, C.M.; Moura G.J.B.; Lisboa E.B.F. & Luz V.L.F. 2014. Distribuição geográfica e considerações ecológicas sobre a fauna de Testudines da Região Nordeste do Brasil. *Sitientibus série Ciências Biológicas* 14: 1 – 20.
- Cheng, H.Y. 1987. A review on annual reproductive and energetic patterns of five taxa of lizards in Taiwan for ten years. *Proceedings of the National Science Council, Republic of China* 11: 313–321.
- Colli, G.R.; Mesquita, D.O.; Rodrigues, P.V. & Kitayama, K. 2003. Ecology of the gecko *Gymnodactylus geckoides amarali* in a Neotropical savanna. *Journal of Herpetology* 37: 694 – 706.
- Cooper-Jr, W.E.; Mendonça, M.T. & Vitt, L.J. 1987. Induction of orange head coloration and activation courtship and aggression by testosterone in the male broad-head skink (*Eumeces laticeps*). *Journal of Herpetology* 21: 96 – 101.
- Costa, H.C. & Bérnils, R.S. 2018. Répteis brasileiros: lista de espécies. *Herpetologia Brasileira* 7: 11 – 57.
- Derickson, W.K. 1976. Lipid storage and utilization in reptiles. *American Zoologist* 16: 711 – 723.
- Díaz-Vega, R.I.; Aravena, P. A. M.; & Rannou, T. A. 2020. Observaciones de campo en la primera población registrada para Chile del geco *Tarentola mauritanica* (Linnaeus 1758)(Squamata, Phyllodactylidae). *Boletín Chileno de Herpetología* 7: 20 – 26.
- Doughty, P. 1997. The effects of “Fixed” clutch sizes on lizard life-histories: reproduction in the Australian Velvet Gecko, *Oedura lesueurii*. *Journal of Herpetology* 31: 266
- Ferreira, A.; Laura, I.A. & Dolder, H. 2002. Reproductive cycle of male green iguanas, *Iguana iguana* (Reptilia: Sauria: Iguanidae), in the Pantanal region of Brazil. *Brazilian Journal of Morphological Sciences* 19: 23 – 28.
- Ferreira A.; Silva, D.N.; Van-Sluys, M. & Dolder, H. 2009. Seasonal changes in testicular and epididymal histology of the tropical lizard, *Tropidurus itambere* (Rodrigues, 1987), during its reproductive cycle. *Brazilian Journal of Biology* 69: 429 – 435.
- Galdino, C.A.B.; Assis V.B.; Kieffer M.C. & Van-Sluys, M. 2003. Reproduction and fat body cycle of *Eurolophosaurus nanuzae* (Sauria; Tropiduridae) from a seasonal montane habitat of southeastern Brazil. *Journal of Herpetology* 37: 687–694.
- Garda, A.A.; Medeiros, P.H.; Lion, M.B.; Brito, M.R.; Vieira, G.H. & Mesquita, D.O. 2014. Autoecology of *Dryadosaura nordestina* (Squamata: Gymnophthalmidae) from Atlantic forest fragments in Northeastern Brazil. *Zoologia (Curitiba)* 31: 418– 425.
- Goldberg, S.R.; Kraus, F. & Bursey, C.R. 2002. Reproduction in an introduced population of the Brown Anole, *Anolis sagrei*, from O’ahu, Hawaii. *Pacific Science* 56: 163–168.
- Gribbins, K.M. 2011. Reptilian spermatogenesis: A histological and ultrastructural perspective. *Spermatogenesis* 1: 250–269.
- Guedes, J.J.; Fantuzzi, D.; Assis, C.L. & Feio, R.N. 2020. Reproductive biology of *Gymnodactylus darwini* (Gray, 1845) (Squamata: Phyllodactylidae) from southeastern Brazil. *Acta Zoologica* 00: 1 – 9.
- Hopwood, D. 1990. Fixation e fixatives. Pp. 21 – 42. *En: Bancroft JD, Stevens A: Theory and practice of histological techniques. Churchill Livingstone (3rd ed.), New York.*
- Hernández-Gallegos, O.; Granados-González, G.; Rheubert, J.L.; Villagrán-Santacruz, M.; Peña-Herrera, E. & Gribbins, K.M. 2019. Lack of spermatogenic variation in a polymorphic lizard, *Sceloporus aeneus* (Squamata: Phrynosomatidae). *Acta Zoologica* 100: 359 – 364.
- Jenssen, T.A.; Lovern, M.B. & Congdon, J.D. 2001. Field-testing the protandry-based mating system for the lizard, *Anolis carolinensis*: does the model organism have the right model? *Behavioral Ecology and Sociobiology* 50: 162–172.
- Kaliozopoulou, A.; Carretero, M.A. & Llorente, G.A. 2007. Multivariate and geometric morphometrics in the analysis of sexual dimorphism variation in Podarcis lizards. *Journal of Morphology* 268: 152 – 165.
- Lima-Junior, S.E.; Cardone, I.B. & Goitein, R. 2002. Determination of a method for calculation of Allometric Condition Factor of fish. *Acta Scientiarum Maringá* 24: 397 – 400.
- Lozano, A.; Ramírez-Bautista, A. & Uribe, M.C. 2014. Oogenesis and ovarian histology in two populations of the viviparous lizard *Sceloporus grammicus* (Squamata: Phrynosomatidae) from the central Mexican Plateau. *Journal of morphology* 275: 949 – 960.
- Lozano, A.; Uribe, M.C. & Ramírez-Bautista, A. 2015. Seasonal and continuous spermatogenesis in the viviparous lizard *Sceloporus grammicus*, a study of two populations in contrasting environments from the Central Mexican Plateau. *Zoologischer Anzeiger* 254: 72 – 85.
- Mamou, R.; Moudilou, E.; Amroun, M. & Exbrayat, J.M. 2017. Reproductive cycle of male wall lizard, *Podarcis vaucheri* (Reptilia: Sauria: Lacertidae), in Djurdjura, Northern Algeria. *Basic and Applied Herpetology* 31: 77 – 89.
- Mandarim-de-Lacerda, C.A. 1995. Métodos quantitativos em morfologia. Eduerj, Rio de Janeiro, Brazil.
- Mayhew, W.W. & Wright, S.J. 1970. Seasonal changes in testicular histology of three species of the lizard genus *Uma*. *Journal of Morphology* 130: 163–186.
- Mesquita, D.O.; Costa, G.C.; Figueredo, A.S.; França, F.G.; Garda, A.A.; Bello-Soares, A.H. & Werneck, F.P. 2015. The autecology of *Anolis brasiliensis* (Squamata, Dactyloidae) in a Neotropical Savanna. *The Herpetological Journal* 25: 233 – 244.
- Migliore, S.; Braz, H.; Barreto-Lima, A. & Almeida-Santos, S. 2017. Reproductive timing and fecundity in the Neotropical lizard *Enyalius perditus* (Squamata: Leiosauridae). *Acta Herpetologica* 12: 187 – 191.
- Moodley, G.K. & Van-Wyk, J.H. 2007. Folliculogenesis and ovarian histology of the oviparous gecko, *Hemidactylus mabouia* (Sauria: Gekkonidae). *African Journal of Herpetology* 56: 115 – 135.
- Norval, G.; Slater, K.; Brown, L.R.; Mao, J. & Goldberg, S.R. 2019. Interrelation of fat body mass, liver mass, and environmental parameters on the reproductive cycle of the Brown Anole (*Anolis sagrei*), an introduced lizard in Taiwan. *Herpetological Conservation and Biology* 14: 67–79.
- Oitaven, L.P.C.; Ribeiro, F.S.; Moura, G.J.B. & Oliveira, J.B. 2019. Parasites of *Gymnodactylus darwini* Gray, 1845 (Squamata, Phyllodactylidae) from an Atlantic Rainforest fragment. *Acta tropica* 192: 123–128.
- Pike, D. A., Andrews, R. M., & Du, W. G. 2012. Eggshell morphology and gekkotan life-history evolution. *Evolutionary Ecology* 26: 847 – 861.
- Pinilla, M.P.R. 1991. Reproductive and fat body cycles of the

- viviparous lizard *Liolaemus huacahuasicus*. *Journal of Herpetology* 25: 205–208.
- Pinilla, M.P.R. 1995. Reproductive and fat body cycles of the oviparous lizard *Liolaemus bitaeniatus* (Sauria: Tropiduridae). *Journal of Herpetology* 29: 256 – 260.
- Ramirez-Bautista, A.; Hernandez-Ramos, D.; Rojas-Martínez, A. & Marshall, J.C. 2009. Fat bodies and liver mass cycles in *Sceloporus grammicus* (Squamata: Phrynosomatidae) from Southern Hidalgo, México. *Herpetological Conservation and Biology* 4: 164 – 170.
- Lara-Resendiz, R.A. 2020. "¿Qué implicaciones ecofisiológicas tiene la actividad nocturna en reptiles "diurnos"?": una revisión". *Acta Biológica Colombiana* 25: 314 – 326.
- Robinson, G. & Gray, T. 1990. Electron microscopy 2: Tissue preparation, sectioning and staining. Pp. 525–562. *En: Theory and practice of histological techniques*. Edinburgh (3rd Ed.). British Library, London, England.
- Salvador, A. 2011 Salamanquesa común *Tarentola mauritanica*. *En: Enciclopedia Virtual de los Vertebrados Españoles*. Salvador, A, A Marco (Eds). Museo Nacional de Ciencias Naturales, Madrid. En línea: < <http://www.vertebradosibericos.org/>>. [Consulta: august 6th, 2021].
- Sánchez-Hernández, P.; Molina-Borja, M. & Ramírez-Pinilla, M.P. 2013. Annual Reproductive Cycle in the Scincid Lizard *Chalcides viridanus* from Tenerife, Canary Islands. *Current herpetology* 32: 170 – 181.
- Santos, L.R.S.; & de Oliveira, C. 2008. Histological aspects and structural characteristics of the testes of *Dendropsophus minutus* (Anura, Hylidae). *Micron* 39: 1266 – 1270.
- Santos, H.S.; Menezes, V.G.; Freire, E.M.X.; Matos, M.H.T. & Ribeiro, L.B. 2020. Correlation between the granulosa cell layer and active caspase-3 expression in ovarian follicles of *Tropidurus hispidus* and *T. semitaeniatus* (Squamata, Tropiduridae): immunohistochemical approach. *Cuadernos de Herpetología* 34: 239 – 246.
- Serrano-Cardozo, V.H.; Ramírez-Pinilla, M.P.; Ortega, J.E. & Cortes, L.A. 2007. Annual reproductive activity of *Gonatodes albogularis* (Squamata: Gekkonidae) living in an anthropic area in Santander, Colombia. *South American Journal of Herpetology* 2: 31 – 38.
- Sexton, O.J. & Brown, K.M. 1977. The reproductive cycle of an iguanid lizard *Anolis sagrei*, from Belize. *Journal of Natural History* 11: 241–250.
- Sylva, D.P.; & Breder, P.R. 1997. Reproduction, gonad histology, and spawning cycles of north Atlantic billfishes (Istiophoridae). *Bulletin of Marine Science* 60: 668 – 697.
- Takashiba, K.S.; Segatelli, T.M.; de Moraes, S.M.F.; & Natali, M.R.M. 2011. Morfologia testicular de ratos Wistar obesos sedentários e submetidos a treinamento físico. *Acta Scientiarum. Health Sciences* 33: 25 – 33.
- Torki, F. 2007. Reproductive cycle of the Snake-eyed Lizard *Ophisops elegans* Ménétriés, 1832 in western Iran. *Herpetozoa* 20: 57 – 66.
- Tumkiratiwong, P.; Meesuk, W.; Chanhom, L.; & Aowphol, A. 2012. Reproductive patterns of captive male and female monocled cobra, *Naja kaouthia* (Lesson, 1831). *Zool Stud* 51: 692 – 700.
- Uribe, M.C.A.; Omana, M.E.M.; Quintero, J.E.G. & Guillette, L.J. 1995. Seasonal variation in ovarian histology of the viviparous lizard *Sceloporus torquatus torquatus*. *Journal of Morphology* 226: 103 – 119.
- Vazzoler, A.E.A.M. 1982. Manual de métodos para estudos biológicos de populações de peixes. Reprodução e crescimento. Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasília, Distrito Federal, Brazil.
- Vazzoler, A.E.A.M. 1996. Biologia da reprodução de peixes teleósteos: teoria e prática. NUPELIA, Maringá, Paraná, Brazil.
- Watling, J.I.; Waddle, J.H.; Kizirian, D. & Donnelly, M.A. 2005. Reproductive phenology of three lizard species in Costa Rica, with comments on seasonal reproduction of neotropical lizards. *Journal of Herpetology* 39:341 – 348.
- Weibel, E.R. 1979. Fleischner Lecture: looking into the lung: what can tell us? *American Journal of Roentgenology* 133: 1021–1031.
- Wiederhecker, H.C.; Pinro, A.C.S. & Colli, G.R. 2002. Reproductive ecology of *Tropidurus torquatus* (Squamata: Tropiduridae) in the highly seasonal cerrado biome of central Brazil. *Journal of Herpetology* 36: 82 – 91.
- Zar, J.H. 1999. Biostatistical analysis. Prentice-Hall, New Jersey, USA.

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

Lucas Henrique Carvalho Siqueira^{1,2}, Jade Lima-Santos³, Karina Maria Pereira Silva¹

¹ Laboratório de Ecologia e Evolução, Instituto Butantan, Av. Vital Brazil 1500, Butantã, São Paulo, SP, 05503-900, Brazil.

² Departamento de Zoologia e Botânica, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho", Rua Cristóvão Colombo, 2265, Jardim Nazareth, São José do Rio Preto, SP, 15054-000, Brazil.

³ Programa de Pós-Graduação em Ecologia e Evolução, Universidade Federal de São Paulo, Rua Artur Riedel, 275, Jd. Eldorado, Diadema, SP, 09972-270, Brazil.

Recibido: 23 Septiembre 2021

Revisado: 11 Marzo 2022

Aceptado: 30 Marzo 2022

Editor Asociado: V. Arzamendia

doi: 10.31017/CdH.2022.(2021-060)

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 semifosorial *Atractus zebrinus* a través 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 reproductivo; 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-

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 Puerto, 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 Puerto, 1993; Giraud 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 ($5 \mu\text{m}$) 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 \pm standard deviation (SD) of the total sampling number and range of distribution.

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

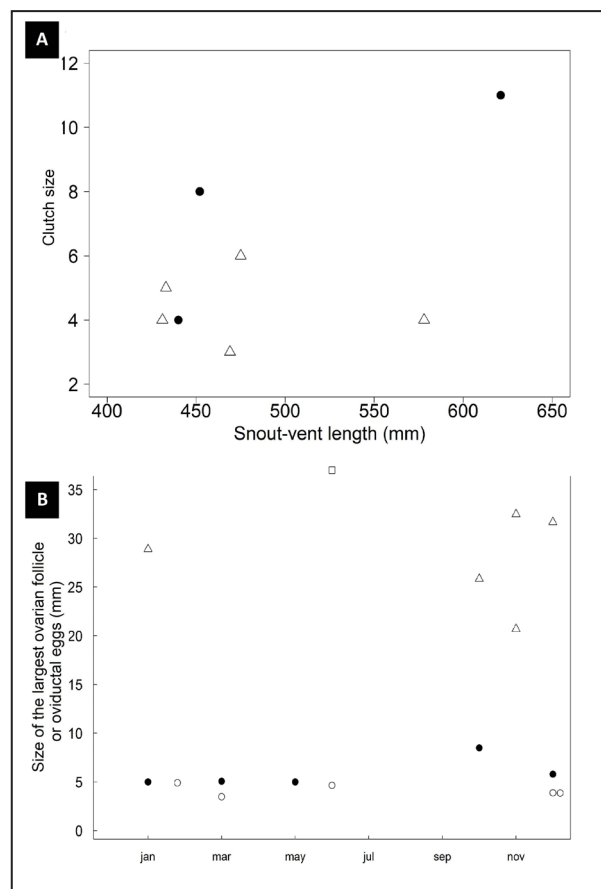


Figure 1. Reproductive traits of female *Atractus zebrinus*. (a) Relationship between maternal snout-vent length and clutch size. (b) Seasonal variation in the size of the largest ovarian follicles/oviductal eggs and timing of eggs found in nature. Note: Black circles = follicles in early vitellogenesis;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

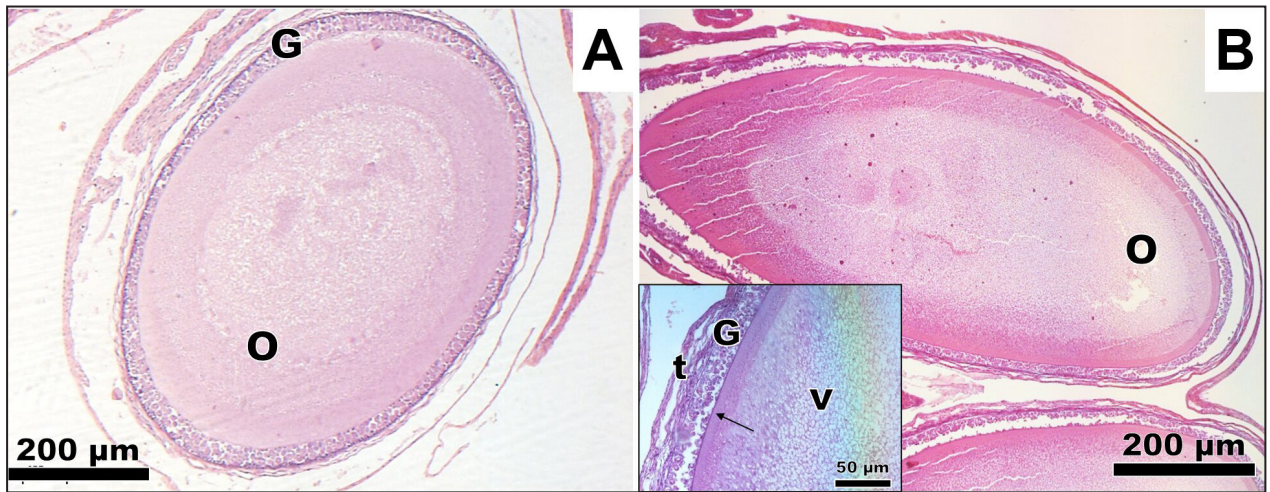


Figure 2. Histology of the ovarian follicles of *Atractus zebrinus* at the onset of vitellogenesis. (a) 5 mm diameter follicle. (b) 8.5 mm diameter follicle. Note the vacuolated ooplasm: G = Granulosa layer; V = vesicles; O = oocyte; t = theca. The arrow indicates the zona radiata. Hematoxylin-eosin.

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)

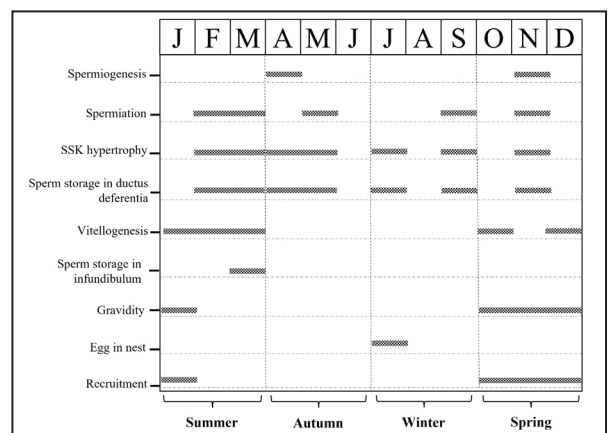


Figure 3. Overview of the reproductive events in male and female *Atractus zebrinus*.

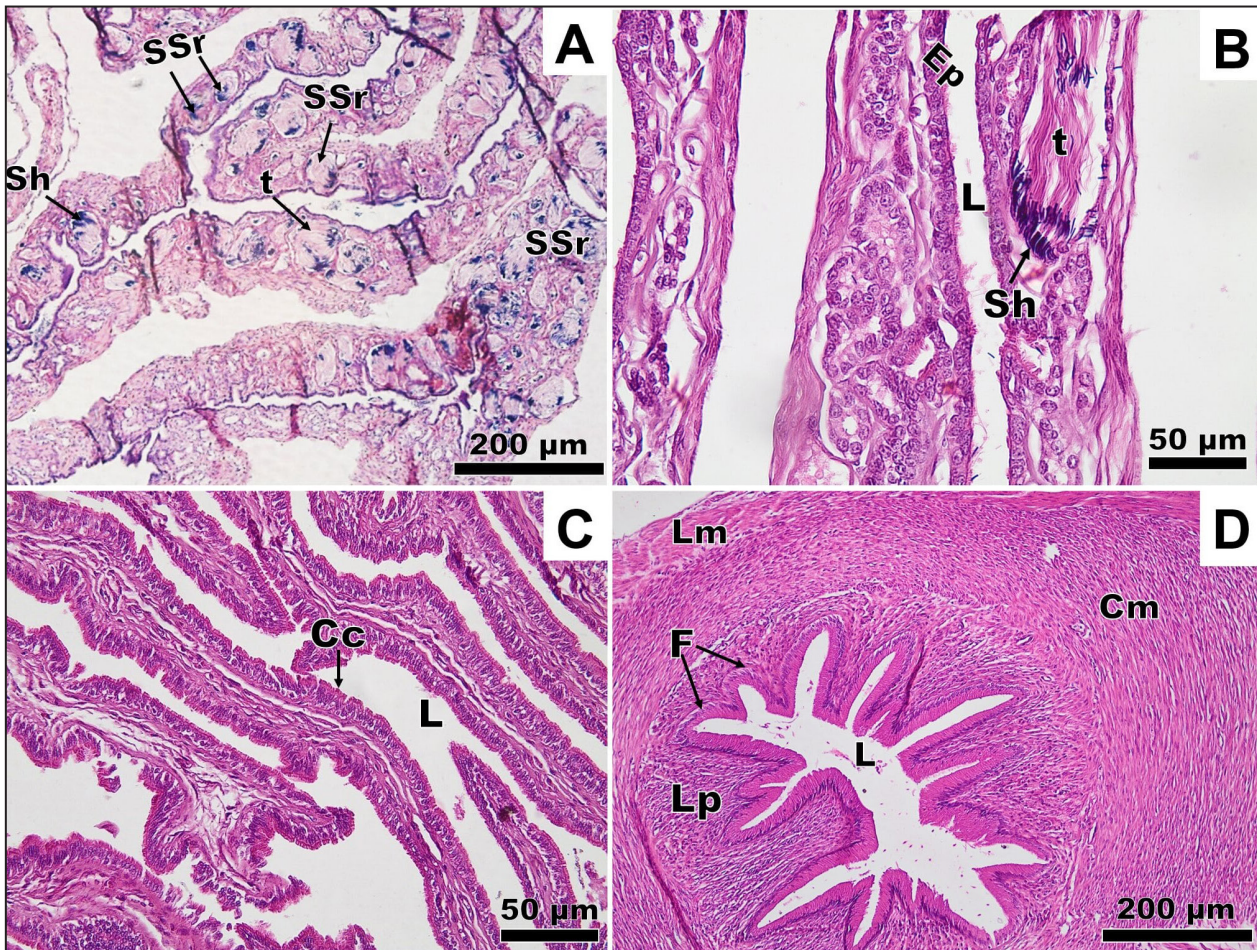


Figure 4. Histology of the reproductive tract of female *Atractus zebrinus*. (a) Posterior infundibulum showing various alveolar glands with sperm. (b) Higher magnification of sperm receptacles. (c) Nonglandular uterus. (d) Pouch showing thick epithelium. *Note:* SSr = Sperm storage receptacles; Sh = 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),

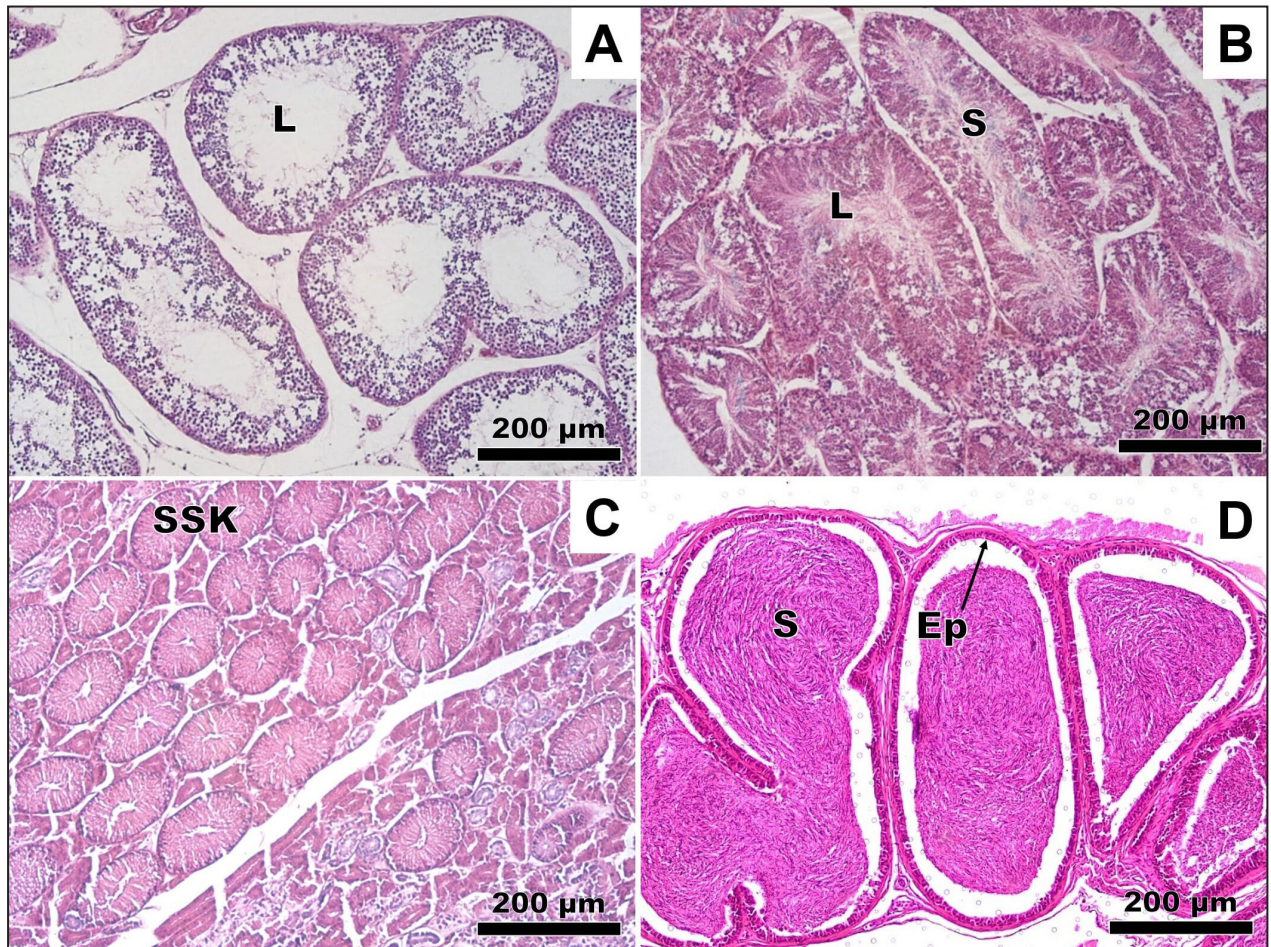


Figure 5. Histology of the reproductive tract of male *Atractus zebrinus*. (a) Seminiferous tubules in spermiogenesis. (b) Seminiferous tubules in spermiation, peak of cell division. (c) Hypertrophy of the sexual segment of the kidney. (d) Ductus deferens with the lumen packed with sperm. Note: L = lumen;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

Acknowledgments

We thank Paulo Passos, Giuseppe Puerto, Felipe Grazziotin, Paulo Roberto Manzani, and Julio Moura Leite for allowing access to specimens under their care. We also thank Valdir Germano for his help in the collection of the Instituto Butantan. We are grateful to Selma Maria Almeida-Santos for providing access to the laboratory of histology and Carlos Jared, Marta Antoniazzi, and Maria José de J. Silva for allowing access to the microscope equipment for photographing the histological slides. We also thank Luciana Sato for her help with the microscope equipment of the Instituto Butantan, Karina R. E. Gomes for providing information on the eggs found in nature, and Henrique B. Braz for comments on the manuscript and language editing.

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.

Literature cited

- Abegg, A.D.; Passos, P.; Mario-Da-Rosa, C.; Azevedo W. Dos S.; Malta-Borges, L. & Bubadué J.M. 2020. Sexual dimorphism, ontogeny and static allometry of a semi-fossorial snake (genus *Atractus*). *Zoologischer Anzeiger* 287: 95-104.
- Abramoff, M.D.; Magalhães P.J. & Ram, S.J. 2004. Image processing with ImageJ. *Biophotonics International* 11: 36-42. <https://doi.org/10.1016/j.jcz.2020.05.008>
- Aldridge, R.D.; Jellen, B.C.; Siegel, D.S. & Wisniewski, S.S. 2011. The Sexual Segment of the Kidney: 477-509. In: Aldridge, R.D. & Sever, D.M. (Eds), *Reproductive Biology and Phylogeny of Snakes*. Science Publishers, Enfield.
- Aldridge, R.D. & Duvall, D. 2002. Evolution of the mating season in the pitvipers of North America. *Herpetological Monographs* 16: 1-25.
- Almeida-Santos, S.M.; Braz, H.B.; Santos, L.C.; Sueiro, L.R.; Barros, V.A.; Rojas, C.A. & Kasperoviczus, K.N. 2014. Biologia reprodutiva de serpentes: recomendações para a coleta e análise de dados. *Herpetologia Brasileira* 3: 14-24
- Alves, F.Q.; Argolo, A.J.S. & Jim, J. 2005. Biologia reprodutiva de *Dipsas neivai* e *Dipsas catesbyi* (Santzen) (Serpentes, Colubridae) no sudeste da Bahia, Brasil. *Revista Brasileira de Zoologia* 22: 573-579.
- Angarita-Sierra, T. & López-Hurtado C.A. 2020. Exploring the reproductive ecology of the tropical semifossorial snake *Ninia atrata*. *Zoological research*, 41: 157. [10.24272/j.issn.2095-8137.2020.015](https://doi.org/10.24272/j.issn.2095-8137.2020.015)
- Arnold, S.J. 1988. Quantitative genetics and selection in natural populations: microevolution of vertebral numbers in the Garter snake *Thamnophis elegans*: 619-637. In: Weir, B.S.; Eisen, E.J.; Goodman, M.M. & Namkoong, G. (Eds), *Proceedings of the Second International Conference on Quantitative Genetics*. Sinauer Associates Inc, Sunderland.
- Balestrin, R.L. & Di-Bernardo, M. 2005. Reproductive biology of *Atractus reticulatus* (Boulenger, 1885) (Serpentes, Colubridae) in southern Brazil. *Herpetological Journal* 15: 195-199.
- Barbo, F.E.; Marques, O.A.V. & Sawaya, R.J. 2011. Diversity, natural history, and distribution of snakes in the municipality of São Paulo. *South American Journal of Herpetology* 6: 135-160.
- Barros, V.A.; Rojas, C.A. & Almeida-Santos, S.M. 2014. Is rainfall seasonality important for reproductive strategies in viviparous Neotropical pit vipers? a case study with *Bothrops leucurus* from Brazil Atlantic Forest. *Herpetological Journal* 24: 67-75.
- Barros, V.A.; Sueiro, L.R. & Almeida-Santos, S.M. 2012. Reproductive biology of the neotropical rattlesnake *Crotalus durissus* from northeastern Brazil: a test of phylogenetic conservatism of reproductive patterns. *Herpetological Journal* 22: 97-104.
- Barros, F.C.; Herrel, A. & Kohlsdorf, T. 2011. Head shape evolution in Gymnophthalmidae: does habitat use constrain the evolution of cranial design in fossorial lizards? *Journal of Evolutionary Biology* 24: 2423-2433.
- Bellini, G.P.; Arzamendia, V. & Giraud, A.R. 2013. Ecology of *Thamnodynastes hypoconia* in subtropical-temperate South America. *Herpetologica* 69: 67-79. [doi: https://doi.org/10.1655/HERPETOLOGICA-D-12-00027](https://doi.org/10.1655/HERPETOLOGICA-D-12-00027)
- Bizerra, A.F.; Marques, O.A.V. & Sazima, I. 2005. Reproduction and feeding of the colubrid snake. *Amphibia-Reptilia* 26: 33-38. [Doi: 10.1163/1568538053693350](https://doi.org/10.1163/1568538053693350)
- Böhm, M. *et al.* 2013. The conservation status of the world's reptiles. *Biological Conservation* 157: 372-385. [doi: org/10.1016/j.biocon.2012.07.015](https://doi.org/10.1016/j.biocon.2012.07.015)
- Braz, H.B.; Kasperoviczus, K.N. & Guedes, T.B. 2019. Reproductive Biology of the Fossorial Snake *Apostolepis gaboi* (Elapomorhini): A Threatened and Poorly Known Species from the Caatinga Region. *South American Journal of Herpetology* 14: 37-47.
- Braz, H.B.; Kasperoviczus, K.N. & Almeida-Santos, S.M. 2014. Reproductive ecology and diet of the fossorial snake *Phalotris lativittatus* in the Brazilian Cerrado. *Herpetological Journal* 24: 49-57.
- Brown, G.P. & Shine, R. 2006. Why do most tropical animals reproduce seasonally? Testing hypotheses on an Australian snake. *Ecology* 87: 133-143.
- Costa, H.C. & Bérnils, R.S. 2018. Répteis do Brasil e suas Unidades Federativas: Lista de espécies. *Herpetologia Brasileira* 8:.
- Crews, D. & Moore, M.C. 1986. Evolution of mechanisms controlling mating behavior. *Science* 231: 121-125.
- Crews, D. & Garstka, W.R. 1982. The Ecological Physiology of a Garter Snake. *Scientific American* 247: 158-168. [doi: org/10.1038/scientificamerican1182-158](https://doi.org/10.1038/scientificamerican1182-158)
- Cyriak, V.P. & Kodandaramaiah, U. 2018. Digging their own macroevolutionary grave: fossoriality as an evolutionary dead end in snakes. *Journal of Evolutionary Biology* 31: 587-598.
- DeNardo, D.F. & Taylor, E.N. 2011. Hormones and reproduction in free-ranging snakes: 265-287. In Aldridge, R.D. & Sever, D.M. (Eds.), *Reproductive Biology and Phylogeny of Snakes*, FL: CRC Press, Boca Raton.
- De Resende, F.C. & Nascimento, L.B. 2015. The Female Reproductive Cycle of the Neotropical Snake *Atractus pantostictus* (Fernandes and Puerto, 1993) from South-eastern Brazil. *Anatomia, histologia, embryologia* 44: 225-235. [doi:org/10.1111/ah.12132](https://doi.org/10.1111/ah.12132)
- Dowling, H.G. 1951. A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1: 97-99. doi.org/10.1080/04416651.1965.9650679
- Fernandes, R.; Freire, E.M.X. & Puerto, G. 2000. Geographic variation of the Brazilian atlantic rain forest snake *Atractus maculatus* (Günther, 1858) with the revalidation of *Rhabdosoma zebrinum* Jan, 1862 (Serpentes: Colubridae). *Boletim do Museu Nacional Nova Série Zoologia* 419: 1-8.
- Fernandes, R. & Puerto, G. 1993. A new species of *Atractus* from Brazil and the status of *A. guentheri* (Serpentes: Colubridae). *Memórias do Instituto Butantan* 55: 7-14.
- Ferreira-Silva, C.; Ribeiro, S.C.; De Alcântara, E.P. & Ávila, R.W. 2019. Natural history of the rare and endangered snake *Atractus ronnie* (Serpentes: Colubridae) in northeastern Brazil. *Phyllomedusa* 18: 77-87.
- Friesen, C.R.; Kahrl, A.F. & Olsson, M. 2020. Sperm competition in squamate reptiles: Sperm competition in lizards and snakes. *Philosophical Transactions of the Royal Society B: Biological Sciences* 375. doi.org/10.1098/rstb.2020.0079
- Friesen, C.R.; Shine, R., Krohmer, R.W. & Mason, R.T. 2013. Not

- just a chastity belt: The functional significance of mating plugs in garter snakes, revisited. *Biological Journal of the Linnean Society* 109: 893-907.
- García-Cobos, D.; Crawford, A.J. & Ramírez-Pinilla, M.P. 2021. Reproductive phenology in a Neotropical aquatic snake shows marked seasonality influenced by rainfall patterns. *Journal of Natural History* 54: 1845-1862.
- Giraud, A.R. & Scrocchi, G.J. 2000. The genus *Atractus* (Serpentes: Colubridae) in north-eastern Argentina. *Herpetological Journal* 10: 81-90.
- Goldberg, S.R. 2004. Reproduction of the coffee snake, *Ninia maculata* (Serpentes: Colubridae), From Costa Rica. *Texas Journal of Science* 56: 81-84.
- Grazziotin, F.G.; Zaher, H.; Murphy, R.W.; Scrocchi, G.; Benavides, M.A.; Zhang, Y. & Bonatto, S.L. 2012. Molecular phylogeny of the New World Dipsadidae (Serpentes: Colubroidea): a reappraisal. *Cladistics* 28: 437-459. doi.org/10.1111/j.1096-0031.2012.00393.x
- Gualdrón-Durán, L.E.; Calvo-Castellanos, M.F. & Ramírez-Pinilla, M.P. 2019. Annual Reproductive Activity and Morphology of the Reproductive System of an Andean Population of *Atractus* (Serpentes, Colubridae). *South American Journal of Herpetology* 14: 58. doi.org/10.2994/sajh-d-16-00054.1
- Junqueira, L.C. & Carneiro, J. 2013. Biologia celular e molecular. Guanabara Koogan, Rio de Janeiro.
- King, R.B. 1989. Sexual dimorphism in snake tail length: sexual selection, natural selection, or morphological constraint? *Biological Journal of the Linnean Society* 38: 133-154.
- Khoury, R. S.; Almeida-Santos, S. M. & Fernandes D. S. 2020. Anatomy of the reproductive system of a population of *Amerotyphlops brongersmianus* from southeastern Brazil (Serpentes: Scolecophidia). *The Anatomical Record* 330: 2485-2496. DOI: 10.1002/ar.24382.
- Krohmer, R.W. 2004. Variation in seasonal ultrastructure of sexual granules in the renal sexual segment of the northern water snake, *Nerodia sipedon sipedon*. *Journal of Morphology* 261: 70-80.
- Levine, B.A.; Schuett, G.W. & Booth, W. 2021. Exceptional long-term sperm storage by a female vertebrate. *PLOS ONE* 16(6): e0252049.
- Lindell, L.E.; Forsman, A. & Merilä, J. 1993. Variation in number of vertebral scales in snakes: effects on body size, growth rate and survival in the Adder, *Vipera berus*. *Journal of Zoology* 1993: 101-115.
- Loebens, L.; Almeida-Santos, S.M. & Cechin, S.Z. 2020. Reproductive biology of the Sword Snake *Tomodon dorsatus* (Serpentes: Dipsadidae) in South Brazil: comparisons within the tribe Tachymenini. *Amphibia-Reptilia* 41: 445-459. doi.org/10.1163/15685381-bja10012
- Loebens, L.; Rojas, C.A.; Almeida-Santos, S.M. & Cechin, S.Z. 2017. Reproductive biology of *Philodryas patagoniensis* (Snakes: Dipsadidae) in south Brazil: Female reproductive cycle. *Acta Zoologica* 99: 105-114.
- Loebens, L.; Cechin, S.Z.; Theis, T.F.; Moura, L.B. & Almeida-Santos, S.M. 2016. Reproductive biology of *Philodryas patagoniensis* (Snakes: Dipsadidae) in south Brazil: Male reproductive cycle. *Acta Zoologica* 98, 329-339. doi: 10.1111/azo.12172
- Lovich, J.E. & Gibbons, J.W.; 1992. A review of techniques for quantifying sexual size dimorphism. *Growth, Development and Aging* 56: 269-281.
- Lutterschmidt, D.I. & Masob, R.T. 2009. Endocrine mechanisms mediating temperature-induced reproductive behavior in red-sided garter snakes (*Thamnophis sirtalis parietalis*). *Journal of Experimental Biology* 212: 3108-3118.
- Manes, M.E.; Noriega, T.; Casal F.C. & Apichela, S. 2007. Ovarian changes during the reproductive cycle of the *Tupinambis merianae* lizard raised in a temperate environment. *Cuadernos de Herpetología* 21: 21-29.
- Marques, O.A.V.; Eterovic, A. & Sazima, I. 2019. Serpentes da Mata Atlântica: guia ilustrado para as florestas costeiras do Brasil. Ponto A Editora, Cotia.
- Marques, O.A.V. & Puerto, G. 1998. Feeding, reproduction and growth in the crowned snake. *Amphibia-Reptilia* 19: 311-318. doi.org/10.1163/156853898X00214
- Marques, O.A.V. 1996. Reproduction, seasonal activity and growth of the coral snake, *Micrurus corallinus* (Elapidae), in the southeastern Atlantic Forest in Brazil. *Amphibia-Reptilia* 17: 277-285. doi.org/10.1163/156853896X00441
- Martins, M. & Oliveira, M.E. 1993. The snakes of the genus *Atractus* Wagler (Reptilia: Squamata: Colubridae) from the Manaus region, central Amazonia, Brazil. *Zoologische Mededelingen* 67: 21-40.
- Mathies, T. 2011. Reproductive cycles of tropical snakes: 511-550. In: Aldridge, R.D. & Sever, D.M. (Eds), Reproductive biology and phylogeny of snakes. CRC Press, Boca Raton.
- Mendonça, F. & Danni-Oliveira, I.M. 2007. Climatologia: Noções básicas e climas do Brasil. Oficina de Textos, São Paulo.
- Meneses-Pelayo, E. & Passos, P. 2019. New Polychromatic Species of *Atractus* (Serpentes: Dipsadidae) from the Eastern Portion of the Colombian Andes. *Copeia* 2019: 250-261. doi.org/10.1643/CH-18-163
- Myers, C.W. 2003. Rare snakes-five new species from eastern Panama: reviews of northern *Atractus* and southern *Geophis* (Colubridae: Dipsadinae). *American Museum Novitates* 2003: 1-47.
- Navas, C.A.; Antoniazzi, M.M.; Carvalho, J.E.; Chaui-Berlink, J.G.; James, R.S.; Jared, C.; Kohlsdorf, T.; Pai-Silva, M.D. & Wilson, R.S. 2004. Morphological and physiological specialization for digging in amphisbaenians, an ancient lineage of fossorial vertebrates. *Journal of Experimental Biology* 207: 2433-2441
- Nogueira, C.C. et al. 2019. Atlas of Brazilian snakes: Verified point-locality maps to mitigate the Wallacean shortfall in a megadiverse snake fauna. *South American Journal of Herpetology*, 14: 1-274. doi.org/10.2994/SAJH-D-19-00120.1.
- Passos, P.; Prudente, A. L.C.; Ramos, L.O.; Caicedo-Portilla, J.R. & Lynch, J.D. 2018. Species delimitations in the *Atractus collaris* complex (Serpentes: Dipsadidae). *Zootaxa*, 4392: 491-520. doi.org/10.11646/zootaxa.4392.3.4
- Passos, P.; Echevarría, L.Y. & Venegas, P.J. 2013. Morphological variation of *Atractus carrioni* (Serpentes: Dipsadidae). *South American Journal of Herpetology* 8: 109-120.
- Passos, P.; Fernandes, R.; Bernils, R.S. & Moura-Leite, J.D. 2010a. Taxonomic revision of the Brazilian Atlantic Forest *Atractus* (Reptilia: Serpentes: Dipsadidae). *Zootaxa* 23: 64-63.
- Passos, P.; Dobiey, M. & Venegas, P.J. 2010b. Variation and natural history notes on giant groundsnake, *Atractus gigas* (Serpentes: Dipsadidae). *South American Journal of Herpetology* 5: 73-82.
- Perkins, M.J. & Palmer, B.D. 1996. Histology and functional morphology of the oviduct of an oviparous snake, *Diadophis*

- punctatus. *Journal of Morphology* 227: 67-79.
- Pizzatto, L.; Cantor, M., Oliveira, J.L; Marques, O.A.V.; Capovilla, V. & Martins, M. 2008. Reproductive Ecology of Dipsadine Snakes, With Emphasis on South American Species. *Herpetologica*, 64: 168-179. doi.org/10.1655/07-031.1
- Pizzatto, L. & Marques, O.A.V. 2002. Reproductive biology of the false coral snake *Oxyrhopus guibei* (Colubridae) from southeastern Brazil. *Amphibia-Reptilia* 23: 495-504. doi.org/10.1163/15685380260462392
- Pizzatto, L. & Marques, O.A.V. 2006. Interpopulational variation in reproductive cycle and activity of the water snake *Liophis miliaris* (Colubridae) in Brazil. *Herpetological Journal* 16: 353-362.
- Pleguezuelos, J.M. & Feriche, M. 1999. Reproductive ecology of the horseshoe whip snake (*Coluber hippocrepis*) in the Iberian Peninsula. *Journal of Herpetology* 33: 202–207. doi.org/10.2307/1565715
- Pough, F.H. 1980. The advantages of ectothermy for tetrapods. *American Naturalist* 115: 92-112.
- R Development Core Team. 2021. R: A language and environment for statistical computing. Retrieved from <https://www.r-project.org>.
- Rivas, J.A. & Burghardt, G.M. 2001. Understanding sexual size dimorphism in snakes: wearing the snake's shoes. *Animal Behavior* 61: 1-6.
- Rojas, C.A.; Barros, V.A. & Almeida-Santos, S.M. 2017. A histological and ultrastructural investigation of the female reproductive system of the water snake (*Erythrolamprus miliaris*): Oviductal cycle and sperm storage. *Acta Zoologica* 2017: 1-12.
- Rojas, C.A, Barros, V.A & Almeida-Santos, S.M. 2015. Sperm storage and morphofunctional bases of the female reproductive tract of the snake *Philodryas patagoniensis* from southeastern Brazil. *Zoomorphology* 134: 577-586.
- Rojas, C.A.; Barros, V.A. & Almeida-Santos S.M. 2013. The reproductive cycle of the male sleep snake *Sibynomorphus mikanii* (Schlegel, 1837) from southeastern Brazil. *Journal of Morphology* 274: 215-228.
- Saint-Girons, H. 1982. Reproductive cycles of male snakes and their relationships with climate and female reproductive cycles. *Herpetologica* 38: 5-16.
- Saint-Girons, H. 1957. Le cycle sexuel chez *Vipera aspis* dans l'ouest de la France. *Bulletin Biologique de la France et de la Belgique* 91: 284–350.
- Sandoval, M.; Palomas, S.; Ayarragaray, M. & Álvarez, B.B. 2009. Nesting site, clutch size and development of *Atractus reticulatus* (Serpentes, Colubridae) from Corrientes, Argentina. *Herpetological Bulletin* 109: 11-16.
- Schuett, G.W.; Harlow, H.J.; Rose, J.D.; Van Kirk, E.A. & Murdoch; W.J. 1997. Annual cycle of plasma testosterone in male copperheads, *Agkistrodon contortrix* (Serpentes, Viperidae): Relationship to timing of spermatogenesis, mating, and agonistic behavior. *General and Comparative Endocrinology* 105: 417-424.
- Sever, D.M. & Hopkins, W.A. 2005. Renal sexual segment of the ground skink, *Scincella laterale* (Reptilia, Squamata, Scincidae). *Journal of Morphology* 266: 46-59.
- Shine, R.; Olsson, M.M.; Moore, I.T.; Lemaster, M.P. & Mason, R.T. 1999. Why do male snakes have longer tails than females? *Proceedings of the Royal Society B* 266: 2147-2151.
- Shine, R. 1994. Sexual size dimorphism in snakes revisited. *Copeia* 1994: 326-346.
- Shine R. 1993. Sexual dimorphism in snakes: 49-86. In: Seigel, R.A. & Collins, J.T. (Eds), *Snakes: Ecology and Behavior*, McGraw-Hill, New York.
- Shine R. 1978. Growth rates and sexual maturation in six species of Australian Elapid snakes. *Herpetologica* 34: 73-79.
- Siegel, D.S.; Miralles, A.; Chabarria, R.E. & Aldridge, R.D. 2011. Female reproductive anatomy: Cloaca, Oviduct, and Sperm storage: 347-409. In: Aldridge R. D.; & Sever, D.M. (Eds.). *Reproductive Biology and Phylogeny of Snakes*. CRC Press, Boca Raton.
- Siegel, D.S. & Sever, D.M. 2008. Sperm Aggregations in female *Agkistrodon piscivorus* (Reptilia, Squamata): A histological and ultrastructural investigation. *Journal of Morphology* 269: 189-206.
- Silva, K.M.P.; Silva, K.B.; Sueiro, L.R.; Oliveira, M.E.E.S. & Almeida-Santos, S.M. 2019a. Reproductive biology of *Bothrops atrox* (Serpentes, Viperidae, Crotalinae) from the Brazilian Amazon. *Herpetologica* 75: 198-207.
- Silva, K.M.P.; Barros, V.A.; Rojas, C.A. & Almeida-Santos, S.M. 2019b. Infundibular sperm storage and uterine muscular twisting in the Amazonian lancehead, *Bothrops atrox*. *The Anatomical Record* 303: 3145-3154. doi.org/10.1002/ar.24309
- Silva, K.M.P.; Braz, H.B.; Kasperoviczus, K.N.; Marques, O.A.V. & Almeida-Santos, S.M. 2020. Reproduction in the pitviper *Bothrops jararacussu*: Large females increase their reproductive output while small males increase their potential to mate. *Zoology*, 142, 125816. <https://doi.org/10.1016/j.zool.2020.125816>
- Taylor, E.N.; DeNardo, D.F. & Jennings, D.H. 2004. Seasonal steroid hormone levels and their relation to reproduction in the Western Diamond-backed Rattlesnake, *Crotalus atrox* (Serpentes: Viperidae). *General and Comparative Endocrinology* 136: 328-337. doi.org/10.1016/j.ygcen.2004.01.008
- Teixeira, V.H.S.; Quintela, F.M. & Loebmann, D. 2020. Reproductive biology of *Erythrolamprus jaegeri coralliventris* (Serpentes: Dipsadidae) in the Brazilian Coastal Pampa. *Anais da Academia Brasileira de Ciências* 92: e20181383. DOI 10.1590/0001-3765202020181383.
- Travaglia-Cardoso, S.R. & Maia, D.C. 2012. Clutch size and incubation period of *Atractus pantostictus* (Fernandes & Puerto, 1993) (Serpentes: Dipsadidae). *Biologia Geral e Experimental* 12: 5-6.
- Uller, T. & Olsson, M. 2008. Multiple paternity in reptiles: Patterns and processes. *Molecular Ecology* 17: 2566-2580. doi.org/10.1111/j.1365-294X.2008.03772.x
- Vieira, S.; Romero De Perez, G. & Ramirez-Pinilla, M.P. 2010. Ultrastructure of the ovarian follicles in the placentotrophic Andean Lizard of the genus *Mabuia* (Squamata: Scincidae). *Journal of Morphology* 271: 738-749.
- Vitt, L.J. 1992. Diversity of Reproductive Strategies Among Brazilian Lizards and Snakes: The Significance of Lineage and Adaptation: 135 – 149: In Hamlet, W.C. (Ed.), *Reproductive Biology of South American Vertebrates II*, Springer-V. doi.org/10.1007/978-1-4612-2866-0_10
- Zaher, H. *et al.* 2019. Large-scale molecular phylogeny, morphology, divergence-time estimation, and the fossil record of advanced caenophidian snakes (Squamata: Serpentes). *PloS One* 14. doi.org/10.1371/journal.pone.0216148
- Zanella, N. & D'agostini, F. 2018. Ecology of the snake *Atractus paraguayensis* (Dipsadidae) in southern Brazil. *Zoologia* 35:

e12487 doi.org/10.3897/zoologia.35.e12487

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.

© 2022 por los autores, licencia otorgada a la Asociación Herpetológica Argentina. Este artículo es de acceso abierto y distribuido bajo los términos y condiciones de una licencia Atribución-No Comercial 4.0 Internacional de Creative Commons. Para ver una copia de esta licencia, visite <http://creativecommons.org/licenses/by-nc/4.0/>

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

Gustavo J. Scrocchi^{1,3}, Alejandro R. Giraudo², Santiago J. Nenda³

¹ Instituto de Herpetología, Unidad Ejecutora Lillo, Fundación Miguel Lillo – CONICET, Miguel Lillo 251, 4000 San Miguel de Tucumán, Tucumán, Argentina.

² Instituto Nacional de Limnología – CONICET, UNL, Ciudad Universitaria, 3000 Santa Fe, Argentina.

³ División Herpetología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" – CONICET, Ángel Gallardo 470, 1405 Buenos Aires, Argentina.

Recibido: 09 Enero 2022

Revisado: 06 Abril 2022

Aceptado: 22 Abril 2022

Editor Asociado: D. Baldo

doi: 10.31017/CdH.2022.(2022-002)

urn:lsid:zoobank.org:act:4C0E9CEC-781A-4821-9CE8-401319C47B16

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, redescrimos 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 Elapomorhini 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). Elapomorhini 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 Elapomorhini 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

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. Puerto 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 Puerto 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 (Puerto 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* (Puerto and Ferrarezzi, 1993). This last arrangement was followed by most of the posterior authors that studied the genus (Giraudó, 2001; Giraudó and Scrocchi, 2002). Recently, Cabral and Cacciali (2015) proposed a new species from Paraguay named *P. normanscottii*. 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. normanscottii* present in western Paraguay (Puerto 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 Puerto 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), Puerto 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.

urn:lsid:zoobank.org:act:4C0E9CEC-781A-4821-9CE8-401319C47B16

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),

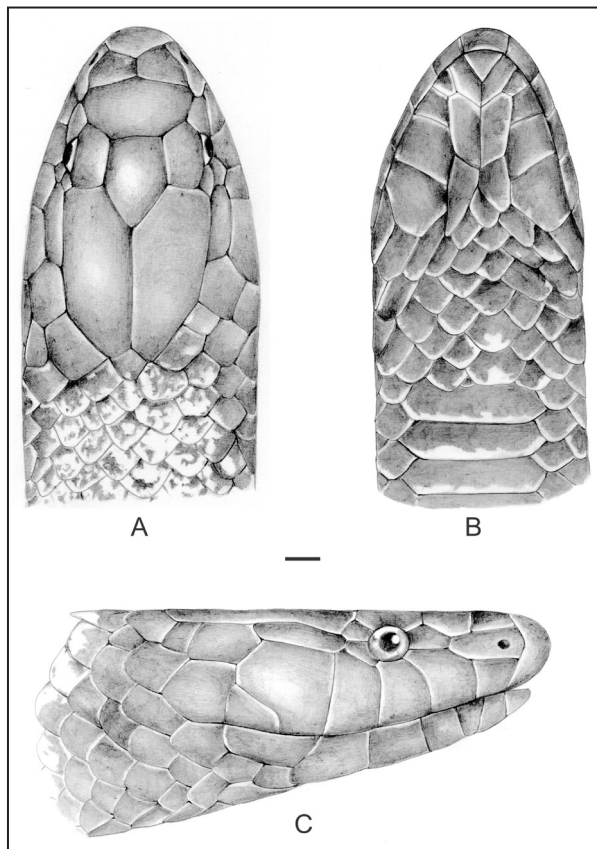


Figure 1. Dorsal (A), ventral (B) and lateral (C) view of the head of the holotype (FML 23858) of *Phalotris illustrator* sp. nov. Scale bar = 1 cm.

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 sub-cylindrical; 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	Supra-labials	Ventrals	Sub-caudals	Head length	Head width	SVL	TL	% TL/SVL	Ros-tral length (mm)	Interna-sal length (mm)	Prefron-tal length (mm)	Fron-tal length (mm)	Pa-rietal 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 "provincia 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, Abalos & Mischis, 1975 (in part).

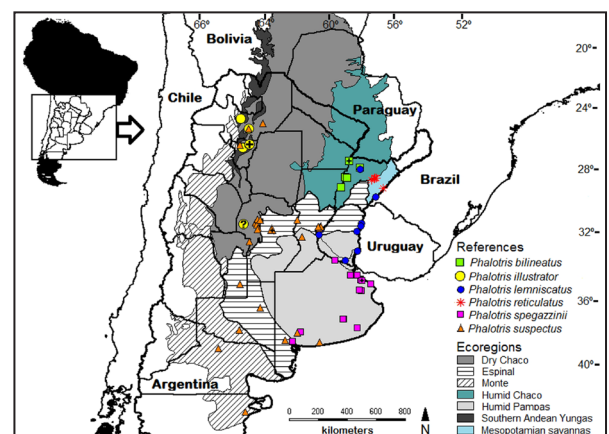


Figure 3. Distribution of the species of *Phalotris bilineatus* group in Argentina. + type localities of *Phalotris illustrator* sp. nov., *P. suspectus*, *P. bilineatus*, *P. bilineatus* and *P. spegazzinii*. The symbol "?" represents doubtful locality based on the confused collection data available.



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 spegazzinii, 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 “Amérique 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, Ceï 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, Giraud, 2001.

Phalotris reticulatus, Giraud and Scrocchi, 2002.

Phalotris reticulatus, Giraud *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. spagazzinii*); 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. spagazzinii*); 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 whitish posterior border resulting in a reticulated lateral pattern in general view; black in *P. spagazzinii* 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 (\bar{X} = 192.00, SD = 4.69, n = 4) ventral scales (188–198 reported for Brazilian populations by Ferrarezzi, 1993a), females 201–207 (\bar{X} = 205.00, SD = 3.46, n = 3; 202–211 reported by Ferrarezzi, 1993a). Subcaudal scales 30–33 (\bar{X} = 31.30, SD = 1.26, n = 4) in males (27–33 reported by Ferrarezzi, 1993a), and 23–27 in females (\bar{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 pro-

vince 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 spegazzinii, 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

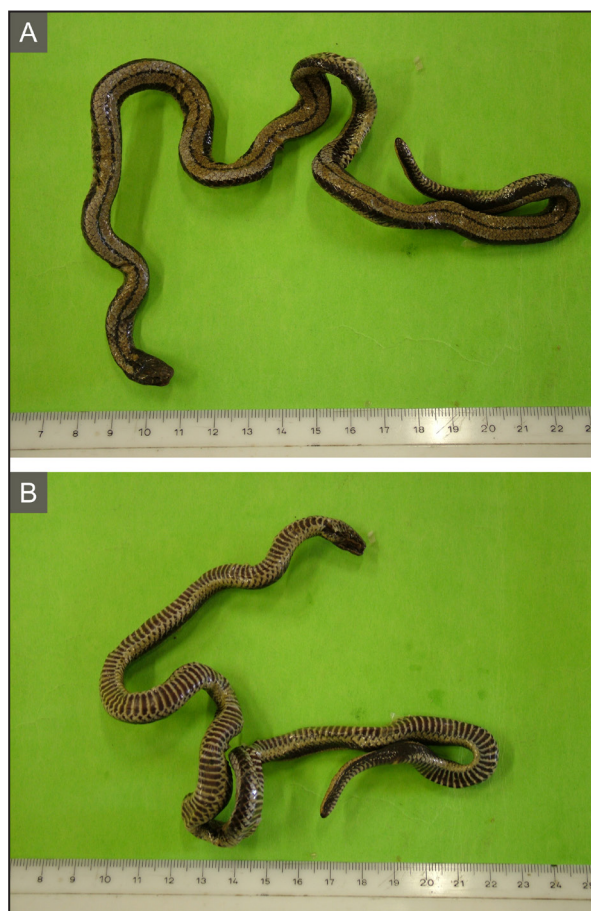


Figure 5. Dorsal (A) and ventral (B) views of the holotype of *Phalotris spegazzinii* (MSNG 30651) from La Plata, Argentina. Photo by G. Doria.

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

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 (\bar{X} = 195.00, SD = 1.15, n = 4) ventral scales, females 201–218 (\bar{X} = 210.09, SD = 5.01, n = 11). Subcaudal scales 29–35 in males (\bar{X} = 31.00, SD = 2.71, n = 4), and 21–25 in females (\bar{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 "Pilar", 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*).

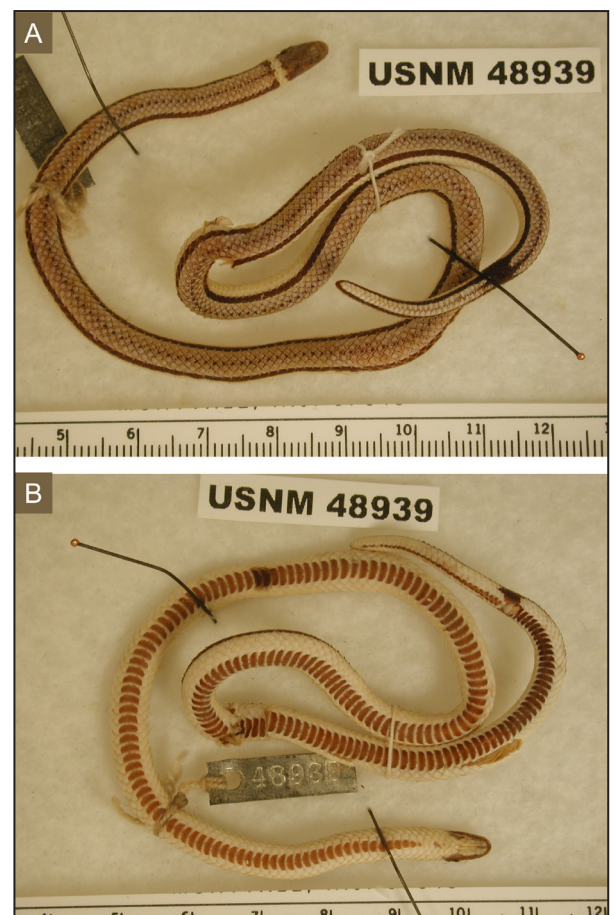


Figure 6. Dorsal (A) and ventral (B) views of the holotype of *Phalotris suspectus* (USNM 48939) from Pilar, Córdoba, Argentina. Photo J.A. Poindexter II.

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 Puerto 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.

Acknowledgments

We thank Blanca Alvarez, Julian Faivovich, Sonia Kretzschmar, Esteban Lavilla, Soledad Palomas, and Jorge Williams for allowing the examination of specimens under their care. We are indebted to Giuliano Doria, Ron Heyer, and James A. Poindexter II for providing us with excellent photos of the types of *Elapomorphus spegazzinii*, and *Elapomorphus suspectus*, respectively; and Eduardo Etchepare and Luis Giambelluca for providing us with photos from live specimens.

Literature cited

- Ábalos, J.W. & Mischis, C.C. 1975. Elenco sistemático de los ofidios argentinos. *Boletín de la Academia Nacional de Ciencias* 51: 55-76.
- Amaral, A. 1924. New genus and species of South American snakes contained in the United States National Museum. *Journal of the Washington Academy of Sciences* 14: 200-202.
- Amaral, A. 1930. Estudos sobre ofidios neotrópicos. XVII. Valor sistemático de várias formas de ofidios neotrópicos. *Memórias do Instituto Butantan* 4: 3-68.
- Aquino, A.L.; Scott, N. & Motte, M. 1996. Lista de los anfibios y reptiles del Museo Nacional de Historia Natural del Paraguay. In: Romero, O. (ed.), *Colecciones de Fauna y Flora del Museo Nacional de Historia Natural del Paraguay*. Museo Nacional de Historia Natural del Paraguay. Dirección de Parques Nacionales y Vida Silvestre. Asunción. 331-400.
- Braz, H.B.; Kasperoviczus, K.N. & Almeida-Santos, S.M. 2014. Reproductive ecology and diet of the fossorial snake

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

	Dorsal Head coloration	Ventral Head coloration	Nuchal collars	Vertebral stripe	Sides of the body	Cloacal black ring	Ventral tail coloration
<i>P. illustrator</i> 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%).
<i>P. bilineatus</i> (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%).
<i>P. lemniscatus</i> (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%).
<i>P. multipunctatus</i> (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 row and decreases to the fifth (100%). With a narrow black stripe on 4th–5th dorsal rows, 1st to 3rd dorsal rows white (100%).	Absent (100%)	Reticulate in black, with a white dot in each subcaudal (100%).
<i>P. normanscotii</i> (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%)	Almost black (100%).	Present (100%)	White (100%)
<i>P. reticulatus</i> (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%).
<i>P. spegazzinii</i> (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%).
<i>P. suspectus</i> (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%).

- Phalotris lativittatus* in the Brazilian Cerrado. *Herpetological Journal* 24: 49-57.
- Bertoni, A.W. 1914. Fauna paraguaya: Catálogos sistemáticos de los vertebrados del Paraguay. 1-83. In: Bertoni, M.S. (ed.), *Descripción Física y Económica del Paraguay*. M. Brossa. Asunción.
- Bertoni, A.W. 1939. Catálogos sistemáticos de los Vertebrados del Paraguay. *Revista de la Sociedad Científica del Paraguay* 4: 3-60.
- Boulenger, G.A. 1889. Description of a new snake and two new fishes obtained by Dr. H. von Ihering in Brazil. *Annals and Magazine of Natural History* 6: 265-267.
- Boulenger, G.A. 1889. Description of a new snake and two new fishes obtained by Dr. H. von Ihering in Brazil. *Annals and Magazine of natural History* 6: 265-267.
- Boulenger, G.A. 1913. Descriptions of a new lizard and a new snake from South America. *Annali del Museo Civico di Storia Naturale Giacomo Doria* 3: 49-50.
- Cabral, H. & Cacciali P. 2015. A New species of *Phalotris* (Serpentes: Dipsadidae) from the Paraguayan Chaco. *Herpetologica* 71(1): 72-77.
- Cabrera, A.L. 1976. *Regiones fitogeográficas argentinas*. *Enciclopedia Argentina de Agricultura y Jardinería* 2: 1-85.
- Cabrera, M.R. 2004. *Las serpientes de Argentina Central*. Publicaciones de la Universidad Nacional de Córdoba. Córdoba, Argentina. 109 pp.
- Cacciali, P. 2009. *Guía para la identificación de 60 serpientes del Paraguay*. Asociación Guyra Paraguay, Asunción, Paraguay. 218 pp.
- Cacciali, P.; Scott N.; Aquino, A.L.; Fitzgerald, L.A. & Smith, P. 2016. The Reptiles of Paraguay: literature, distribution, and an annotated taxonomic checklist. *Special Publications of the Museum of Southwestern Biology* 11: 1-373.
- Carreira, S.; Meneghel, M. & Achaval, F. 2005. *Reptiles de Uruguay*. Montevideo, D.I.R.A.C. Facultad de Ciencias, Universidad de la República. 639pp.
- Cei, J.M. 1993. Reptiles del noroeste, nordeste y este de la Argentina. Herpetofauna de las selvas subtropicales, puna y pampas. *Museo Regionale di Scienze Naturali di Torino*, Monografía 14, 1-929.
- Cope, E.D. 1861. [No title]. *Proceedings of the Academy of Natural Sciences of Philadelphia* 1861: 524.
- Cope, E.D. 1885. Twelfth contribution to the herpetology of tropical America. *Proceedings of the American Philosophical Society* 22: 167-194.
- Costa, H.C. & Bérnils R.S. 2014. Répteis brasileiros: lista de espécies. *Herpetologia Brasileira* 3: 74-84.
- Costa, H.C. & Bérnils, R.S. 2015. Répteis brasileiros: lista de espécies 2015. *Herpetologia Brasileira* 4: 75-93.
- Costa, H.C. & Bérnils, R.S. 2018. Répteis do Brasil e suas unidades federativas: lista de espécies. *Herpetologia Brasileira* 7: 11-57.
- Dowling, H.G. 1951. A proposed standard system of counting ventrals in snakes. *British Journal of Herpetology* 1: 97-99.
- Duméril, A.M.C.; Bibron, G. & Duméril, A. 1854. *Erpétologie Générale ou histoire naturelle Complète des Reptiles*. Tome Septième. Deuxième partie. Librairie Encyclopédique de Roret, Paris, 1536 pp.
- Entiauspe-Neto, O.M.; Koch, C.; Guedes, T. B. & Tiutenko A. 2020. Revisiting the taxonomic status of *Apostolepis sanctaeritae*, a forgotten Neotropical dipsadid snake. *Salamandra* 56: 329-341.
- Ferrarezzi, H. 1993a. Sistemática Filogenética de *Elapomorphus*, *Phalotris* e *Apostolepis* (Serpentes: Colubridae: Xenodontinae). Unpubl. MSc. Dissertation, Universidade de São Paulo, São Paulo, 277 pp.
- Ferrarezzi, H. 1993b. Nota sobre o genero *Phalotris* com revisão do grupo *nasutus* e descrição de três novas especies (Serpentes, Colubridae, Xenodontinae). *Memórias do Instituto Butantan* 55: 21-38.
- Gatti, C. 1955. Las culebras venenosas del Paraguay. *Revista Médica del Paraguay* 1: 81-100.
- Giraud, A.R. "2001" (2004). *Serpientes de la Selva Paranaense y del Chaco Húmedo*. L.O.L.A. Buenos Aires. 289 pp.
- Giraud, A.R.; Arzamendia, V.; Bellini, G.; Bessa, C.; Calamante, C.; Cardozo, G.; Chiaraviglio, M.; Costanzo, B.; Etchepare, E.; Di-Cola, V.; Di-Pietro, D.; Kretschmar, S.; Nenda, S.J.; Rivera, P.; Rodríguez, M.E.; Scrocchi, G. & Williams, J. 2012. Categorización del estado de conservación de las Serpientes de la República Argentina. *Cuadernos de Herpetología* 26: 303-326.
- Giraud, A.R. & Scrocchi, G. 2002. Argentinean snakes: an annotated checklist. *Smithsonian Herpetological Information Service* 132: 1-53.
- Grazziotin, F.G.; Zaher, H.; Murphy, R.W.; Scrocchi, G.; Benavides, M.A.; Zhang, Y.P. & Bonatto, S.L. 2012. Molecular phylogeny of the new world Dipsadidae (Serpentes: Colubroidea): a reappraisal. *Cladistics* 28: 437-459.
- Harvey, M.B. 1999. Revision of Bolivian *Apostolepis* (Squamata: Colubridae). *Copeia* 1999: 388-409.
- Jan, G. 1862. Enumerazione sistematica delle specie d'ofici del grupo Calamaridae. *Archivio de Zoologia, Anatomia ed Fisiologia* 2: 1-76.
- Jan, G. & Sordelli, F. 1865. *Iconographie Générale des ophidiens*. Volume I, Livraison 14. J.B. Bailliére et Fills, Paris.
- Koslowky, J. 1898. Enumeración sistemática y distribución geográfica de los reptiles argentinos. *Revista del Museo de La Plata* 8: 161-200.
- Lema, T. 1970. Sobre o status de *Elapomorphus bilineatus* Duméril, Bibron & Duméril, 1854, curiosa serpente subterránea. *Iheringia, Série Zoologia* 38: 89-117.
- Lema, T. 1978a. O status de *Elapomorphus suspectus* Amaral 1924 (Ophidia: Colubridae). *Comunicações do Museu de Ciências da PUCRS, Série Zoologia* 16/17: 1-10.
- Lema, T. 1978b. Invalidação de *Elapomorphus bollei* Mertens 1954 e o status de *Elapomorphus spegazzinii* Boulenger 1913. (Ophidia: Colubridae). *Comunicações do Museu de Ciências da PUCR, Série Zoologia* 16/17: 11-16.
- Lema, T. 1979. Sobre a validade dos nomes *Elapomorphus bilineatus* Duméril, Bibron & Duméril, 1854 e *E. lemniscatus* Duméril, Bibron & Duméril, 1854 (Ophidia, Colubridae). *Iheringia, Série Zoologia* 54: 77-81.
- Lema, T. 1984. Sobre o gênero *Elapomorphus* Wiegmann, 1843 (Serpentes, Colubridae, Elapomorphinae). *Iheringia, Série Zoologia* 64: 53-86.
- Lema, T. 1989. Notas sobre a biologia de duas espécies de *Elapomorphus* Wiegmann, 1843 (Serpentes, Colubridae, Elapomorphinae). *Iheringia, Série Zoologia* 69: 61-69.
- Lema, T. & Deiques C.H. 2010. Description of a new genus for allocation of *Elapomorphus lepidus* and the status of *Elapomorphus wuchereri* (Serpentes: Dipsadidae: Xenodontinae: Elapomorphini). *Neotropical Biology and Conservation* 5: 113-119.
- Martins, L.A. & Lema, T. 2017. The distribution of *Phalotris tricolor* group in Argentina and Paraguay, with notes on its

G. Scrocchi *et al.* — Taxonomy of *Phalotris bilineatus* group

- taxonomy (Serpentes, Elapomorhini). *Neotropical Biology and Conservation* 12: 100-108.
- Mertens, R. 1954. Neue Eidechsen aus Südwest-Afrika. *Senckenbergiana* 34: 175-183.
- Olson, D.M.; Dinerstein, E.; Wikramanayake, E.D.; Burgess, N.D.; Powell, G.V.; Underwood, E.C. & Loucks, C.J. 2001. Terrestrial ecoregions of the world: a new map of life on earth a new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *BioScience* 51: 933-938.
- Passos, P.; Chiessi, A.; Torres-Carvajal, O. & Savage, J.M. 2009. Testing species boundaries within the *Atractus occipitoalbus* complex (Serpentes: Dipsadidae). *Herpetologica* 65: 384-403.
- Passos, P. & Prudente, A.L. 2012. Morphological variation, polymorphism and taxonomy of the *Atractus torquatus* complex (Serpentes: Dipsadidae). *Zootaxa* 3407: 2-21.
- Peters, J.A. 1960. The snakes of the subfamily Dipsadinae. *Miscellaneous Publications Museum of Zoology, University of Michigan* 114: 1-224.
- Peters, J.A. & Orejas-Miranda, B. 1970. Catalogue of the Neotropical Squamata. Part I. Snakes. *United States National Museum Bulletin* 297: 1-347.
- Peters, W.C.H. 1860. Drei neue Schlangen des k. zoologischen Museums aus America und Bemerkungen über die generelle Unterscheidung von anderen bereits bekannten Arten. *Monatsberichte der Königlich Akademie der Wissenschaft Berlin* 1860: 517-521.
- Puerto, G. & Ferrarezzi H. 1993. Uma nova espécie de *Phalotris* Cope, 1862, com comentários sobre o grupo *bilineatus* (Serpentes: Colubridae: Xenodontinae). *Memórias do Instituto Butantan* 55: 39-46.
- Pyron, R.A., Burbrink, F.T. & Wiens, J.J. 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology* 13: 1-93.
- Reati, G. 1996. Serpientes de la Provincia de Córdoba, Argentina. In: Biodiversidad de la Provincia de Córdoba: Fauna. Vol. I. 1996, pp. 239-254.
- Sabaj, M.H. 2016. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. Version 6.5 (16 August 2016). Electronically accessible at <http://www.asih.org/>, American Society of Ichthyologists and Herpetologists, Washington, DC.
- Schouten, G. 1931. Contribuciones al conocimiento de la fauna herpetológica del Paraguay y de los países limítrofes. *Revista de la Sociedad Científica del Paraguay* 3: 5-32.
- Schouten, G. 1937. Fauna herpetológica del Paraguay. Sociedad Argentina de Patología Regional del Norte. *Novena Reunión* 2: 1218-1232.
- Scrocchi, G.J.; Abdala C.C.; Nori J. & Zaher H. 2010. Reptiles de la provincia de Río Negro, Argentina. Editora Fondo Rionegrino, Viedma, Río Negro, Argentina. 252 pp.
- Scrocchi, G.J.; Aguer, I.; Arzamendia, V.; Cacivio, P.; Carcacha, H.; Chiaraviglio, M.; Giraudo, A.R.; Kretzschmar, S.; Leynaud, G.; López, M.S.; Rey, L.; Waller, T. & Williams, J. 2000. Categorización de las serpientes de Argentina, 75-93. In: Lavilla, E., Richard, E. & Scrocchi, G. (eds.). Categorización de los anfibios y reptiles de la República Argentina. Asociación Herpetológica Argentina, San Miguel de Tucumán, Tucumán, Argentina.
- Scrocchi, G.J.; Moreta, J.C. & Kretzschmar, S. 2006. Serpientes del Noroeste Argentino. Fundación Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina. 178 pp.
- Serié, P. 1936. Nueva enumeración sistemática de los ofidios argentinos. Obra del Cincuentenario. *Revista del Museo de La Plata* 2: 33-68.
- Smith, P.; Brouard, J. P.; & Cacciali, P. 2022. A new species of *Phalotris* (Serpentes, Colubridae, Elapomorhini) from Paraguay. *Zoosystematics and Evolution* 98: 77-85.
- Strauch, A. 1884. Bemerkungen über die Schlangengattung *Elapomorphus* aus der Familie der Calamariden. 2nd. Ed. *Bulletin de l'Académie impériale des sciences de St.-Petersbourg* 29: 541-590.
- Talbot, J.J. 1979. Una nueva lista sistemática de reptiles del Paraguay. *Informes Científicos*, Instituto de Ciencias Básicas, Universidad Nacional de Asunción 2: 76-94.
- Uetz, P.; Aguilar, P. & Hošek, J. 2022. *The Reptile Database*. Available from <http://www.reptile-database.org> (Accessed April 13, 2022).
- Wallach, V.; Williams, K.L. & Boundy, J. 2014. Snakes of the world. A catalogue of living and extinct species. CRC Press. Taylor & Francis Group. Boca Raton, London, Nueva York. 1227 pp.
- Wiegmann, A.F.A. (In Fitzinger, L.J.F.J. 1843. *Systema Reptilium. Fasciculus Primus*. Braumüller et Seidel, Viena, Austria.
- Wiens, J.J. & Servedio, M.R. 2000. Species delimitation in systematics: inferring diagnostic differences between species. *Proceedings of the Royal Society of London B: Biological Sciences*, 267: 631-636.
- Williams, J.D. & Francini, F. 1991. A checklist of the Argentine snakes. *Bollettino del Museo Regionale di Scienze Naturali di Torino* 9: 55-90.
- Williams, J.D.; Vera, D.G. & Di Pietro, D.O. 2021. Lista comentada de las serpientes de la Argentina, con referencias a su sistemática, distribución geográfica, dieta, reproducción, potencial peligrosidad y etimologías. *Revista del Museo de La Plata* 6(1):26-124.
- Zaher, H.; Grazziotin, F.G.; Cadle, J.E.; Murphy, R.W.; Moura-Leite, J.C.D. & Bonatto, S.L. 2009. Molecular phylogeny of advanced snakes (Serpentes, Caenophidia) with an emphasis on South American Xenodontines: a revised classification and descriptions of new taxa. *Papéis Avulsos de Zoologia* 49: 115-153.

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 (-29.716666° -57.083333°): CENAI 3184; **ENTRE RÍOS: Departamento Colón:** Parque Nacional El Palmar, Jardín de la Portada (-31.853055° -58.3225°): PNP 26; **Departamento Concordia:** INTA El Alambrado, near Concordia (-31.381408° -58.115306°): INALI 1116; Puerto Yerúa (-31.5314° -58.0153°): MACN 44597 (former CENAI 3739); **Departamento Diamante:** Diamante (-32.066666° -60.65°): INALI 4042, 5508; **Departamento Gualeguaychú:** Ñandubaysal beach (-33.066229° -58.389108°): INALI 3986; **Departamento Islas del Ibicuy:** Villa Paranacito (-33.7° -58.683333°): MACN 44590 (former CENAI 2145); **Departamento Paraná:** Paraná (-31.744444° -60.5175°): MACN 7822.

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

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

(-37.318889° -59.134722°): MACN 12193-96, 24838.

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

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

Cristiane Nikely S. Palmeira^{1,2,3}, Ubiratan Gonçalves^{1,2}, Marcos J. Matias Dubeux^{1,2,4}, Luana Rodrigues Lima^{1,2}, Carolina Lambertini⁵, Anyelet Valencia-Aguilar⁶, Thomas Stuart Jenkinson⁷, Timothy Yong James⁷, Luís Felipe Toledo⁵, Tamí Mott^{1,2}

¹ Laboratório de Biologia Integrativa, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, Avenida Lourival Melo Mota SN, Maceió, Alagoas, 57072-970, Brazil.

² Laboratório de Herpetologia, Departamento de Zoologia, Museu de História Natural, Universidade Federal de Alagoas, Avenida Amazonas SN, Maceió, Alagoas, 57010-020, Brazil.

³ Laboratório de Genética Toxicológica, Departamento de Biologia Celular e Genética, Programa de Pós-Graduação em Desenvolvimento e Meio Ambiente, Universidade Federal do Rio Grande do Norte, Centro de Biociências, Campus Universitário, 59078-970, Natal, RN, Brazil.

⁴ Laboratório de Herpetologia, Departamento de Zoologia, Programa de Pós-Graduação em Biologia Animal, Universidade Federal de Pernambuco, Centro de Biociências, Av. Professor Moraes Rego SN, 50670-901, Recife, Pernambuco, Brazil.

⁵ Laboratório de História Natural de Anfíbios Brasileiros, Instituto de Biologia, Universidade Estadual de Campinas, Rua Monteiro Lobato 255, Campinas, São Paulo, 13083-862, Brazil.

⁶ Laboratório de Herpetologia, Departamento de Biodiversidade, Universidade Estadual Paulista, Avenida 24A, Rio Claro, São Paulo, 13506-900, Brazil.

⁷ Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan, 48109, United States of America.

Recibida: 09 Septiembre 2020

Revisada: 13 Julio 2021

Aceptada: 19 Octubre 2021

Editor Asociado: C. Canedo

doi: 10.31017/CdH.2022.(2020-065)

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 km² 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

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

Originally the Atlantic Forest extended for ca. 1300 km² 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 km², 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 northern 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 km² and mainly covered by semideciduous forest (18,88 km²), sand dunes (0,82 km²) and lagoons (0,65 km²) (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).

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.

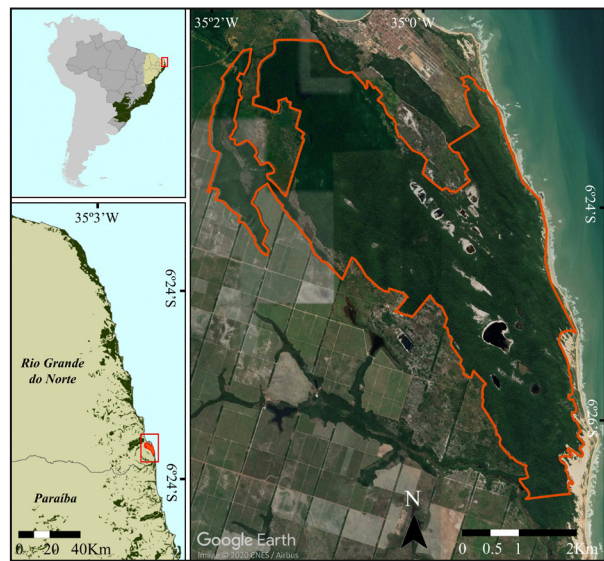


Figure 1. Satellite image of the Private Natural Heritage Reserve Mata Estrela (with boundaries shown in orange). Inset map is South America with rectangle showing the study area.

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), *Dendropsophus branneri* (Cochran, 1948) (89), *Pleurodema diplo-lister* (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, 2020³, 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 km², 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 km², 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 Jundiá, 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 Jundiá, 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 at a Private Natural Heritage Reserve Mata Estrela, Baía Formosa, Rio Grande do Norte state, Brazil.

Species	Conservation status		Enviroments		Geographic distribution
	Forest	Open areas	Forest	Open areas	
BUFONIDAE					
<i>Rhinella granulosa</i> (Spix, 1824)	Least Concern (LC)		Sandy soil	Sandy soil and clayey	In the Brazil, Pará, Maranhão to Piauí, Ceará, and Alagoas, south to Rio Grande do Sul and Espírito Santo. Inland Paraguay to Amazonian and eastern Bolivia; and southwest to northern and central Argentina and northern Uruguay.
<i>Rhinella diptycha</i> (Cope, 1862)	Data Deficient (DD)		Sandy soil and clayey	Sandy soil and clayey, streams, temporary and permanent ponds and weirs edge	
CRAUGASTORIDAE					
<i>Pristimantis ramagii</i> (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					
<i>Boana albomarginata</i> (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.
<i>Boana crepitans</i> (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.
<i>Boana ranceps</i> (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.
<i>Boana</i> sp.	–		Tree branches	–	PE, AL, SE, BA, MG, ES, RJ, RN and PB.
<i>Dendropsophus branneri</i> (Cochran, 1948)	Least Concern (LC)		–	Vegetation in temporary and permanent ponds	Colombia, Venezuela, Guianas, Trindade, Ecuador, Peru, Bolivia, Paraguay, Uruguay and Argentina. In all of Brazil.
<i>Dendropsophus minutus</i> (Peters, 1872)	Least Concern (LC)		–	Vegetation in temporary and permanent ponds	Northeast to the extreme South of Brazil. Suriname, French Guiana, Paraguay, Argentina, Bolivia and Uruguay.
<i>Dendropsophus nanus</i> (Boulenger, 1889)	Least Concern (LC)		–	Vegetation in temporary and permanent ponds.	From RJ to RN.
<i>Dendropsophus oliveirai</i> (Bokermann, 1963)	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.
<i>Scinax fuscomarginatus</i> (A. Lutz, 1925)	Least Concern (LC)		–	Vegetation in temporary and permanent ponds	

<i>Scinax nebulosus</i> (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.
<i>Scinax x-signatus</i> (Spix, 1824)	Least Concern (LC)	-	Vegetation in temporary ponds	Colombia, Venezuela andd Suriname. Eastern, south and southeast of Brazil.
LEPTODACTYLIDAE				
<i>Adenomera hylaedactyla</i> (Cope, 1868)	Least Concern (LC)	-	-	Colombia, Venezuela, Guianas, Ecuador, Peru and Bolivia. From south to north, eastern and central of Brazil.
<i>Leptodactylus fuscus</i> (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.
<i>Leptodactylus macrosternum</i> 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.
<i>Leptodactylus mystaceus</i> (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
<i>Leptodactylus natalensis</i> A. Lutz, 1930	Least Concern (LC)	-	Herbaceous vegetation with soggy soil and temporary ponds	Coastal region of Brazil: MA, RN to RJ
<i>Leptodactylus podicipinus</i> (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.
<i>Leptodactylus troglodytes</i> A. Lutz, 1926	Least Concern (LC)	Herbaceous vegetation with soggy soil	Herbaceous vegetation with soggy soil and temporary ponds	Northeast Brazil to MG.
<i>Leptodactylus vastus</i> (Lutz 1930)	Least Concern (LC)	-	Edge of temporary ponds	Northeast Brazil
<i>Physalaemus cuvieri</i> Fitzinger, 1826	Least Concern (LC)	-	Herbaceous vegetation with soggy soil	Northeast, central and southern Brazil; Argentina, Paraguay, Bolivia and Venezuela.
<i>Pleurodema diplolister</i> (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.
<i>Pseudopaludicola mystacalis</i> (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.
<i>Pseudopaludicola pocoto</i> 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				
<i>Pithecopus gonzagai</i> Andrade, Haga, Ferreira, Recco-Pimentel, Toledo, and Bruschi, 2020.	Not Evaluated (NE)	-	Vegetation in temporary and permanent ponds and vegetation of dams	RN, PB, PE, AL, CE, PI and MA.

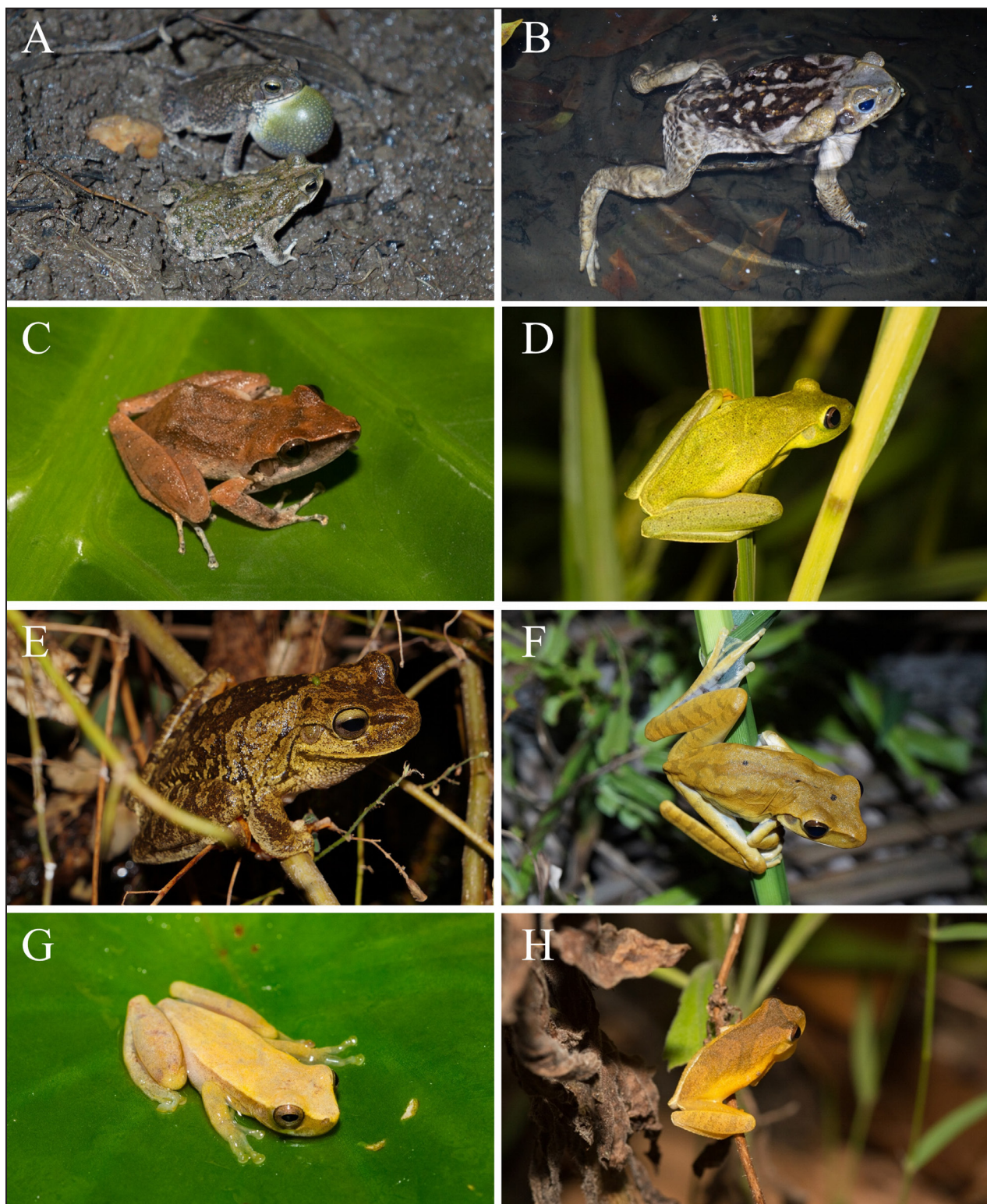


Figure 3. Anurans of the Private Natural Heritage Reserve Mata Estrela, municipality of Baía Formosa, Rio Grande do Norte State, Brazil. **A.** *Rhinella granulosa* (Spix, 1824). **B.** *R. diptycha* (Cope, 1862). **C.** *Pristimantis ramagii* (Boulenger, 1888). **D.** *Boana albomarginata* (Spix, 1824).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

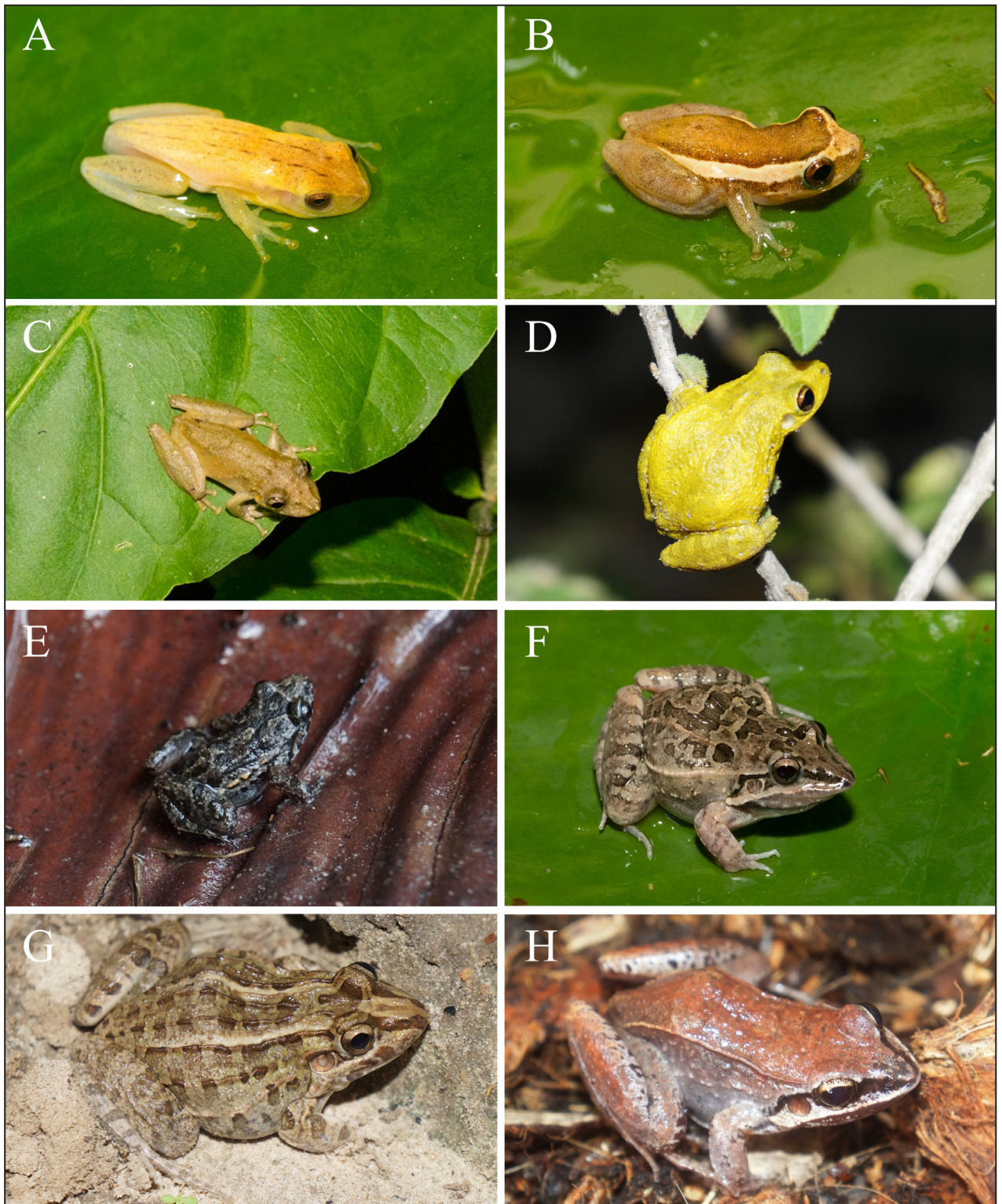


Figure 4. Anurans of the Private Natural Heritage Reserve Mata Estrela, municipality of Baía Formosa, Rio Grande do Norte State, Brazil. **A.** *Dendropsophus nanus* (Boulenger, 1889). **B.** *D. oliveirai* (Bokermann, 1963). **C.** *Scinax nebulosus* (Spix, 1824). **D.** *S. fuscovarius* (Lutz, 1925). **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.

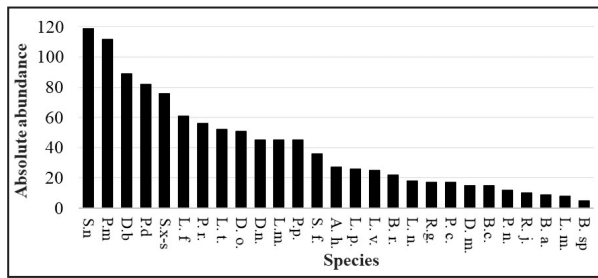


Figure 6. Total number of anurans recorded in the Private Natural Heritage Reserve Mata Estrela, municipality of Baía Formosa, Rio Grande do Norte State, Brazil between June 03 – 04, 2015 ($N = 1122$). *Scinax nebulosus*, *Pseudopaludicola mystacalis*, *Dendropsophus branneri*, *Pleurodema diplolister*, *Scinax x-signatus*, *Leptodactylus fuscus*, *Pristimantis ramagii*, *Leptodactylus troglodytes*, *Dendropsophus oliveirai*, *Dendropsophus nanus*, *Leptodactylus macrosternum*, *Pseudopaludicola pocoto*, *Scinax fuscocomarginatus*, *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.

Acknowledgments

We thank Grupo Farias (Unidade: Baía Formosa) for allowing us to access the area to conduct this study. TM thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq # 309904/2015-3 and 312291/2018-3). MJMD thanks Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco (FACEPE # IBPG-1117-2.04/19). This work was also supported by São Paulo Research Foundation (FAPESP #2016/25358-3; FAPESP+NSF #2011/51694-7), CNPq (#405285/2013-2; #300896/2016-6), the University of Michigan's Graham Sustainability Institute, and by the *Coordination for the Improvement of Higher Education Personnel* (CAPES - Finance

Code 001). The project which place under the licenses SISGEN AC56BCD and CEUA 36/2015.

Literature cited

- Alvares, C.A.; Stape, J.L.; Sentelhas, P.C.; Gonçalves, J.L.M. & Sparovek, G. 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22: 711–728. DOI: 10.1127/0941-2948/2013/0507.
- AmphibiaWeb. 2021. <<https://amphibiaweb.org>> University of California, Berkeley, CA, USA. Accessed 16 July 2021.
- Baillie, J.E.M.; Griffiths, J.; Turvey, S.T.; Loh, J. & Collen, B. 2010. *Evolution Lost: Status and Trends of the World's Vertebrates*. Zoological Society of London. London.
- Barbosa, A.R. & Alves, I.T.L.S. 2014. Diversidade e uso de hábitat da anurofauna em um fragmento de um brejo de altitude. *Gaia Scientia* 8: 215–25.
- Barbosa, V.N.; Pereira, E.N. & Maranhão, E.S. 2017. Anfíbios da Estação Ecológica de Caetés Paulista, Pernambuco - atualização da lista de espécies. *Revista de Ciências Ambientais* 11: 39–49. DOI: 10.18316/rca.v11i2.3003.
- Brassaloti, R.A.; Rossa-Feres, D.C. & Bertoluci, J. 2010. Anurofauna da Floresta Estacional Semidecidual da Estação Ecológica dos Caetés, Sudeste do Brasil. *Biota Neotropica* 10(1): 275–291. DOI: 10.1590/S1676-06032010000100024.
- Cardoso, A.J.; Andrade, G.V. & Haddad, C.F.B. 1989. Distribuição espacial em comunidades de anfíbios (Anura) no sudeste do Brasil. *Revista Brasileira de Biologia* 49: 241–249.
- CFMV - Conselho Federal de Medicina Veterinária. 2012. Resolução do Conselho Federal de Medicina Veterinária Nº 1000 de 11 de maio de 2012. Dispõe sobre procedimentos e métodos de eutanásia em animais e dá outras providências. Available in: <<http://portal.cfmv.gov.br/lei/index/id/326>> Accessed on: 2 March 2020.
- Coelho, H.E.A. & Oliveira, R.S. 2010. Anurofauna de um fragmento de Mata Atlântica em Lauro de Freitas - Bahia. *Revista Virtual* 6: 52–60.
- Colwell, R.K. 2016. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9.10. User's guide and application published online. Available in: <http://viceroy.eeb.uconn.edu/estimates>. Accessed on: 2 March 2020.
- Condez, T.H.; Sawaya, R.J. & Dixo, M. 2009. Herpetofauna dos remanescentes de Mata Atlântica da região de Tapirai e Piedade, SP, sudeste do Brasil. *Biota Neotropica* 9: 157–186. DOI: 10.1590/S1676-06032009000100018.
- Dorado, A.; Melero, M.G.; Gomes, E.P.C.; Damato, M. & Cavana, D. 2006. Unidades de vegetação da Mata Estrela, município de Baía Formosa – RN. In: Anais III Simpósio Regional de Geoprocessamento e Sensoriamento Remoto. Resumo expandido, Geonordeste, Aracaju, 1–5.
- Dubeux, M.J.M.; Gonçalves, U.; Nascimento, F.A.C. & Mott, T. 2020. Anuran amphibians of a protected area in the northern Atlantic Forest with comments on topotypic and endangered populations. *Herpetology Notes* 13: 61–74.
- EMPARN. 2019. Governo do Estado do Rio Grande do Norte. Empresa de Pesquisa Agropecuária do Rio Grande do Norte – EMPARN. Monitoramento Pluviométrico - Análise de chuvas no ano (RN). Available in: <<http://meteorologia.emparn.rn.gov.br:8181/monitoramento/2019.htm>>. Accessed on: 25 March 2020.
- Fonseca, W.L.; Silva, J.D.; Abegg, A.D.; Rosa, C.M. & Bernarde,

C. Palmeira *et al.* — Anurans in a fragment in the Northern Atlantic Forest

- P.S. 2019. Herpetofauna of Porto Walter and surrounding areas, Southwest Amazonia, Brazil. *Herpetology Notes* 12: 91-107.
- Frost, D.R. 2021. Amphibian Species of the World: An Online Reference. Version 6.1. Available in: <http://research.amnh.org/herpetology/amphibia/index.html>. Accessed on: 16 July 2021.
- Gambale, P.G., Woitovicz-Cardoso, M., Vieira, R.R., Batista, V.G., Ramos, J., Bastos, R.P. 2014. Composição e riqueza de anfíbios anuros em remanescentes de Cerrado do Brasil Central. *Iheringia, Série Zoologia* 104: 50-58.
- Gotelli, N.J. & Colwell, R.K. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* 4: 379-391.
- Govindin, J.L.S. & Miller, E.S. 2015. Práticas sociais e simbólicas: comunidade de pescadores e unidade de conservação em Baía Formosa/RN. *Sociedade & Natureza* 27: 125-139. DOI: 10.1590/1982-451320150109.
- Haddad, C.F.B. & Sazima, I. 1992. Anfíbios anuros da Serra do Japi: 188-211. In: Morellato, L.P.C. (ed.), *História Natural da Serra do Japi: Ecologia e preservação de uma área florestal no Sudeste do Brasil*. Editora da Unicamp/ FAPESP. Campinas.
- Haddad, C.F.B.; Toledo, L.F.; Prado, C.P.A.; Loebmann, D.; Gasparini, J.L. & Sazima, I. 2013. Guia dos Anfíbios da Mata Atlântica: diversidade e biologia. Anolis Books. São Paulo.
- Heyer, W.R.; Donnelly, M.A.; McDiarmid, R.W.; Hayek, L.C. & Foster, M.S. 1994. Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institution. Washington.
- ICMBio – Instituto Chico Mendes de Conservação da Biodiversidade. 2018. Biodiversidade. Fauna Brasileira. Lista de Espécies Ameaçadas. Available in: <http://www.icmbio.gov.br/portal/biodiversidade/fauna-brasileira/lista-de-especies.html>. Accessed on: 20 March 2020.
- IUCN 2022. The IUCN Red List of Threatened Species. Version 2021-3. <<https://www.iucnredlist.org>> Accessed on: 27 April 2022.
- Júnior, A.P.P.; Campos, C.E.C. & Araújo, A.S. 2013. Composição e diversidade de anfíbios anuros do campus da Universidade Federal do Amapá. *Biota Amazônia* 3: 13-21. DOI: 10.18561/2179-5746/biotaamazonia.v3n1p13-21.
- Krebs, C.J. 1999. *Ecological Methodology*. Addison Wesley Educational Publishers. Inc., Menlo Park. Califórnia.
- Leite-Filho, E.; Oliveira, F.A.; Eloi, F.J.; Liberal, C.N.; Lopes, A.O. & Mesquita, D.O. 2017. Evolutionary and Ecological Factors Influencing an Anuran Community Structure in an Atlantic Rainforest Urban Fragment. *Copeia* 105: 64-74. DOI: 10.1643/CH-15-298
- Lips, K.R.; Burrows, P.A.; Mendelson, J.R. & Parra-Olea, G. 2005. Amphibian population declines in Latin America: a synthesis. *Biotropica* 37: 222-226. DOI: 10.1111/j.1744-7429.2005.00029.x.
- Maffei, F.; Ubaid, F.K. & Jim, J. 2011. Anurofauna em área de cerrado aberto no município de Borebi, estado de São Paulo, Sudeste do Brasil: uso do habitat, abundância e variação sazonal. *Biota Neotropica* 11: 221-233. DOI: 10.1590/S1676-06032011000200023.
- Magalhães, F.M.; Dantas, A.K.B.P.; Brito, M.R.M.; Medeiros, P.H.; Oliveira, A.F.S.; Pereira, T.C.S.O.; Queiroz, M.H.C.; Santana, D.J.; Silva, W.P. & Garda, A.A. 2013. Anurans from an Atlantic Forest-Caatinga ecotone in Rio Grande do Norte State, Brazil. *Herpetology Notes* 6: 1-10.
- Marinho, J.R.D.; Souza, J.B.; Lira, V. M.; Paiva, L.L.; Danino, G.S.; Coutinho, P.W.R.; Mata, T.C.; Medeiros, D.C.; Lopes, D.A.; Demartelaere, A.C.F.; Junior, S.M.; Preston, H.A.F.; Mattos, K.M.C.; Nunes, S.K.S.; Silva, T.P.P.; Preston, W.; Silva, R.M. 2021. Economic analysis and financial viability the production *Manihot esculenta* (Crantz) in rural settlements the municipality Macaíba-RN. *Brazilian Journal of Development, Curitiba* 7: 43363-43404
- Melo, A.S.; Pereira, R.A.S.; Santos, A.J.; Shepherd, G.J.; Machado, G.; Medeiros, H.F. & Sawaya, R.J. 2003. Comparing species richness among assemblages using sample units: Why not use extrapolation methods to standardize different sample sizes? *Oikos* 101: 398-410. DOI: 10.1034/j.1600-0706.2003.11893.x.
- Morato, S.A.A.; Lima, A.M.X.; Staut, D.C.P.; Faria, R.G.; Souza-Alves, J.P.; Gouveia, S.F. & Silva, M.J. 2011. Amphibians and reptiles of the Refúgio de Vida Silvestre Mata do Junco, municipality of Capela, state of Sergipe, northeastern Brazil. *Check List* 7: 756-762. DOI: 10.15560/11015
- Moraes, R.A.; Sawaya, R.J.; Barrella, W. 2007. Composição e diversidade de anfíbios anuros em dois ambientes de Mata Atlântica no Parque Estadual Carlos Botelho, SP. *Biota Neotropica* 7:1-10.
- Oliveira-Filho, F.X.; Miranda, N.O.; Medeiros, J.F.; Silva, P.C.M.; Mesquita, F.O. & Costa, T.K.G. 2016. Compactação de solo cultivado com cana-de-açúcar em Baía Formosa, Rio Grande do Norte. *Revista Ceres* 63: 715-723. DOI: 10.1590/0034-737x201663050017.
- Palmeira, C.N.S. & Gonçalves, U. 2015. Anurofauna de uma localidade na Mata Atlântica setentrional, Alagoas, Brasil. *Boletim do Museu de Biologia Mello Leitao* 37: 149-171.
- Pazinato, D.M.M.; Silva, D.E.; Corrêa, L.L.C. & Cappellari, L.H. 2013. Diversidade de répteis em uma área da região central do Rio Grande do Sul, Brasil. *Perspectiva* 37: 115-122.
- Pereira, E.A.; Neves, M.O.; Hote, P.S.; Santana, D.J. & Feio, R.N. 2016. Anurans of the municipality of Barão de Monte Alto, state of Minas Gerais, southeastern Brazil. *Check List* 12: 1-13. DOI: 10.15560/12.5.1977.
- Pombal-Jr JP, & Gordo M 2004. Anfíbios anuros da Juréia: 243-256. In: Marques, O.A.V. & Duleba, W. (eds), *Estação Ecológica Juréia-Itatins. Ambiente físico, flora e fauna*. Holos editora. Ribeirão Preto.
- Protázio, A.S.; Albuquerque, R.L.; Falkenberg, L.M. & Mesquita, D.O. 2015. Niche differentiation of an anuran assemblage in temporary ponds in the Brazilian semi-arid Caatinga: influence of ecological and historical factors. *Herpetological Journal* 25: 109-121.
- Ribeiro, M.C.; Metzger, J.P.; Martensen, A.C.; Ponzoni, F.J. & Hirota, M.M. 2009. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation* 142: 1141-1153. DOI: 10.1016/j.biocon.2009.02.021.
- Ribeiro-Júnior, M.A.; Gardner, T.A. & Ávila-Pires, T.C.S. 2008. Evaluating the Effectiveness of Herpetofaunal Sampling Techniques across a Gradient of Habitat Change in a Tropical Forest Landscape. *Journal of Herpetology* 42: 733-749. DOI: 10.1670/07-097R3.1.
- Roberto, I.J.; Oliveira, C.R.; Araújo-Filho, J.A.; Oliveira, H.F. & Ávila, R.W. 2017. The herpetofauna of the Serra do Urubu mountain range: a key biodiversity area for conservation in the Brazilian Atlantic forest. *Papéis Avulsos de Zoologia* 57:

- 347–373. DOI: 10.11606/0031-1049.2017.57.27.
- Rossa-Feres, D.C.; Garey, M.V.; Caramaschi, U.; Napoli, M.F.; Nomura, F.; Bispo, A.A.; Brasileiro, C.R.A.; Thomé, M.T.C.; Sawaya, R.J.; Conte, C.E.; Cruz, C.A.G.; Nascimento, L.B.; Gasparini, J.L.; Almeida, A.P. & Haddad, C.F.B. 2017. Anfíbios da Mata Atlântica: lista de espécies, histórico dos estudos, biologia e conservação: 237–314. *In*: Monteiro-Filho, E.L.A. & Conte, C.E. (orgs), Revisões em Zoologia: Mata Atlântica Editora. UFPR. Curitiba.
- Santana, D.J.; Mangia, S.; Silveira-Filho, R.; Barros, L.C.S.; Andrade, I.; Napoli, M.F.; Juncá, F.A. & Garda, A.A. 2015. Anurans from the Middle Jaguaribe River Region, Ceará State, Northeastern Brazil. *Biota Neotropica* 15: 1–8. DOI: 10.1590/1676-06032015001715.
- Santana, G.G.; Vieira, W.L.; Pereira-Filho, G.A.; Delfim, F.R.; Lima, Y.C. & Vieira, K.S. 2008. Herpetofauna em um fragmento de Floresta Atlântica no estado da Paraíba, Região Nordeste do Brasil. *Biotemas* 21: 75–84. DOI: 10.5007/2175-7925.2008v21n1p75.
- Santos, A.J. 2004. Estimativas de riqueza em espécies: 19–41. *In*: Cullen Jr, L.; Rudran, R. & Valladares-Pádua, C. (orgs.), Métodos de estudos em biologia da conservação e manejo da vida silvestre. Universidade Federal do Paraná (UFPR). Curitiba.
- Santos, E.J. & Conte, C.E. 2016. Diversity of anurans in dry forest fragments of a subtropical region in Brazil. *Anais da Academia Brasileira de Ciências* 88: 1923–1940. DOI: 10.1590/0001-3765201620150698.
- Stuart, S.N.; Chanson, J.S.; Cox, N.A.; Youn, B.E.; Rodrigues, A.S.L. & Fischman, W.R.W. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1788–1786. DOI: 10.1126/science.1103538.
- Tabarelli, M.; Aguiar, A.V.; Ribeiro, M.C.; Metzger, J.P. & Peres, C.A. 2010. Prospects for biodiversity conservation in the Atlantic Forest: Lessons from aging human-modified landscapes. *Biological Conservation* 143: 2328–2340. DOI: 10.1016/j.biocon.2010.02.005.
- Verdade, V.K.; Valdujo, P.H.; Carnaval, A.C.; Schiesari, L.; Toledo, L.F.; Mott, T.; Andrade, G.V.; Eterovick, P.C.; Menin, M.; Pimenta, B.V.S.; Nogueira, C.; Lisboa, C.S.; Paula, C.D. & Silvano, D.L. 2012. A leap further: the Brazilian Amphibian Conservation Action Plan. *Alytes* 29: 28–43.
- Vieira, W.L.S.; Arzabe, C. & Santana, G.G. 2007. Composição e distribuição espaço-temporal de anuros no Cariri paraibano, Nordeste do Brasil. *Oecologia Brasiliensis* 11: 383–396. DOI: 10.4257/oeco.2007.1103.08.
- Vilela, B.; Nascimento, F.A. & Vital, M.V.C. 2018. Impacts of climate change on small-ranged amphibians of the northern Atlantic Forest. *Oecologia Australis* 22: 130–143. DOI: 10.4257/oeco.2018.2202.03.
- Wake, D.B. & Vredenburg, V.T. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences* 105: 11466–11473. DOI: 10.1073/pnas.0801921105.
- Wells, K.D. 2007. The Ecology and behavior of amphibians. The University of Chicago Press, Ltd.. London.

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

Flávio José da Silva¹, Emerson Gonçalves Dias², Marcelo Nogueira de Carvalho Kokubum^{3,4,5}, Ednilza Maranhão dos Santos⁶

¹ Universidade Federal Rural de Pernambuco, Mestrando no Programa de Pós-Graduação em Biodiversidade, Rua Manoel de Medeiros, s/n, Recife, Pernambuco, Brasil.

² Universidade Federal de Pernambuco, Doutorando no Programa de Pós-Graduação em Biologia Animal, Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife, Pernambuco, Brasil.

³ Universidade Federal de Campina Grande, Centro de Saúde e Tecnologia Rural, Unidade Acadêmica de Ciências Biológicas, Laboratório de Herpetologia, Av. dos Universitários, s/n, Santa Cecília, CEP 58700-970, Patos, PB, Brasil.

⁴ Programa de Pós-Graduação em Ciências Florestais, Av. dos Universitários, s/n, Santa Cecília, CEP 58700-970, Patos, PB, Brasil.

⁵ Programa de Pós-Graduação em Ecologia e Conservação, Universidade Estadual da Paraíba, CEP 58429-500, Campina Grande, PB, Brasil.

⁶ Universidade Federal Rural de Pernambuco, Professora do Departamento de Biologia, Laboratório Interdisciplinar de Anfíbios e Répteis, Rua Manoel de Medeiros, s/n, Recife, Pernambuco, Brasil.

Recibida: 09 Noviembre 2020

Revisada: 17 Agosto 2021

Acceptada: 01 Mayo 2022

Editor Asociado: D. Baldo

doi: 10.31017/CdH.2022.(2020-105)

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 produziram 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 anúncio 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.

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 (LHUFCEG-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).

Parâmetros	Canto de soltura			Canto de anúncio	
	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

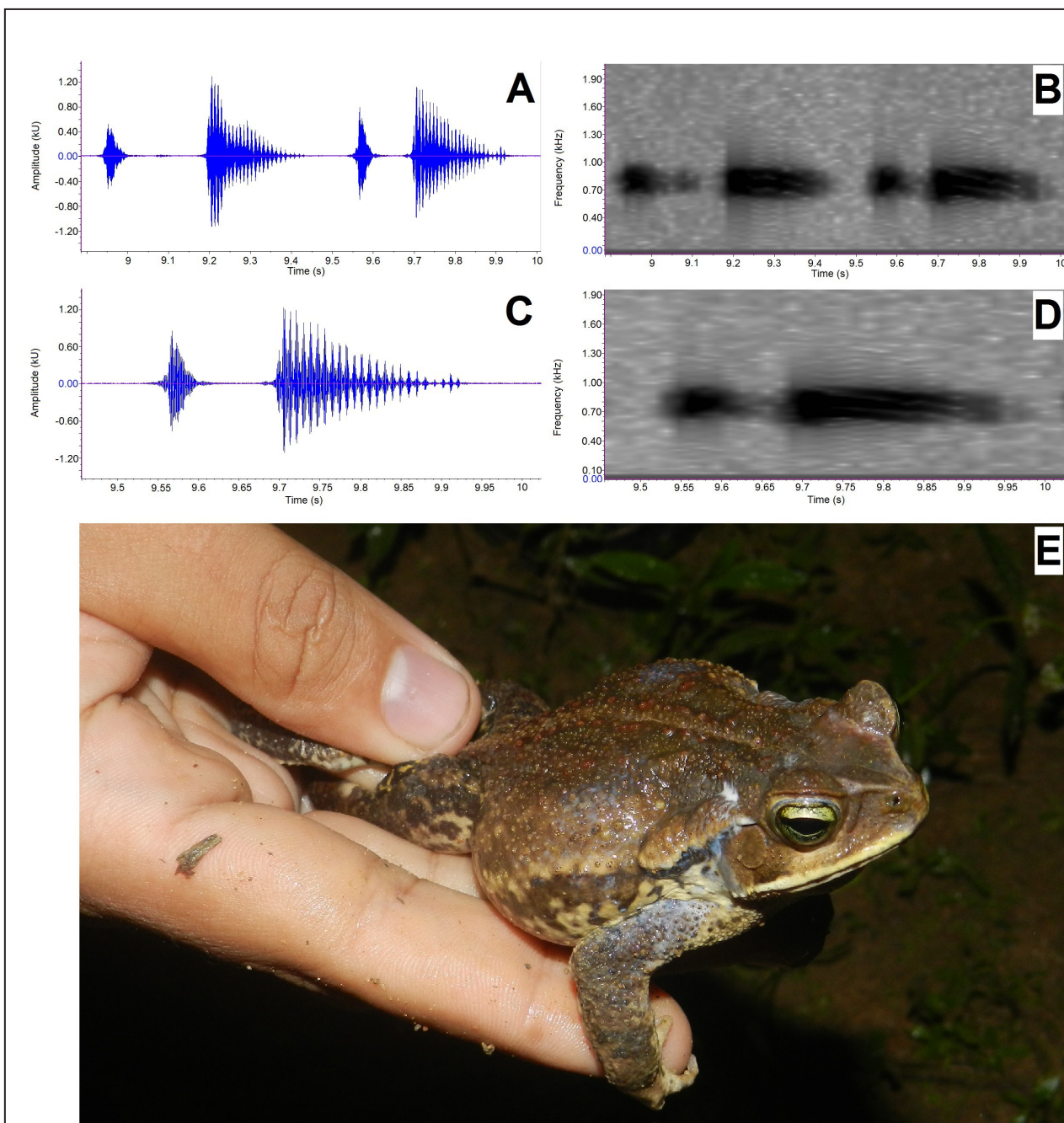


Figura 1. Canto de soltura de um macho adulto de *Rhinella crucifer* (UFRPE-SLIAR 299). **A)** Oscilograma de duas vocalizações consecutivas. **B)** Espectrograma (kHz) demonstrando a presença de harmônicos nas últimas notas do canto. **C)** Oscilograma de canto único, vê-se a curta nota inicial seguida 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 s (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).

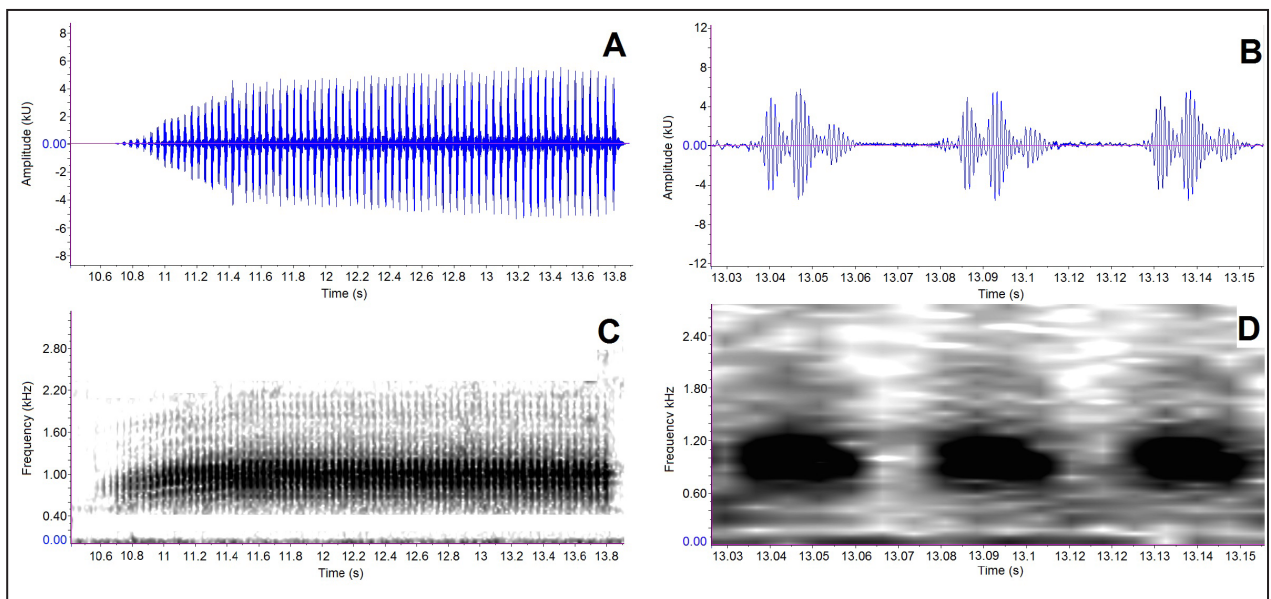


Figura 2. Canto de anúncio de um macho adulto de *Rhinella crucifer* (UFRPE-SLIAR 297). **A)** Canto composto por notas curtas e rápidas. **B)** Conjunto de três notas com três pulsos parcialmente fusionados. **C)** Oscilograma do canto de anúncio. **D)** Conjunto de três notas com ausência de harmônicos.

Entre os espécimes postulados, os mais próximos acusticamente de *Rhinella crucifer* foram indivíduos de *Rhinella 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).

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)
<i>Rhinella crucifer</i> *	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	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	-	-
<i>R. ornata</i>	1.62±0.679	-	Média de 2 (2–8)	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)
<i>R. crucifer</i> *	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)
<i>R. henselii</i> [†]	0.25 ± 0.04 (0.21 – 0.31) (n = 7)	-	-	-	-	-	-	-	980±20 (950–1010)(n=7)	-	-
Canto de anúncio	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)
<i>Rhinella ornata</i> [‡]	4–7	-	60–150	0.03–0.06	-	-	-	-	960–1750	-	-
<i>Rhinella casconii</i> [§]	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) (n=12)	1550.5±497.8 (1134.9–2275.6) (n=12)
<i>Rhinella inopina</i> [¶]	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)	-	-
<i>Rhinella ornata</i> [¶]	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; $n = 32$]).

Agradecimentos

Agradecimentos especiais a comunidade local do Refúgio Ecológico Matas de Siriji pelo acolhimento. A Gestão de Unidade de Conservação da Agência Estadual de Meio Ambiente pela autorização das atividades de pesquisa. Ao Dr. I.J. Roberto pelo grande auxílio e sugestões no manuscrito. Ao Cornell Lab of Ornithology pelo atencioso suporte técnico na análise de dados.

Referências bibliográficas

Andrade, S.P.; Rocha, C.F.; Victor-Junior, E.P. & Vaz-Silva, W. 2015. Advertisement call of *Rhinella inopina* Vaz-Silva,

Valdujo & Pombal, 2012 (Anura: Bufonidae) from the type-locality, northeastern Goiás State, Central Brazil. *Zootaxa* 3931: 448-450.

- Baldissera, F.A.B.; Caramaschi, U. & Haddad, C.F.B. 2004. Review of the *Bufo crucifer* species group, with descriptions of two new related species (Amphibia: Anura: Bufonidae). *Arquivos do Museu Nacional* 62: 255-282.
- Batista, V.G.; Ramalho, W.P.; Amaral, D.F.; Maciel, N.M. & Bastos, R.P. 2016. The advertisement and aggressive calls of *Rhinella abei* (Baldissera, Caramaschi, and Haddad, 2004) (Anura: Bufonidae) from Campo Largo, Paraná, Brazil. *Zootaxa* 4107: 595-597.
- Batista, V.G.; Oda, F.H.; do Amaral, D.F.; Costa, N.D.Q.; Maciel, N.M. & Bastos, R.P. 2017. Release and distress calls of *Rhinella abei* (Baldissera, Caramaschi & Haddad, 2004) and *Rhinella icterica* (Spix, 1824). *Herpetozoa* 30: 100-105.
- Bioacoustics Research Program. 2020. Raven Pro: interactive sound analysis software (Version 1.5). Ithaca, NY, The Cornell Lab of Ornithology. Available from <http://www.birds.cornell.edu/raven>.
- Castellano, S.; Tonttini, L.; Giacoma, C.; Lates, A. & Balletto, E. 2002. The evolution of the release and advertisement calls in the Green Toads (*Bufo viridis* complex). *Biological Journal of the Linnean Society* 77: 379-391.
- Costa, F.R.; Moura, P.H.A. & Nunes, I. 2020. On the courtship, breeding behavior and vocalization of *Rhinella ornata* (Spix, 1824) (Anura: Bufonidae): a well-marked escalated behaviour in a lek-like system. *Acta Ethologica* 23: 69-77.
- Frost, D.R. 2020. *Rhinella crucifer*. Amphibian species of the world: an online reference. Version 6.1. Disponível em: <http://research.amnh.org/herpetology/amphibia/index.html>. Último acesso: 20 de maio de 2020.
- Grenat, P.R. & Martino, A.L. 2013. The release call as a diagnostic character between cryptic related species *Odontophrynus cordobae* and *O. americanus* (Anura: Cycloramphidae). *Zootaxa* 3635: 583-586.
- Guerra, C.; Baldo, D.; Rosset, S.; Borteiro, C. & Kolenc, F. 2011. Advertisement and release calls in neotropical toads of the *Rhinella granulosa* group and evidence of natural hybridization between *R. bergi* and *R. major* (Anura: Bufonidae). *Zootaxa* 3092: 26-42.
- Guerra, V. 2020. Relationship between body size and release call parameters in *Rhinella* species (Anura: Bufonidae), and description of the release call of *Rhinella ocellata* (Günther, 1858). *Herpetology Notes* 13: 191-197.
- Heyer, W.R.; Rand A.S.; Da Cruz C.A.G.; Peixoto O.L. & Nelson C.E. 1990. Frogs of Boracéia. *Arquivos de Zoologia* 31: 231-410.
- Köhler, J.; Jansen, M.; Rodríguez, A.; Kok, P.J.R.; Toledo, L.F.; Emmrich, M.; Glaw, F.; Haddad, C.F.B.; Rödel, M.O. & Vences, M. 2017. The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa* 4251: 1-124.
- Leary, C.J. 2001. Investigating opposing patterns of character displacement in release and advertisement vocalizations of *Bufo fowleri* and *Bufo americanus* (Anura: Bufonidae). *Canadian Journal of Zoology* 79: 1577-1585.
- Lingnau, R. 2009. Distribuição temporal, atividade reprodutiva e vocalizações em uma assembléia de anfíbios anuros de uma Floresta Ombrófila Mista em Santa Catarina, sul do Brasil. (Tese de Doutorado). Pontifícia Universidade Católica do Rio Grande do Sul. Porto Alegre.

- Lourenço, A.C.C.; Baeta, D. & de Abreu, A.C.L. 2010. Tadpole and advertisement call of *Rhinella pombali* (Baldissera, Caramaschi & Haddad, 2004) (Amphibia: Anura: Bufonidae). *Zootaxa* 2370: 65-68.
- Lutz, A. 1934. Notas sobre espécies brasileiras do gênero *Bufo*. *Memórias do Instituto Oswaldo Cruz* 28: 111-159.
- Mângia, S.; Carvalho, P.S.; Almeida, E.P.; Cavalcanti, L.; Simões, C.R. & Santana, D.J. 2017. Release call of *Scinax eurydice* (Bokermann, 1968) (Anura: Hylidae) and advertisement call of northeastern populations. *Herpetology Notes* 10: 237-243.
- Martof, B.S. & Thompson E.F.J. 1958. Reproductive behavior of the chorus frog, *Pseudacris nigrita*. *Behaviour* 13: 243-258.
- McClelland, B.E. & Wilczynski, W. 1989. Release call characteristics of male and female *Rana pipiens*. *Copeia* 1989: 1045-1049.
- Oliveira, R.M.; Ruas, D.S.; Mendes, C.V.D.M. & Sole, M. 2014. Advertisement call of *Rhinella crucifer* (Wied-Neuwied, 1821) (Anura: Bufonidae) from southern Bahia, Brazil. *Zootaxa* 3784: 97-98.
- Pereira, E.; Santos, E.M. & Rodrigues, M.F. 2016. Guia dos anfíbios do Parque Estadual De Dois Irmãos. Editora da UFRPE. Recife.
- Pereyra, M.O.; Blotto B.L.; Baldo, D.; Chaparro J.C.; Ron, S.R.; Elias-Costa, A.J.; Iglesias, P.P.; Venegas, P.J.; Thomé, M.T.C.; Ospina-Sarria, J.J.; Maciel, N.M.; Rada, M.; Kolenc, F.; Borteiro, C.; Rivera-Correa, M.; Rojas-Runjaic, F.J.M.; Moravec, J.; de la Riva, I.; Wheeler W. C.; Castroviejo-Fisher, S.; Grant, T.; Haddad, C.F.B. & Faivovich, J. 2021. Evolution in the Genus *Rhinella*: a total evidence phylogenetic analysis of neotropical true toads (Anura: Bufonidae). *Bulletin of the American Museum of Natural History* 447: 1-156.
- Roberto, I.J.; Brito, L. & Thomé, M.T.C. 2014. A new species of *Rhinella* (Anura: Bufonidae) from northeastern Brazil. *South American Journal of Herpetology* 9: 190-199.
- Spix, J.B. 1824. Animalia nova sive Species novae Testudinum et Ranarum quas in itinere per Brasiliam annis MDCCCXVII–MDCCCXX jussu et auspiciis Maximiliani Josephi I. Bavariae Regis. F.S. Hübschmann. München.
- Thomé, M.T.C.; Zamudio, K.R.; Giovanelli, J.G.R.; Haddad, C. F.B.; Baldissera J.F. A. & Alexandrino, J. 2010. Phylogeography of endemic toads and post-Pliocene persistence of the Brazilian Atlantic Forest. *Molecular Phylogenetics and Evolution* 55: 1018-1031.
- Thomé, M.T.C.; Zamudio, K.R.; Haddad, C.F. & Alexandrino, J. 2012. Delimiting genetic units in Neotropical toads under incomplete lineage sorting and hybridization. *BMC Evolutionary Biology* 12: 242.
- Toledo, L.F.; Martins, I.A.; Bruschi, D.P.; Passos, M.A.; Alexandre, C. & Haddad, C.F.B. 2014. The anuran calling repertoire in the light of social context. *Acta Ethologica* 18: 87-99.
- Vaz-Silva, W.; Valdujo, P.H. & Pombal J.J.P. 2012. New species of the *Rhinella crucifer* group (Anura: Bufonidae) from the Brazilian Cerrado. *Zootaxa* 3265: 57-65.
- Vieira, R.R.S.; Bastos, R.P. & Gambale, P.G. 2014. The release call of *Rhinella mirandaribeiroi* (Gallardo, 1965) (Anura: Bufonidae). *Herpetology Notes* 7: 543-545.

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

Patrick Ribeiro Sanches¹, Fillipe Pedroso-Santos², Carlos Eduardo Costa-Campos³

¹ Instituto Nacional de Pesquisas da Amazônia, Programa de Pós-Graduação em Ecologia (Biologia), Manaus, Amazonas, Brasil.

² Universidade Federal do Amapá, Programa de Pós-Graduação em Biodiversidade Tropical, Macapá, Amapá, Brasil.

³ Laboratório de Herpetologia, Departamento de Ciências Biológicas e da Saúde, Universidade Federal do Amapá, Campus Marco Zero do Equador, CEP 68903-419, Macapá, Amapá, Brasil.

Recibida: 02 Febrero 2021

Revisada: 11 Agosto 2021

Aceptada: 19 Octubre 2021

Editor Asociado: C. Canedo

doi: 10.31017/CdH.2022.(2021-023)

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

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 ($r_s = -0.3714$, $p = 0.47$), body mass and clutch size ($r_s = -0.6667$, $p = 0.15$), and body mass and egg diameter ($r_s = -0.1029$, $p = 0.85$) as well as egg diameter and SVL ($r_s = 0.1449$, $p = 0.49$) (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 berthaltutzae* (Hartmann *et al.*, 2010), *D. ebraccatus* (Touchon and Warkentin, 2008), *D. haddadi* (Toledo *et al.*, 2012; Mageski *et al.*, 2014;

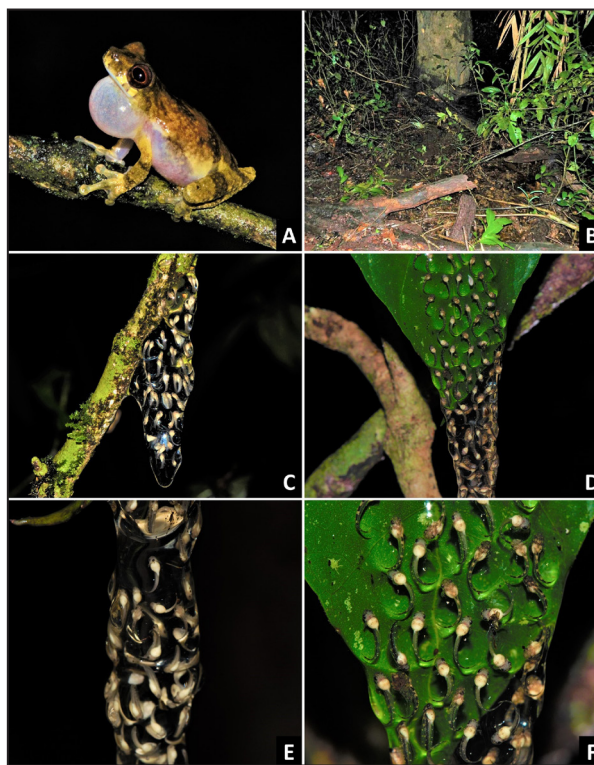


Figure 1. *Dendropsophus counani*: (A) calling male; (B) reproductive habitat located in municipality of Serra do Navio, Amapá state, Brazil, where frogs were sampled; (C), (D), (E) and (F) mature clutch on the leaves and tree branches hanging above water.

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).

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-

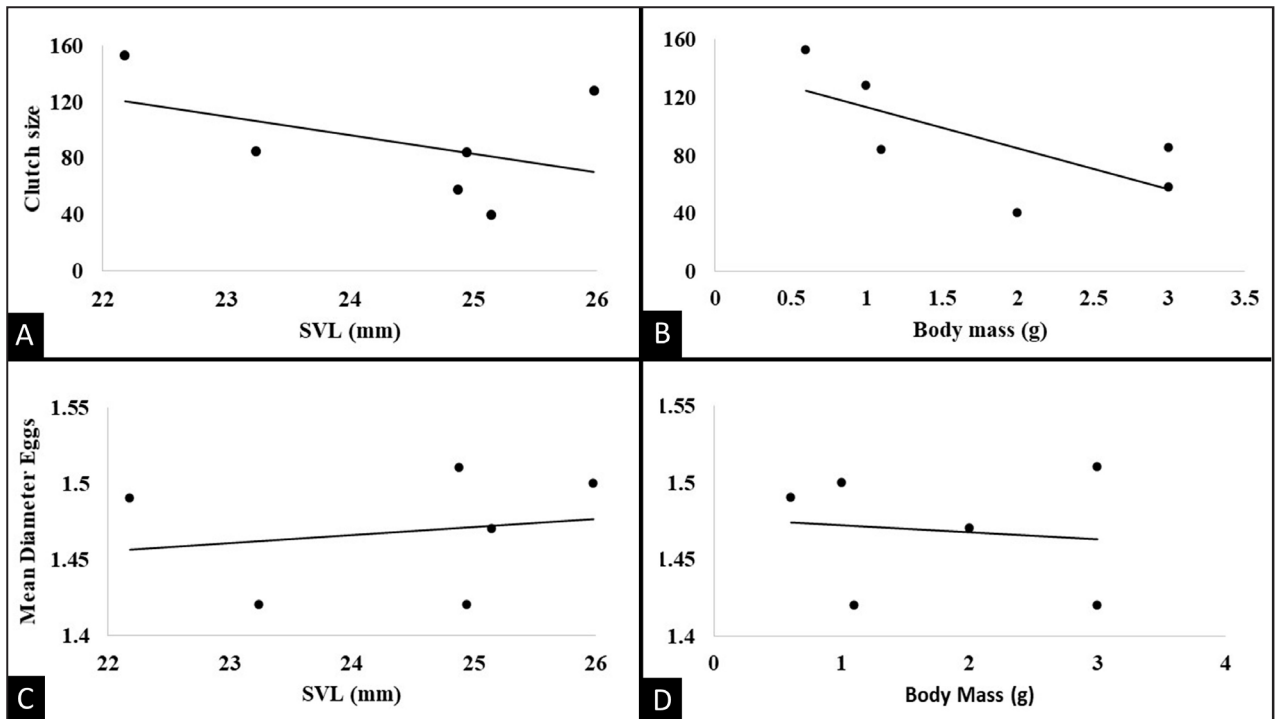


Figure 2. Relationship between (A) clutch size and SVL; (B) clutch size and body mass; (C) mean diameter eggs and SVL, and (D) mean diameter eggs and body mass in *Dendropsophus counani* females from a primary forest in eastern Amazon.

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

This study was carried out as part of the Project "Natural history of anurans of eastern Amazon". We are thankful to Antoine Fouquet for identifying the *Dendropsophus counani*. We thank the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for providing collection permits (SISBIO #48102-2) and Christoph Jaster (PARNA Montanhas

do Tumucumaque) for logistical support during the fieldwork.

Literature cited

- Crump, M.L. 1974. Reproductive strategies in a tropical anuran community. *Miscellaneous publication, University of Kansas publications, Museum of Natural History* 61: 1-68.
- Duellman, W.E. 1970. The Hylid frogs of Middle America. Society for the Study of Amphibians and Reptiles. New York. Ithaca.
- Emlen, S.T. & Oring, L.W. 1977. Ecology, Sexual Selection, and the Evolution of Mating Systems. *Science* 197: 215-223.
- Fouquet, A.; Orrico, V.D.D.; Ernst, R.; Blanc, M.; Martinez, Q.; Vacher, J.P.; Rodrigues, M.T.; Ouboter, P.; Jairam, R. & Ron, S. 2015. A new *Dendropsophus* Fitzinger, 1843 (Anura: Hylidae) of the parviceps group from the lowlands of the Guiana Shield. *Zootaxa* 4052: 39-64.
- Haddad, C.F.B. & Prado, C.P. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. *BioScience* 55: 207-217.
- Hartmann, M.T.; Hartmann, P.A. & Haddad, C.F.B. 2010. Reproductive modes and fecundity of an assemblage of anuran amphibians in the Atlantic rainforest, Brazil. *Iheringia Série Zoologia* 100: 207-215.
- Loyola, R.D.; Becker, C.G.; Kubota, U.; Haddad, C.F.B.; Fonseca, C.R. & Lewinsohn, T.M. 2008. Hung out to dry: choice of priority ecoregions for conserving threatened Neotropical anurans depends on life-history traits. *PlosOne* 3: e2120.
- Mageski, M.; Silva-Soares, T. & Ferreira, R.B. 2014. Hábito bromelígena de *Dendropsophus haddadi* (Anura: Hylidae) em ambiente de Mata Atlântica no sudeste do Brasil. *Boletim do Museu de Biologia Mello Leitão* 34: 97-100.

Sanches *et al.* — Breeding biology of *Dendropsophus counani*

- Orrico, V.G.D.; Grant, T.; Faivovich, J.; Rivere-Correa, M.; Rada, M.A.; Lyra, M.L.; Cassini, C.S.; Valdujo, P.H.; Schargel, W.E.; Machado, D.J.; Wheeler, W.C.; Barrio-Amorós, C.; Loebmann, D.; Moravec, J.; Zina, J.; Solé, M.; Sturaro, M.J.; Peloso, P.L.V.; Suarez, P. & Haddad, C.F.B. 2021. The phylogeny of Dendropsophini (Anura: Hylidae: Hylinae). *Cladistics* 37: 73-105.
- Sanabria, E.A.; Quiroga, L.B. & Acosta, J.C. 2005. Dieta de *Leptodactylus ocellatus* (Linnaeus, 1758) (Anura: Leptodactylidae) en un humedal del oeste de Argentina. *Revista Peruana de Biología* 12: 473-477.
- Silva, N.R.; Neto, J.A.; Prado, C.P.A. & Mott, T. 2019. Reproductive biology of *Dendropsophus haddadi* (Bastos and Pombal, 1994), a small treefrog of the Atlantic forest. *Herpetology Notes* 12: 319-325.
- Toledo, L.F.; Garey, M.V.; Costa, T.R.; Lourenço-de-Moraes, R.; Hartmann, M.T. & Haddad, C.F.B. 2012. Alternative reproductive modes of Atlantic forest frogs. *Journal of Ethology* 30: 331-336.
- Touchon, J.C. & Warkentin, K.M. 2008. Reproductive mode plasticity: aquatic and terrestrial oviposition in a treefrog. *Proceedings of the Natural Academy of Sciences* 105: 7495-7499.
- Wells, K.D. 1977. The social behaviour of anuran amphibians. *Animal Behaviour* 25: 666-693.
- Zar, J.H. 1999. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River.

© 2022 por los autores, licencia otorgada a la Asociación Herpetológica Argentina. Este artículo es de acceso abierto y distribuido bajo los términos y condiciones de una licencia Atribución-No Comercial 4.0 Internacional de Creative Commons. Para ver una copia de esta licencia, visite <http://creativecommons.org/licenses/by-nc/4.0/>

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

Antônio Rafael Lima Ramos^{1,2}, John Allyson Andrade Oliveira¹, Margarida Maria Xavier da Silva^{1,2}, Robson Victor Tavares^{1,3}, Diva Maria Borges-Nojosa^{1,2,3}

¹ Núcleo Regional de Ofiologia, Centro de Ciências, bloco 905. Av. Humberto Monte, Universidade Federal do Ceará, Pici, 60440-900, Fortaleza, Ceará, Brazil.

² Programa de Pós-graduação em Sistemática, Uso e Conservação da Biodiversidade, Departamento de Biologia, bloco 902. Av. Humberto Monte, Universidade Federal do Ceará, Pici, 60440-900, Fortaleza, Ceará, Brazil.

³ Programa de Pós-graduação em Ecologia e Recursos Naturais, Departamento de Biologia, bloco 902. Av. Humberto Monte, Universidade Federal do Ceará, Pici, 60440-900, Fortaleza, Ceará, Brazil.

Recibida: 03 Julio 2021

Revisada: 24 Agosto 2021

Acceptada: 03 Diciembre 2021

Editor Asociado: T. Guedes

doi: 10.31017/CdH.2022.(2021-052)

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

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. klugei* 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 autotomy 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*

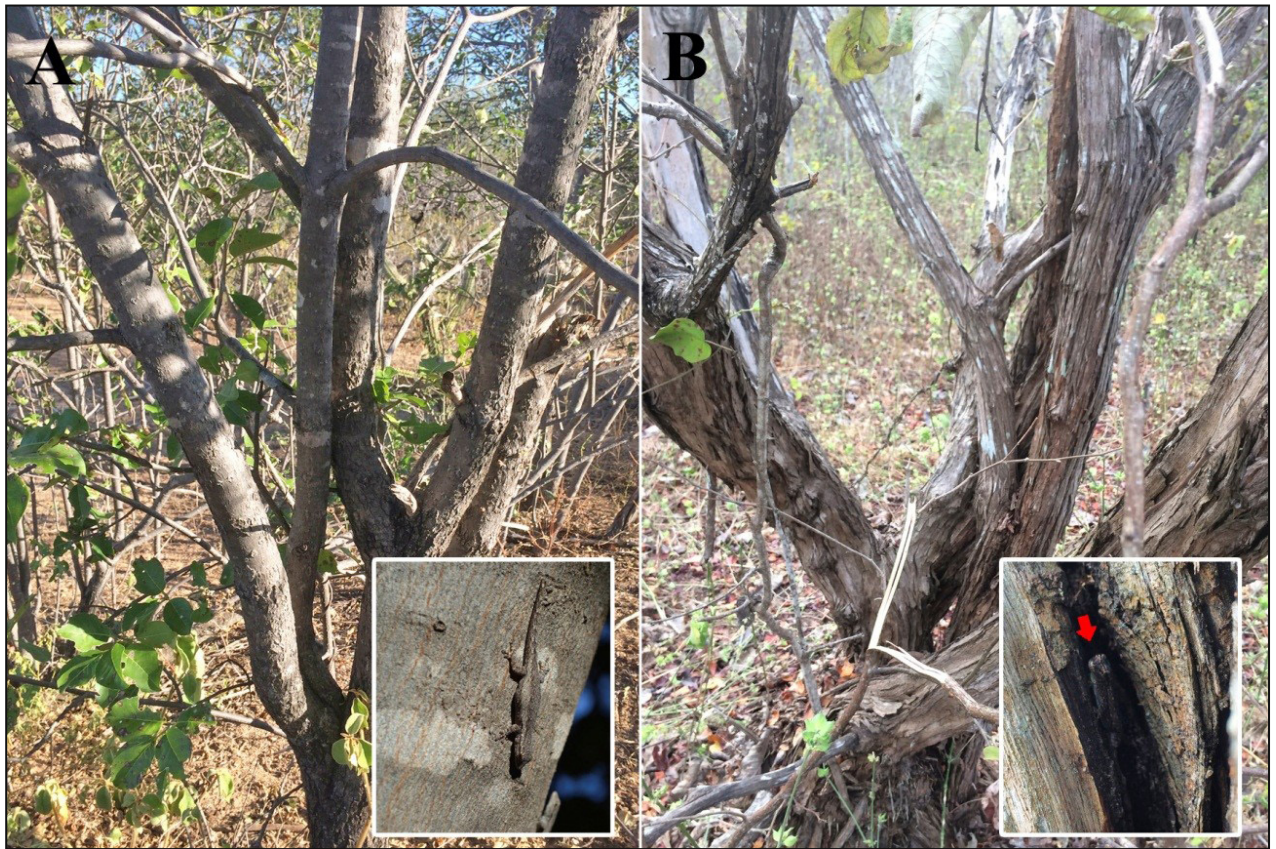


Figure 1. Specimens of trees with smooth (A) and rough (B) trunks with details for the use of the respective trunk types by *L. klugei*.

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)

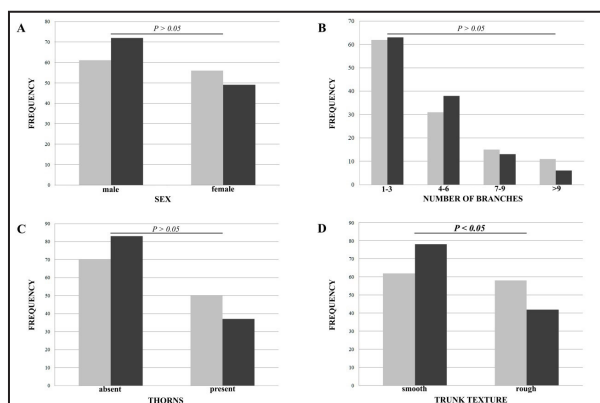


Figure 2. Frequency of lizards with tails intact (light gray bars) and autotomized (dark gray bars) in relation to sex (A), number of branches available at chest level (B), thorns (C) and trunk texture (D) with the respective significance values of the G-test.

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.

Acknowledgments

We are grateful to the Núcleo Regional de Ofiologia at the Universidade Federal do Ceará (NUROF-

UFC), which provided logistical support for the development of our research. This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Projeto Universal 430030 / 2016-9). Collect permits N° 64284-3 issued by SISBio - ICMBio.

Literature cited

- Bateman, P.W., Fleming, P.A. 2009. To cut a long tail short a review of lizard caudal autotomy studies carried out over the last 20 years. *Journal of Zoology* 277: 1-14.
- Bateman, P.W. & Fleming, P.A. 2011. Frequency of tail loss reflects variation in predation levels, predator efficiency, and the behaviour of three populations of brown anoles. *Biological Journal of the Linnean Society* 103: 648-656.
- BeVier, G.T.; Brock, K.M. & Foufopoulos, J. 2021. Life on an island: the effects of insularity on the ecology and home range of the Aegean Wall Lizard (*Podarcis erhardii*). *Herpetological Conservation and Biology* 16: 394-404.
- Chapple, D.G. & Swain, R. 2004. Inter-population variation in the cost of autotomy in the metallic skink (*Niveoscincus metallicus*). *Journal of Zoology* 264: 411-418.
- Clause, A.R. & Capaldi, E.A. 2006. Caudal autotomy and regeneration in lizards. *Journal of Experimental Zoology* 305: 965-973.
- Cooper, W.E.Jr. & Whiting, M.J. 2007. Universal optimization of flight initiation distance and habitat-driven variation in escape tactics in a Namibian lizard assemblage. *Ethology* 113: 661-672.
- Costa A.C.G.L. 2014. História Natural de *Lygodactylus klugei* (Squamata, Gekkonidae) em Patos, Paraíba, Brasil. Undergraduate Thesis, Universidade Federal de Campina Grande, Patos, Paraíba, Brasil.
- Cuadrado, M.; Martín, J. & López, P. 2001. Camouflage and escape decisions in the common chameleon *Chamaeleo chamaeleon*. *Biological Journal of the Linnean Society* 72: 547-554.
- Daniels, C.B. 1985. Economy of autotomy as a lipid conserving mechanism: an hypothesis rejected for the gecko *Phyllodactylus marmoratus*. *Copeia* 1985: 468-472.
- Donihue, C.M.; Brock, K.M.; Foufopoulos, J. & Herrel, A. 2016. Feed or fight: testing the impact of food availability and intraspecific aggression on the functional ecology of an island lizard. *Functional Ecology* 30: 566-575.
- Duckett, P.E. & Stow, A.J. 2011. Levels of dispersal and tail loss in an Australian gecko (*Gehyra variegata*) are associated with differences in forest structure. *Australian Journal of Zoology* 59: 170-176.
- Fleming, P.A.; Verburgt, L.; Scantlebury, M.; Medger, K. & Bateman, P.W. 2009. Jettisoning ballast or fuel? Caudal autotomy and locomotory energetics of the Cape dwarf gecko *Lygodactylus capensis* (Gekkonidae). *Physiological and Biochemical Zoology* 82: 756-765.
- Fleming, P.A. & Bateman, P.W. 2012. Autotomy, tail regeneration and jumping ability in Cape Dwarf Geckos (*Lygodactylus capensis*) (Gekkonidae). *African Zoology* 47: 55-59.
- Fleming, P.A.; Valentine, L.E. & Bateman, P.W. 2013. Telling tails: selective pressures acting on investment in lizard tails. *Physiological and Biochemical Zoology* 86: 645-658.
- Galdino, C.; Ventura, S. & Moreira, G. 2017. Unveiling a spatial

- tail breakage outbreak in a lizard population. *Amphibia-Reptilia* 38: 238-242.
- Gordeev, D.A.; Ananjeva, N.B. & Korost, D.V. 2020. Autotomy and regeneration in Squamate reptiles (Squamata, Reptilia): defensive behavior strategies and morphological characteristics (using computer microtomography methods). *Biology Bulletin* 47: 389-398.
- Grolle, E.K.; Lopez, M.C. & Gerson, M.M. 2014. Flight Initiation Distance Differs between Populations of Western Fence Lizards (*Sceloporus occidentalis*) at a Rural and an Urban Site. *Bulletin of the Southern California Academy of Sciences* 113: 42-46.
- Higham, T.E.; Lipsett, K.R.; Syme, D.A. & Russell, A.P. 2013. Controlled chaos: three-dimensional kinematics, fiber histochemistry, and muscle contractile dynamics of autotomized lizard tails. *Physiological and Biochemical Zoology* 86: 611-630.
- Itescu, Y.; Schwarz, R.; Meiri, S. & Pafilis, P. 2017. Intraspecific competition, not predation, drives lizard tail loss on islands. *Journal of Animal Ecology* 86: 66-74.
- Lin, J.-W.; Chen, Y.-R.; Wang, Y.-H.; Hung, K.-C. & Lin, S.-M. 2017. Tail regeneration after autotomy revives survival: a case from a long-term monitored lizard population under avian predation. *Proceedings of the Royal Society B: Biological Sciences* 284: doi:10.1098/rspb.2016.2538
- Medger, K.; Verburt, L. & Bateman, P.W. 2008. The influence of tail autotomy on the escape response of the Cape Dwarf Gecko, *Lygodactylus capensis*. *Ethology* 114: 42-52.
- Nunes, J.V.; Elisei, T. & Sousa, B.M. 2012. Anti-predator behaviour in the Brazilian lizard *Tropidurus itambere* (Tropiduridae) on a rocky outcrop. *Herpetological Bulletin* 120: 22-28.
- Pafilis, P.; Sagonas, K.; Kapsalas, G.; Foufopoulos, J. & Valakos, E.F. 2017. Sex does not affect tail autotomy in lacertid lizards. *Acta Herpetologica* 12: 19-27.
- Passos, D.C.; Galdino, C.A.B.; Bezerra, C.H. & Zanchi, D. 2013. Indirect evidence of predation and intraspecific aggression in three sympatric lizard species from a semi-arid area in northeastern Brazil. *Zoologia* 30: 467-469.
- Pianka, E.R. & Huey, R.B. 1978. Comparative ecology, resource utilisation and niche segregation among gekkonid lizards in the southern Kalahari. *Copeia* 1978: 691-701.
- R Development Core Team. 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <https://www.R-project.org/>. Last accessed: 05 May 2021.
- Recoder, R.; Teixeira Junior, M.; Camacho, A. & Rodrigues, M.T. 2012. Natural history of the tropical gecko *Phyllolopezus pollicaris* (Squamata, Phyllodactylidae) from a sandstone outcrop in Central Brazil. *Herpetology Notes* 5: 49-58.
- Salido, C.A. & Vicente, N.S. 2019. Sex and refuge distance influence escape decision in a *Liolaemus* lizard when it is approached by a terrestrial predator. *Behaviour* 156: 909-925.
- Savvides, P.; Poliviou, V.; Stavrou, M.; Sfenthourakis, S. & Pafilis, P. 2018. Insights into how predator diversity, population density and habitat type may affect defensive behaviour in a Mediterranean lizard. doi: 10.1080/03949370.2018.1477836.
- Sokal, R.R. & Rohlf, F.J. 1995. Biometry: the principles of statistics in biological research 3rd ed. Freeman and Company. New York.
- Sousa, H.C.; Costa, B.M.; Morais, C.J.S.; Pantoja, D.L.; de Queiroz, T.A.; Vieira, C.R. & Colli, G.R. 2016. Blue tales of a blue-tailed lizard: ecological correlates of tail autotomy in *Micrablepharus atticolus* (Squamata, Gymnophthalmidae) in a Neotropical savannah. *Journal of Zoology* 299: 202-212.
- Smith, G.R. & Ballinger R.E. 2001. The ecological consequences of habitat and microhabitat use in lizards: a review. *Contemporary Herpetology* 2001: 1-13.
- Tanner, D. & Perry, J. 2007. Road effects on abundance and fitness of Galapagos lava lizards (*Microlophus albemarlensis*). *Journal of Environmental Management* 85: 270-278.
- Vanhooydonck, B.; Andronescu, A.; Herrel, A. & Irschick, D.J. 2005. Effects of substrate structure on speed and acceleration capacity in climbing geckos. *Biological Journal of the Linnean Society* 85: 385-393.
- Vitt, L.J. 1983. Tail loss in lizards: the significance of foraging and predator escape modes. *Herpetologica* 39: 151-162.
- Vitt, L.J. & Ballinger, R. 1982. The adaptive significance of a complex caudal adaptation in the tropical gekkonid lizard *Lygodactylus klugei*. *Canadian Journal of Zoology* 60: 2587-2582.
- Vitt, L.J. & Zani, P.A. 1997. Ecology of the nocturnal lizard *Thecadactylus rapicauda* (Sauria: Gekkonidae) in the Amazon region. *Herpetologica* 53: 165-179.
- Vitt, L.J.; Shepard, D.B.; Caldwell, J.P.; Vieira, G.H.C.; França, F.G.R. & Colli, G.R. 2007. Living with your food: geckos in termitaria of Cantão. *Journal of Zoology* 272: 321-328.

Appendix. Material examined from Coleção Herpetológica da Universidade Federal de Ceará (CHUFC): Aiuaíba (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).

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

Carlos Santiago Rosales-Martínez¹, Carlos Daniel Bello-Sánchez², Marco Antonio López-Luna^{3,4}, Armando H. Escobedo-Galván⁵, Fabio G. Cupul-Magaña⁵

¹ Justo Sierra 2807, Colonia Vallarta Norte, Guadalajara 44690, Jalisco, México.

² Cocoteros 116-A, Fraccionamiento Paseos Universidad II, Puerto Vallarta 48280, Jalisco, México.

³ División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco, Carr. Villahermosa-Cárdenas km 0.5 Villahermosa 86039, Tabasco, México.

⁴ Instituto de Neuroetología, Universidad Veracruzana, Av. Dr. Luis Castelazo, Industrial de las ánimas, Xalapa Enríquez 91190, Veracruz, México.

⁵ Centro Universitario de la Costa, Universidad de Guadalajara, Av. Universidad 203, Delegación Ixtapa, Puerto Vallarta 48280, Jalisco, México.

Recibida: 20 Agosto 2021

Revisada: 20 Octubre 2021

Aceptada: 08 Noviembre 2021

Editor Asociado: J. Goldberg

doi: 10.31017/CdH.2022.(2021-055)

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

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:

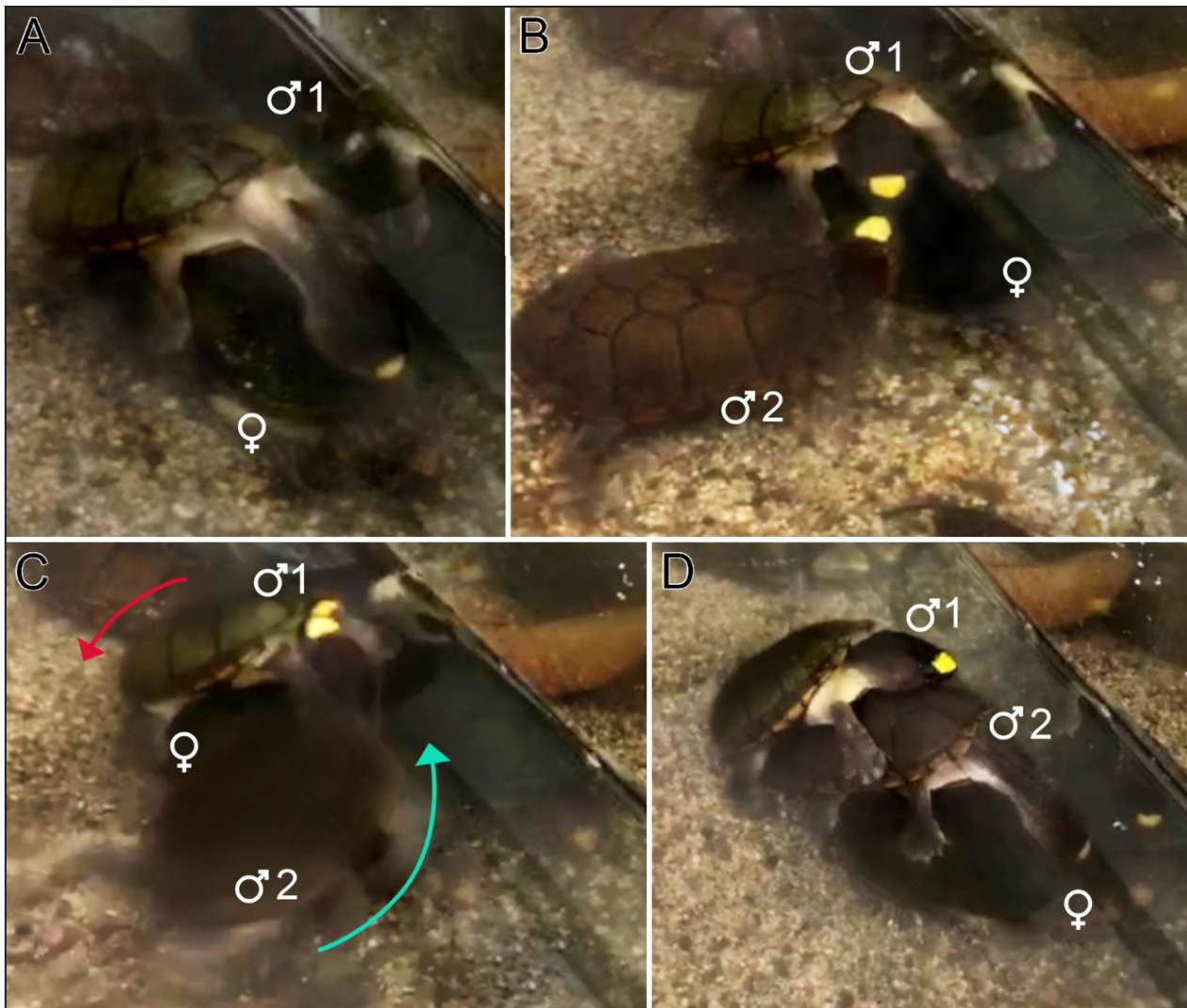


Figure 1. Reproductive combat behavior sequence between two captive *Kinosternon vogti* males. A) Male #1 in mount of female #1. B) Confrontation between males. C) Slam of male #2 (the direction is indicated by the green arrow) on male #1 to knock him off the mount of female #1 (the red arrow indicates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

(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 x 10 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.

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. 1B). 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

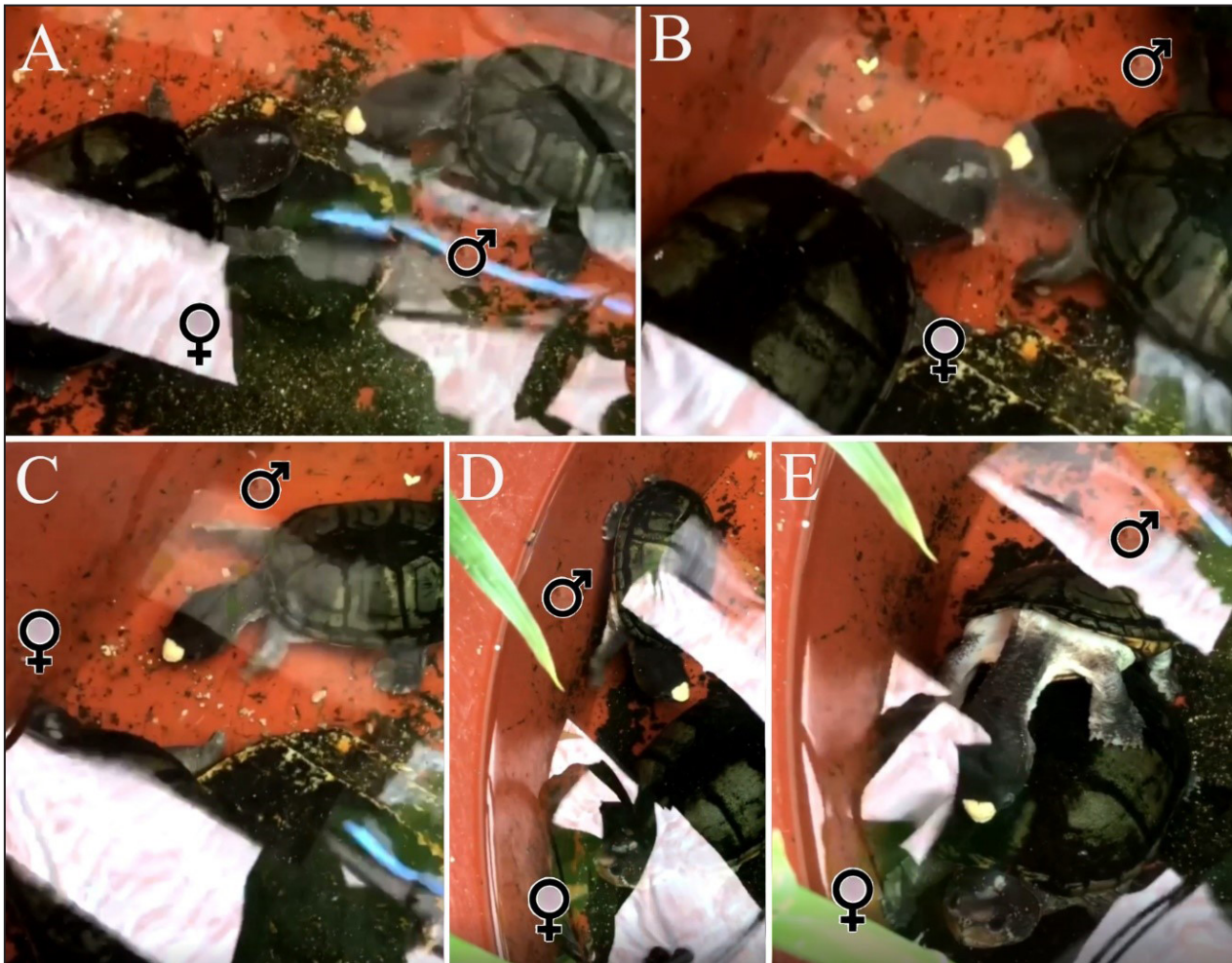


Figure 2. *Kinosternon vogti* female #2 and male #3, courtship and mount. A) Male block the advancement of the female. B) Female bites the male's rostral scale. C) Male in titillation posture. D) Approximation of the male to the back of the female. E) Male in mount on the female. Images from video available 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 x 15.88 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 traits observed in Montaña-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 Vogt, 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.

Acknowledgments

To UMA Reptilario Cipactli (INE/CITES/DGVS-CR-IN-0610-JAL/00) of the Centro Universitario de la Costa of the Universidad de Guadalajara, and the Centro de Investigación y Conservación de Especies Amenazadas CICEA (INE/CITES/DFYFS-CR-IN-0023-TAB/99) of the División Académica de Ciencias Biológicas of the Universidad Juárez Autónoma de Tabasco for facilities information and the videos. We thank the comments and the recommendations from the anonymous reviewers. Special thanks to Taggart Butterfield for his comments and who kindly corrected the English of the final version of the manuscript. We are also grateful to SEMARNAT for providing the collecting permit (FAUT-0064).

Literature cited

- Berry, J.F. & Shine, R. 1980. Sexual size dimorphism and sexual selection in turtles (Order Testudines). *Oecologia* 44: 185-191.
- Ewert, M.A. 1985. Embriology of the turtles: 75-267. *In*: Gans, C.; Billet, F. & Maderson, P.F.A. (eds.), *Biology of the Reptilia*. John Wiley and Sons, New York.
- García-Oliva, F.; Camou, A. & Maass, J.M. 2002. El clima de la región central de la costa del Pacífico mexicano: 3-10. *In*: Noguera, F.A.; Vega-Rivera, J.H.; García-Aldrete, A.N. & Quesada-Avendaño, M. (eds.), *Historia natural de Chamela*. Instituto de Biología-UNAM, México.
- Iverson, J.B. 1996. Notes on the natural history of the Oaxaca Mud Turtle, *Kinosternon oaxacae*. *Journal of Herpetology* 20: 119-123.
- Kramer, M. & Burghardt, G.M. 2010. Precocious courtship and play in Emydid turtles. *Ethology* 104: 38-56.
- Legler, J.M. & Vogt, R.C. 2013. *The turtles of Mexico: Land and freshwater forms*. University of California Press, Berkeley.
- Liu, Y-x.; Davy, C.M.; Shi, H-T. & Murphy, R.W. 2013. Sex in the half-shell: A review of the functions and evolution of courtship behavior in freshwater turtles. *Chelonian Conservation and Biology* 12: 84-100.
- Loc-Barragán, J.A.; Reyes-Velasco, J.; Woolrich-Piña, G.A.; Grünwald, C.I.; Venegas de Anaya, M.; Rangel-Mendoza, J.A. & López-Luna, M.A. 2020. A new species of Mud Turtle of genus *Kinosternon* (Testudines: Kinosternidae) from the Pacific Coastal plain of Northwestern Mexico. *Zootaxa* 4885: 509-529.
- López-Luna, M.A.; Cupul-Magaña, F.G.; Escobedo-Galván, A.H.; González-Hernández, A.J.; Centenero-Alcalá, E.; Rangel-Mendoza, J.A.; Ramírez-Ramírez, M.M. & Cazarez-Hernández, E. 2018. A distinctive new species of Mud Turtle from Western Mexico. *Chelonian Conservation and Biology* 17: 2-13.
- Mahmoud, I.Y. 1967. Courtship behavior and sexual maturity in four species of Kinosternid turtles. *Copeia* 1967: 314-319.
- Montaño-Ruvalcaba, C.; Loc-Barragán, J.A.; Grünwald, C.I. & Reyes-Velasco, J. 2020. *Kinosternon vogti* (Vallarta Mud Turtle). *Reproduction*. *Herpetological Review* 51: 315-316.
- Packard, G.C.; Packard, M.J.; Miller, K. & Boardman, T.J. 1987. Influence of moisture, temperature, and substrate on snapping turtle eggs and embryos. *Ecology* 68: 983-993.
- Ramírez-Ramírez, M.M.; López-Luna, M.A.; Escobedo-Galván, A.H. & Cupul-Magaña, F.G. 2019. *Kinosternon vogti* (Vallarta Mud Turtle). *Diet*. *Herpetological Review* 50: 258-259.
- Rosales-Martínez, C.S.; Bello-Sánchez, C.D.; Centenero-Alcalá, E. & Cupul-Magaña, F.G. 2021. *Kinosternon vogti* (KINOSTERNIDAE). *Revista Latinoamericana de Herpetología* 4: 232-233.
- Secretaría de Medio Ambiente y Recursos Naturales. 2019. *Modificación del Anexo Normativo III, Lista de especies en riesgo de la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo*, publicada el 30 de diciembre de 2010. *Diario Oficial de la Federación* del jueves 14 de noviembre de 2019. Available at: https://www.dof.gob.mx/nota_detalle.php?codigo=5578808&fecha=14/11/2019. Last access: 13 May 2021.

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

Fillipe Pedroso-Santos¹, Vinícius A. M. B. de Figueiredo², Carlos E. Costa-Campos²

¹Programa de Pós-Graduação em Biodiversidade Tropical, Universidade Federal do Amapá, 68903-419, Macapá, Amapá, Brazil.

²Laboratório de Herpetologia, Departamento de Ciências Biológicas e da Saúde, Universidade Federal do Amapá, 68903-419, Macapá, Amapá, Brazil.

Recibida: 20 Junio 2021

Revisada: 12 Octubre 2021

Aceptada: 26 Octubre 2021

Editor Asociado: C. Borteiro

doi: 10.31017/CdH.2022.(2021-061)

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

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).

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.

Acknowledgments

We are indebted to Ambiental Consultoria for logistical support during the fieldwork and for the authorization to photograph the individual. Claudio Borteiro and an anonymous reviewer for helpful comments on the manuscript. This study is part of the project “Natural history of anuran amphibians

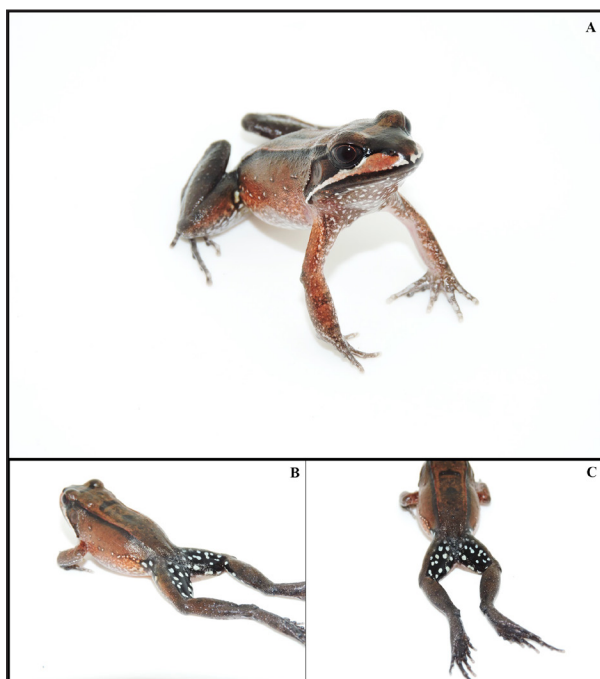


Figure 1. (A) Usual posture of *Leptodactylus rhodomystax*. (B) Postero-lateral and (C) posterior view of stretching limbs posture evidencing hidden aposematism.

from the eastern Amazon". FPS received a fellowship from CNPq during this study (#134760/2018-2).

Literature cited

- Bertoluci, J.; Brassaloti, R.A.; Sawakuchi, H.O.; Ribeiro Jr, J.W. & Woehl Jr, G. 2007. Defensive behavior with stiff-legged posture in the Brazilian tree toads *Dendrophryniscus brevipollicatus* and *D. leucomystax* (Anura, Bufonidae). *Alytes* 25: 1–2.
- Borteiro, C.; Rosset, S.D.; Kolenc, F.; Barrasso, D.A.; Lescano, J.N. & Baldo, D. 2018. Stereotyped defensive behaviours in frogs of the genus *Odontophrynus* (Amphibia: Anura: Odontophrynidae). *Current Herpetology* 37: 172–179.
- Barrio-Amorós, C.L.; Rojas-Runjaic, F.J.M. & Señaris, J.C. 2019. Catalogue of the amphibians of Venezuela: illustrated and annotated species list, distribution, and conservation. *Amphibian & Reptile Conservation* 13: 1–198.
- Brodie, E.D. & Brodie Jr, E.D. 1999. Predator-prey arms races. *BioScience* 49: 557–568.
- Duellman, W.E. & Trueb, L. 1994. *Biology of amphibians*. Baltimore: John Hopkins University. 696 pp.
- de Sá, R.O.; Grant, T.; Camargo, A.; Heyer, W.R.; Ponssa, M.L. & Stanley, E. 2014. Systematics of the Neotropical Genus *Leptodactylus* Fitzinger, 1826 (Anura: Leptodactylidae): Phylogeny, the Relevance of Non-molecular Evidence, and Species Accounts. *South American Journal of Herpetology* 9: 1–128.
- Dreher, C.E.; Cummings, M.E. & Pröhl, H. 2015. An analysis of predator selection to affect aposematic coloration in a poison frog species. *PLoS ONE* 10: e0130571.
- Ferreira, R.B.; Lourenço-de-Moraes, R.; Zocca, C.; Duca, C. & Beard, K.H. 2019. Antipredator mechanisms of post-metamorphic anurans: a global database and classification system. *Behavioral Ecology and Sociobiology* 2019: 1–21.
- Ferrante, L.; Najar, T. & Kaefer, I.L. 2020. Four new anuran defence behaviours observed in the cane toad *Rhinella marina*. *Ethology Ecology & Evolution* 6: 590–595.
- Figueiredo-de-Andrade, C.A. & Silveira, L.S. 2018. The defensive behaviour of *Rhinella pygmaea* (Myers & Carvalho, 1952) (Anura: Bufonidae). *Herpetology Notes* 11: 205–207.
- Frost, D. R. (ed.). 2021. Amphibian Species of the World: an Online Reference. Version 6.0 (1 April 2020). Electronic Database accessible at <https://amphibiansoftheworld.amnh.org/index.php>. American Museum of Natural History, New York, USA. Captured on 09 June 2021.
- Gally, M.; Zina, J.; Mira-Mendes, C.V. & Solé, M. 2014. Legs-interweaving: An unusual defense behavior of anurans displayed by *Agalychnis aspera* (Peters, 1983). *Herpetology Notes* 7: 623–625.
- Greenbaum, E. 2004. The influence of prey-scent stimuli on predatory behavior of the North American copperhead *Agkistrodon contortrix* (Serpente: Viperidae). *Behavioral Ecology* 15: 345–350.
- Johnson, J.A. & Brodie Jr, E.D. 1975. The selective advantage of the defensive posture of the newt, *Taricha granulosa*. *The American Midland Naturalist* 93: 139–148.
- Kolenc, F.; Borteiro, C.; Baldo, D.; Ferraro, D.P. & Prigioni, C.M. 2009. The tadpoles and advertisement calls of *Pleurodema bibroni* Tschudi and *Pleurodema kriegi* (Müller), with notes on their geographic distribution and conservation status (Amphibia, Anura, Leiuperidae). *Zootaxa* 1969: 1–35.
- Mângia, S. & Santana, D.J. 2013. Defensive behavior in *Rhinella granulosa* (Spix, 1824) (Amphibia: Anura: Bufonidae). *Herpetology Notes* 6: 45–46.
- Mira-Mendes, C.V.; Ruas, D.S.; Castro, I.; Solé, M. & Baumgarten, J.E. 2016. Defensive behaviours in the Bahia forest frog *Macrogenioglottus alipioi* Carvalho, 1946 (Anura: Odontophrynidae), with a review of the stiff-legged posture. *Herpetology Notes* 9: 91–94.
- Rubbo, M.J.; Townsend Jr, V.R.; Smyers, S.D. & Jaeger, R.G. 2003. An experimental assessment of invertebrate/vertebrate predation: the interaction between wolf spider (*Gladicosa pulchra*) and terrestrial salamanders (*Ambystoma maculatum*). *Journal of Zoology* 261: 1–5.
- Rebouças, R.; Carollo, A.B.; Freitas, M.O.; Lambertini, C.; Santos, R.M.N. & Toledo, L.F. 2019. Is the conspicuous dorsal coloration of the Atlantic Forest pumpkin toadlets aposematic? *Salamandra* 55: 39–47.
- Rocha, R. & López-Baucells, A. 2014. Predation attempt of *Hypsiboas boans* (Anura: Hylidae) by *Helicops angulatus* (Squamata: Dipsadidae) with notes on defensive behavior. *Alytes* 30: 78–81.
- Sazima, I. 1978. Convergent defensive behavior of two leaf-litter frogs of southeastern Brazil. *Biotropica* 10: 158.
- Toledo, L.F.; Sazima, I. & Haddad, C.F.B. 2010. Is it all death feigning? Case in anurans. *Journal of Natural History* 44: 31–32.
- Toledo, L.F.; Sazima, I. & Haddad, C.F.B. 2011. Behavioural defences of anurans: an overview. *Ethology Ecology and Evolution* 23: 1–25.

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

Ana Luiza Sangel Soeiro¹, Anna Klara Matos Guerreiro¹, Aline Emanuele Oliveira-Souza¹, Maria Madalena Salviano Santana¹, Carlos Eduardo Costa-Campos^{1,2}

¹Universidade Federal do Amapá, Departamento de Ciências Biológicas e da Saúde, Laboratório de Herpetologia, Macapá, AP, CEP: 68.903-419, Brazil.

²Programa de Pós-Graduação em Biodiversidade e Biotecnologia-Rede BIONORTE, Universidade Federal do Amapá, Macapá, AP, CEP: 68.902-280, Brazil.

Recibida: 11 Diciembre 2021

Revisada: 11 Marzo 2021

Aceptada: 21 Abril 2022

Editor Asociado: P. Peltzer

doi: 10.31017/CdH.2022.(2021-067)

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).

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. *Dendropsophus haraldschultzi* was sampled in a seasonally flooded area (regionally referred to as the “ressaca” area) surrounding human settlements in the municipality of Santana (0.0365°S, 51.1626°W). *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: $B = 1 / \sum p_i^2$, 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 (B_{sta}), which limits the index to a scale from 0 to 1 according to the following equation: $B_{sta} = (B-1) / (n-1)$, 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* (B_{sta} = 0.82) and *D. minutus* (B_{sta} = 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	<i>Dendropsophus haraldschultzi</i> (N = 32)				<i>Dendropsophus minutus</i> (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).

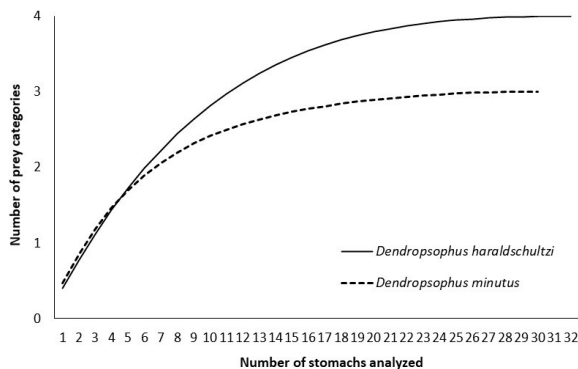


Figure 1. Accumulation curve showing the number of prey categories relative to the number of stomachs containing prey items analyzed in the diet of *Dendropsophus haraldschultzi* and *D. minutus* in anthropogenic environments in eastern Amazonia.

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.

Acknowledgments

The authors are grateful to *colleagues* from the Laboratório de Herpetologia who helped and collaborated

in anuran sampling and two anonymous reviewers for their helpful comments on the manuscript. We would like to thank the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for providing a collection permit (SISBIO #48102-2) and Christoph Jaster (PARNA Montanhas do Tumucumaque) for logistical support during the fieldwork in the municipality of Serra do Navio.

Literature cited

- Abegg, A.D.; Rosa, C.M. & Borges, L.M. 2014. Predation of *Dendropsophus minutus* (Anura: Hylidae) by *Aglaotenus oblongus* (Araneae: Lycosidae). *Herpetology Notes* 7: 605-606.
- Böning, P.; Wolf, S.; Upton, K.; Menin, M.; Venegas, P.J. & Lötters, S. 2017. Amphibian diversity and its turnover in floating meadows along the Amazon river. *Salamandra* 53: 379-388.
- Castro, I.M.; Rebouças, R. & Solé, M. 2016. Diet of *Dendropsophus branneri* (Cochran, 1948) (Anura: Hylidae) from a cocoa plantation in southern Bahia, Brazil. *North-Western Journal of Zoology* 12: 159-165.
- Ceron, K.; Moroti, M.T.; Benício, R.A.; Balboa, Z.P.; Marçola, Y.; Pereira, L.B. & Santana, D.J. 2018. Diet and first report of batracophagy in *Leptodactylus podicipinus* (Anura: Leptodactylidae). *Neotropical Biodiversity* 4: 69-73.
- Colwell, R.K. 2013. EstimateS: Statistical estimation of species richness and shared species from 279 samples. Available at: <http://purl.oclc.org/estimates>. Last access 10 March 2022.
- Crump, M.L. & Scott Jr., N.J. 1994. Visual Encounter Surveys: 84-92. In: Heyer, W.R.; Donnelly, M.A.; McDiarmid, R.W.; Hayek, L.C. & Foster, M.S. (eds.), *Measuring and Monitoring Biological Diversity: standard methods for amphibians*. Smithsonian Institution Press. Washington D.C.
- Figueiredo, V.A.M.B.; Tavares-Pinheiro, R.; Sanches, P.R.; Pedroso-Santos, F.; Sousa, J.C.; Corrêa, J.G.; França, P.F. & Costa-Campos, C.E. 2021. *Sphaenorhynchus lacteus* (Orinoco Lime Treefrog) and *Dendropsophus haraldschultzi* (Haralds Treefrog). Microhabitat use. *Herpetological Review* 52: 123.
- Fonseca-Pérez, K.A.; Molina, C. & Tárano, Z. 2017. Diet of *Dendropsophus microcephalus* and *Scarthyla vigilans* (Anura: Hylidae) at a locality in north-western Venezuela with notes on microhabitat occupation. *Papéis Avulsos de Zoologia* 57: 93-104.
- Griffiths, R.A. & Mylotte, V.J. 1987. Microhabitat selection and feeding relations of smooth and warty newts, *Triturus vulgaris* and *T. cristatus*, at an upland pond in mid-Wales. *Holarctic Ecology* 10: 1-7.
- Hödl, W. 1977. Call Differences and Calling Site Segregation in Anuran Species from Central Amazonian Floating Meadows. *Oecologia* 28: 351-363.
- Huey, R.B. & Pianka, E.R. 1981. Ecological consequences of foraging mode. *Ecology* 62: 991-999.
- IUCN 2022. The IUCN Red List of Threatened Species. Version 2021-3. Available at: <https://www.iucnredlist.org>. Last access: 14 March 2022.
- Krebs, C.J. 2004. *Ecological Methodology*. Second Edition. Benjamin/Cummings. New York.
- Leivas, P.T.; Mayer, T.B.; Leivas, F.W.T. & Fávoro, L.F. 2018. Trophic niche of *Dendropsophus minutus* (Anura: Hylidae) in southern Brazil. *Phyllomedusa* 17: 267-272.
- López, J.A.; Scarabotti, P.A.; Medrano, M.C. & Ghirardi, R. 2009. Is the red spotted green frog *Hypsiboas punctatus* (Anura: Hylidae) selecting its preys? The importance of prey availability. *Revista de Biología Tropical* 57: 847-857.
- Menin, M.; Rossa-Feres, D.C. & Giaretta, A.A. 2005. Resource use and coexistence of two syntopic hylid frogs (Anura, Hylidae). *Revista Brasileira de Zoologia* 22: 61-72.
- Menin, M.; Santos, R.S.; Borges, R.E. & Piatti, L. 2015. Notes on the diet of seven terrestrial frogs in three agroecosystems and forest remnants in Northwestern São Paulo State, Brazil. *Herpetology Notes* 8: 401-405.
- Menin, M.; Almeida, A.P. de; Pedroso-Santos, F.; Sanches, P.R. & Costa-Campos, C.E. 2020. Description of the tadpole of *Dendropsophus haraldschultzi* (Bokermann, 1962) (Anura: Hylidae), with comments on reproductive biology. *Zootaxa* 4780: 594-600.
- Michelin, G.; Ceron, K. & Santana, D. J., 2020. Prey availability influences the diet of *Scinax fuscomarginatus* in a Cerrado area, Central Brazil. *Animal Biodiversity and Conservation* 43: 169-175.
- Moroti, M.T.; Soares, P.T.; Pedrozo, M.; Provet, D.B. & Santana, D.J. 2021. The effects of morphology, phylogeny and prey availability on trophic resource partitioning in an anuran community. *Basic and Applied Ecology* 50: 181-191.
- Moser, C.F.; Oliveira, M.; Dutra-Araújo, D.; Farina, R.K. & Tozetti, A.M. 2019. Diet and trophic niche overlap of *Boana bischoffi* and *Boana marginata* (Anura: Hylidae) in southern Brazil. *Biota Neotropica* 19: e20180542.
- Parmelee, J.R. 1999. Trophic ecology of a tropical anuran assemblage. *Occasional Papers of the Museum of Natural History, the University of Kansas* 11: 1-59.
- Petrozzi, F.; Akani, G.C.; Eniang, E.A.; Ajong, S.N.; Funk, S.M.; Fa, J.E.; Amadi, N.; Dendi, D. & Luiselli, L. 2021. Generalist, selective or 'mixed' foragers? Feeding strategies of two tropical toads across suburban habitats. *Journal of Zoology* 315: 288-300.
- Pinkas, L.; Oliphant, M.S. & Iverson, I.L.K. 1971. Food habits of albacore, bluefin tuna, and bonito in Californian waters. *Fisheries Bulletin* 152: 11-105.
- Rafael, J.A.; Melo, G.; Carvalho, C.; Casari, C.A. & Constantino, R. 2012. *Insetos do Brasil: Diversidade e Taxonomia*. Holos. Ribeirão Preto, Brasil.
- Sanches, P.R.; Pedroso-Santos, F.; Corrêa, J.G.; França, P.F.; Sousa, J.C.; Cantuária, P.C. & Costa-Campos, C.E. 2019. Anurans associated with the bromeliad *Bromelia goeldiana* L.B.Sm on a floodplain in northern Brazil, eastern Amazon. *Herpetology Notes* 12: 431-433.
- Sanches, P.R.; Pedroso-Santos, F. & Costa-Campos, C.E. 2019. Diet of *Adenomera hylaedactyla* (Cope, 1868) (Anura: Leptodactylidae) from an urban area in southern Amapá, eastern Amazon. *Herpetology Notes* 12: 841-845.
- Sanches, P.R.; Pedroso-Santos, F. & Costa-Campos, C.E. 2021. Diet of *Dendropsophus counani* (Anura: Hylidae) during breeding season in the Eastern Amazonia. *Cuadernos de Herpetología* 35: 327-331.
- Santana, D.J.; Ferreira, V.G.; Crestani, G.N. & Neves, M.O. 2019. Diet of the Rufous Frog *Leptodactylus fuscus* (Anura, Leptodactylidae) from two contrasting environments. *Herpetozoa* 32: 1-6.
- Santos, E.M.; Almeida, A.V. & Vasconcelos, S.D. 2004. Feeding habits of six anuran (Amphibia: Anura) species in a

- rainforest fragment in Northeastern Brazil. *Iheringia, Série Zoologia* 94: 433-438.
- Silva, I.C.O.; Soares, P.; Ribas, A.C.A.; Santana, D.J.; Campião, K.M. & Tavares, L.E.R. 2021. *Scinax fuscovarius* (Anura, Hylidae) diet assessment at Serra da Bodoquena, Mato Grosso do Sul, Brazil. *North-Western Journal of Zoology* 17: 29-33.
- Taigen, T.L. & Pough, F.H. 1983. Prey preference, foraging behavior, and metabolic characteristics of frogs. *American Naturalist* 122: 509-520.
- Toft, C.A. 1980. Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. *Oecologia* 45: 131-141.
- Toft, C.A. 1981. Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. *Journal of Herpetology* 15: 39-144.
- Van Sluys, M. & Rocha, C.F.D. 1998. Feeding habitats and microhabitat utilization by two syntopic Brazilian Amazonian frogs (*Hyla minuta* and *Pseudopaludicola* sp. (gr. *falripes*). *Revista Brasileira de Biologia* 58: 559-562.

© 2022 por los autores, licencia otorgada a la Asociación Herpetológica Argentina. Este artículo es de acceso abierto y distribuido bajo los términos y condiciones de una licencia Atribución-No Comercial 4.0 Internacional de Creative Commons. Para ver una copia de esta licencia, visite <http://creativecommons.org/licenses/by-nc/4.0/>

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

Victor M. Pardo¹, Luciana L. Castillo¹, Ana C. Ochoa^{1,2}, Lucía Martínez Retta¹

¹ PROICO 2-2818, Ecología de mamíferos de San Luis: Procesos ecológicos y valoración de la fauna autóctona, Secretaría de Ciencia y Técnica, FQByF, Universidad Nacional de San Luis (UNSL). Ejército de Los Andes 950 (5700), San Luis, Argentina.

² Instituto Multidisciplinario de Investigaciones Biológicas-CCT San Luis, Argentina.

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 xerófila o halófila, 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

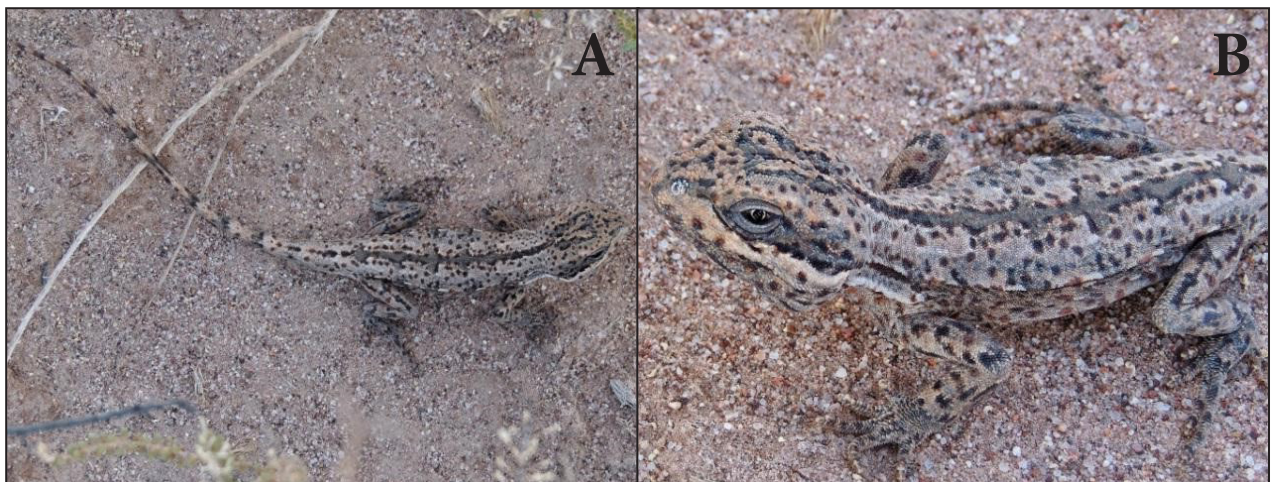


Figura 1. Fotografías del ejemplar de *Leiosaurus jaguaris* registrado en el Parque Nacional Talampaya, provincia de la Rioja, Argentina (en 2020). (A) Vista dorsal general. (B) Vista dorso-lateral de la parte anterior del cuerpo.

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

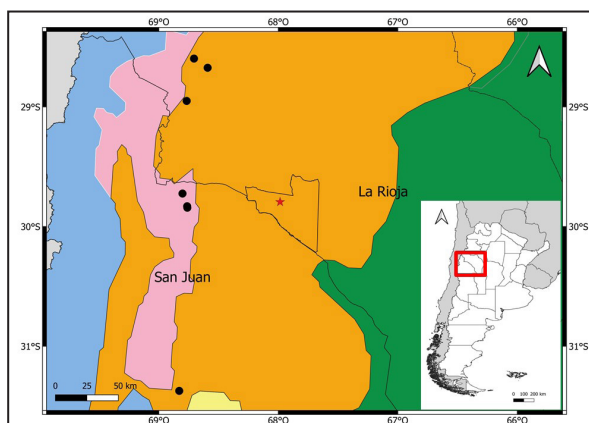


Figura 2. Mapa donde se indican los sitios donde se ha registrado a *L. jaguaris*. Los puntos negros representan los registros anteriores; la estrella roja representa el registro de este trabajo. En colores se representan las ecorregiones según Burkart *et al.* (1999): Chaco seco (Verde), Monte de Sierras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

unidad de hábitat (psamófilo, dominado por dunas de arenas), quedando planteada la incógnita acerca de la posible simpatria de estas dos especies en el área protegida.

Agradecimientos

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.

Literatura citada

- Abdala, C.S.; Acosta, J.L.; Acosta, J.C.; Álvarez, B.B.; Arias, F.; Ávila, L.J.; Blanco, M.G.; Bonino, M.; Boretto, J.M.; Brancatelli, G.; Breitman, M.F.; Cabrera, M.R.; Cairo, S.; Corbalán, V.; Hernando, A.; Iburgüengoytia, N.R.; Kacolis, F.; Laspiur, A.; Montero, R.; Morando, M.; Pelegrín, N.; Pérez, C.H.F.; Quinteros, A.S.; Semhan, R.V.; Tedesco, M.E.; Vega, L. & Zalba, S.M. 2012. Categorización del estado de conservación de las lagartijas y anfisbenas de la República Argentina. *Cuadernos de Herpetología* 26: 215-248.
- APN (Administración de Parques Nacionales). 2001. Plan de Manejo del Parque Nacional Talampaya. Fase II.
- APN (Administración de Parques Nacionales). 2019. Plan de Gestión del Parque Nacional Talampaya.
- Burkart, R.; Bárbaro, N.O.; Sánchez, R.O. & Gómez, D.A. 1999. Eco-regiones de la Argentina. Administración de Parques

Nacionales. PRODIA.
Kass, C.; Kass, N.A.; Velasco, M.A.; Juri, M.D.; Williams, J.D & Kacoliris, F.P. 2018. Inventory of the herpetofauna of Talampaya National Park, a World Heritage Site in Argentina. *Neotropical Biology and Conservation* 13: 202-211.
Laspiur, A. & Acosta, J.C. 2015. New records of *Leiosaurus jaguaris* LASPIUR, ACOSTA & ABDALA, 2007, extend

the known distribution in the Argentine Precordillera. *Herpetozoa* 27: 176-179.
Laspiur, A.; Acosta, J.C. & Abdala, C.S. 2007. A new species of *Leiosaurus* (Iguania: Leiosauridae) from central-western Argentina. *Zootaxa* 1470: 47-57.

Recibida: 17 Marzo 2021

Revisada: 09 Agosto 2021

Aceptada: 19 Octubre 2021

Editor Asociado: S. Quinteros

doi: 10.31017/CdH.2022.(2021-014)

© 2022 por los autores, licencia otorgada a la Asociación Herpetológica Argentina. Este artículo es de acceso abierto y distribuido bajo los términos y condiciones de una licencia Atribución-No Comercial 4.0 Internacional de Creative Commons. Para ver una copia de esta licencia, visite <http://creativecommons.org/licenses/by-nc/4.0/>

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

Sâmia Caroline Melo Araújo^{1,3}, Lucas Rafael Uchôa^{1,3}, Felipe Paixão de Sena^{2,3}, Carina Uchôa Barbosa³, Alan Pablo Antonio Gonçalves Campêlo de Sousa³, Etielle Barroso de Andrade³

¹ Programa de Pós-Graduação em Biodiversidade, Ambiente e Saúde-PPGBAS, Universidade Estadual do Maranhão, Caxias, Maranhão, Brazil.

² Programa de Pós-Graduação em Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

³ Grupo de Pesquisa em Biodiversidade e Biotecnologia do Centro-Norte Piauiense-BIOTECPI, Instituto Federal do Piauí, *Campus* Pedro II, Piauí, Brazil.

Locality.— Brazil, state of Piauí, municipality of Pedro II, Grotta 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 collected 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 (Lehr *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



Figure 1. Juvenile of *Drymoluber brazili* (CBP1189; 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, *Drymoluber 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.

Acknowledgments

The authors are grateful to Marta Angélica Pereira for preparing the specimen and Henrique Caldeira Costa for confirming the species identification. We thank the Instituto Federal de Educação, Ciência e Tecnologia do Piauí for providing the grant PROAGRUPAR-INFRA (#077 - 07/05/2018), and to Instituto Chico Mendes de Conservação da Biodiversidade by provide the collection permission by provide the collection permission (MMA/ICMBio/SISBIO 61838-4/20). SCMA thanks the Universidade Estadual do Maranhão for fellowship (#01/2020 PPG-UEMA) and LRU thanks the Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão for the fellowship (#BM-01016/21).

Literature cited

- Amaral, A. 1930. Estudos Sobre Ophidios Neotrópicos. XXII – Sobre a espécie *Coluber dichrous* (Peters) Boulenger, 1894. *Memórias do Instituto Butantan* 4: 333-337.
- Costa, H.C.; Moura, M.R. & Feio, R.N. 2013. Taxonomic revision of *Drymoluber* Amaral, 1930 (Serpentes: Colubridae). *Zootaxa* (Online) 3716: 349-394.
- Dal Vechio, F.; Teixeira-Junior, M.; Recoder, R.S.; Rodrigues, M.T. & Zaher, H. 2016. The herpetofauna of Parque Nacional da Serra das Confusões, state of Piauí, Brazil, with a regional species list from an ecotonal area of Cerrado and Caatinga. *Biota Neotropica* 16: e20150105.

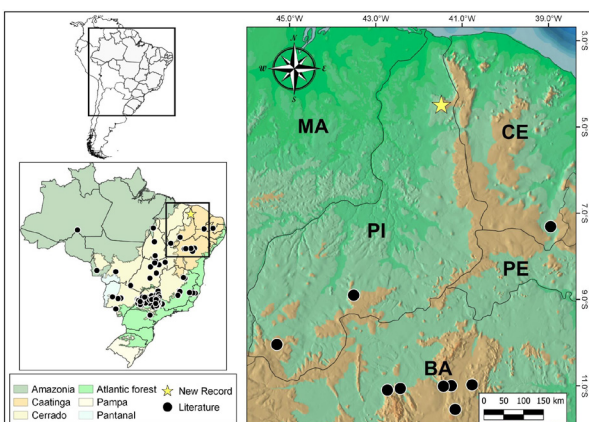


Figure 2. Geographic distribution of *Drymoluber brazili* based on literature records (Nogueira, 2001; Lehr *et al.*, 2004; Moreira *et al.*, 2009; Freitas *et al.*, 2012; Ribeiro *et al.*, 2012; Costa *et al.*, 2013; França and Braz, 2013; Costa *et al.*, 2014; Dal Vechio *et al.*, 2016; Nogueira *et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

- França, F.G.R.; Mesquita, D.O. & Colli, G.R. 2006. A checklist of snakes from Amazonian Savannas in Brazil, housed in the Coleção Herpetológica da Universidade de Brasília, with new distribution records. *Occasional Papers* 17: 1-13.
- Freitas, M.A.; França, D.P.F. & Veríssimo, D. 2012. Distribution extension of *Drymoluber brazili* (Gomes, 1918) (Serpentes: Colubridae) for the state of Piauí, Brazil. *Check List* 8: 168-169.
- Gomes, J.F. 1918. Contribuição para o conhecimento dos ofídios do Brasil. III (1). *Memorial do Instituto Butantan* 1: 57-83.
- Guedes, T.B.; Nogueira, C. & Marques, O.A.V. 2014. Diversity, natural history, and geographic distribution of snakes in the Caatinga, Northeastern Brazil. *Zootaxa* 3863: 1-93.
- IBGE. 2012. Manual Técnico da Vegetação Brasileira. Sistema Fitogeográfico, Inventário das formações florestais e campestres, Técnicas e manejo de coleções botânicas. Procedimentos para mapeamentos. 2. ed. Available in: <https://biblioteca.ibge.gov.br/visualizacao/monografias/GEBIS%20%20RJ/ManuaisdeGeociencias/Manual%20Tecnico%20da%20Vegetacao%20Brasileira%20n.1.pdf>. Accessed: 10 May, 2021.
- Lehr, E.; Carrillo, N. & Hocking, P. 2004. New species of *Drymoluber* (Reptilia: Squamata: Colubridae) from Southeastern Peru. *Copeia* 1: 46-52.
- Moreira, L.A.; Fenolio, D.B.; Silva, H.L.R. & Silva-Júnior, N.J. 2009. A preliminary list of the Herpetofauna from termite mounds of the cerrado in the Upper Tocantins river valley. *Papéis Avulsos de Zoologia* 49: 183-189.
- Nogueira, C.C.; Argôlo, A.J.S.; Arzamendia, V.; Azevedo, J.A.; Barbo, F.E.; Bérnils, R.S.; Bolochio, B.E.; Borges-Martins, M.; Brasil-Godinho, M.; Braz, H.; Buononato, M.A.; Cisneros-Heredia, D.F.; Colli, G.R.; Costa, H.C.; Franco, F.L.; Giraudo, A.; Gonzalez, R.C.; Guedes, T.; Hoogmoed, M.S.; Marques, O.A.V.; Montingelli, G.G.; Passos, P.; Prudente, A.L.C.; Rivas, G.A.; Sanchez, P.M.; Serrano, F.C.; Silva-Júnior, N.J.; Strüssmann, C.; Vieira-Alencar, J.P.S.; Zaher, H.; Sawaya, R.J. & Martins, M. 2019. Atlas of Brazilian snakes: verified point-locality maps to mitigate the Wallacean shortfall in a megadiverse snake fauna. *South American Journal of Herpetology* 14(sp1): 1-274.
- Nogueira, C.C. 2001. New records of Squamate Reptiles in Central Brazilian Cerrado II: Brasília Region. *Herpetological Review* 32(4): 285-287.
- Peters, W. 1863. Über einige neue oder weniger bekannte Schlangenarten des zoologischen Museums zu Berlin. *Monatsberichte der königlich Akademie der Wissenschaften zu Berlin* 29: 272-289.
- Ribeiro, S.C. & Mesquita, D.O. 2014. *Drymoluber brazili* (Brazil's woodland racer): Defensive behaviour. *Herpetological Bulletin* 129: 30-30.
- Ribeiro, S.C.; Roberto, I.J.; Sales, D.L.; Ávila, R.W. & Almeida, W.D.O. 2012. Amphibians and reptiles from the Araripe bioregion, northeastern Brazil. *Salamandra*, 48(3): 133-146.
- Santos, A.J.; Costa, C.A.; Sena, F.P.; Araújo, K.C. & Andrade, E.B. 2019. New record and geographic distribution of *Proceratophrys caramaschii* Cruz, Nunes, and Juncá, 2012 in the state of Piauí, northeastern Brazil (Anura: Odontophrynidae). *Herpetology Notes* 12: 675-679.

Recibida: 18 Mayo 2021
 Revisada: 09 Diciembre 2021
 Aceptada: 21 Diciembre 2021
 Editor Asociado: P. Passos

doi: 10.31017/CdH.2022.(2021-037)

© 2022 por los autores, licencia otorgada a la Asociación Herpetológica Argentina. Este artículo es de acceso abierto y distribuido bajo los términos y condiciones de una licencia Atribución-No Comercial 4.0 Internacional de Creative Commons. Para ver una copia de esta licencia, visite <http://creativecommons.org/licenses/by-nc/4.0/>

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

Freddy A. Grisales-Martínez^{1,2}, Beatriz E. Rendón-Valencia¹

¹ Servicios Ambientales y Geográficos S.A., Calle 11B No. 40A-130 Piso 4, Medellín, Colombia.

² Grupo Herpetológico de Antioquia, Instituto de Biología, Universidad de Antioquia, Calle 67 # 53-108, Bloque 7-121, A.A. 1226, Medellín, Colombia.

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

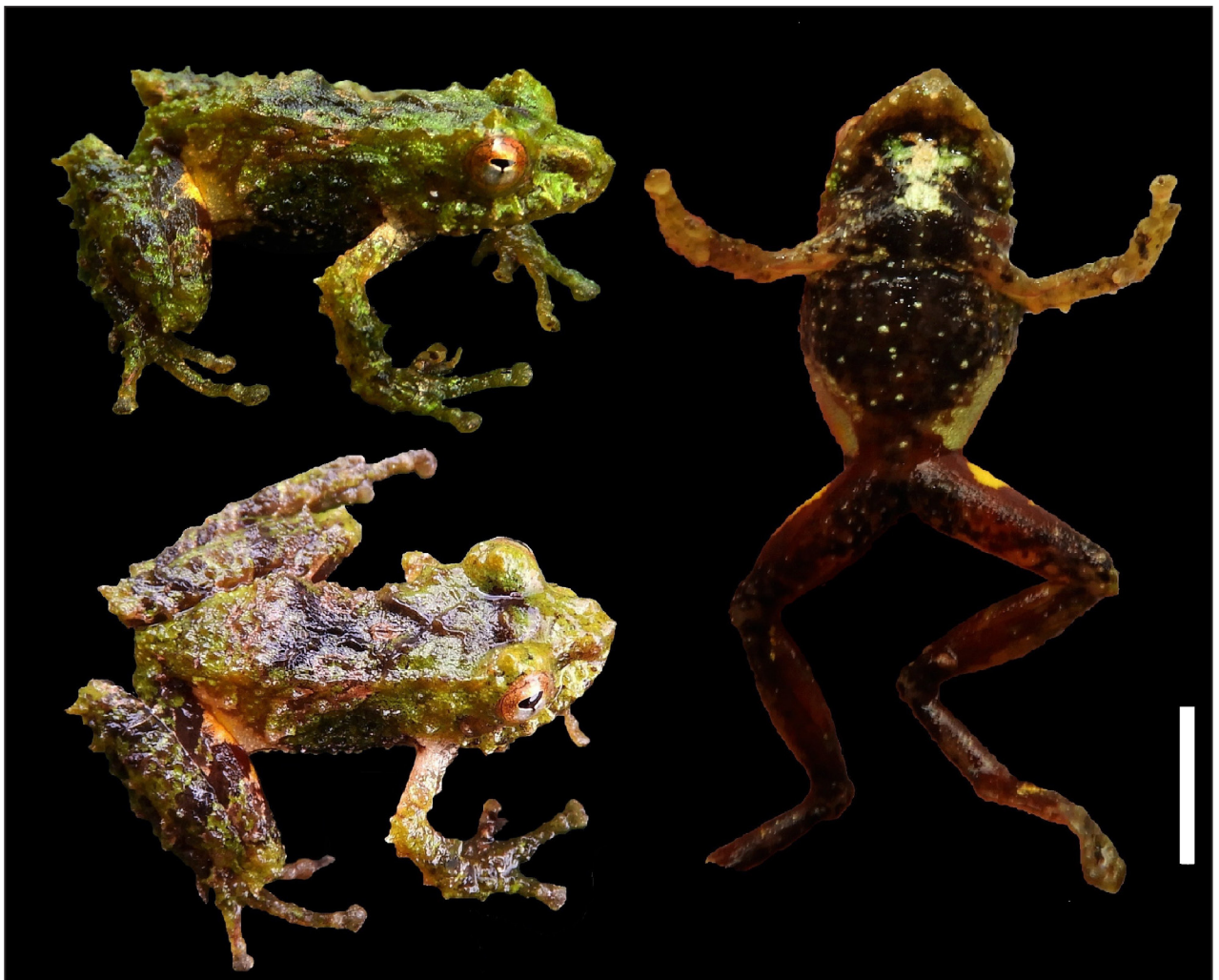


Figure 1. Female of *Pristimantis quantus* (MHUA-A 11308), from Natural Reserve El Centello, north of the western Andes, Jardín municipality, Antioquia departament, Colombia. Scale bar 0,5 mm.

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 Bolívar-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 Serranía 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 Bolívar-García, 2010). The estimated extent of occurrence (EOO) of *P. quantus* is approximately 65 km² and coincides with the protected area Reserva Natural Comunitaria Cerro El Ingles, owned by the Serranía foundation (Castro-Herrera and Bolívar-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 Bolívar-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 Bolívar-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 (Serra-

nía 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.

Acknowledgments

The authors would like to thank: Divier Idárraga, Daniel Arango, Marisella López, Ana María Gutierrez and Verónica Sánchez for support in field work; Yadis Alvarez y Ana Benavides for logistic support; Mauricio Rivera Correa and anonymous reviewers for their comments on the manuscript; the Museo de Herpetología de la Universidad de Antioquia (MHUA) for providing us workspace; the Fundación Jardín Botánico de Medellín Joaquín Antonio Uribe for financial support for this work.

Literature cited

- Acevedo, A.A.; Franco, R. & Carrero, D.A. 2016. Diversity of Andean amphibians of the Tamá National Natural Park in Colombia: a survey for the presence of *Batrachochytrium dendrobatidis*. *Animal Biodiversity and Conservation* 39: 1-10.
- Acosta Gálvis, A.R. 2021. Batrachia. Lista de los Anfibios de Colombia. Version 2021-11. Available in: (<http://www.batrachia.com>). Accessed on 25 May 2021.
- Aguilar-López, J.L.; Ortiz-Lozada, L.; Pelayo-Martínez, J.; Mota-Vargas, C.; Alarcón-Villegas, L.E. & Demeneghi-Calatayud, A.P. 2020. Diversidad y conservación de anfibios y reptiles en un área protegida privada de una región altamente transformada en el sur de Veracruz, México. *Acta Zoológica Mexicana* 36: 1-14.
- Armesto, L.O. & Señaris, J.C. 2017. Anuros del norte de los Andes: patrones de riqueza de especies y estado de conservación. *Papeis Avulsos de Zoología* 57: 491-526.
- Cardona-Botero, V.E.; Viáfara-Vega, R.A.; Valencia-Zuleta, A.; Echeverry-Bocanegra, A.; Hernández-Córdoba, O.D.; Jaramillo-Martínez, A.F.; Gálvis-Cruz, R.; Gutiérrez-Zúñiga, J.A. & Castro-Herrera, F. 2013. Diversidad de la herpetofauna en el Valle del Cauca (Colombia): un enfoque basado en la distribución por ecorregiones, altura y zonas de vida. *Biota Colombiana* 14: 156-232.
- Castro-Herrera, F. & Vargas-Salinas, F. 2008. Anfibios y reptiles en el departamento del Valle del Cauca, Colombia. *Biota Colombiana* 9: 251 - 277
- Castro-Herrera, F. & Bolívar-García, W. 2010. Libro Rojo de los Anfibios del Valle del Cauca. Feriva Impresores SA. Cali, Colombia.
- Cuellar-Valencia, O.M.; Arriaga-Jaramillo, F.G.; García-Gómez, I., Ceballos-Castro, I., Bolívar-García, W.; Velásquez-Trujillo, D.A.; Ortiz-Baez, A.S. & Ospina-Sarria, J.J. 2021. Two new species of *Pristimantis* (Anura: Strabomantidae) from the Serranía de los Paraguas: a priority site for conservation of amphibians in Colombia. *Herpetologica* 77: 72-84.

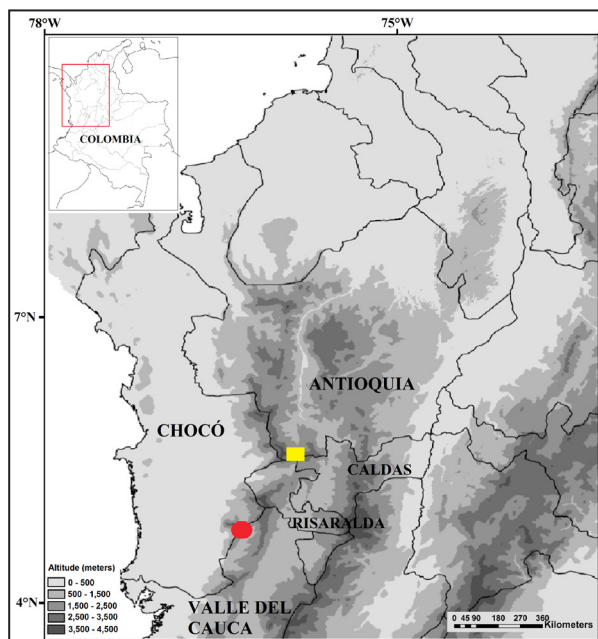


Figure 2. Distribution map of *Pristimantis quantus*. Yellow square correspond to the new locality reported by the present study, and the red circle to the type locality.

Grisales-Martínez & Rendón Valencia — New record of *Pristimantis quantus* in Colombia.

- Frost, D.R. 2021. Amphibian species of the world: an online reference. Version 6.1. New York: American Museum of Natural History. Available in (<http://research.amnh.org/herpetology/amphibia/index>). Accessed on 20 May 2021.
- García-R, J.C.; Posso-Gómez, C.E. & Cardenas-Henao, H. 2015. Diet of direct-developing frogs (Anura: Craugastoridae: *Pristimantis*) from the Andes of western Colombia. *Acta Biológica Colombiana* 20: 79-87.
- Gonzalez-Duran, G.A.; Targino, M.; Rada, M. & Grant, T. 2017. Phylogenetic relationships and morphology of the *Pristimantis leptolophus* species group (Amphibia: Anura: Brachycephaloidea), with the recognition of a new species group in *Pristimantis* Jiménez de la Espada, 1870. *Zootaxa* 4243: 42-74.
- Hedges, S.B.; Duellman, W.E. & Heinicke, M.P. 2008. New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* 1737: 1-182.
- Holdridge, L.R. 1967. Life Zone Ecology. Revised ed. Tropical Science Center, San José, Costa Rica.
- IUCN SSC 2021. *Pristimantis quantus*: The IUCN Red List of Threatened Species. Version 2021-1. Available in: (<https://www.iucnredlist.org>). Accessed on 7 June 2021.
- Lannoo, M.J. 2005. Amphibian declines: the conservation status of United States species. California, USA: University of California Press.
- Lynch, J.D., & Duellman, W.E. 1997. Frogs of the genus *Eleutherodactylus* (Leptodactylidae) in western Ecuador: systematic, ecology, and biogeography. *Natural History Museum, University of Kansas* 23: 1-236.
- Lynch, J.D. 1998. New Species of *Eleutherodactylus* from The Cordillera Occidental of western Colombia with synopsis of the distribution of species in Western Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 22: 117-148.
- Rivera-Correa, M.; González-Durán, G.A.; Saldarriaga-Gómez, A.; & Duarte-Marín, S. (2021). Biodiversity in the Andean Mountains: Two new rain frogs of the genus *Pristimantis* (Anura: Craugastoridae) from the northern Cordillera Central in Colombia. *Zootaxa*, 5040: 334-364.
- Stuart, S.N.; Hoffmann, M.; Chanson, J.; Cox, N.; Berridge, R.; Ramani, P. & Young, B. 2008. Threatened amphibians of the world. Lynx Edicions. Zusammenarbeit mit IUCN, Conservation International und NatureServe.

Recibida: 31 Agosto 2021
Revisada: 04 Octubre 2021
Aceptada: 13 Octubre 2022
Editor Asociado: A. Jerez

doi: 10.31017/CdH.2022.(2021-057)

© 2022 por los autores, licencia otorgada a la Asociación Herpetológica Argentina. Este artículo es de acceso abierto y distribuido bajo los términos y condiciones de una licencia Atribución-No Comercial 4.0 Internacional de Creative Commons. Para ver una copia de esta licencia, visite <http://creativecommons.org/licenses/by-nc/4.0/>

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

María Paula Cabrera¹, Eduardo Vargas², Juan Carlos Stazonelli¹

¹ Instituto de Vertebrados, Fundación Miguel Lillo. Miguel Lillo 251 (4000) S. M. de Tucumán, Argentina.

² Secretaría de Biodiversidad y Desarrollo Sustentable. Ministerio de Ambiente de Jujuy. República de Siria 147, San Salvador de Jujuy, Jujuy, Argentina.

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; Ceí, 1993; Carreira *et al.*, 2005) y se la encuentra en Sudamérica desde hace casi 500 años (Carranza y Arnold, 2006).

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.

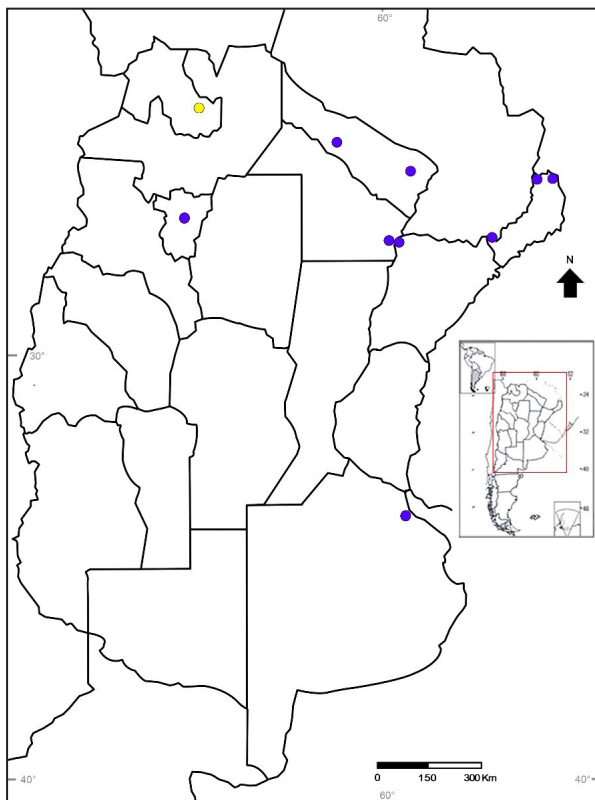


Figura 1. Registros de distribución de *Hemidactylus mabouia* (puntos azules) en la Argentina. El punto amarillo representa el primer registro de la especie para la provincia de Jujuy.



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.

Literatura citada

- Álvarez, B.B.; Aguirre, R.H.; Céspedes, J.A.; Hernando, A.B. & Tedesco, M.E. 2002. Atlas de Anfibios y Reptiles de las Provincias de Corrientes, Chaco y Formosa (Argentina) I. (Anuros, Cecílicos, Saurios, Amphisbénidos y Serpientes). Corrientes: Universidad Nacional del Nordeste.
- Álvarez, B.B.; García, J.R.; Céspedes, J.A.; Hernando, A.B.; Zaracho, V.H.; Calamante, C.C. & Aguirre, R.H. 2009. Herpetofauna, provinces of Chaco and Formosa, Chaco Oriental region, north-eastern Argentina. *Check List* 5: 74-82.
- Álvarez-Romero, J.G.; Medellín, R.A.; Oliveras de Ita, A.; Gómez de Silva, H. & Sánchez, O. 2008. Animales exóticos en México: una amenaza para la biodiversidad. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Instituto de Ecología, UNAM, Secretaría de Medio Ambiente y Recursos Naturales, México, D.F.
- Anjos, L.A. & Rocha, C.F.D. 2008. The *Hemidactylus mabouia* Moreau de Jonnès, 1818 (Gekkonidae) lizard: an invasive alien species broadly distributed in Brazil. *Natureza & Conservação* 6: 96-207.
- Baldo, D.; Borteiro C.; Brusquetti, F.; García, J.E. & Prigioni, C. 2008. Reptilia, Gekkonidae, *Hemidactylus mabouia*, *Tarentola mauritanica*: Distribution extension and anthropogenic dispersal. *Check List* 4: 434-438.
- Carranza, S. & Arnold, E.N. 2006. Systematics, biogeography, and evolution of *Hemidactylus* geckos (Reptilia: Gekkonidae) elucidated using mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 38: 531-545.
- Carreira, S.; Achaval, F. & Umpiérrez, S. 2005. *Hemidactylus mabouia*. *Herpetological Review* 36: 468.

- Cei, J.M. 1993. Reptiles del noroeste, nordeste y este de Argentina. Herpetofauna de las Selvas Subtropicales, Puna y Pampas. Monografía XIV. Torino: Museo Regionale di Scienze Naturali Bollettino.
- Cyriac, V.P. & Umesh, P.K. 2021. Natural history of the gecko *Hemidactylus prashdi*: demography, spatial partitioning, diet, and reproduction in a human-altered habitat. *Herpetological Conservation and Biology* 16: 325-336.
- Federico, L. & Cacivio, P.M. 2000. *Hemidactylus mabouia*. *Herpetological Review* 31: 53.
- Genise, J.F. & Montanelli, S.B. 1991. Primer hallazgo de *Hemidactylus mabouia* (Moreau de Jones) en la Argentina. *Boletín de la Asociación Herpetológica Argentina* 7: 23.
- Kluge, A.G. 1969. The evolution and geographical origin of the New World *Hemidactylus mabouia*-*brookii* complex (Gekkonidae, Sauria). *Miscellaneous Publications of the Museum of Zoology, University of Michigan* 138: 1-78.
- Krysko, K.L. & Daniels, K. 2005. A key to the Geckos (Sauria: Gekkonidae) of Florida. *Caribbean Journal of Science* 41: 28-36.
- Powell, R.; Crombie, R.I. & Boos, H.E.A. 1998. *Hemidactylus mabouia* (Moreau de Jonnès). *Catalogue of American Amphibians and Reptiles* 674: 1-11.
- Scrocchi, G.J.; Stazzonelli, J.C. & Cabrera, M.P. 2019. Nuevas citas de Squamata (Gekkonidae, Phyllodactylidae y Dipsadidae) para la provincia de Tucumán, Argentina. *Cuadernos de Herpetología* 33: 75-78.
- Torres, P.J.; Escalante, O & Cardozo, D. 2018. First record of the invasive *Hemidactylus mabouia* (Moreau de Jonnès, 1818) (Squamata, Gekkonidae), in the dry Chaco, Argentina. *Check List* 14: 633-63.
- Vanzolini, P.E. 1968. Lagartos Brasileiros da Família Gekkonidae (Sauria). *Arquivos de Zoologia* 17: 1-84.
- Vanzolini, P.E. 1978. On South American *Hemidactylus* (Sauria, Gekkonidae). *Papéis Avulsos de Zoologia* 31: 307-343.
- Williams, J.D. 1988. Hallazgo de *Hemidactylus turcicus* (L. 1758) (Lacertilia: Gekkonidae) en Argentina. *Boletín de la Asociación Herpetológica Argentina* 4: 9-10.

Recibida: 28 Diciembre 2021

Revisada: 07 Abril 2022

Aceptada: 12 Abril 2022

Editor Asociado: J. Goldberg

doi: 10.31017/CdH.2022.(2021-068)

© 2022 por los autores, licencia otorgada a la Asociación Herpetológica Argentina. Este artículo es de acceso abierto y distribuido bajo los términos y condiciones de una licencia Atribución-No Comercial 4.0 Internacional de Creative Commons. Para ver una copia de esta licencia, visite <http://creativecommons.org/licenses/by-nc/4.0/>

Defensive behaviors of <i>Leptodactylus rhodomystax</i> (Anura: Leptodactylidae) from northern Brazil Fillipe Pedroso-Santos, Vinícius A. M. B. de Figueiredo, Carlos E. Costa-Campos	101
Diet composition of two hylid species (<i>Dendropsophus haraldschultzei</i> and <i>D. minutus</i>) in anthropogenic environments in eastern Amazonia Ana Luiza Sangel Soeiro, Anna Klara Matos Guerreiro, Aline Emanuele Oliveira-Souza, Maria Madalena Salviano Santana, Carlos Eduardo Costa-Campos	105
 NOVEDADES ZOOGEOGRÁFICAS	
<i>Leiosaurus jaguaris</i> Laspiur, Acosta & Abdala, 2007 (Squamata: Leiosauridae): Primer registro para el Parque Nacional Talampaya, provincia de La Rioja, Argentina Victor M. Pardo, Luciana L. Castillo, Ana C. Ochoa, Lucía Martínez Retta	111
New record and updated distribution map for <i>Drymoluber brazili</i> (Gomes, 1918) (Serpentes: Colubridae) Sâmia Caroline Melo Araújo, Lucas Rafael Uchôa, Felipe Paixão de Sena, Carina Uchôa Barbosa, Alan Pablo Antonio Gonçalves Campêlo de Sousa, Etielle Barroso de Andrade	115
New distribution record of endangered and endemic frog <i>Pristimantis quantus</i> (Lynch, 1998) in western Andes of Colombia Freddy A. Grisales-Martínez, Beatriz E. Rendón-Valencia	119
Primera cita de <i>Hemidactylus mabouia</i> (Moreau de Jonnès, 1818) en la provincia de Jujuy, Argentina María Paula Cabrera, Eduardo Vargas, Juan Carlos Stazzonelli	123

CUADERNOS *de* HERPETOLOGÍA

VOLUMEN 36 - NÚMERO 1 - MAYO 2022

ojs.aha.org.ar - aha.org.ar

VOLUMEN 36 - NÚMERO 1

TRABAJOS

- Hannemania* sp. *larvae* (Acari: Leeuwenhoekiidae) in *Leptodactylus luctator* (Anura: Leptodactylidae) in the Pampa biome, southern Brazil
Juliana H. Wolter, Carolina S. Mascarenhas, Bruna M. Chaviel, Ana Beatriz D. Henzel, Ricardo R. C. Silva, Frank Lira³, Gertrud Müller 5
- Herpetofauna of an urban environmental protection area in an Amazon forest remnant of Amapá state, northern Brazil
Rodrigo Tavares-Pinheiro, Vinícius A. M. B. de Figueiredo, Fernanda S. Melo, Aline E. Oliveira-Souza, Abdiel Pinheiro-Freitas, Carlos E. Costa-Campos 11
- Lipidic cycle, condition factor and reproductive cell maturation in *Gymnodactylus darwini* Gray, 1845 (Squamata, Phyllodactylidae) from a fragment of Atlantic Forest in northeastern Brazil
Leonardo P. C. Oitaven, Márcio F. Chaves, José L. de Araújo, Felipe S. Ribeiro, Geraldo J.B. de Moura 21
- Reproductive strategies of the semifossorial snake *Atractus zebrius* from the Atlantic Forest
Lucas Henrique Carvalho Siqueira, Jade Lima-Santos, Karina Maria Pereira Silva 33
- Taxonomic notes on the *Phalotris bilineatus* group (Serpentes: Dipsadidae: Elapomorhini), with the description of a new species from northwestern Argentina
Gustavo J. Scrocchi, Alejandro R. Giraud, Santiago J. Nenda 47
- NOTAS
- 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
Cristiane Nikely S. Palmeira, Ubiratan Gonçalves, Marcos J. Matias Dubeux, Luana Rodrigues Lima, Carolina Lambertini, Anyelet Valencia-Aguilar, Thomas Stuart Jenkinson, Timothy Yong James, Luís Felipe Toledo, Tamí Mott 65
- O canto de soltura de *Rhinella crucifer* (Wied-Neuwied, 1821), no Estado de Pernambuco, Nordeste do Brasil
Flávio José da Silva, Emerson Gonçalves Dias, Marcelo Nogueira de Carvalho Kokubum, Ednilza Maranhão dos Santos 77
- Breeding activity of *Dendropsophus counani* (Anura: Hylidae) in eastern Brazilian Amazonia
Patrick Ribeiro Sanches, Fillipe Pedroso-Santos, Carlos Eduardo Costa-Campos 85
- Tail-break frequency inferences on *Lygodactylus klugei* (Smith, Martin & Swain, 1977) (Squamata: Gekkonidae) in northeastern Brazil
Antônio Rafael Lima Ramos, John Allyson Andrade Oliveira, Margarida Maria Xavier da Silva, Robson Victor Tavares, Diva Maria Borges-Nojosa 89
- First observations on courtship and nesting behavior of *Kinosternon vogti* (Testudines: Kinosternidae)
Carlos Santiago Rosales-Martínez, Carlos Daniel Bello-Sánchez, Marco Antonio López-Luna, Armando H. Escobedo-Galván, Fabio G. Cupul-Magaña 95

continúa en el reverso de contratapa



Miembro de Publication Integrity & Ethics

Revista de la Asociación Herpetológica Argentina

Indizada en:

Zoological Record, Directory of Open Journals, Latindex, Periódica, Ebsco, Academic Journal Database, Biblat. e-revistas, Cite Factor, Universal Impact Factor, Sedcir, InfoBase Index.