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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 trasparente más una alta visibilidad internacional de sus trabajos. Para los lectores, se garantiza el acceso libre a los artículos. Los idiomas aceptados son castellano, portugués e inglés.

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Trabajo

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Estudios sobre anuros de Argentina durante la década 2010-2020: tendencias y vacíos de información

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ABSTRACT
Scientific knowledge may present taxonomic, geographic and thematic biases, which could have negative consequences on conservation decisions, especially in highly threatened groups such as amphibians. This work aimed to explore the scientific production of anurans from Argentina during the 2010-2020 decade, to understand the current state of knowledge and identify possible biases and information gaps. Our results showed that the production of scientific knowledge on anurans from Argentina remained constant during the studied period. Nonetheless, we observed taxonomic, geographic and topic biases. The number of studies was higher in areas with higher human population density and on topics related to morphology, as well as for common species with medium body sizes, general habits, with a broad geographic distribution, and occurring in rural-urban areas. Our results represent a baseline to clarify and evidence the different biases of scientific knowledge about anurans from Argentina.

Key words: Literature review, Geographic bias, Taxonomic bias, Research topics bias.

RESUMEN
La producción del conocimiento científico puede presentar sesgos taxonómicos, geográficos y temáticos, los que a su vez podrían tener consecuencias negativas al momento de tomar decisiones en conservación, sobre todo en grupos altamente amenazados como son los anfibios. El objetivo de este trabajo fue explorar la producción científica sobre anuros de Argentina durante la década 2010-2020, a fin de establecer el estado actual del conocimiento e identificar posibles sesgos y vacíos de información. Nuestros resultados sugieren que la producción del conocimiento científico en anuros de Argentina se mantuvo constante en el período estudiado. Se observaron sesgos en cuanto a la distribución geográfica de las publicaciones, en las especies más estudiadas y en los temas abordados. El número de estudios fue mayor en provincias con una densidad de su población más alta y en temas relacionados con morfología, así como para especies comunes con tamaños corporales medianos, de hábitos generalistas, con una amplia distribución geográfica, y con ocurrencia en zonas rurales-urbanas. Estos resultados constituyen un punto de partida para esclarecer y evidenciar los diferentes sesgos del conocimiento científico sobre anuros de Argentina.

Palabras clave: Revisión bibliográfica, Sesgo geográfico, Sesgo taxonómico, Sesgo en temas de investigación.

Introducción

La producción del conocimiento científico, representada por la literatura publicada con revisión de pares, en general no presenta una distribución uniforme en cuanto a la representatividad de las especies estudiadas, las áreas geográficas y las áreas temáticas analizadas (e.g., Clark y May, 2002; Pawar, 2003; Troudet *et al*., 2017; Phaka *et al*., 2022). Estas asimetrías o sesgos, están relacionadas al esfuerzo

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de investigación, es decir cuán intensamente se estudia una especie o un tema (di Marco *et al.*, 2017), y pueden deberse a numerosos factores. Por un lado, a factores intrínsecos de las especies, como ser características de las historias de vida, rasgos y requerimientos (e.g., rareza ecológica, *sensu* Giraudo *et al.*, 2012), ocurrencia geográfica, accesibilidad logística a los sitios y cercanía a poblaciones humanas (Reddy y Dávalos, 2003; Brooke *et al.*, 2014; Ducatez y Lefebvre, 2014; Ibáñez-Álamo *et al.*, 2017). Así, algunas especies son inherentemente más fáciles de estudiar que otras, ya sea porque tienen una amplia distribución o habitan en lugares accesibles logísticamente, o son fáciles de localizar e identificar debido a su tamaño, colores llamativos o porque son más activas durante el día (Pawar, 2003; Troudet *et al.*, 2017; dos Santos *et al.*, 2020). Otros factores pueden estar relacionados con los intereses personales de cada investigador, la realidad política y el sistema de investigación vigentes, los cuales contribuyen con el grado de inversión económica, la infraestructura y la cantidad de investigadores, impactando directamente en la producción científica (Collen *et al.*, 2008; Martin *et al.*, 2012; Amano y Sutherland, 2013). Finalmente, la apreciación social positiva de especies consideradas como “especies carismáticas” podría también favorecer su sobrerrepresentación en la literatura científica (Wilson *et al.*, 2007; Jarić *et al.*, 2014; Donaldson *et al.*, 2016).

La última Categorización del estado de conservación de anfibios de Argentina reportó 175 especies de anfibios, correspondiente a 171 anuros y 4 cecilias, ubicando a Argentina como el décimo país con mayor diversidad de la Región Neotropical (Vaira *et al.*, 2012, 2017). Este grupo constituye uno de los más amenazados a nivel mundial, con al menos un tercio de las especies conocidas incluidas bajo alguna categoría de amenaza (Stuart *et al.*, 2004; IUCN, 2022), y con especies de anfibios que a nivel nacional carecen de información para evaluar su estado de conservación (Vaira *et al.*, 2018). Desde la constitución de la Asociación Herpetológica Argentina en la década de 1980, y gracias al continuo aporte de la comunidad científica dedicada al estudio de los anfibios, se realizaron importantes contribuciones en el país con respecto al conocimiento de este grupo. Sin embargo, varios trabajos mencionan la existencia de vacíos de información para muchas de las especies presentes en Argentina (e.g., Lavilla y Heatwole, 2010; Vaira *et al.*, 2017, 2018). Actualmente, no se conoce en detalle cómo se estructura el conocimiento científico referido a anfibios anuros en cuanto a proporciones y tendencias de la producción científica generada en Argentina.

Dado que la existencia de vacíos y sesgos en la información, pueden ocasionar impactos contraproducentes al momento de desarrollar propuestas y políticas de conservación basadas en el conocimiento científico generado, resulta importante identificarlos (Clark y May, 2002; Pawar, 2003). El objetivo de este trabajo fue explorar la producción de conocimiento científico sobre los anfibios anuros de Argentina durante el período 2010-2020, a fin de describir el conocimiento actual en cuanto a las especies estudiadas, la distribución geográfica de las publicaciones científicas y los temas abordados, así como destacar los sesgos y vacíos de información existentes.

Materiales y métodos

Se efectuó una búsqueda bibliográfica exhaustiva de artículos científicos publicados en revistas periódicas con referato relacionados con anuros de Argentina durante el período 2010-2020. Para la búsqueda se utilizó la base de datos de Google Scholar$^{(R)}$, un buscador gratuito que comparte características comunes con otros buscadores como Scirus de Elsevier y bases de datos bibliográficas como WoS y Scopus (Giles, 2005). Empleamos los términos “amphibian” OR “anuran” OR “Amphibia” OR “Anura” OR “anuros” OR “ranas” AND “Argentina”. Además, se realizó una búsqueda por separado para cada especie, incluyendo sinonimias. Se aplicaron filtros para cada año a fin de cubrir la mayor cantidad de artículos publicados.

Publicaciones y temáticas principales de investigación

Se recopilaron datos sobre el año de publicación, tipo de trabajo (artículo, nota y revisión), nacionalidad de la revista en la que fue publicado (categorías: no latinoamericana y latinoamericana, discriminando además dentro de esta categoría, a las revistas argentinas), idioma de publicación (inglés o español), y nombres y afiliaciones de los autores, tomando en cuenta la primera afiliación mencionada en caso de presentar más de una.

Se reporta el número total de publicaciones registradas durante el período estudiado y la proporción de publicaciones por año, así como la mediana de publicaciones registrada en la década estudiada y su respectivo rango. En base a las publicaciones que presentaron datos sobre la ubicación geográfica donde se realizó el estudio, se determinó el número de artículos realizados en cada provincia. En base a las afiliaciones informadas, se estimó el número de autores por provincia, y se exploró su relación con los datos sobre el total de recursos humanos pertenecientes al Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) reportadas para el año 2020 (https://cifras.conicet.gov.ar/publica/) mediante un análisis de correlación por rangos de Spearman. Se exploró la relación entre la afiliación de los autores y la provincia donde se desarrolló cada trabajo, identificando la proporción de publicaciones con al menos un autor de la misma provincia donde se efectuó el estudio y aquellas donde ningún autor indicó afiliación en la provincia donde se desarrolló el estudio.

Las publicaciones fueron clasificadas según su temática principal, teniendo en cuenta la información presente en el título y en los objetivos. En función de la diversidad de artículos obtenidos, se definieron 16 temas principales: Aportes a la Conservación; Bioacústica; Comportamiento; Desarrollo; Diversidad y Distribución; Ecología; Ecotoxicología; Etnoherpetología, Farmacología; Fisiología; Genética; Hematología e Histología; Morfología; Patologías (e.g., parásitos, hongos, malformaciones); Reproducción y Sistemática. Se describió el número de publicaciones realizadas por tema y su distribución por provincia, evaluando la similitud entre provincias en cuanto a los temas realizados en las mismas mediante un análisis de agrupamientos jerárquicos, buscando la máxima homogeneidad dentro de cada grupo (Borcard *et al.*, 2011). Para ello se empleó el método de Ward, usando la distancia Euclidiana como medida de similitud.

Por último, se estimaron valores de riqueza de especies por provincia en base a la información indicada en la última categorización de anfibios de Argentina (Vaira *et al.*, 2012), y actualizada siguiendo la bibliografía pertinente, la información otorgada en *Amphibian Species of the World* (Frost, 2023) y consultas a especialistas. Con ello se evaluó la correlación entre la medida de riqueza de especies y la cantidad de publicaciones y temas realizados por provincia mediante un análisis de correlación por rangos de Spearman (McDonald, 2014).

Esfuerzo de investigación por especie

Las especies de anuros registradas fueron actualizadas en cuanto a cambios nomenclaturales siguiendo la información del sitio de referencia en línea *Amphibian Species of the World* (Frost, 2023) y bibliografía pertinente. Basados en la información recabada, se consideraron para este trabajo 174 especies de anuros, incluída la especie introducida *Litobathes catesbeianus* (Tabla 1). Para este estudio, se tomaron en cuenta aquellas especies descritas hasta el año 2020, inclusive. Las modificaciones taxonómicas posteriores fueron consideradas al momento de realizar los análisis.

Asumiendo que las especies más estudiadas tendrán mayor número de publicaciones (e.g., da Silva *et al.*, 2020), se estimó el esfuerzo de investigación por especie como la proporción entre el número de publicaciones de una especie sobre el total de publicaciones en la década analizada (N° Publ. sp_i/Total Publ.). Además, se estimó el sesgo taxonómico por provincia (sP) y por tema (sT) relacionando el número de provincias (nP) y temas (nT) que abarcaron las publicaciones de una especie en relación al número de provincias que abarca su distribución y los 16 temas definidos en esta publicación, respectivamente. Se estimó nP y nT mediante los números de Hill (${}^qD$; Jost, 2006), los cuales están parametrizados por q, que determina la sensibilidad de la medida D a la abundancia relativa de las unidades, en este caso las publicaciones. Para el análisis, se consideró un valor de $q = 0$, el cual no toma en cuenta las abundancias relativas y equivale al número efectivo de provincias y temas que abarcan las publicaciones de una especie determinada, y $q=1$ que considera las abundancias relativas y permite examinar si existe una equitatividad en la distribución de las abundancias de las publicaciones dentro de cada provincia o tema. Los valores de sP y sT varían entre 1 (no hay sesgo) a 0 (vacío de información).

Por último, se exploró el posible efecto de diferentes variables sobre el esfuerzo de investigación de cada especie mediante modelos lineales generalizados (MLG) con una distribución de errores binomial negativa y una función de enlace log (Zuur *et al.*, 2009). Las variables independientes consideradas se basaron en el trabajo de da Silva *et al.* (2020), y fueron las siguientes:

1) *Estado de conservación:* definido a nivel nacional según la última categorización Argentina de la Asociación Herpetológica Argentina (AHA; Vaira *et al.*, 2012) y a nivel global según la UICN (UICN, 2022). Para la categorización nacional de la AHA se consideraron 5 estados; IC: insuficientemente conocidas, NA: no amenazadas, VU: vulnerables, AM: amenazadas y EP: en peligro de extinción; por

otro lado, para la categorización global de la UICN se consideraron también 5 estados; DD: deficiente de datos, LC: preocupación menor, VU: vulnerable, EN: amenazada y CR: en peligro crítico. Se incluyeron ambos métodos, dado que se encuentran a distintas escalas (local-AHA y global-UICN), por lo que no son necesariamente equiparables.

2) *Tamaño corporal de adultos*: fueron obtenidos a partir de los criterios utilizados para realizar la última Categorización de Anfibios de Argentina (Giraudo *et al.*, 2012; Vaira *et al.*, 2012) resumidos en tres categorías: *tamaño pequeño:* especies con un tamaño corporal menor a 80 mm, *tamaño medio*: tamaño corporal entre 81 a 160 mm y *tamaño grande:* especies con un tamaño corporal mayor a 161 mm.

3) *Rareza ecológica*: definida como el grado de especialización de las especies, tomando en cuenta tres dimensiones: hábitat, sustrato y recurso trófico (ver Giraudo *et al.*, 2012). Esta variable se basó en los datos utilizados para realizar la última Categorización de Anfibios de Argentina (Giraudo *et al.*, 2012; Vaira *et al.*, 2012) y presentó tres categorías: *generalistas*: en cuanto al uso del hábitat, sustrato y del recurso trófico; *intermedio*: la especie es especialista en al menos una de las dimensiones y generalista en las otras; *especialista*: la especie es especialista en más de dos de las dimensiones mencionadas previamente.

4) *Rango de distribución nacional:* en base a los datos incluidos en la Categorización de Anfibios de Argentina (Giraudo *et al.*, 2012; Vaira *et al.*, 2012), se determinaron tres categorías de distribución: *Amplia*: la especie presenta una distribución continua en el 50% del territorio argentino; *Intermedia*: presenta poblaciones aisladas o endemismos regionales y ocupan menos del 50% territorio argentino, y *endémica*: comprende especies con endemismos dentro de ecorregiones o micro-endemismos.

5) *Presencia de la especie en ambientes antrópicos:* considerada como una medida de cuán accesibles son las poblaciones de la especie para los investigadores. Con base a información presentada en la web de UICN (UICN, 2022), se definieron tres categorías: *natural*: especies que se encuentran solamente en ambientes naturales; *rural*: especies que se encuentran en ambientes rurales o levemente antropizados y *urbano*: especies que se han registrado en ambientes urbanos.

Todos los análisis y gráficos se realizaron en el programa R v4.1.3 (R Core Team, 2022). El paquete *tidyverse* (Wickham *et al.*, 2019) se utilizó para el manejo y procesamiento de las bases de datos. Se utilizó la función "glm.nb" del paquete *MASS* 7.5 (Venables y Ripley, 2002) para el análisis de regresión con distribución binomial negativa, y la función "cor" del paquete *stats* (R Core Team, 2022) para el análisis de correlación de Spearman. Los números de Hill fueron estimados con el paquete *hillR* (Li, 2018). Los gráficos se realizaron con el paquete *ggplot2* (Wickham, 2016), el paquete *rgdal* (Bivand *et al.*, 2022), el paquete *ComplexHeatmap* (Gu *et al.*, 2016), y el paquete *packcircles* (Bedward *et al.*, 2020).

Resultados

Artículos y temáticas principales de investigación

Luego de filtrar las publicaciones obtenidas de la búsqueda bibliográfica, se descartaron cinco publicaciones inaccesibles al texto completo, se obtuvo un total de 704 artículos científicos para el período 2010-2020, con una mediana de 65 publicaciones por año, con un mínimo de 36 en el año 2010, y un máximo de 86 publicaciones en el año 2020 (Fig. 1). El número acumulado de publicaciones sugiere una producción constante de trabajos sobre anuros de Argentina desde el 2010 al 2020 (Fig. 1).

Con respecto a los tipos de publicaciones científicas, 590 correspondieron a artículos (84%), 100 a notas cortas (14%) y 14 a revisiones (2%). En cuanto a la nacionalidad de las revistas donde los artículos fueron publicados, se registraron 216 revistas científicas, de las cuales 173 fueron no lati-

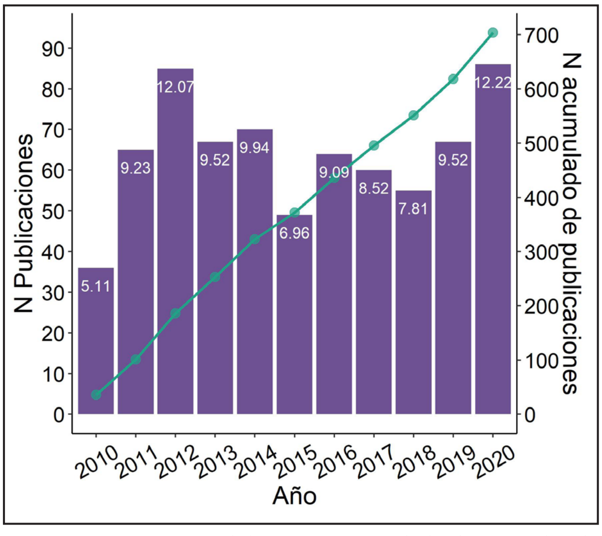

Figura 1. Número anual (barras) y acumulado (línea verde) de publicaciones sobre la anurofauna de Argentina registradas en 216 revistas periódicas con referato para el período 2010-2020. Los valores blancos en cada barra expresan el porcentaje respecto al total de publicaciones en la década.

noamericanas (80%) y 43 fueron latinoamericanas (20%). De estas últimas, Cuadernos de Herpetología fue la revista más frecuente, seguida de la Revista Mexicana de Biodiversidad, Revista de Biología Tropical y Phyllomedusa. Considerando la relación artículos-nacionalidad de la revista, 556 investigaciones fueron publicadas en revistas internacionales (80%) y 148 en revistas latinoamericanas (20%). Considerando las revistas latinoamericanas, 65 estudios fueron publicados en revistas científicas argentinas (44%), con 46 artículos publicados en Cuadernos de Herpetología particularmente. Por último, en cuanto al idioma en el que se redactaron los artículos, 610 fueron escritos en inglés (87%) y 94 en español (13%).

Los estudios fueron llevados a cabo en la mayor parte del país, exceptuando Tierra del Fuego, Antártida e Islas del Atlántico, donde no existen registros de especies de anuros (Fig. 2a). El número de publicaciones fue relativamente alto en las provincias de Buenos Aires, Córdoba, Santa Fe y Corrientes, con más de 90 publicaciones cada una, y un número relativamente bajo en las provincias de La Pampa, Santa Cruz y la Rioja, con menos de 20 publicaciones en cada una.

La mayor parte de los autores presentaron su afiliación en las provincias de Buenos Aires, seguida por Tucumán, Santa Fe y Córdoba (Fig. 2b) y no se registraron autores provenientes de las provincias de Catamarca, Chaco, Formosa, y La Pampa (Fig. 2). Este patrón en el número de autores por provincia coincidió con el número de recursos humanos reportado por CONICET para el año 2020 (coeficiente de correlación de Spearman r_s=0,81, p<0,001). Con respecto a los autores y sus afiliaciones, se registraron 951 autores, de los cuales 698 indicaron una afiliación dentro de Argentina en algún momento entre los años 2010-2020. De de estos, 370 autores participaron de una sola publicación durante el período de tiempo analizado y 70 autores participaron en diez o más publicaciones. En aquellas provincias con mayor cantidad de investigadores, los estudios fueron efectuados principalmente dentro de las mismas provincias (Fig. 2). Los estudios realizados en Catamarca, Chaco, Formosa, La Pampa y Santa Cruz fueron efectuados en su totalidad por autores residentes en otras provincias (Fig. 2). Se observó una relación significativa y positiva entre la riqueza de especies de anuros y el número de publicaciones de cada provincia (coeficiente de correlación de Spearman r_s= 0,698, p<0,001).

Las áreas temáticas de Morfología, Ecotoxicología, Diversidad y Distribución, Ecología y Patologías fueron las que presentaron una mayor cantidad de estudios realizados durante el período analizado (Fig. 3). Se pudieron registrar en todas las provincias estudios referidos a Morfología, Aportes a la Conservación y Patologías, seguidos por estudios de Diversidad y Distribución y Sistemática. El área de la EtnoHerpetología presentó una sola publicación realizada en la provincia de Buenos Aires; le siguió el área de Comportamiento, Desarrollo y Farmacología con 15 publicaciones cada uno (Fig. 3). Considerando la distribución y similitud entre las provincias en cuanto a los temas de estudio, se pudo observar tres grupos geográficos: 1) Buenos Aires - Santa Fe: presentaron una similitud en cuanto a las áreas temáticas Ecotoxicología y Morfología, además, las publicaciones realizadas en este grupo cubrieron casi en su totalidad los temas de estudio definidos en este trabajo; 2) Provincias del norte del país: presentaron una similitud en temas de Morfología y Diversidad y Distribución, y se observa una disminución en los temas abordados en las seis provincias incluídas en este grupo, y 3) Provincias de la zona centro-sur: se puede observar una disminución más marcada en los temas abordados en las nueve provincias presentes en este grupo (Fig. 3). Se observó correlación positiva y significativa entre el número de temas de estudio realizados en una provincia y la riqueza de especies de anuros presente en la misma (coeficiente de correlación de Spearman r_s= 0,626, p<0,001).

De las 704 publicaciones registradas, se determinó que 220 publicaciones (31%) fueron de tipo experimental. Por otro lado, 117 publicaciones (16,5%) utilizaron ejemplares depositados previamente en colecciones herpetológicas, y el 32% de los estudios que obtuvieron sus datos o individuos en campo, depositaron ejemplares en alguna colección biológica institucionalizada de Argentina.

Esfuerzo de investigación por especie

Todas las especies citadas para Argentina hasta el año 2020 inclusive fueron incluidas en algún artículo científico durante la década analizada, además de la especie introducida *Lithobates catesbeianus,* con una mediana de 11 publicaciones, y un rango entre 1 a 206 publicaciones. Se registró un sesgo taxonómico marcado, con sólo el 4% de las especies (cinco especies) representando un poco más del 70% de las publicaciones realizadas en el periodo de tiempo analizado (Fig. 4). El 47% de las especies presentaron

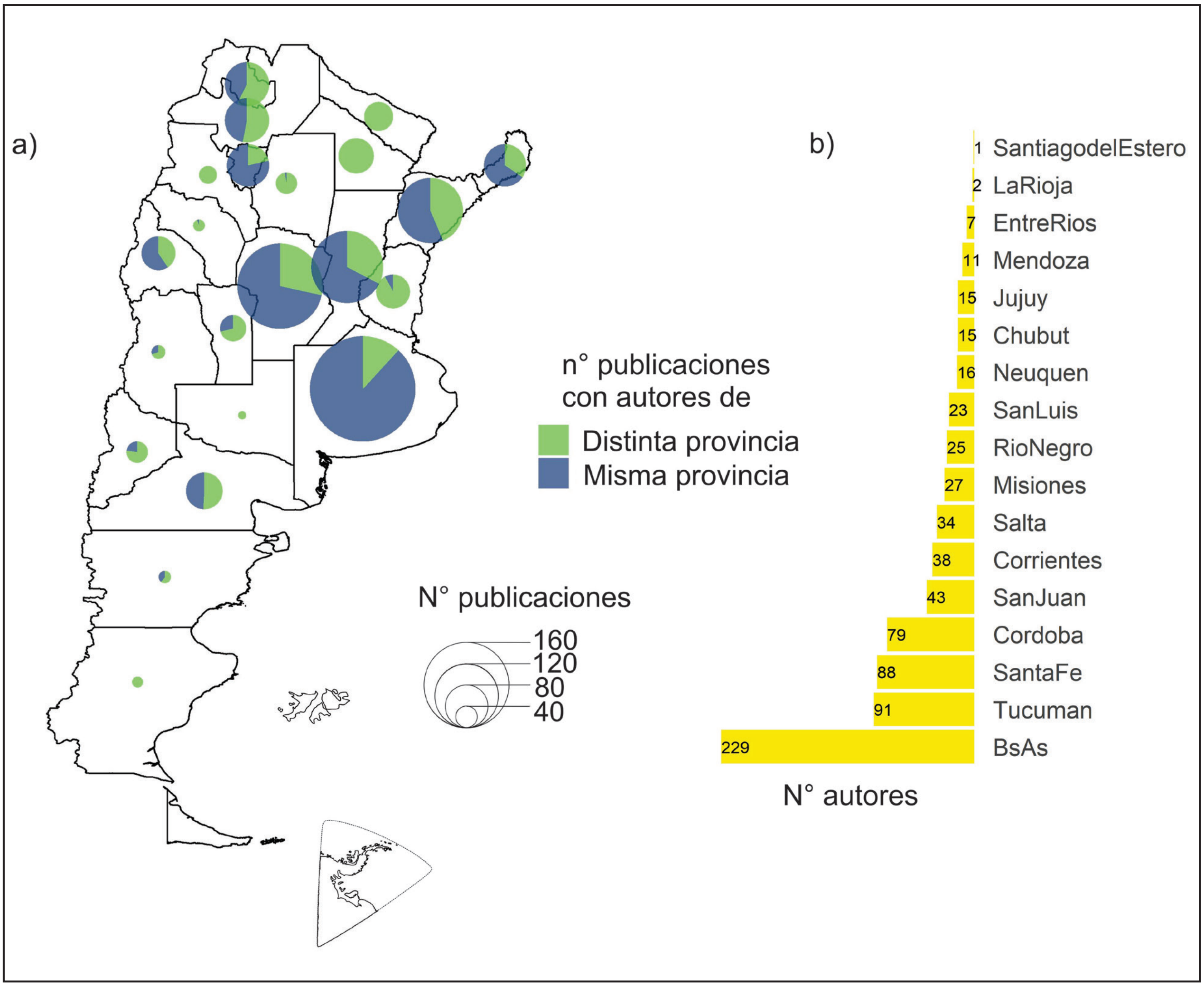

Figura 2. a) Número de publicaciones realizadas durante el período 2010-2020 en las distintas provincias de Argentina, indicando la proporción de estudios realizados por al menos un autor de la misma provincia y aquellos realizados sólo por autores de otra provincia. b) Número de autores por provincia que publicaron durante el mismo período.

que abarcaron más temas, con valores de $^0D>0,8$. El 22,9% de las especies presentó trabajos en al menos el 50% de los temas, y el 14,7% presentó publicaciones sólo en un tema (Tabla 1). Al tomar en cuenta la distribución de las abundancias de publicaciones por tema, se pudo observar que sólo el 4,09% de las especies presentaron valores de $^1D> 0,5$, con el máximo valor de $^1D=0,6$ registrado para la especie *Melanophryniscus rubriventris*.

Los modelos lineales generalizados mostraron diferencias significativas en cuanto a la cantidad de publicaciones científicas de una especie entre las categorías de las variables independientes seleccionadas (Fig. 5). El 60% de las especies de anuros se encontró bajo la categoría No amenazada a nivel nacional (Vaira *et al.*, 2012); estas especies fueron incluidas significativamente en más publicaciones menos de 10 publicaciones durante el período 2010-2020. El 44,5% presentó entre 10 y 40 publicaciones, el 7,5% presentó entre 40 y 80 publicaciones y sólo un 1% presentó más de 80 publicaciones, las cuales pertenecieron a las familias Bufonidae e Hylidae (Fig. 4). Las especies de las familias Brachycephalidae, Centrolenidae, Hemiphractidae, y Rhinodermatidae, representadas por una sola especie, fueron todas incluidas en menos de diez publicaciones, mientras que todas las especies de las familias Ceratophryidae y Phyllomedusidae fueron incluidas en más de diez publicaciones (Fig. 4).

El sapo común, *Rhinella arenarum,* fue incluído en alrededor del 30% de todas las publicaciones registradas. De las 206 publicaciones en las que esta especie fue incluida, 128 fueron exclusivas para la especie y 119 fueron estudios experimentales. El

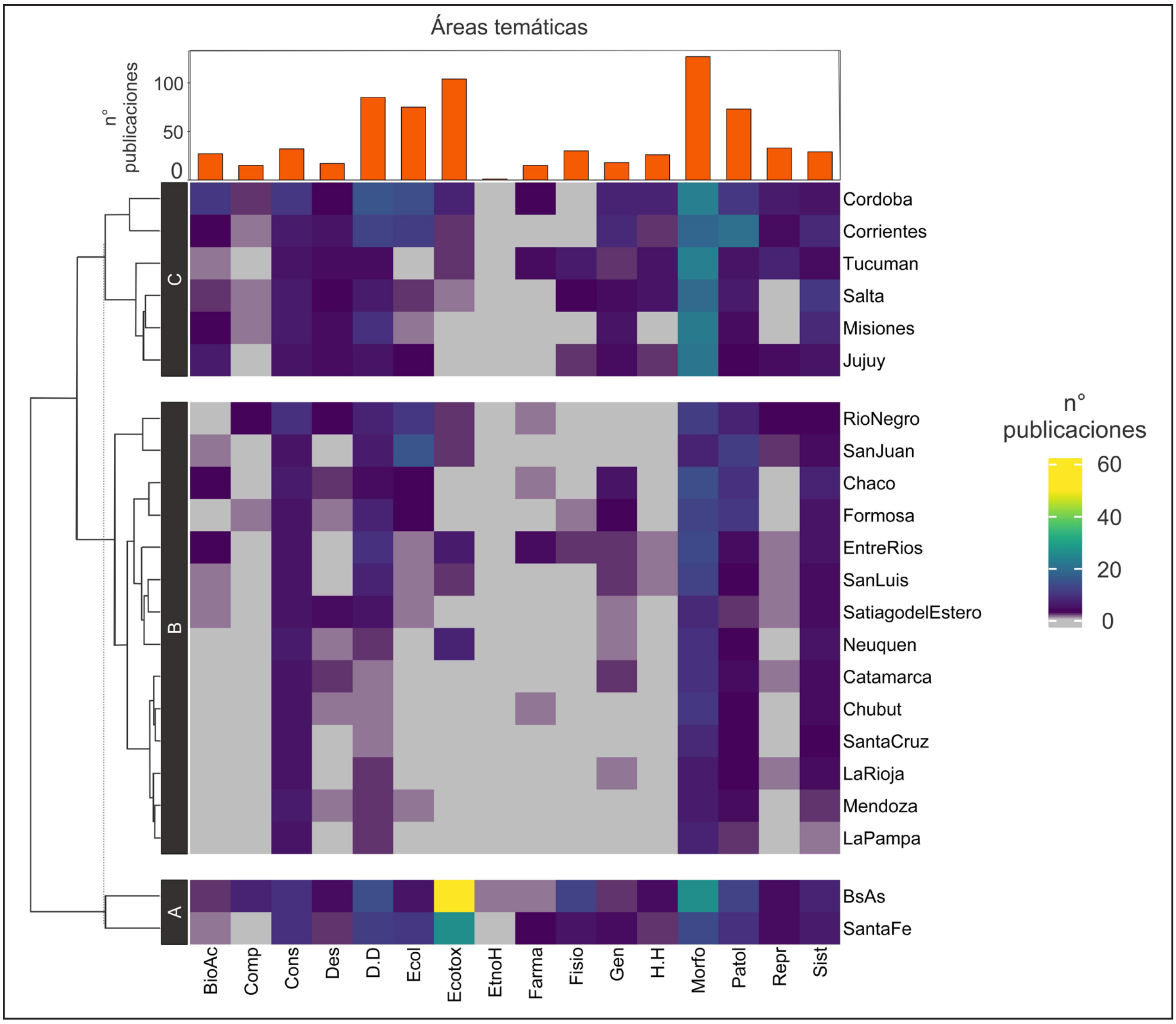

Figura 3. Mapa de calor de la cantidad de publicaciones en las distintas temáticas por cada provincia. Se agrupa a las provincias mediante un análisis de conglomerado jerárquico empleando el método de Ward, con una distancia Euclidiana como medida de similitud. Se obtuvieron tres grupos: A) Grupo Bs As- Santa Fe, Grupo B) Sur-Centro del país y C) Norte-Centro del país. Las barras naranjas denotan el número total de publicaciones según tema en el período 2010-2020.

34% de las publicaciones que presentó la especie fue en Ecotoxicología, el 12% fue en Diversidad y Distribución y el 11% en Morfología. *Boana pulchella, Leptodactylus latinasus*, *L. luctator* y *L. macrosternum* presentaron entre 70 y 80 publicaciones cada una durante el período estudiado, representando alrededor del 45% de las publicaciones de la década. *Boana pulchella* se incluyó en 22 publicaciones en las que aparece como el único organismo de estudio, y fue la segunda especie más utilizada en estudios experimentales. *Leptodactylus latinasus, L. luctator* y *L. macrosternum* fueron la única especie del estudio en sólo 6 (8,4%), diez (13,8%) y diez (14,3%) publicaciones, respectivamente (Tabla 1). Estas cuatro especies presentaron un mayor número de publicaciones en las áreas de Diversidad y Distribución, Morfología, Ecotoxicología y Patologías.

Todas las especies presentaron estudios en toda su distribución (sP, ^{0}D) (Tabla 1). *Rhinella arenarum*, a pesar de ser la especie que más publicaciones presentó durante el período, fue la especie con menos equitatividad de publicaciones por provincia (^{1}D=0,59), con una dominancia de publicaciones en las provincias de Buenos Aires y Córdoba. En cuanto a los valores estimados de sesgo por temas para cada especie (sT), se observa que ninguna especie presentó estudios en todos los temas; *Boana pulchella*, *R. arenarum* y *Leptodactylus macrosternum* fueron las científicas que aquellas que se encontraban bajo alguna categoría de amenaza (Fig. 5). Asimismo, el 68% de las especies incluidas bajo la categoría No amenazada (LC) según los criterios de la UICN, presentó significativamente más publicaciones en relación con aquellas que se encontraban en las otras categorías de amenaza (Fig. 5). El 43% de las especies fueron clasificadas como generalistas, y el 32% fueron clasificadas como de amplia distribución (Vaira *et al.*, 2012). Las especies generalistas o semi generalistas (categoría *intermedia* de rareza ecológica) y con una amplia distribución presentaron un número significativamente mayor de publicaciones científicas que aquellas especies clasificadas como especia-

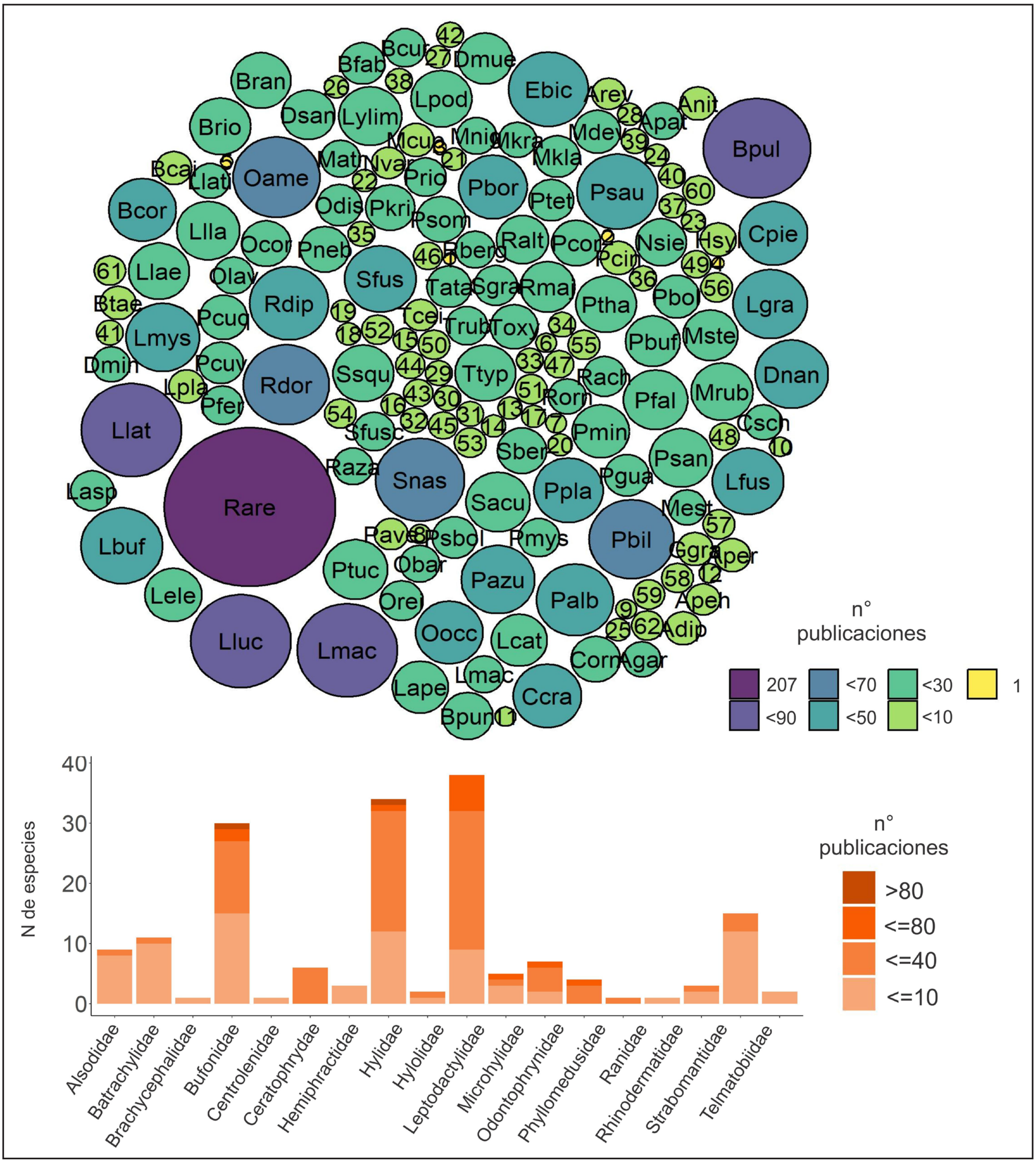

Figura 4. Gráfico de burbujas representativo, donde el tamaño refleja el esfuerzo de investigación registrado por especie durante el período en estudio. Las barras naranjas denotan número de especies por Familia que presentaron menos de 10 publicaciones, entre 10 y 40 publicaciones, entre 40 y 80 publicaciones y más de 80 publicaciones durante el período 2010-2020.

listas y endémicas (Fig. 5). El 85% de las especies se encontró dentro de la categoría de tamaño pequeño (< 80mm), un 13% se encontró dentro de la categoría de tamaño medio (81 a 160 mm) y solamente un 2% se encontró dentro de la categoría de tamaño grande (>161 mm). Las especies con tamaños medios presentaron un número significativamente mayor de artículos científicos que las especies de menor tamaño (Fig. 5). Por último, el 80% de las especies fueron registradas en ambientes urbanos o rurales, presentando significativamente mayor cantidad de artículos que aquellas que se encuentran solamente en ambientes naturales (Fig. 5).

Discusión

Los resultados de este estudio sugieren que la producción científica referida a los anfibios anuros de Argentina durante el período 2010-2020 fue constante. Pese a ello, se observaron diferentes sesgos en cuanto a la distribución de publicaciones en las provincias del país, en las áreas del conocimiento abordadas y en cuanto a las especies más estudiadas.

Artículos y temáticas principales de investigación

La producción científica desarrollada durante 2010-2020 cubrió todo el territorio argentino, y presentó tendencias y sesgos en la concentración geográfica del número de publicaciones realizadas. Además de la relación positiva esperada con la riqueza de especies, se evidenció que las provincias con un mayor número de publicaciones y de autores se corresponden con los grandes centros urbanos y de mayor densidad poblacional, como ser el caso de Buenos Aires, Córdoba, Santa Fe y Tucumán. Estas provincias presentan, conjuntamente, la mayor cantidad de centros de investigación y de unidades académicas y universitarias, con una extensa trayectoria en

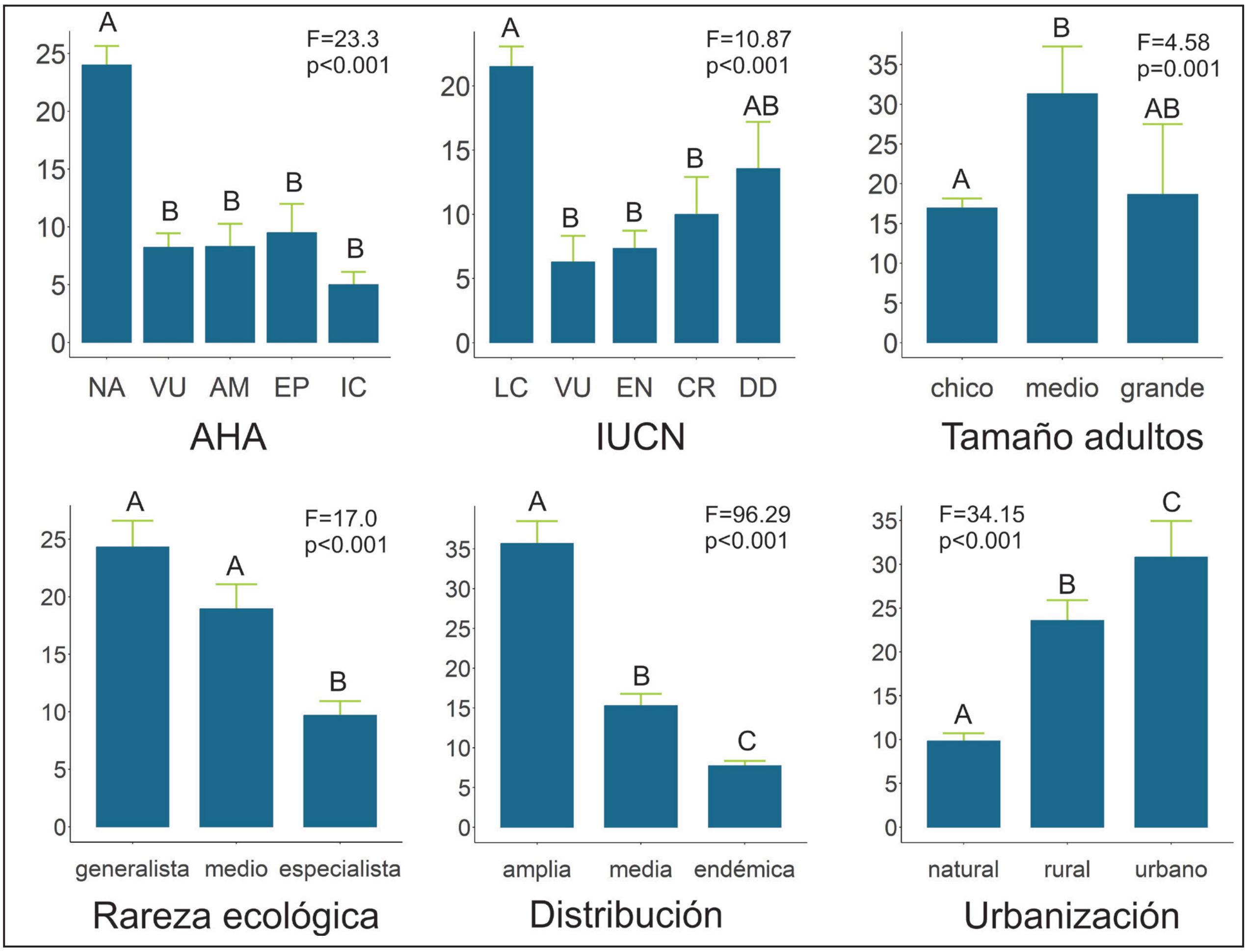

Figura 5. Resultado de las regresiones lineales generalizadas con distribución binomial negativa relacionando el número de publicaciones de las especies con variables independientes: Categorías de Conservación según AHA, IUCN, Tamaño de adultos, Rareza ecológica, Distribución según Vaira *et al.* (2012) y ocurrencia en ambientes antropizados (IUCN, 2022). Las barras representan valores medios ± error estándar. Las letras diferentes indican diferencias estadísticamente significativas (p<0,05) a partir del análisis post hoc.

ciencias. Es así que el patrón observado en número de autores por provincia se correspondió de forma significativa con el número de recursos humanos de CONICET reportados en el 2020, debiendo considerar que no todos los investigadores pertenecen a este sistema científico. Por otro lado, las investigaciones llevadas a cabo en aquellas provincias que registran pocos o ningún investigador residente dependieron, en gran medida, de autores con afiliación en otras provincias. De esta forma, tomando en cuenta que trasladarse a otra provincia conlleva un alto costo debido al aumento en el requerimiento de diferentes recursos (e.g., humanos, económicos, tiempo, infraestructura, traslado, entre otros), es esperable que los investigadores elijan más frecuentemente efectuar sus estudios en la misma provincia donde residen, reforzando el patrón de sesgo en el número de publicaciones por provincia. Lo mencionado previamente nos sugiere que el sesgo observado en el número de publicaciones por provincia es esperable, y que el patrón de número de publicaciones por provincia observado estaría reflejando la disparidad de investigadores activos en el área de herpetología en cada provincia, así como la escasez de centros de investigación y universitarios con carreras afines a las Ciencias Biológicas en ciertas provincias del país. Las políticas para la promoción del progreso científico y tecnológico en Argentina tienen un limitado alcance territorial, por lo que existen aún desigualdades entre las provincias en cuanto a recursos, capacidades científico-tecnológicas, desarrollo socio-económico y alcance de las políticas públicas (MCT, 2022; Niembro y Starobinsky, 2021). Es así que las provincias del NEA y NOA (salvo Tucumán), junto con Santa Cruz, son consideradas de bajos recursos en cuanto a inversión y personal, con menores competencias para acceder a programas de financiamiento, los que podrían representar factores que contribuyen a la baja producción científica observada en ciertas provincias o zonas geográficas (Ciocca y Delgado, 2017; Beigel *et al*, 2018; Niembro y Starobinsky, 2021). Parte de los objetivos planteados en el Plan Nacional de Ciencia, Tecnología e Innovación 2030 (MCT, 2022) proponen distintas políticas, estrategias e instrumentos orientados a revertir estas desigualdades y promover el desarrollo técnico-científico en distintas partes del país, algo que repercutirá favorablemente a futuro sobre el patrón de distribución de la producción de conocimiento científico observado en este estudio.

La investigación científica es dinámica y el interés hacia ciertos temas puede aumentar o disminuir con el tiempo (Griffiths y Steyvers, 2004; Mane y Börner, 2004). Nuestros resultados indicaron que la distribución geográfica de los temas presentó una mayor predominancia de estudios en Diversidad y Distribución y Ecología en las provincias del norte del país, que puede vincularse posiblemente a la mayor diversidad de especies de anfibios presentes en las mismas (Vaira *et al.*, 2012). Por otro lado, en aquellas provincias con un fuerte desarrollo agroindustrial, como Buenos Aires, Santa Fe y Córdoba, se registró una mayor cantidad de estudios en Ecotoxicología. Se observó una escasa producción científica en temas como EtnoHerpetología, la cual es un área de conocimiento tradicionalmente más relacionada con las Ciencias Sociales, así como en Comportamiento y en Hematología e Histología. Aunque no siempre es sencillo definir cuáles de las áreas temáticas con vacíos de información identificados representan direcciones fructíferas para la investigación futura, consideramos que pueden representar oportunidades para investigaciones en áreas de conocimiento poco exploradas en la Herpetología argentina. Además de los intereses personales de cada investigador/a, otros de los posibles factores que podrían explicar esta tendencia son la desigual distribución y cantidad de investigadores en las distintas disciplinas (Zuk, 2016), la fuerte tradición en líneas de estudio específicas marcada por las escuelas de investigación establecidas históricamente en distintas provincias del país, y el establecimiento de líneas de investigación prioritarias y temas estratégicos planteados por los gobiernos nacionales, provinciales y por cada Instituto de investigación. Un ejemplo de esto es que en los últimos años se incentivaron estudios relacionados a la biodiversidad, calidad y cuidado del ambiente, impacto ambiental de las actividades productivas, mitigación y adaptación al cambio climático, entre otros (ver Temas estratégicos CONICET, 2021 - https://convocatorias.conicet.gov.ar/wp-content/uploads/sites/3/Listado-de-Temas-Estrategicos-2021.pdf), lo que podría explicar la importante cantidad de estudios de la anurofauna de Argentina relacionados con Diversidad y Distribución, Ecología y Ecotoxicología durante la década analizada. Por el conotrario, el área de Aportes a la Conservación no presentó un número relativamente alto de publicaciones, pero sí fue de las pocas áreas de conocimiento que se llevan a cabo en todas las provincias. Por otro lado, la clasificación realizada en esta publicación fue en base al tema principal de las publicaciones, por lo que muchas de las referidas a las áreas de Diversidad y Distribución, Ecología y Ecotoxicología previamente mencionadas pueden estar también relacionadas al área de Aportes a la Conservación, dado que la información que se genera a partir de las mismas podría representar herramientas útiles para el desarrollo de medidas efectivas de conservación.

El bajo uso de material presente en colecciones biológicas podría deberse, por un lado, a la naturaleza *per se* de los estudios, por ejemplo, estudios experimentales u observacionales que requieren especímenes vivos y no necesariamente material de museo. Por otro lado, ciertos estudios de áreas temáticas como la Morfología, Hematología e Histología y Desarrollo, pueden requerir la destrucción total del material analizado, lo cual puede constituir una restricción para emplear material de museo depositado o de poder depositarlo, eventualmente, en alguna colección biológica. Por otro lado, propiciar la digitalización y carga en repositorios nacionales de todos los datos de colección de los ejemplares depositados en los museos podría aumentar el uso del material que se encuentra depositado en los museos, dado que permitiría que los mismos estén fácilmente disponibles para los investigadores, reduciendo así los costos de traslado para visitar las colecciones distantes (Meineke *et al*., 2018).

Por último, la alta proporción de publicaciones en el idioma inglés se relaciona con que actualmente, el número de publicaciones y el factor de impacto de la revista en las que los artículos son publicados, representan una medida de la productividad científica (Van Dijk *et al*., 2014; Lindner *et al*., 2018). Gran parte de las revistas de alto impacto, y por ende de mayor visibilidad, se publican en idioma inglés, por lo que su dominancia en la comunicación científica a nivel internacional y nacional es esperable. De hecho, cerca del 98% de las investigaciones a nivel global son publicadas en este idioma (Montgomery, 2004; Guardiano *et al*., 2007; Hamel, 2007; Ramírez-Castañeda, 2020). Resulta interesante remarcar que, dentro de las publicaciones en revistas editadas en países latinoamericanos registrados, 31% se publicaron en Cuadernos de Herpetología, lo cual denota el interés y la relevancia de esta revista argentina, sugiriendo una maduración de la disciplina en el país (Salatino, 2019).

Especies de anuros utilizadas en los estudios

Los resultados de este estudio evidenciaron un fuerte sesgo taxonómico, patrón que coincide con otras revisiones referidas tanto a anfibios (Schiesari *et al*., 2007; da Silva *et al*., 2020) como a otros grupos taxonómicos (Bonnet *et al*., 2002; Clark y May, 2002; Fisher *et al*., 2010; Brooke *et al*., 2014, Ducatez y Lefevre 2014; Ducatez, 2019; Piccolo *et al*., 2020, Ellison *et al*., 2021). Este sesgo taxonómico puede explicarse en función de las relaciones que se observaron entre el número de publicaciones que presentaba una especie y sus características intrínsecas. En particular, aquellas especies que se encontraron bajo la categoría de No Amenazadas presentaron más publicaciones que aquellas bajo algún grado de amenaza, un patrón ya observado en otros grupos taxonómicos (Jarić *et al*., 2014; Yarwood *et al*., 2019, Tensen, 2018, Piccolo *et al*., 2020). Frecuentemente, las especies incluidas en alguna categoría de amenaza presentan ciertas restricciones geográficas (e.g., distribución reducida, con presencia restringida en áreas remotas o poco accesibles, da Silva *et al.,* 2020), las cuales pueden contribuir a que sean poco estudiadas o que la probabilidad de registrarlas en distintos estudios sea relativamente baja. Asimismo, observamos que las especies generalistas (*sensu* Giraudo *et al.,* 2012), con una amplia distribución y que se encuentran en sitios cercanos a ciudades o zonas rurales (i.e., especies más accesibles) presentaron una cantidad relativamente mayor de publicaciones que las especialistas y endémicas. Estas especies generalistas suelen ser más fáciles de encontrar y registrar en diferentes hábitats, lo que aumenta la probabilidad de ser registradas en diversos estudios. Estas relaciones entre el esfuerzo de investigación, rango de distribución y rasgos e historias de vida de las especies fueron también registradas para otros taxones (e.g., Ducatez y Lefebvre, 2014; Ibáñez-Alamo *et al.,* 2017; Yarwood *et al*., 2018).

Considerando el tamaño corporal de las especies de anfibios presentes en Argentina, resulta interesante destacar que a pesar de que en nuestro país se observa una menor proporción de especies de tamaño intermedio respecto a la de especies de tamaño pequeño, las primeras registraron mayor cantidad de publicaciones durante el período estudiado. La ausencia de diferencia significativa en el número de publicaciones entre especies de tamaño grande con respecto a especies pequeñas y medianas podría deberse a un artefacto del análisis, por ejemplo, la inclusión en la categoría de tamaño corporal grande de especies muy comunes y abundantes, como el sapo común (*Rhinella arenarum*) y de especies caracterizadas por su rareza ecológica y consideradas amenazadas (e.g., spp. de la familia Telmatobiidae), ocasionaría una mayor variación intragrupo que intergrupo en relación a la cantidad de estudios registrados, disminuyendo la potencia de los análisis empleados para encontrar diferencias significativas entre las distintas clases de tamaño. Esta relación positiva entre tamaño corporal y el esfuerzo de investigación ha sido registrada en otro estudio con anfibios (da Silva *et al.*, 2020) y para otros grupos taxonómicos (e.g., Brooke *et al.*, 2014; Tensen, 2018), y generalmente se explica considerando que las especies de mayor tamaño corporal suelen ser relativamente más fáciles de encontrar y manipular que aquellas de menor tamaño.

Al examinar cuáles fueron las especies con un mayor esfuerzo de investigación y aquellas con vacíos de información, se identificó al sapo común, *Rhinella arenarum,* como la especie con mayor cantidad de publicaciones registradas durante el período estudiado. Las características de esta especie coinciden con las identificada como relacionadas con un alto número de publicaciones, es abundante en ambientes urbanos dentro de su amplia área de distribución, de tamaño relativamente grande y fácil de localizar y colectar (Ibáñez-Brooke *et al.*, 2014; Jarić *et al.*, 2014; Álamo *et al.*, 2017; dos Santos *et al.*, 2020). Esta especie además fue frecuentemente empleada para estudios experimentales como 'especie modelo', principalmente en el área de Ecotoxicología (Candioti *et al.*, 2010; Lajmanovich *et al.*, 2011, 2019; Aronzon *et al.*, 2011, 2020; Cervino *et al.*, 2017, entre otros) y Farmacología (Cervino *et al.*, 2017; Zapata-Martínez *et al.*, 2017), lo cual se ve reflejado en la gran cantidad de estudios incluyendo a esta especie registrados durante el período relevado. El uso de especies modelos en estudios experimentales puede resultar pragmático al momento de comparar resultados (e.g., Schiesari *et al.*, 2007). De todas maneras, es necesario remarcar la inclusión del sapo común (*R. arenarum*) en publicaciones dentro del área de la Ecotoxicología llevadas a cabo a nivel de comunidad y en otras áreas del conocimiento, como por ejemplo Diversidad y Distribución y Morfología, lo cual indicaría que se está generando también conocimiento de base sobre esta especie.

No podemos definir fehacientemente un número mínimo de publicaciones para considerar que una especie presenta vacíos de información, dado que esto no sólo depende del número de publicaciones *per se*. El caso del sapo común es un buen ejemplo, a pesar que fue la especie con más publicaciones (casi el 30% del total registrado), tuvo la menor equitatividad en publicaciones por provincia y por temas, con una alta proporción de estudios experimentales que tuvieron a esta especie como ´especie modelo´, lo cual posiblemente aumentó el número total de publicaciones. De esta forma, no podemos afirmar que esta especie no presenta vacíos de información en ciertas provincias y, más seguramente, en ciertos temas. Consideramos entonces necesario complementar el número de publicaciones que presenta una especie con la cantidad y equitatividad de publicaciones por provincia y por temas abordados al momento de evaluar vacíos de información. De esta forma, dado que todas las especies presentaron publicaciones en las distintas provincias dentro de su distribución, seleccionamos de forma arbitraria aquellas especies con menos de 5 publicaciones durante la década analizada, y con igual o menos del 25% de los temas abordados, identificando 25 especies que podrían estar presentando vacíos de información (Tabla 1). La mayoría de estas especies fueron especialistas, de pequeño tamaño y con una distribución geográfica, características que identificadas como relacionadas a una baja cantidad de publicaciones. Otras especies fueron descriptas recientemente, como *Scinax fontanarrosai* (Baldo *et al.*, 2019), o presentan problemas taxonómicos, como *Rhinella* cf. *cerradensis*, *Crossodactylus dispar, Rhinella gnustae* y *Chiasmocleis albopunctata* (Ferraro *et al.*, 2018), lo que posiblemente impacta sobre el número de publicaciones y/o temas abordados por las mismas. De todas maneras, dado que este estudio se presenta a una escala amplia a fin de observar patrones en número de publicaciones de todas las especies y provincias de Argentina, sería necesario llevar estudios dirigidos a nivel de especie para identificar asertivamente estos vacíos de información.

Las contribuciones al conocimiento taxonómico de los anfibios anuros han sido numerosas en años recientes, y todavía existen importantes revisiones que seguramente propiciarán más cambios al listado de los Anfibios de la República Argentina (ver Ferraro *et al.*, 2018). De esta forma, las acciones conjuntas entre investigadores y especialistas del país como, por ejemplo, la Categorización del Estado de Conservación de Anfibios de Argentina, son necesarias dado que resultan en un inventario actualizado y accesible, con una taxonomía adecuada y actualizada, así como de la información sobre su distribución dentro del país. Esta lista puede servir entonces para definir líneas prioritarias en cuanto a temas y especies.

Nuestros resultados constituyen un punto de partida para esclarecer y evidenciar los diferentes sesgos y vacíos del conocimiento científico sobre la anurofauna del país, esperando sea útil para comenzar a discutir una propuesta integral sobre la importancia y consecuencias de estos sesgos en la información científica y sus efectos potenciales sobre la conservación de este grupo de vertebrados.

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Anexo

Tabla 1. Lista de especies actualizada hasta 2022, incluyendo el código, el número de publicaciones durante el período 2010-2020 (N°). esfinv: esfuerzo de investigación (n° publicaciones spp/ n° total publicaciones), sP: sesgo por provincia y sT: sesgo por temas, estimados en base a números de Hill (qD) (Jost, 2006), con q=0 (no toma en cuenta abundancias relativas) y q=1 (toma en cuenta abundancias relativas). * especies detectadas en Argentina o descriptas en 2022 ~ especie invasora. # especies con posible extensión a Argentina, pero sin datos de distribución.

	Especies	**Código**	**N°**	**esfinv**	**sP**		**sT**	
					q=0	**q=1**	**q=0**	**q=1**
	Alsodidae							
1	*Alsodes coppingeri (*)*							
2	*Alsodes gargola*	Agar	10	0,014	1	0,957	0,313	0,287
3	*Alsodes neuquensis*	Aneu	8	0,012	1	1	0,188	0,172
4	*Alsodes pehuenche*	Apeh	8	0,012	1	1	0,313	0,250
5	*Alsodes verrucosus*	Aver	3	0,004	1	1	0,063	0,063
6	*Eupsophus calcaratus*	Ecal	5	0,007	1	0,969	0,188	0,162
7	*Eupsophus emiliopugini*	Eemi	4	0,006	1	1	0,063	0,063
8	*Eupsophus roseus*	Eros	5	0,007	1	1	0,125	0,125
9	*Eupsophus vertebralis*	Ever	3	0,004	1	1	0,063	0,063
10	*Limnomedusa macroglossa*	Lmac	13	0,019	1	0,898	0,188	0,166
	Batrachylidae							
11	*Atelognathus nitoi*	Anit	9	0,013	1	0,979	0,125	0,123
12	*Atelognathus patagonicus*	Apat	13	0,017	1	1	0,313	0,260
13	*Atelognathus praebasalticus*	Apra	4	0,006	1	1	0,063	0,063
14	*Atelognathus reverberii*	Arev	8	0,012	1	0,945	0,188	0,149
15	*Atelognathus solitarius*	Asol	5	0,007	1	1	0,125	0,110
16	*Batrachyla antartandica*	Bant	4	0,006	1	1	0,063	0,063
17	*Batrachyla fitzroya*	Bfit	3	0,004	1	1	0,063	0,063
18	*Batrachyla leptopus*	Blep	8	0,010	1	0,960	0,188	0,172
19	*Batrachyla taeniata*	Btae	8	0,012	1	0,990	0,125	0,110
20	*Chaltenobatrachus grandisonae*	Cgra	4	0,006	1	1	0,125	0,110
21	*Hylorina sylvatica*	Hsyl	9	0,013	1	0,979	0,188	0,172
	Brachycephalidae							
22	*Ischnocnema henselii*	Ihen	4	0,006	1	1	0,125	0,110
	Bufonidae							
23	*Melanophryniscus atroluteus*	Matr	11	0,016	1	0,966	0,313	0,251
24	*Melanophryniscus cupreuscapularis*	Mcup	9	0,013	1	1	0,250	0,211

25	*Melanophryniscus devincenzii*	Mdev	14	0,020	1	0,980	0,375	0,308
26	*Melanophryniscus diabolicus*	Mdia	6	0,009	1	1	0,250	0,217
27	*Melanophryniscus estebani*	Mest	10	0,014	1	1	0,313	0,272
28	*Melanophryniscus fulvoguttatus*	Mful	6	0,009	1	1	0,125	0,118
29	*Melanophryniscus klappenbachi*	Mkla	14	0,020	1	0,945	0,375	0,311
30	*Melanophryniscus krauczuki*	Mkra	10	0,014	1	1	0,250	0,201
31	*Melanophryniscus nigricans*	Mnig	11	0,016	1	1	0,375	0,323
32	*Melanophryniscus rubriventris*	Mrub	27	0,039	1	0,932	0,688	0,602
33	*Melanophryniscus stelzneri*	Mste	21	0,029	1	0,993	0,500	0,420
34	*Nannophryne variegata*	Nvar	8	0,012	1	1	0,188	0,172
35	*Rhinella achalensis*	Rach	13	0,019	1	0,810	0,375	0,318
36	*Rhinella altiperuviana*	Ralt	16	0,023	1	0,956	0,375	0,308
37	*Rhinella arenarum*	Rare	206	0,298	1	0,591	0,813	0,503
38	*Rhinella azarai*	Raza	11	0,016	1	0,990	0,375	0,308
39	*Rhinella bergi*	Rberg	11	0,016	1	0,927	0,375	0,341
40	*Rhinella bernardoi*	Rber	7	0,010	1	1	0,188	0,172
41	*Rhinella* cf. *cerradensis*	Rcerr	1	0,001	1	1	0,063	0,063
42	*Rhinella diptycha*	Rdip	44	0,064	1	0,957	0,500	0,257
43	*Rhinella dorbignyi*	Rdor	57	0,081	1	0,834	0,688	0,452
44	*Rhinella gallardoi*	Rgal	4	0,006	1	1	0,125	0,110
45	*Rhinella gnustae*	Rgnu	3	0,004	1	1	0,063	0,063
46	*Rhinella icterica*	Rict	5	0,007	1	1	0,125	0,110
47	*Rhinella major*	Rmaj	20	0,029	1	0,943	0,438	0,386
48	*Rhinella ornata*	Rorn	11	0,016	1	0,909	0,313	0,258
49	*Rhinella papillosa*	Rpap	9	0,013	1	0,910	0,250	0,232
50	*Rhinella rubropunctata*	Rrub	3	0,004	1	1	0,063	0,063
51	*Rhinella rumbolli*	Rrum	6	0,009	1	0,945	0,250	0,217
	Centrolenidae							
52	*Vitreorana uranoscopa*	Vura	6	0,009	1	1	0,188	0,172
	Ceratophryidae							
53	*Ceratophrys cranwelli*	Ccra	36	0,051	0,917	0,869	0,625	0,493
54	*Ceratophrys ornata*	Corn	17	0,025	1	0,859	0,563	0,440
55	*Chacophrys pierottii*	Cpie	34	0,049	1	0,890	0,500	0,391
56	*Lepidobatrachus asper*	Lasp	13	0,019	1	0,973	0,375	0,334
57	*Lepidobatrachus laevis*	Llae	25	0,036	1	0,931	0,500	0,442
58	*Lepidobatrachus llanensis*	Llla	30	0,043	1	0,963	0,563	0,468
	Hemiphractidae							
59	*Gastrotheca christiani*	Gchr	7	0,010	1	0,986	0,188	0,139
60	*Gastrotheca chrysosticta*	Gchry	7	0,010	1	1	0,188	0,139
61	*Gastrotheca gracilis*	Ggra	9	0,013	1	0,986	0,188	0,139
	Hylidae							
62	*Aplastodiscus perviridis*	Aper	9	0,013	1	1	0,250	0,210
63	*Boana albopunctata*	Balb	6	0,007	1	1	0,188	0,162
64	*Boana caingua*	Bcai	8	0,012	1	0,973	0,250	0,211
65	*Boana cordobae*	Bcor	32	0,045	1	0,933	0,688	0,598
66	*Boana curupi*	Bcur	12	0,017	1	1	0,375	0,292

67	*Boana faber*	Bfab	13	0,019	1	1	0,375	0,304
68	*Boana marianitae*	Bmar	4	0,006	1	1	0,188	0,162
69	*Boana pulchella*	Bpul	80	0,116	1	0,767	0,875	0,530
70	*Boana punctata*	Bpun	17	0,025	1	0,886	0,313	0,279
71	*Boana raniceps*	Bran	24	0,035	1	0,939	0,375	0,259
72	*Boana riojana*	Brio	25	0,036	1	0,974	0,563	0,491
73	*Boana stellae*	Bste	1	0,001	1	1	0,125	0,125
74	*Dendropsophus minutus*	Dmin	11	0,016	1	0,960	0,188	0,163
75	*Dendropsophus nanus*	Dnan	39	0,056	1	0,912	0,500	0,314
76	*Dendropsophus sanborni*	Dsan	20	0,029	1	0,903	0,438	0,265
77	*Itapotihyla langsdorffii*	Ilan	5	0,007	1	1	0,188	0,162
78	*Lysapsus limellum*	Llim	29	0,041	1	0,847	0,500	0,331
79	*Nyctimantis siemersi*	Nsie	18	0,025	1	0,889	0,438	0,373
80	*Pseudis minuta*	Pmin	24	0,035	1	0,847	0,438	0,360
81	*Pseudis platensis*	Ppla	34	0,049	1	0,946	0,625	0,480
82	*Scinax acuminatus*	Sacu	30	0,043	1	0,976	0,438	0,336
83	*Scinax aromothyella*	Saro	7	0,010	1	1	0,250	0,217
84	*Scinax berthae*	Sber	17	0,025	1	0,976	0,313	0,211
85	*Scinax castroviejoi (#)*							
86	*Scinax fontanarrosai*	Sfon	4	0,006	1	0,994	0,188	0,162
87	*Scinax fuscovarius*	Sfus	37	0,054	1	0,937	0,500	0,330
88	*Scinax fuscomarginatus*	Sfusc	12	0,017	1	0,894	0,375	0,308
89	*Scinax granulatus*	Sgra	13	0,019	1	0,922	0,250	0,192
90	*Scinax nasicus*	Snas	57	0,082	1	0,924	0,563	0,353
91	*Scinax perereca*	Sper	7	0,010	1	1	0,188	0,149
92	*Scinax similis*	Ssim	7	0,010	1	1	0,188	0,171
93	*Scinax squalirostris*	Ssqu	26	0,038	1	0,947	0,563	0,308
94	*Scinax uruguayus*	Suru	4	0,006	1	1	0,063	0,063
95	*Trachycephalus dibernardoi*	Tdib	5	0,007	1	1	0,188	0,162
96	*Trachycephalus typhonius*	Ttyp	26	0,038	1	0,992	0,375	0,281
	Hylodidae							
97	*Crossodactylus dispar*	Cdis	3	0,004	1	1	0,063	0,063
98	*Crossodactylus schmidti*	Csch	12	0,017	1	1	0,188	0,166
	Leptodactylidae							
99	*Adenomera diptyx*	Adip	9	0,013	1	0,951	0,375	0,334
100	*Leptodactylus apepyta*	Lape	22	0,032	1	0,978	0,375	0,277
101	*Leptodactylus bufonius*	Lbuf	46	0,067	1	0,936	0,563	0,375
102	*Leptodactylus elenae*	Lele	23	0,033	1	0,984	0,250	0,168
103	*Leptodactylus furnarius*	Lfur	7	0,010	1	1	0,125	0,123
104	*Leptodactylus fuscus*	Lfus	36	0,052	1	0,958	0,438	0,254
105	*Leptodactylus gracilis*	Lgra	39	0,056	1	0,952	0,438	0,197
106	*Leptodactylus labyrinthicus*	Llab	7	0,010	1	1	0,125	0,123
107	*Leptodactylus latinasus*	Llat	71	0,103	1	0,934	0,688	0,395
108	*Leptodactylus laticeps*	Llati	10	0,014	1	0,995	0,125	0,123
109	*Leptodactylus luctator*	Lluc	72	0,104	1	0,838	0,750	0,450
110	*Leptodactylus macrosternum*	Lmac	70	0,101	1	0,921	0,813	0,495

111	*Leptodactylus mystacinus*	Lmys	38	0,055	1	0,922	0,625	0,321
112	*Leptodactylus plaumanni*	Lpla	9	0,013	1	1	0,250	0,224
113	*Leptodactylus podicipinus*	Lpod	27	0,039	1	0,931	0,313	0,245
114	*Physalaemus albonotatus*	Palb	41	0,059	1	0,797	0,625	0,471
115	*Physalaemus biligonigerus*	Pbil	50	0,072	1	0,876	0,438	0,307
116	*Physalaemus carrizorum*	Pcar	5	0,007	1	1	0,188	0,162
117	*Physalaemus cuqui*	Pcuq	17	0,025	1	0,919	0,375	0,261
118	*Physalaemus cuvieri*	Pcuv	14	0,020	1	0,893	0,313	0,280
119	*Physalaemus fernandezae*	Pfer	12	0,017	1	0,841	0,313	0,285
120	*Physalaemus henselii*	Phen	4	0,006	1	0,990	0,125	0,110
121	*Physalaemus riograndensis*	Prio	12	0,017	1	0,963	0,188	0,148
122	*Physalaemus santafecinus*	Psan	27	0,039	1	0,852	0,688	0,585
123	*Pleurodema borellii*	Pbor	33	0,048	1	0,887	0,625	0,516
124	*Pleurodema bufoninum*	Pbuf	21	0,030	1	0,904	0,500	0,429
125	*Pleurodema cinereum*	Pcin	9	0,013	1	0,945	0,250	0,217
126	*Pleurodema cordobae*	Pcor	17	0,025	1	0,625	0,563	0,495
127	*Pleurodema guayapae*	Pgua	14	0,020	1	0,968	0,375	0,333
128	*Pleurodema kriegi*	Pkri	18	0,026	1	1	0,438	0,402
129	*Pleurodema marmoratum*	Pmar	5	0,007	1	1	0,125	0,103
130	*Pleurodema nebulosum*	Pneb	17	0,025	1	0,927	0,438	0,336
131	*Pleurodema somuncurense*	Psom	17	0,025	1	1	0,563	0,442
132	*Pleurodema thaul*	Ptha	26	0,038	1	0,794	0,563	0,456
133	*Pleurodema tucumanum*	Ptuc	29	0,042	1	0,929	0,563	0,389
134	*Pseudopaludicola boliviana*	Pbol	14	0,020	1	0,866	0,313	0,273
135	*Pseudopaludicola falcipes*	Pfal	28	0,041	1	0,914	0,500	0,330
136	*Pseudopaludicola mystacalis*	Pmys	12	0,017	1	0,933	0,375	0,308
	Microhylidae							
137	*Chiasmocleis albopunctata*	Calb	1	0,001	1	1	0,063	0,063
138	*Dermatonotus muelleri*	Dmue	21	0,030	1	0,939	0,375	0,333
139	*Elachistocleis bicolor*	Ebic	47	0,068	1	0,931	0,563	0,352
140	*Elachistocleis haroi*	Ehar	7	0,010	1	0,826	0,250	0,198
141	*Elachistocleis skotogaster*	Esko	5	0,007	1	0,883	0,125	0,123
	Odontophrynidae							
142	*Odontophrynus americanus*	Oame	54	0,077	1	0,882	0,750	0,444
143	*Odontophrynus asper* (*)							
144	*Odontophrynus cordobae*	Ocor	17	0,025	1	0,825	0,625	0,575
145	*Odontophrynus lavillai*	Olav	11	0,016	1	0,942	0,375	0,331
146	*Odontophrynus occidentalis*	Oocc	35	0,045	1	0,861	0,500	0,390
147	*Odontophrynus reigi*	Orei	13	0,019	1	0,980	0,375	0,291
148	*Proceratophrys avelinoi*	Pave	9	0,013	1	1	0,188	0,172
149	*Proceratophrys bigibbosa*	Pbig	3	0,004	1	1	0,063	0,063
	Phyllomedusidae							
150	*Phyllomedusa boliviana*	Pbol	15	0,022	1	1	0,188	0,149
151	*Phyllomedusa sauvagii*	Psau	47	0,068	1	0,891	0,500	0,367
152	*Phyllomedusa tetraploidea*	Ptet	14	0,020	1	1	0,250	0,210
153	*Pithecopus azureus*	Pazu	38	0,055	1	0,917	0,563	0,454

	Ranidae							
154	*Lithobates catesbeianus (~)*	Lcat	22					
	Rhinodermatidae							
155	*Rhinoderma darwinii*	Rdar	4	0,006	1	1	0,063	0,063
	Strabomantidae							
156	*Oreobates barituensis*	Obar	10	0,014	1	0,986	0,313	0,287
157	*Oreobates berdemenos*	Ober	4	0,006	1	0,785	0,250	0,237
158	*Oreobates discoidalis*	Odis	15	0,022	1	1	0,375	0,341
	Telmatobiidae							
159	*Telmatobius atacamensis*	Tata	14	0,020	1	1	0,188	0,139
160	*Telmatobius ceiorum*	Tcei	8	0,012	1	1	0,063	0,063
161	*Telmatobius contrerasi*	Tcon	4	0,006	1	1	0,063	0,063
162	*Telmatobius hauthali*	Thau	4	0,006	1	1	0,063	0,063
163	*Telmatobius huayra (#)*							
164	*Telmatobius hypselocephalus*	Thyp	4	0,006	1	1	0,063	0,063
165	*Telmatobius laticeps*	Tlat	7	0,010	1	1	0,063	0,063
166	*Telmatobius marmoratus*	Tmar	5	0,007	1	1	0,063	0,063
167	*Telmatobius oxycephalus*	Toxy	17	0,025	1	0,877	0,188	0,160
168	*Telmatobius pinguiculus*	Tpin	5	0,007	1	1	0,063	0,063
169	*Telmatobius pisanoi*	Tpis	6	0,009	1	1	0,063	0,063
170	*Telmatobius platycephalus*	Tpla	5	0,007	1	1	0,063	0,063
171	*Telmatobius rubigo*	Trub	12	0,017	1	1	0,188	0,172
172	*Telmatobius schreiteri*	Tsch	7	0,010	1	0,877	0,125	0,110
173	*Telmatobius scrocchii*	Tscr	6	0,009	1	1	0,063	0,063
174	*Telmatobius stephani*	Tste	6	0,009	1	1	0,063	0,063

Trabajo

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Anuran detectability and calling phenology in urban ponds from the Brazilian Savanna

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ABSTRACT
Over the last century, urban development has steadily resulted in wetland destruction, stream flow disturbance, and land clearing. Small intermittent wetlands are easily overlooked in large cities, and the secretive nature of amphibian species in these habitats can hinder see the big picture about the persistence of populations in urban habitats. Here, we determined anuran detectability associated with site-specific and survey-specific variables. We also identified the reproductive modes and described the calling activity along a four-year period in urban ponds of the Brazilian Cerrado ecoregion. We recorded 16 anuran species, but only nine were detected at levels suitable for modeling. Seven reproductive modes were observed in the assemblages, and although breeding choruses were more common in the rainy season, calling activity was also recorded in the dry season for 11 species. Anuran detectability was related to pond water depth, rainfall for the previous 24h, and day-of-year. Our results showed interplay between species-specific traits and water availability. We highlight that urban ponds can harbor a considerable proportion of anuran diversity; however, urbanization seems to constraint even generalist species recorded for the Cerrado ecoregion.

Key words: Temporary Wetlands; Cerrado; Amphibians; Water availability; Reproductive modes.

RESUMO
Ao longo do último século, o desenvolvimento urbano resultou constantemente na destruição de áreas úmidas, alteração do curso de riachos e desmatamento. Pequenas áreas úmidas intermitentes são facilmente ignoradas nas grandes cidades, e a natureza inconspícua das espécies de anfíbios nestes habitats pode esconder o quadro geral sobre a persistência das populações em ambientes urbanos. Aqui, determinamos a detectabilidade de anuros associada a variáveis específicas do local e da amostragem. Nós também identificamos a diversidade de modos reprodutivos e atividade de vocalização ao longo de um período de quatro anos em lagoas urbanas do Cerrado. Registramos 16 espécies de anuros, mas apenas nove foram detectadas em níveis adequados para a modelagem. Sete modos reprodutivos foram observados nas assembleias e, embora coros reprodutivos tenham sido mais comuns na estação chuvosa, a atividade de vocalização também foi registrada na estação seca para 11 espécies. A detectabilidade de anuros foi relacionada à profundidade da água, precipitação nas 24h anteriores e dia do ano. Nossos resultados mostraram uma interação entre traços específicos das espécies e a disponibilidade hídrica. Destacamos que as lagoas urbanas podem abrigar uma porção considerável da diversidade de anuros. Entretanto, a urbanização parece restringir até mesmo espécies generalistas registradas para a ecorregião do Cerrado.

Palavras Chave: Áreas Úmidas Temporárias; Cerrado; Anfíbios; Disponibilidade hídrica; Modos reprodutivos.

Introduction

Rapid urban development is a major threat for biodiversity, presenting unprecedented challenges to many plant and animal species. Ongoing habitat loss and fragmentation, human disturbances (e.g. heat islands, exposure to harmful chemicals, noise, and light pollution), and changes in microbiota interactions are just a glimpse of how urbanization might change the profile of biodiversity in cities (Babini *et al.*, 2015a; Murray *et al.*, 2019). In urban environments, small wetlands (< 1ha) are often viewed as

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wastelands and easily overlooked, mainly the intermittent ones. Nevertheless, these apparently isolated habitats are important refuges for biodiversity and the persistence of local populations of wetland-associated organisms, such as plants, invertebrates, and amphibians (Semlitsch and Bodie, 1998; Deane *et al.*, 2017; Hutto and Barrett, 2021).

As Brazil hosts a mega diversity of amphibians (Segalla *et al.*, 2021), it is unsurprising that some species can thrive in urban areas (e.g. Ferreira *et al.*, 2010; Menin *et al.*, 2019; Dorigo *et al.*, 2021). The challenge is that the Brazilian territory encompasses different forest and non-forest ecoregions, which reflect in patterns of species endemism and phylogenetic endemism across the country (Vasconcelos *et al.*, 2019). Among these highly diverse systems, the Cerrado ecoregion is a mosaic encompassing a broad spectrum of vegetation types—such as grasslands, open and dense savannas, and dry forests—affected by a strong seasonal climate (Del-Claro and Torezan-Silingardi, 2019). Despite the Cerrado harbors several endemic species of amphibians (Azevedo *et al.*, 2016), local assemblages show predominance of widely-distributed species that are considered habitat generalist with generalized breeding requirements (Brasileiro *et al.*, 2005; Gambale *et al.*, 2014; Santoro and Brandão, 2014; Ferreira *et al.*, 2017). Such use of multiple types of habitats and generalized reproductive modes (i.e. aquatic eggs deposited directly in the water or in foam nests on the water) have been pointed as adaptations to unpredictable habitats and dry environments (Gomez-Mestre *et al.*, 2012; Santoro and Brandão, 2014), but they also seem key to amphibian survival and successful reproduction in human-altered landscapes (Hamer and McDonnell, 2008; Ferreira *et al.*, 2017).

Amphibians inhabiting urban areas have received little attention, mainly in megadiverse tropical areas. Available information about aspects of reproductive ecology in the city's wetlands indicated issues related to habitat split, reduction in body size, and assemblage homogenization with decreases in arboreal amphibian species (Acosta *et al.*, 2005; Babini *et al.*, 2015a; Murray *et al.*, 2019). Still, research into urban ecology is in its beginnings in tropical environments. The lack of information about species occurrence, phenology, and population fluctuations hinders forecasts about impacts on environments that still resist urbanization. Two other subtle issues must be recognized here. First, species inventories and studies on amphibian-habitat relationships are often based on either short-term data or derived from random samplings focused on the breeding season. However, temporary unsuitable weather or breeding site conditions, infrequent or unpredictable species breeding behavior may jeopardize abundance estimates and observed patterns (Greenberg *et al.*, 2017; Leão Pompeu *et al.*, 2020). Second, detection probability estimates for amphibians are often low (< 0.5) and high variability in the detection for species in the same community has been reported in different environments (Barata *et al.*, 2017; Ribeiro *et al.*, 2018; Asad *et al.*, 2020).

Although imperfect detection may depend on a myriad of conditions that vary geographically, it is notable that amphibian detectability depends on the individual species traits, survey conditions, aquatic vegetation, and hydroperiod (Moreira *et al.*, 2016; Ribeiro *et al.*, 2018; Asad *et al.*, 2020). In addition, amphibians often exhibit periodic cycles (i.e. phenology) of calling activity/reproduction that go along in concert with temperature, precipitation, and pond water availability (e.g. Andrade *et al.*, 2019; Souza *et al.*, 2020). A major issue is trade-offs and synergies between phenology and detectability. For example, shifts in phenology can be masked by imperfect detection if calling activity and detectability were linked to distinct environmental factors (Lowe *et al.*, 2016). Only a few studies on amphibians from rapidly urbanizing regions have incorporated detection probabilities to understand species-habitat relationships (Hamer, 2018; Hamer *et al.*, 2021). Although water availability seems to affect detectability at species level in urban areas, overall assemblage patterns are also depending on temperature and day-of-year.

Here we provide information on the anuran amphibian assemblages associated to temporary ponds in urban areas of the Brazilian Cerrado ecoregion. First, we determined detectability associated with site-specific and survey-specific variables. We expected seasonal variation in detectability (i.e. higher values during rainy periods), although some species could be detected regardless of season (Guimarães *et al.*, 2014; Barata *et al.*, 2017). Then, we identified the reproductive modes and described the calling activity over a four-year period (2015-2019).

Materials and methods

Study area

Cuiabá is the fourth largest city in Midwestern Brazil that together with Várzea Grande—a satellite city—forms an urban agglomeration with a population of approximately 913 thousand people (IBGE 2020). Cuiabá has a tropical savanna climate, with an annual mean temperature of 26.8°C and annual mean precipitation of 1495 mm, concentrated from November to March (~70%; Souza *et al.*, 2013). Fifty-five species of amphibians inhabit the Greater Cuiabá area (Dorado-Rodrigues *et al.*, 2018) and many of these are known to breed in aquatic environments, although some species have terrestrial reproduction.

We conducted our fieldwork in sites located at the Universidade Federal de Mato Grosso (15.612114°S, 56.068240°W). Three small temporary ponds (Fig. 1), located 0.28–0.8 km apart, were selected to represent different types of habitats available for amphibians in urban areas of Cuiabá: i) feed by irregular inundation by headwater streams during heavy rains; ii) feed by surface runoff along roads and pluvial drainage associated to rain; iii) feed exclusively by precipitation. In the middle of the rainy season, all ponds support aquatic vegetation and small fish schools are found in the ponds feed by flood or pluvial drainage. All sampling sites were close to human infrastructure (i.e. roads, buildings). The vegetation in these areas consisted mostly of shrubs, grasses, and sparse trees of native and exotic species. Traffic noises, constant movement of people, and night lighting associated with vehicle headlights and street lighting are widespread in all ponds surrounding areas.

Field methods

We surveyed each site 56 times: seven times in the dry season (May to September) and seven times in the rainy season (November to March) over four consecutive years (2015-2019). Between January and March 2015 we surveyed our ponds, as a pilot study, to test ideas and the feasibility of our sampling design. All ponds were surveyed on the same night between 18:00h and 22:00h and the areas were sampled in random order. Two trained field observers followed two different 50-m transects perpendicular to the edge of the pond. Each transect was sampled by only one observer during 20 minutes. We registered the presence of all amphibians, visually (distinguishing between adult and juvenile frogs) or acoustically. Calling activity for each species heard was classified in two groups: i) call of individuals can be distinguished, with little overlapping, ii) full chorus, calls are constant, continuous and overlapping. All individuals were identified in the field and returned to the ponds. The species were grouped into their respective reproductive modes following the classification proposed by Nunes-de-Almeida *et al.* (2021).

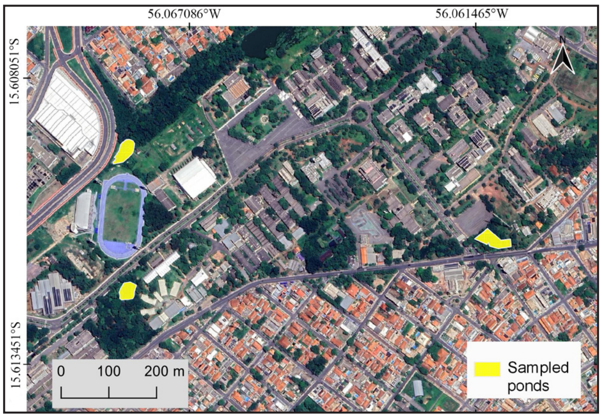

Figure 1. Geographical location of the three sampled ponds in the urban area of Cuiabá, Mato Grosso State, Brazil.

Data on survey-specific factors thought to influence detection, colonization and local extinction probabilities were collected (Roloff *et al.*, 2011; Moreira and Maltchik, 2015). These include day-of-year, rainfall in the last 24-hour period, air temperature, pond water depth, hydroperiod, presence of superficial water, and time since the last rain. We transformed the calendar dates of each sampling into day-of-year (i.e. count the number of a given day in the year). To measure precipitation during the study period, we used a single rain gauge placed in the center of the study area. The temperature was measured at the beginning of each pond sampling. The water column depth corresponded to average values of four random measures using a ruler.

Data analyses

For our analysis of detection estimates, we used a multi-season occupancy model that explicitly accounts for imperfect detection (MacKenzie *et al.*, 2006). The study consisted of eight primary sampling periods (seasons) and within each primary period seven surveys by pond (sampling nights). Only species with a detection probability ≥ 0.15 were modeled (O'Connell *et al.*, 2006). Ten out of the 12 species recorded during our pilot study were present in more than one pond, so we assumed a constant occupancy probability (ψ) over the study. We choose not assesses factors associated with pond occupancy, because the closeness among sampled ponds. Still, colonization (γ) and extinction (ε) probabilities may change among the primary sampling periods. We built models where γ was related to pond hydroperiod (i.e. number of samplings events with water in each season) and ε was related to species abundance in the previous season.

We followed a two-step process for building occupancy models (Roloff *et al.*, 2011). First, we modeled those variables that we thought would influence detection probabilities (d), including rainfall, pond water depth, presence of superficial water, air temperature, day-of-year and time since the last rain while holding other parameters constant (ψ, γ, ε). As we expected that detection probability may reach a peak at a particular date (e.g. onset of the rainy season), we included day-of-year as a quadratic term. We used a correct Akaike's information criteria (AICc; Burnham and Anderson, 2002) to rank candidate models for each species. Second, we examined occupancy models with the best model for detection probability (i.e., ψ (.), γ (hydroperiod), ε (abundance), d (best covariate)). Then, we conducted a goodness-of-fit-test based on 999 simulations to detect if violations of model assumptions had occurred and to evaluate the fit of the most supported model to the data. Variance inflation factor ($\hat{c}$) values > 1 indicate overdispersion, while values much higher than 1 (i.e. > 4) indicate a lack of fit (MacKenzie and Bailey, 2004). Analyses were carried out using the R statistical computing environment (R Core Team, 2021), and its package unmarked (Fiske and Chandeler, 2011). Juveniles can achieve large numbers for some anuran species and their emergence may be decoupled from adult breeding season (López *et al.*, 2011; López *et al.* 2017). Because such abundance shift could bias the detectability, we analyzed the data in two ways: i) all individuals; ii) excluding juveniles.

Results

We recorded a total of 16 species in the study area belonging to 10 genera and four families (Bufonidae, Hylidae, Leptodactylidae, and Microhylidae; Figs. 2-3). Here, individuals recorded as *Adenomera* sp. correspond to a species not yet formally described, with populations present in Cuiabá and Chapada dos Guimarães municipalities (C. Strüssmann, pers. communication). During the four years of the study period, we recorded a total of 3789 individuals, of which about 10% corresponded to juveniles (N = 325).

We could not confirm the breeding activity (i.e. amplectant pairs, eggs, juveniles) of six species (*Boana geographica*, *B. raniceps*, *Scinax fuscovarius*, *Adenomera* sp., *Leptodactylus syphax*, and *Pseudopaludicola motorzinho*) of the 16 species observed in calling activities. Three species corresponded to 65% of all amphibians recorded during the surveys (*Dendropsophus nanus*, *Leptodactylus fuscus*, and *Physalaemus albonotatus*; Table 1). Although, adult abundance recorded in the dry season was around 30% of the abundance recorded in the rainy season, juveniles were seen in similar numbers both in the dry and rainy seasons (Table 1). Hydroperiod was quite variable, with environments retaining water from a few days to more than seven months in some years.

Calling phenology

We recorded calling individuals for most species (11 of all 16 species) both in the dry and rainy seasons (Table 1). Even so, some species exhibited notable inter-annual variation in the phenology (see Supplementary 1). For example, calling records of three species (*D. nanus*, *Scinax nasicus*, and *Elachistocleis bicolor*) were sparse in the two first years of monitoring, increasing from the end of 2016. On the other hand, *L. syphax* callings were seldom recorded after December 2016. Except for *Adenomera* sp., calling activity of leptodactylids was common in both seasons. However, breeding choruses were registered only in the rainy season, mainly between November and February. Between March and August, observations of *Leptodactylus elenae* and *L. labyrinthicus* corresponded mainly to visual registers of individuals with small sizes and calling activity was hardly detected. For *L. fuscus*, we also registered a reduction in the calling activity during the transition from the rainy to dry season, but both adults and juveniles were often sighted. Breeding choruses of species belonging to Microhylidae family were recorded from November to January, although sparse callings of *E. bicolor* were recorded in the dry season. While breeding choruses of *S. nasicus* were recorded only in the rainy season, *D. nanus* also formed breeding choruses in the middle of the dry season (Supplementary 1). Juveniles of *D. nanus* and *S. nasicus* were often sight in the beginning of dry season. We registered seven reproductive modes (Table 1), *sensu* Nunes-de-Almeida *et al.* (2021), as follow: i) three modes with eggs deposited in the water (Mode 1, 2, and 3); ii) two modes with floating foam nests (Mode 45 and 50); iii) two modes with terrestrial foam nest in constructed burrows (Mode 62 and 64).

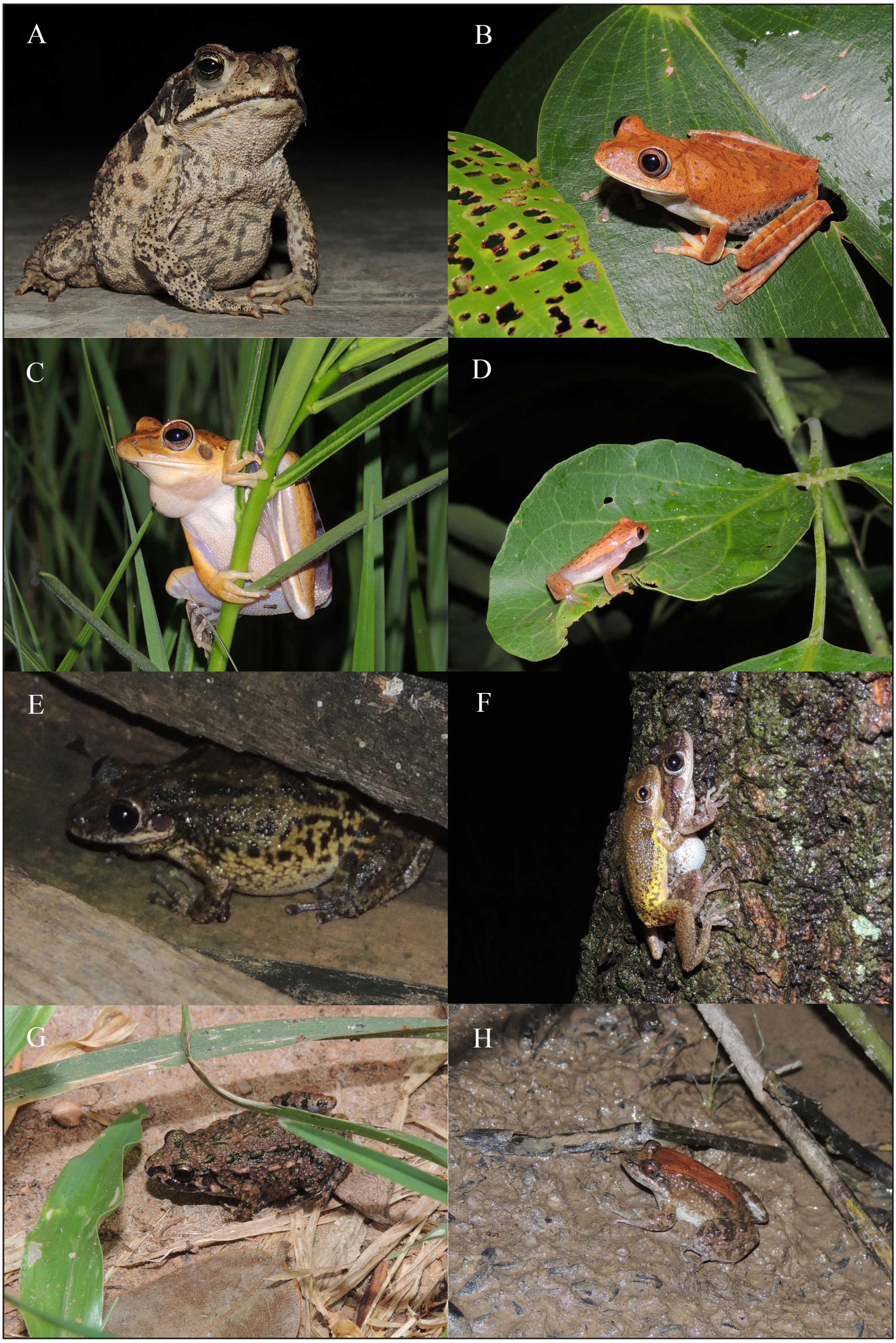

Figure 2. Amphibians recorded at urban ponds from Cuiabá, Mato Grosso, Brazil. A-*Rhinella diptycha*, B-*Boana geographica*, C-*Boana raniceps*, D-*Dendropsophus nanus*, E-*Scinax fuscovarius*, F-*Scinax nasicus*, G-*Adenomera* sp., H-*Leptodactylus brevipes*.

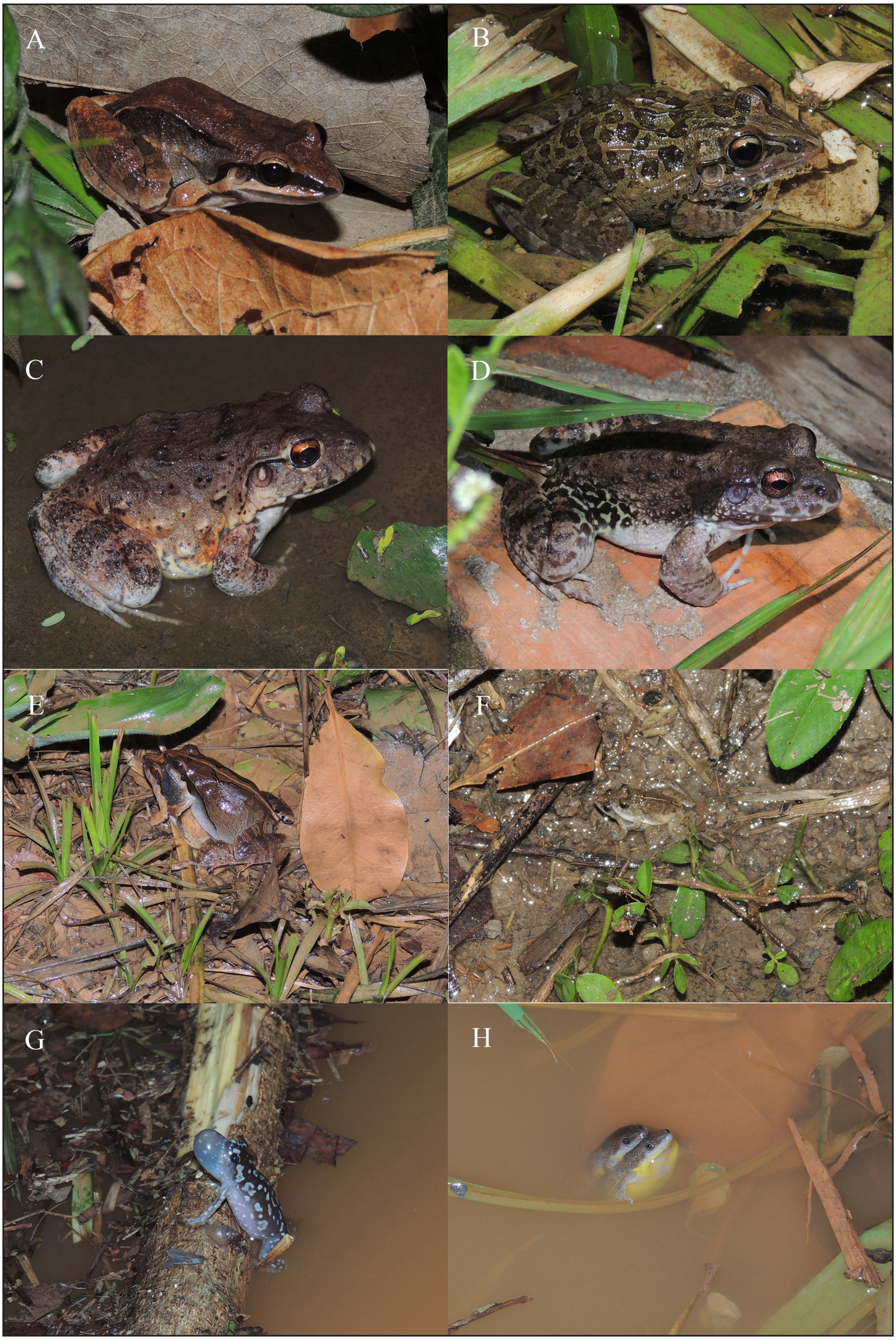

Figure 3. Amphibians recorded at urban ponds from Cuiabá, Mato Grosso, Brazil. A-*Leptodactylus elenae*, B-*Leptodactylus fuscus*, C-*Leptodactylus labyrinthicus*, D-*Leptodactylus syphax*, E-*Physalaemus albonotatus*, F-*Pseudopaludicola motorzinho*, G-*Chiasmocleis albopunctata*, H-*Elachistocleis bicolor*.

Table 1. Amphibian species recorded in temporary ponds in the urban area of Cuiabá, Mato Grosso State, Brazil, from 2015 to 2019. Ab: total abundance—adults (juveniles). (+): distinguishable calls. (*): full chorus. Rm: reproductive mode

Family/ Species	Dry season		Rainy Season		Detection(N)	Rm
	Ab	Calling	Ab	Calling		
Bufonidae						
Rhinella diptycha	13		26(11)	+	31	2
Hylidae						
Boana geographica			1	+	1	2
Boana raniceps			2	+	1	3
Dendropsophus nanus	267(114)	+*	518(83)	+*	84	1
Scinax fuscovarius			3	+	4	2
Scinax nasicus	137(18)	+	157(3)	+*	101	3
Leptodactylidae						
Adenomera sp.	2	+	92	+*	24	62
Leptodactylus brevipes	75	+	180(7)	+*	60	50
Leptodactylus elenae	3(4)	+	162(2)	+*	29	64
Leptodactylus fuscus	105(14)	+	553(19)	+*	145	64
Leptodactylus labyrinthicus	9(4)	+	103(7)	+*	66	50
Leptodactylus syphax	2	+	15	+	13	50
Physalaemus albonotatus	185(12)	+	552(21)	+*	138	45
Pseudopaludicola motorzinho			2	+	1	2
Microhylidae						
Chiasmocleis albopunctata	6	+	108(4)	+*	17	1
Elachistocleis bicolor	23	+	162(3)	+*	60	1

Detection probability

Seven species were deemed unsuitable for unbiased modeling because of low detection: *Adenomera* sp., *Chiasmocleis albopunctata*, *B. geographica*, *B. raniceps*, *L. syphax*, *P. motorzinho*, and *S. fuscovarius*. Models for *Rhinella diptycha* failed to converge and were discarded. Colonization and extinction rates were not related to any investigated variables for the nine species modeled (Table 2). Overall, the best models for detection probability included survey-specific variables related to water availability, although the relationship varied from species to species. The best model for each species received overwhelming support in all cases (i.e. $\Delta AICc > 2 \equiv$ evidence ratio > 2.7). Although none model showed a lack of fit, we found evidence for overdispersion for some leptodactylid species (Table 2).

Pond water depth showed positive effects on detectability for three species: *D. nanus*, *Leptodactylus brevipes*, and *P. albonotatus*. Our data suggested that *D. nanus* and *P. albonotatus* can be detected at levels ≥ 0.70 even at shallow water conditions (depth ≈ 10 cm), whereas *L. brevipes* require deeper habitats (Fig. 4A). Rainfall in the last 24-hour period influenced positively in the detection probability for *E. bicolor* and *S. nasicus* (Fig. 4B). Detectability of three *Leptodactylus* species exhibited association with day-of-year. *Leptodactylus elenae* and *L. labyrinthicus* had lower detection values around day-of-year 70 (~ 10 Mar), while *L. fuscus* had a detection of about 0.50 in the same period (Fig. 4C). The slope for increase in the detection was slightly species dependent, with the two species in the *L. fuscus* group being detected at levels ≥ 0.90 from day-of-year 200 (late July, at middle of the dry season). Even after juvenile exclusion, the best models exhibited similar results for all species analyzed (see Supplementary 2).

Discussion

While we observed variation in detectability among months for some species, our results did not seem directly related to seasonal rainfall distribution. It is interesting to note that detection probabilities recorded here were a reflection of the interplay between species-specific traits and water availabi-

Table 2. Parameters contained in the best models of detection probability for eight species of anurans in Cuiabá, Mato Grosso State, Brazil. Evidence ratio: ratio of Akaike weights between the two best ranked models

Species and model	**Evidence ratio**	**ĉ**	**P**		
			γ	**ε**	**d**
Dendroposophus nanus					
ψ(.), γ(hydroperiod), ε(abundance), d(depth)	>100	0.8	0.23	0.96	<0.001
Scinax nasicus					
ψ(.), γ(hydroperiod), ε(abundance), d(rainfall)	3.7	0.9	0.99	0.94	0.008
Leptodactylus brevipes					
ψ(.), γ(hydroperiod), ε(abundance), d(depth)	>100	0.7	0.34	0.56	<0.001
Leptodactylus elenae					
ψ(.), γ(hydroperiod), ε(abundance), d(day-of-year)	>100	1.8	0.98	0.75	<0.001
Leptodactylus fuscus					
ψ(.), γ(hydroperiod), ε(abundance), d(day-of-year)	30	0.9	0.99	0.95	<0.001
Leptodactylus labyrinthicus					
ψ(.), γ(hydroperiod), ε(abundance), d(day-of-year)	>100	1.4	0.97	0.58	<0.001
Physalaemus albonotatus					
ψ(.), γ(hydroperiod), ε(abundance), d(depth)	>100	0.7	0.99	0.96	<0.001
Elachistocleis bicolor					
ψ(.), γ(hydroperiod), ε(abundance), d(rainfall)	14.1	1.0	0.95	0.3	<0.001

lity. Similar to previous studies in Cerrado areas (Santoro and Brandão, 2014; Vaz-Silva *et al.*, 2020), the anuran assemblages from urban areas of Cuiabá were characterized by the predominance of reproductive modes where eggs are deposited directly in the water without the protection of foam. Still, we also observed strategies involving aquatic (floating on the water or laid in constructed depressions) and terrestrial foam nests. Most species were continuously detected along the four years, and the exceptions were few species that were detected only once (*Boana geographica*, *B. raniceps*, and *Pseudopaludicola motorzinho*) or at few occasions (N=4; *Scinax fuscovarius*).

Here, many species detected at levels suitable for modeling are widely distributed and considered generalist species (Brasileiro *et al.*, 2005; Valdujo *et al.*, 2012). Despite being able to occupy different types of habitats, these species may exhibit distinct behavioral traits that in turn reflect on the visual detection. Previous studies highlighted the importance of specific ecological responses of amphibian species to deal with the low environmental heterogeneity in urban wetlands (Acosta *et al.*, 2005; Babini *et al.*, 2015a). So, results from our study may be explained by: i) species different calling behaviors; ii) microhabitat where to lay their eggs; iii) adults and post-metamorphic juveniles' movement patterns. Although *Elachistocleis bicolor* and *Scinax nasicus* have distinct life habits (fossorial x scansorial), both species are explosive breeders associated to heavy rains (Prado *et al.*, 2005). In this sense, the positive association between detectability and rainfall in the last 24-hour period was not unexpected. Species with prolonged breeding behavior were more closely associated with changes in water depth. *Dendropsophus nanus* and *Physalaemus albonotatus*, which eggs are deposited on the surface of the water, showed a fast increase in the detection rates following pond filling. On the other hand, *Leptodactylus brevipes*, which used constructed depressions at edges of flooded areas, showed a smoother increase. Although *L. brevipes* was generally observed in shallow areas—we observed adults attending tadpole schools in several occasions—the depression construction may be somewhat dependent of pond depth, as the water inside the depression should probably permeate from the adjacent pond.

Intriguingly, some species showed low detectability at the end of the rainy season with increasing towards the dry season. The apparent overdispersion identified in the models for *Leptodactylus elenae* and *L. labyrinthicus* indicates a possible clumping in the observed records. At first glance, increasing detectability could be attributed to juveniles migrating from the pond. However, the patterns observed here

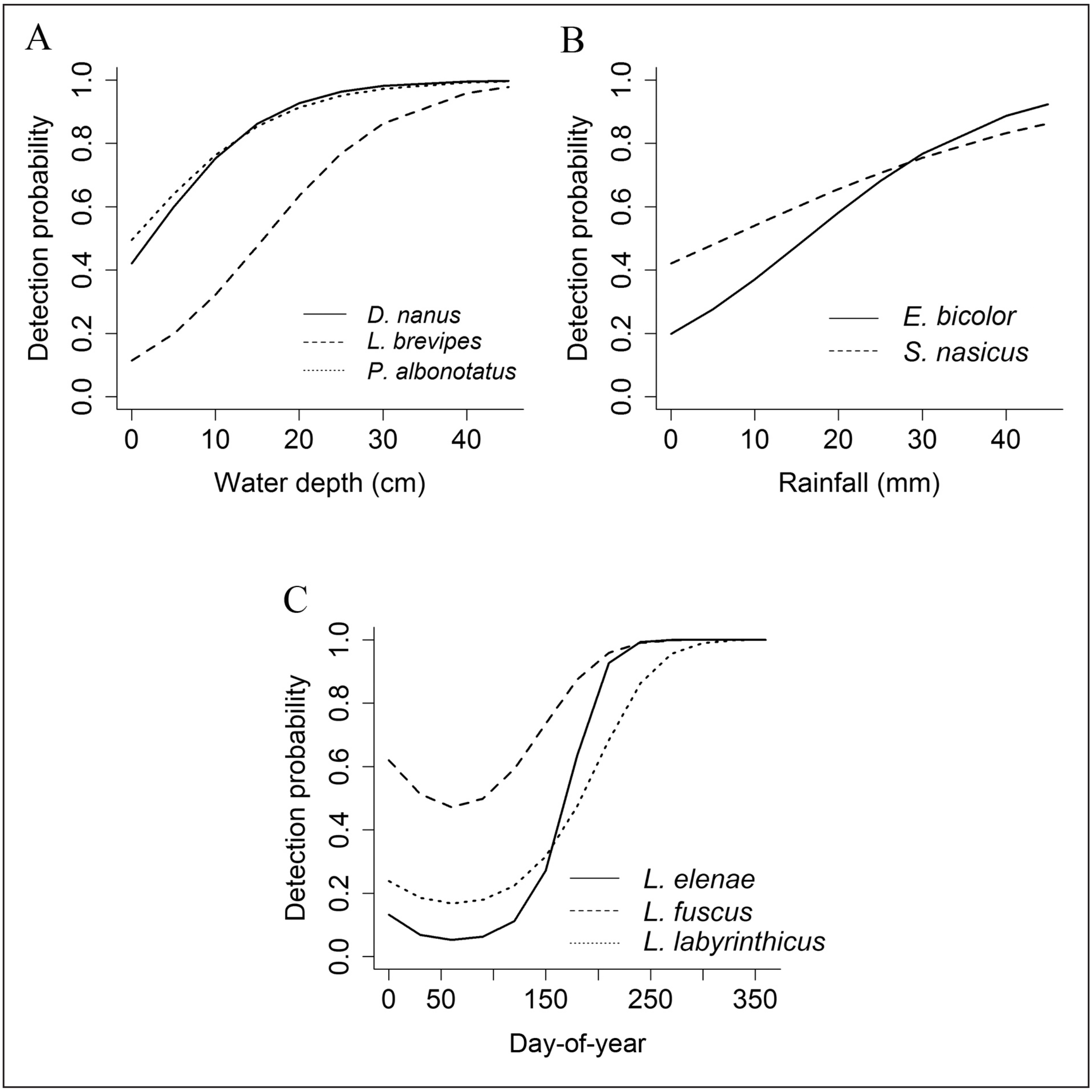

Figure 4. Relationship between detection probability of eight anuran species and (A) pond water depth, (B) rainfall in the last 24-hour period, (C) Day-of-year, at urban ponds from Cuiabá, Mato Grosso, Brazil, between 2015-2019. Only species with significant association are shown in each panel.

remained after the juvenile's exclusion. Movement ecology of amphibians is currently poorly understood, but species' behavioral differences seem to play a major role in key movement decisions, with some generalist species having less motivation to leave the natal pond (Pittman *et al.*, 2014; Bredeweg *et al.*, 2019). Studies have observed that many species recorded here seem engage in dispersal during the transition from the rainy to dry season (Watling *et al.*, 2009; Moreira *et al.*, 2017). On the other hand, the relative high detectability along the year suggests that both juveniles and adults of *L. fuscus* are less motivated to departure from the ponds. As species responses and phenology may exhibit large differences between wet and dry years (e.g. Lowe *et al.*, 2016; Hamer, 2018), our findings reinforce the need for long-term studies encompassing annual variation along both wet and drought periods.

Also noteworthy is the low number of juvenile records, except for *D. nanus*. Studies on recruitment in amphibian assemblages have reported that the juvenile records often exceed the total number of adults (Hocking et al., 2008; López *et al.*, 2011). With that in mind, we presume that the low recruitment indicates that many individuals might not successfully metamorphose in the studied ponds. We can only speculate on impacts on tadpole mortality or metamorphosis rates, such as altered hydroperiod and contaminants (Babini *et al.*, 2015b; Brannely *et al.*, 2019). Because of lack of data, we were not able to determine detectability in juveniles. Still, we cannot rule out detection juvenile' bias that may act simultaneously with other mechanisms.

In line with accumulating evidence of urban amphibian assemblages (Acosta *et al.*, 2005; Babini *et al.*, 2015a; Menin *et al.*, 2019; Dorigo *et al.*, 2021), our results showed that intermittent ponds in urban areas can harbor a considerable proportion of anuran diversity. Despite the small size and accentuated degradation of surrounding environments, we found 30% of all anuran species recorded for Cuiabá municipality. The overall species richness (16 spp.) is lower compared to species richness recorded in Cerrado remnants affected by human activities (~30 spp.), such as crops or hydropower plants (Araujo and Almeida-Santos, 2011; Morais *et al.*, 2012; Silva et *al.*, 2015). In the case of Neotropical amphibians, an emerging trend for surveys in a range of urban areas—from rainforests to non-forest biomes—is similar richness. For instance, short-term studies performed in different Amazonian ecoregions reported between 15–20 species (Knispel and Barros, 2011; Pereira Jr *et al.*, 2013; Menin *et al.*, 2019). Considering only primary data, similar results were also reported for areas in the Cerrado (17–20 spp. Ferreira *et al.*, 2017; Ganci *et al.*, 2022), Dry Chaco (8–12 spp. Acosta *et al.*, 2005; Palavecino and Garcia, 2008; Babini *et al.*, 2015a), Humid Chaco (15 spp. Pesci *et al.* 2018), Pantanal and Uruguayan savanna ecoregions (16 spp. Ávila and Ferreira, 2004; Entiauspe-Neto and Freitas, 2016).

To conclude, detection rates of amphibians were related to water availability in urban wetlands, and they were highly variable between species. This study showed some occurrence/phenology patterns that may not be apparent in typical snapshot survey studies. It is essential that monitoring programs account for imperfect detection considering species individually and include assessments encompassing seasonality (i.e. both conditions ideal for detecting species and drought periods). Widespread amphibian species, as recorded here, are also challenged by widespread threats that likely alter the local environment in distinct ways (Nowakowski *et al.*, 2018). Rapid climate change, reduced resources, and man-made stress call for a multidisciplinary approach to maximize the persistence of amphibian populations also in urban landscapes. Future studies can combine behavioral and morphological traits to anticipate what will happen and help maintain ecosystem services associated with amphibians (e.g. biological control, nutrient cycling). We hope that our results highlight the role of temporary/ephemeral ponds as a refuge for biodiversity. Isolated urban wetlands are not expendable, because their importance for freshwater biodiversity is far from the shallow now.

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SUPPLEMENTARY FILES

Supplementary materials cited in this article are available upon request from Corresponding Author (https://doi.org/10.6084/m9.figshare.21747686.v1)

Supplementary 1. Occurrence and calling activity of each anuran species registered at three urban ponds from Cuiabá, Mato Grosso State, Brazil, between 2015–2019.

Supplementary 2. Parameters contained in the best models of detection probability for eight species of anurans in Cuiabá, Mato Grosso State, Brazil. Only adult data were included in the analyses.

Trabajo

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Relationships between body growth indices and environmental factors on the reproductive cycle of the *Gymnodactylus geckoides* Spix, 1825 (Squamata, Gymnophthalmidae) in Northeast Brazil

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ABSTRACT

In this study, we analyzed the energy and reproductive cycles of female and male *Gymnodactylus geckoides* in the Caatinga area of northeast Brazil. We investigated whether these proxies of body condition, such as reproductive cells maturation and cellular structures changed in response to variation in abiotic and biotic factors (i.e., humidity, temperature, seasonality, body temperature, growing rate and gonad volume), using individuals stored under scientific conditions collected between September 2018 and December 2021. The condition factor showed an isometric growth pattern in the population studied. Meanwhile, the lipid, hepatic, and gonad factors correlated with body growth and showed monthly and seasonal variations, as well as reproductive cell maturation and cellular structure morphology. The cycles displayed constant replacement of energy reserves and mature reproductive cells, indicating constant and acyclic reproduction in *G. geckoides*. Energy reserves appear to be used for many reproductive activities, including meeting, gestation, and egg laying that occur at different frequencies during different periods in the dry and rainy seasons. Therefore, the reproductive cycle is likely to be strongly controlled by biotic factors, which are modeled using abiotic factors and environmental conditions (environmental patterns which proportionate greater resource availability). Our study is the first to investigate energy cycles and reproductive strategies in *G. geckoides*. It has shown that this

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species stores greater amounts of energy during the rainy season and then depletes these reserves during the dry period, since the rainy season correspond to the increase in energy consumption, mainly because of gestation and egg laying.

Key words: Brood; Cell Development; Endogenous Factors; Energy Cycle; Exogenous Factors; Gecko; Reproductive Tasks.

Introduction

Terrestrial animals, especially ectotherms such as Squamata, use biochemical mechanisms to build (Pinilla, 1995; Schaffner, 1998) and accumulate energy reserves within a relatively short time span (Derickson, 1976; Norval *et al.,* 2019). In lizards, the accumulation and use of energy reserves, that is,the energy cycle, vary temporally and ontogenetically. They are mostly directed toward vital activities, including body growth, survival, and reproductive activity (Derickson, 1976; Cheng, 1987; Galdino *et al.,* 2003; Ramirez-Bautista *et al.,* 2006; Norval *et al.,* 2019; Paz *et al.,* 2019). Although lizards have efficient feeding strategies that facilitate energy storage,the energy cycle is ultimately influenced by abiotic factors including temperature, precipitation, and photoperiod (Benabib, 1994; Ramirez-Bautista and Vitt, 1997; Norval *et al.,* 2019).

Environmental variations, mainly seasonal and annual, often correlate with variations in the natural traits of a population (Sexton and Brown 1977; Benabib, 1994). Food availability and abiotic factors, such as temperature, precipitation, and humidity, can affect growth rates and reproductive strategies. This includes the time and duration of the reproductive event, brood size and frequency, and the size at which individuals reach sexual maturity (Ramirez-Bautista and Vitt, 1997; Oitaven *et al.,* 2022). Lizard populations and individuals can also show variation in different proxies for energy reserves, including the growth investment comparing morphology and weight, condition factor (K1) (Le Cren, 1951; Galdino *et al.,* 2003; Oitaven *et al.,* 2022), as well as many index, which represents association of individuals' weight with some energetical reserves stored in the body, like: the lipid index (RFB) (Derickson, 1976; Sexton and Brown, 1977; Paz *et al.,* 2019), the hepatic index (RWL) (Schaffner, 1998; Ramírez-Bautista *et al.,* 2006; Norval *et al.,* 2019), and the gonadosomatic index (RGS) (Pinilla, 1995; Ramirez-Bautista and Vitt, 1997; Sánchez-Hernández *et al.,* 2013).

Previous studies on Neotropicallizards have shown that energy cycles are primarily regulated by food availability, patterns of reproductive investment, and variations in abiotic factors (Derickson, 1976; Cheng, 1987; Benabib, 1994; Galdino *et al.,* 2003; Ramirez–Bautista *et al.,* 2003; Ramírez-Bautista *et al.,* 2009; Norval *et al.,* 2019; Paz *et al.,* 2019; Guedes *et al.,* 2020, Oitaven *et al.,* 2022). In sexually mature individuals, energy reserves tend to diminish as reproductive activities demand high investments in spermatogenesis, oogenesis, gestation, and gonadal development (Ramírez-Bautista and Vitt, 1997; Ramirez–Bautista *et al.,* 2006; Paz *et al.,* 2019).

The reproductive cycle of lizards is associated with several factors, such as photoperiod, precipitation, temperature, growth rate, and resource availability (Ferreira *et al.,* 2002; Oitaven *et al.,* 2022). Therefore, delineation of the reproductive cycle could be associated with many factors that influence several processes in this cycle, including gametogenesis, follicular growth, mating, sperm storage, ovulation, gestation, and egg hatching (Newlin, 1976; Moodley y Van–Wyk, 2007).

Mathies (2011) analyzed the reproductive cycle of squamata groups in females and males, at the individual and population levels. At the individual level, the cycle can be classified as discontinuous with total gonad regression and interruption of the gamete production process, continuous with partial gonad regression and regression of the gamete production process, or acyclic without gonad regression and continuous gamete production process. At the population level, continuous and non-continuous reproductive cycles could display a synchronic and/or semi-synchronic seasonal pattern. This can occur with reproductive events concentrated during a season or a time range, being synchronized or not between females and males, or in a non-seasonal pattern with the reproductive events distributed throughout the year, without synchrony between females and males (Sherbroke, 1975; Mathies, 2011).

Giventhe high diversity of lizards in the tropical region, information on indices of energy cycles, as well as reproductive cycles, is scarce for most species, especially in dry tropical forests. This is the case for *Gymnodactylusgeckoides*Spix, 1825, an endemic lizard that is widely distributed in the Caatinga domain (Alcântara *et al.,* 2019; Oitaven *et al*., 2022b), with LC status, regarding IUCN list (IUCN, 2023), as well as local population from Pernambuco state (SEMAS, 2017; Costa and Bérnils, 2018). Given that the Caatinga domain has a marked seasonal climate, we hypothesized that body indices representing different energy reserves, as well as the reproductive cycle and reproductive cell maturation, vary seasonally with precipitation, humidity, and air temperature.We further hypothesized that they are associated with several vital activities and bodily structures in *G. geckoides*. In this study, we aimed to describethe morphological cellular structures and the energetic and reproductive cycles of *G. geckoides*. We then related the patterns and cycles offemales and males to biotic and abiotic factors.

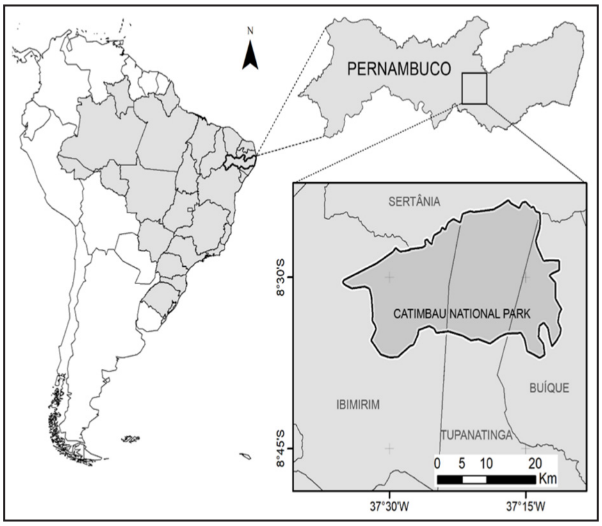

Figure 1. Map of Brazil depicting the state of Pernambuco and the location of the Catimbau National Park (study area), a conservation unit in the Caatinga biome whose area extends over four municipalities.

Materials and methods

Study area

The study was conducted in the Catimbau National Park (692781.38E; 9050296.71S), which is a remnant of the Caatinga. This park is located in the Ipanema Valley in the central region of Pernambuco, Brazil. It encompasses an area of approximately 62,300 ha in the municipalities of Buíque, Sertânia, Ibimirim, and Tupanatinga (Fig. 1) (Ferreira, 2010; Oitaven *et al*., 2022b). The local climate receives rainfall ranging from 0 to 256 mm. We defined the dry and rainy seasons as constant processes. Dry season (September to February) had reduced rainfall levels (4–94 mm) and a slightly air temperature variation (24–33 °C) compared to rainy season. The rainy season (March to August), in turn, had higher precipitation levels (38 – 188 mm) withlow air temperaturesvariations (26–30 °C). However, air temperatures seem not display higher variance between both seasons(see Supplementary Material 1). We obtained data on the monthlyaverageair temperature (°C) and rainfall (mm) levels from the Pernambuco Water and Climate Agency database (APAC) based on meteorological stations near (less than 70 Km) the study site (APAC, 2019).

Data collection

We captured lizards monthly between September 2018 and August 2019 using the active search method.This involved searching for individuals on different substrates and collecting them manually. Surveys were carried out during hourly intervals over three days, being conducted during the day (from 9:00 h to 14:00 h) and at night (from 16:00 h to 20:00 h), with collections and observations made by a pair of observers, totalizing 360h/men of sampling effort. Since *G. geckoides* did not display external morphological differences (Oitaven *et al*., 2022b), we were just able to classify individuals as sexually analyzing the sexual gonads. Females were considered sexually mature when having either vitellogenic follicles or eggs in the oviduct. We considered vitellogenic follicles to be those follicles showing a yellowish color and diameter > 0.3 mm. Follicles with diameter < 2.1 mm were arbitrarily regarded as small, whilst those with diameter > 2.1 mm were regarded as large. Males were sexually mature when having enlarged testes, contorted epididymis, and sperm in the testes and/or epididymis (Colli *et al.,* 2003). To estimate gonad volume, we obtained the length and width of the ovaries, testes and eggs using a digital caliper (± 0.01 mm).

During the study period, 77 sexually mature individuals were captured. Given that the region of Picuí, Paraíba, has climatic conditions similar to those of our study area, with limited amounts of rainfall and constant high temperatures, we added 31 individuals captured in 2021 from the Picuí region to our sample size. We also included 54 specimens collected in 2014 in our sample from Catimbau National Park. This increased the sample size to

162 individuals, comprising 66 females and 96 males. The mean number of individuals collected per month from 2018 to 2021 was eight (range = 4 – 15) (see Supplementary Material 2). We euthanized the individuals collected using intramuscularanesthesia liquid ketamine, a barbituric composed by Ketamine Hydrochloride (10 mg/Kg) and Methedomidine (0.1-0.3mg/Kg).

To evaluate the influence of abiotic factors at a current situation, we measured, the air temperature and humidity at the time of collection of individuals with a digital thermos-hygrometer with accuracy to the nearest ± 0.1°C (Colli *et al.,* 2003). We weighed each individual collected using a digital scale with accuracy to the nearest ± 0.001 g and measured the snout–vent length (SVL) using a digital caliper with accuracy to the nearest ± 0.01 mm. Given that geckos accumulate lipid reserves in the caudal region (Vitt *et al.,* 2007), the caudal region of each specimen was moved through a cross-section and the lipid reserves were weighed separately, includingthe tail mass bonded to the abdominal region of some individuals, with the aim to calculate the lipid index (RFB). We made the same procedure, through a ventromedial incision to remove the liver and gonads and calculate the hepatic (RWL) and gonadosomatic index (RGS), respectively. We weighed all structures using the same digital scale (± 0.001g).

We dissected the individuals to examine their lipid, hepatic, and gonad reserves and compared to total weight (WT) of the individual. The energy reserves were estimated using the following formulas: to RFB (RFB = WFB (100) /WT), where "WFB" is the lipidic bodies weight, to RWL (RWL = WL (100) /WT), where "WL" is the liver weight, and to RGS (RGS = WG (100) /WT), where "WG" is the gonad weight (Ramirez-Bautista andVitt, 1997; Schaffner, 1998; Wootton, 1999).

Reproduction

To analyze the influence of biotic and abiotic factors, regarding females and males, as well as seasonality, we sorted the individuals according to collection period and sex. For both female and male,the gonad volume was estimated using the ellipsoid formula ($V = 4/3\pi a^2 b$), where "V" is the gonad volume, "a" is half the smallest gonad diameter, and "b" is half the longest gonad diameter (Selby, 1965). The volume of eggs was measured using the formula for an ovoid sphere ($V = \pi w^2 l/6$), where "V" is the egg volume, "w" is the egg width, and "l" is the egg length (Ramirez-Bautista y Vitt, 1997). We calculated the condition factor (K1) using the Allometric Method, following the expression K1 = w/Lb, where "W" is the total mass and "L" is the specimen standard length. To estimate the "b" coefficient, we performed a simple mass–length ratio equation (W = aLb) (Le Cren, 1951; Galdino *et al.,* 2003).

Histological procedures

The gonads were fixed in Bouin's solution (71% picric acid, 24% formaldehyde, and 5% glacial acetic acid) for 24 h and dehydrated in aseries ofincreasing alcohol solutions for 30 min at each of six stages: 70%, 80%, 90%, 95%, 100%, and 100% (Hopwood, 1990). Subsequently, we embedded the samples in paraffin and sectioning the gonads to obtain 5 µm thick histological sections. These were colored in Hematoxylin and Eosin and prepared on slides for analysis (Robinson y Gray, 1990; Aguilar-Morales *et al.,* 1996).We analyzed both cuts, as well as quantitative sliders, using a Nikon E800 microscope, taking digital pictures with a Nikon DS-Ri camera, with all cellular measurements, as well as the measure of dispersion, using the NIS Elements System ver. 4.60 (Laboratory Imaging, 2016) program. We performed these procedures on 77 sexually mature individuals, of which 39 were females and 38 males.

Regarding females, we identified reproductive cell types and cellular structures according to Gribbins (2011), Lozano *et al.* (2014), and Santos *et al.* (2020). We analyzed the reproductive cells and cellular structures of the internal and peripheral layers of the ovary, including granulosa, oocyte, and germinal bed diameters. The male spermatogenic cycle was based on theMandarim-de-Lacerda (1995) and Weibel (1979) precepts. We calculated the volume density (Vv) of primary and secondary spermatocytes and sperms as reproductive cell types, representing the individual's reproductive maturation state (Torki, 2007). We also measured the following cellular structures: seminiferous tube diameter (STD), seminiferous epithelium height (SEH), diameter of ductus deferens (DEF), diameter of epididymal ductus (DEP) and epididymal ductus height (DEH) (N = 8 by structure, 40 individuals) (Hernández-Franyutti y Uribe, 2012; Lozano *et al.,* 2015). We calculated the sample using Hally's formula (1964) and corrected the data according to Mandarim-de-Lacerda (1995) to provide an accurate stereological precept.

This study has shown histological changes in both sexes, taking into account the catch period, that is, the onset of the dry season (ODS), the end of the dry season (EDS), the onset of the rainy season (ORS), and the end of the rainy season (ERS). Finally, to determine the reproductive period of the species, we quantified the profile density (Qa) by counting several testing grounds in the testing area (TA) for each individual. We obtained the results using the mean of their respective profiles with the following formula:$Qa= \sum profiles/TA\ (mm^2)$ (Mandarim-de-Lacerda, 1995), analyzing reproductive cellular types of seminiferous tubes, using 40× magnification microscopic lenses (Santos y Oliveira, 2007). We then analyzedthe cellular structures and randomly counted the cells between individuals (Lozano *et al.,* 2015).

Data analysis

Prior to statistical analysis, we checked the data for normal distribution using the Shapiro–Wilk test, with the data of males and females combined. Given that the data were not normally distributed, we used the Kruskal–Wallis test to test for monthly variation in the indices used as proxies for energy reserves. This was then followed by post-hoc pairwise comparisons (Tukey) to check which samples differed significantly (Ramirez-Bautista and Vitt, 1997; Norval *et al.,* 2019). To control for the effects on the response variables of variation in body condition between individuals, we performed linear regressions (r^2) between SVL and body mass, and each body variable, including lipid, hepatic, and gonadal masses. We used linear regression not just to verify the investment of growth in *G. geckoides*, as well as the growth of energetical reserves and gonads (Ramírez-Bautista y Vitt, 1997; Paz *et al.,* 2019).

To analyze monthly variation, we only used data from months in which three or more individuals of each sex were captured (Ramirez-Bautista *et al.,* 2006; Ramirez-Bautista *et al.,* 2009). To test for seasonal variations in body indices and gonad volume, we performed an analysis of variance (ANOVA) with dry and rainy periods as predictors (Zar, 1999; Ramirez-Bautista *et al.,* 2006).

We tested the influence of abiotic factors and body indices on the presence of individuals in each period using generalized linear models (GLM) in the *DHARMa*, *readxl*, and *mice* packages (Zuur *et al.,* 2009; Villamar-Duque *et al.,* 2019). We modeled generalized linear "mixed" models (GLMM) to control for the influence of sampling periods and between-individual variation. Since the reproduction process, as well as the arrival of lizards are associated with abiotic factors (Paz *et al.,* 2019),we used body indices and gonad volume as predictor variables in a model fitted to verify the absence or presence of individuals through the seasons. Air temperature, humidity, and precipitation were used as predictor variables in models fitted to test the influence of abiotic factors on body indices (Zuur *et al.,* 2009).

We compared differences/variation in cellular density values and cellular structure morphology using the Kruskal–Wallis test and, if necessary, the Dunn test, for females and males. In females, wecompared the relationship between environmental factors including temperature, humidity, and precipitation and body factors including body temperature, SVL, gonad volume, and K1 using generalizedlinearmodels (GLM), with environmental and body factors as predictors. For the GLM analysis, we used the *DHARMa*, *readxl*, and *mice* packages. In males, we first used the ANOVA test to analyze variations in the reproductive cellular types and structures according to annual, as well as seasonal cycles. We then used GLM analysis to compare cellular structure measurements and reproductive cells at different stages, that is, primary and secondary spermatocytes and sperm, with abiotic and biotic factors (Zar, 1999). We considered the effect to be statistically significant when $p < 0.05$.All the analyses were performed using the R program (R Development Core Team 2019) (Zar, 1999; Zuur *et al.,* 2009).

Results

Growth rate and energy cycles

The angular regression coefficient (b) of the mass–length ratio equation was 2.85. This value, which is close to the Condition Factor (K1), indicates a similar investment in body mass and length, that is, isometric growth in the population of *Gymnodactylusgeckoides* sampled (Fig. 2). The mean SVL of females was 41.40 ± 4.43 mm (range:28.55 – 47.25 mm, n = 66).Meanwhile, the mean SVL of males was 40.22 ± 3.63 mm (range: 29.50 – 45.74 mm, n = 96). The minimum female and male SVL at sexual maturity were 32.84 mm and 32.11 mm, respectively. The mean gonad volume was 4.67 ± 4.52 mm^3 for females (range: 0.3 – 22.42 mm^3, n = 64), and 7.82 ± 5.96 mm^3 for males (range: 1.49 – 32.78 mm^3, n = 95). Large follicles were found in females

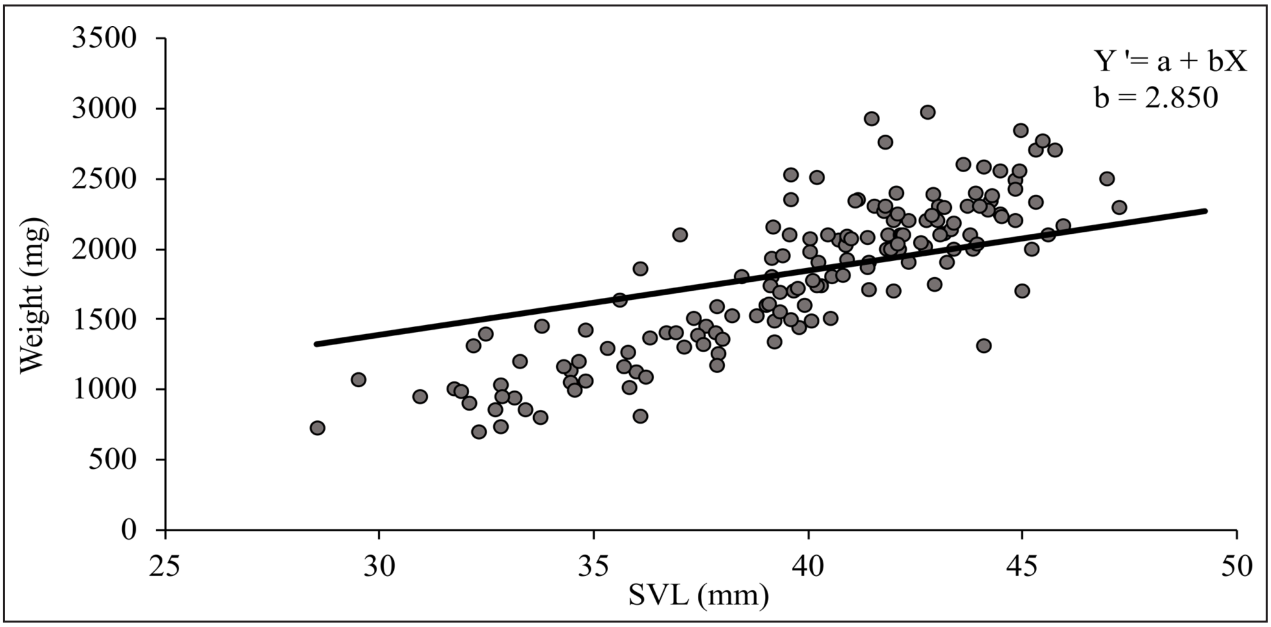

Figure 2. Relationship between the snout-vent length (SVL) and the body weight of individuals of *G. geckoides*Spix, 1825 collected in Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, between September 2018 and September 2021.

throughout the year, particularly during dry periods (September–February). Eggs were found during both seasons, but predominantly in rainy periods (March–August) (Fig. 3A). They were always in a fixed brood of one egg per female, with a mean volume of 295.40 ± 120.12 mm^3 (range: 112.33 – 434.19 mm^3, n = 6). Males presented similar gonad cycles, with larger testes during the dry period (September–December), reduced testes at the end of the dry season (January–February), and growing testes during the rainy season, except in May and June, without recrudescence (Fig. 3B).

Body length had a positive, significant effect on gonad volumein females (β±SE = 0.074 ± 0.027, z = 2.723, $p < 0.01$) and males (β±SE = 0.091 ± 0.020, z = 4.488, $p < 0.001$). This indicated that gonad growth is proportional to the SVL of the individuals. In females, SVL was positively and significantly correlated with lipid and hepatic reserves, but not with gonadal conditions. Similarly, the body mass of females was positively and significantly correlated with lipid and hepatic reserves but not with gonadal conditions. In males, SVL was positively and significantly correlated with lipid, hepatic, and gonadal reserves. Body mass of males also showed a positive and significant correlation with lipid, hepatic, and gonadal reserves (See supplementary material 3).

Females showed a reduction in lipid reserves (RFB) prior to the beginning of the rainy season (Fig. 4A). However, they did not show significant seasonal variation in RFB (Kruskal–Wallis = 2.32; p = 0.50). In contrast, the males showed an increase in RFB prior to the beginning of the rainy season and showed a significant variation in RFB between the ending of rainy season and onset of dry season (Kruskal–Wallis = 22.33; p = 0.02). Hepatic reserves (RWL) decreased slightly at the end of the dry season and the beginning of the rainy season, followed by an increase at the end of this period, especially in females (Fig. 4B). In females, the main variation in RWLoccurred at the end of the period of abundant rainfall, especially in June (Kruskal–Wallis = 17.01; p = 0.02) and August (Kruskal–Wallis = 17.01; $p < 0.01$). Males, in turn, showed no significant variation in RWL (Kruskal–Wallis = 4.86; p = 0.85). Regarding gonadal reserves (RGS), both sexes experienced a decrease at the end of the dry season and beginning of the rainy season. This was then followed by an increase at the end of the dry season (Fig. 4C). Females showed a significant decrease in RGS by November for both years (Kruskal–Wallis = 20.63; $p < 0.01$), and males showed a significant decrease in RGS in the driest months (October and February) (Kruskal–Wallis = 17.22; p = 0.03).

ANOVAs showed higher concentrations of energy reserves in females and males in periods coinciding with the end of abundant rainfall and the beginning of the dry season. The volume of sexual gonads in females did not show significant seasonal variation. In contrast, the males had enlarged, more robust gonads during periods with high precipitation levels (Table 1). The generalized linear models showed no influence from body indices or gonad volume on the seasonal appearance of individuals of either sex (Supplementary Material 4). Regarding abiotic factors, the models showed significant negative and positive effects of temperature and precipitation on the RFB of females, with negative and positive effects of these variables, respectively. However, humidity showed a significant negative effect on RGS, which was associated with a reduction in gonadal reserves (Table 2).

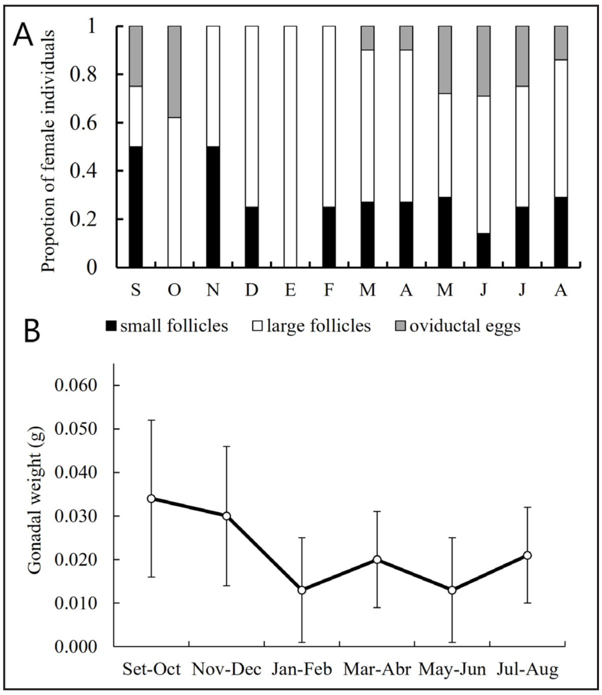

Figure 3. Annual variation in gonads of *G. geckoides* from Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, between 2018 and 2021. A) Monthly proportions of females presenting small follicles (diameter < 2.10 mm), large follicles (diameter > 2.10 mm), and oviductal eggs. B) Monthly variation in the weight of testes (in g); the white circles are the adjusted means, and the vertical bars denote the associated standard error.

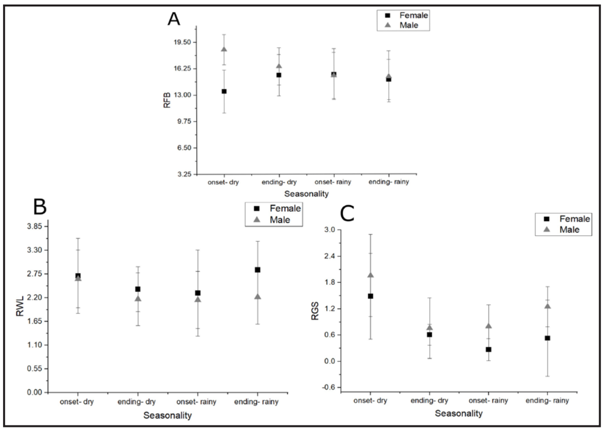

Figure 4. Seasonal variation in different types of energetic reserves in female and male *G. geckoides*Spix, 1825 from Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, from September 2018 to September 2021.A) Liposomal ratio (RFB); B) Hepatosomatic ratio (RWL); C) Gonadosomatic ratio (RGS). Triangles and squares are the adjusted means, and the vertical bars denote the associated standard error.

Cellular variation: females

In *G. geckoides*, the ovary comprises oocytes, granulosa cells, thecalcells, and germinaltissue. Ovaries in the previtellogenic and vitellogenic stages were simultaneously recorded during all sampling months (Figs. 5 and 6). During pre-vitellogenic stages, III and IV, oocytes displayed average diameters of 290 ± 45.6 µm and835 ± 75.0 µm respectively. In those stages, the thickness of the yolk membrane and granulosa layer reached average diameters of 4.75 ± 0.7 µm and50.45 ± 10.7 µm respectively. Regarding the granulosa layer cells, small cells were more abundantat the periphery of follicles (Figs. 5A–B). The germinal bed was substantially thicker, mainly during the earlier stages, with germinal cells having an ovoid or spherical shape with hyaline cytoplasm. During vitellogenesis, the germinal bed tended to become homogeneous and reduced (Figs. 5A–B).

Follicular growth continues to advance during the vitellogenic stage. At earlier stages of vitellogenesis, V and VI, *G. geckoides* displayed ovaries with a mean oocyte diameter of 1100 ± 318 µm. The granulosa layer, as well as the germinal bed, displayed reductions in thickness, reaching diameter averages of 21.1 ± 3.5 µm and 40.8 ± 6.6 µm, respectively. The granulosa was modified into a single layer containing only small cells with small cores (Fig. 6B). Meanwhile, the thecal region was composed of fibroblasts, collagen fibers, and blood vessels (Figs 6A–B). At these stages, changes in the zonapellucida were recorded, which displayed two layers, that is, a homogeneous layer known as the hyaline band and an internal striated layer known as the radiata zone. A mean oocyte diameter of 3.98 ± 0.94 mm was recorded at these stages, with lipid storage maximized and occupying almost the entire ooplasma space (Fig. 6A). The zona pellucida zone was highly reduced, as well as the granulose, consisting of a single layer composed of only spherical

Table 1. Summary of results of the Analysis of Variance (ANOVA) performed to test for seasonal differences in body indices and gonad volume, according to precipitation levels, in female and male *G. geckoides* Spix, 1825 from Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, from September 2018 to September 2021. Body indices: liposomal ratio (RFB), hepatosomatic ratio (RWL), gonadosomatic ratio (RGS). An asterisk (*) indicates a statistically significant difference.

	Seasonal variation (Precipitation level)				
	Df	Sum Sq	Mean Sq	F-value	P-value
Females					
RFB	36	170.7	56.90	2.79	0.05*
RWL	36	5.98	1.99	2.97	< 0.01*
RGS	36	12.94	4.31	3.21	0.03*
Gonadal volume	36	8.30	2.75	0.10	0.95
Males					
RFB	91	30.3	10.08	1.43	0.23
RWL	91	1.74	0.58	1.35	0.26
RGS	91	3.88	1.29	4.58	< 0.001*
Gonadal volume	91	161.62	4.44	2.58	< 0.001*

cells, displaying a mean diameter of 4.68 ± 0.56 µm. Meanwhile, the thecal layer showed an increase in vascularization (Fig. 6B).

The abiotic factors seemed to be strongly influenced by the germinal bed. This structure appeared to display a greater size during periods of higher humidity and precipitation, indicating increased cellular activity, mainly during periods of stronger rainfall. Despite the slight range of variance between seasons, germinal bed also seems to be positively influenced while temperature increase (Table 3). Biotic factors, in turn, have had a strong influence on oocyte diameter and germinal bed. Significant positive effects were recorded regarding the increase in oocyte and germinal bed diameters with an increase in body temperature. The germinal bed also had a significant positive influence on condition factors (K1) such as gonad volume (Table 3). The granulosa layer was not significantly influenced by any biotic or abiotic factors.*G. geckoides* displayed constant ovary maturation, with reproductive activities increasing or decreasing according to the season (Supplementary Material 5).

Cellular variation: males

The testes of *G. geckoides* consisted of paired, symmetric, and ovoid organs located in the celomatic cavity, whereas the hemipenis had a horn shape, also in pairs (Fig. 7A–B). Seminiferous tubes remained spermatogenetically active throughout the year without quiescence. Reproductive cells during the primary stage (Spt1) were located at the periphery of the seminiferous tubes, whereas cells in the secondary stage (Spt2) were located closer to the lumen (Fig. 8B). At the end of the maturation stage, most sperms (SPZ) occupied the lumen region in the seminiferous tubes (Fig. 8B). Spermatids (SEP) were located comprising SPZ at the lumen region (Fig. 8C). Deferens ducts lacked sexually mature reproductive cells in the lumen (Figs. 8D–E).The epididymal ducts, in turn, always contained mature reproductive cells in the lumen (Figs. 8D–F).

Regarding seasonality, significant increases in STD and SEH were recorded during the rainy season, whereas at the end of the rainy season, there was a significant reduction in DEF and an increase in DEP and DEH (Table 4). Mature reproductive cells (SPZ) also showed a significant increase at the end of the rainy season an dat the onset of the dry season, with the opposite trend observed in the earlier stages of reproductive cell maturation (Spt1 and Spt2) (Table 4). Despite constant reproductive cell maturation, the ANOVA test showed significant variations in cellular structure measurements and the number of reproductive cells in different stages. This indicated variations in reproductive activity in *G. geckoides* males for seasonal and annual cycles (Table 4). GLM analysis revealed a significant increase in diameter of DEP associated to the increase of mature cells (SPZ) at the seminiferous tubes ($\beta \pm SE = 0.065 \pm 0.009$, $z = 6.638$, $p < 0.001$).

According to our correlation analysis, biotic and abiotic factors seem to be strongly related to reproductive cell maturation and cellular structure measurements including diameter and height (Table 5). Regarding biotic factors, gonad volume and body temperature revealed a rather range of significant correlation. Considering significant correlation, a positive relationship was recorded between gonad volume and STD, as well as between gonadal volume and cell types analyzed (Spt1, Spt2, and SPZ), indicating a range of structures, as well as many mature reproductive cells, as the gonad volume increases. Body temperature, in turn, showed apositive relationship to Spt1 and Spt2, whereas it was recorded negative relationship with several cellular structures (SEH, DEF, DEP and DEH), as well as SPZ. K1, in turn, just revealed a significant negative relationship

Table 2. Summary of results of the Generalized Linear Model (GLM) fitted to test the influence of abiotic environmental factors on different body indices and gonad volume of female and male *G. geckoides* Spix, 1825 from Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, from September 2018 to September 2021. Body indices: liposomal ratio (RFB), hepatosomatic ratio (RWL), gonadosomatic ratio (RGS).

	Females (n = 61)				**Males (n = 97)**			
	Estimate	**Std. Error**	**z-value**	**p-value**	**Estimate**	**Std. Error**	**z-value**	**p-value**
RFB								
(Intercept)	4.333	0.926	4.679	<0.001	18.926	6.887	2.748	<0.001
Temperature	-0.067	0.034	-1.983	0.047	-0.148	0.214	-0.694	0.489
Humidity	-0.016	0.017	-0.949	0.342	-0.014	0.030	-0.460	0.646
Precipitation	0.001	0.019	2.774	<0.001	0.019	0.056	0.339	0.735
RWL								
(Intercept)	3.271	1.593	2.053	0.040	4.341	1.146	3.789	<0.001
Temperature	-0.013	0.049	-0.273	0.785	-0.043	0.029	-1.460	0.147
Humidity	-0.007	0.007	-1.035	0.305	-0.014	0.008	-1.760	0.081
Precipitation	0.004	0.013	0.035	0.972	-0.003	0.005	-0.644	0.520
RGS								
(Intercept)	1.297	0.629	2.059	0.044	1.861	0.784	2.372	<0.01
Temperature	-0.001	0.017	-0.085	0.932	-0.015	0.002	-0.751	0.454
Humidity	-0.009	0.004	-2.044	0.045	-0.006	0.005	-1.061	0.291
Precipitation	-0.004	0.003	-1.367	0.177	-0.004	0.003	-1.059	0.292
Gonadal Volume								
(Intercept)	17.442	9.733	1.792	0.050	-4.888	1.744	-2.802	<0.01
Temperature	-0.328	0.301	-1.089	0.280	0.103	0.050	2.032	0.042
Humidity	-0.067	0.047	-1.410	0.164	0.047	0.025	1.917	0.050
Precipitation	-0.117	0.079	-1.477	0.145	0.003	0.001	3.165	<0.001

with DEH, with a significant and positive correlation, mainly regarding the final stage of sexually mature cells (SPZ), indicates constant maturation (Table 5).

Regarding abiotic factors, temperature, and humidity significantly influenced cellular structure and reproductive cell maturation, respectively. Our results displayed a reduction in reproductive cell maturation rates during periods of higher temperature and humidity, despite the constant reproductive cell maturation process, which indicates an optimization of reproductive activity during certain periods at the study site (Table 5).

Discussion

Our results have shown that individuals of *Gymnodactylus geckoides* maintain relatively constant values of indices, displaying variations throughout the year. Females and males have shown similar patterns of investment in energy reserves after attaining sexual maturity. Both cycles could be attributed to the different reproductive roles of each sex, mainly regarding reproductive process like mating, gestation and laying eggs, and to different sexual responses to abiotic factors such as temperature, humidity, and precipitation (e.g. Sexton and Brown, 1997;Norval*et al.*,2019).

There was no significant variation in the total mass of the population sampled of *G. geckoides* throughout the year. This indicated that individuals constantly gained mass, a pattern confirmed by the condition factor (K1). Isometric growth was characterized by similar energy investment in both the gonads and body size (Cox *et al.,* 2009).Given that gonad growth is accompanied by an increase in SVL, investing in body growth is the key for an individual to reach sexual maturity (Sexton and Brown, 1977; Ramirez-Bautista *et al.,* 2006; Oitaven *et al.*,2022). The lack of lipid reserves in body parts other than the tail indicates that the tail region stores the most important lipid reserves in this species (Paz *et al.,*

Figure 5. Ovarian follicles on maturation process at previtellogenic (A) and vitellogenic phase(B) in *G.geckoides*: (A) Polymorphic and multilayered epithelium, with granulosalayer (G), with double layers,containing pyriform (PyC), intermediate (Ic) and small cells (Sc)containing spherical nucleus and a nucleolus; (B) theepithelium is composed by a single layer of small cuboid cells (SC), with vacuoles zones (asterisk)during the previtellogenic phase progress. GB: germinalbed, YM: vitellinemembrane,Oop: ooplasm. H-E-stained Scale bar: 50 µm.

2019). Given that all types of reserves analyzed showed a direct relationship with SVL and weight, it can be inferred that the availability of food resources in Catimbau National Park is sufficient to allow *G. geckoides*toaccumulate the energy reserves necessary to sustain vital activities.

Abiotic factors and seasonality are significantly related to reproductive cell maturation, mainly in the earlier stages. The body temperature in *Gymnodactylus* genus is strongly associated with environmental temperatures (Vitt, 1986; Colli*et al.*, 2003). Therefore, since there was a positive and significant relationship between Spt1 and body temperature, the reproductive cell maturation process tended to be optimized at higher temperatures within certain limits. This corresponds to pattern temperature in the Caatinga domain. The epididymal and diferential ducts also seem to increase their composts production, which is responsible for storage and sperm maturation, probably leaded by optimized cell maturation. This situation facilitates the meeting process during the dry season, whereas gestation

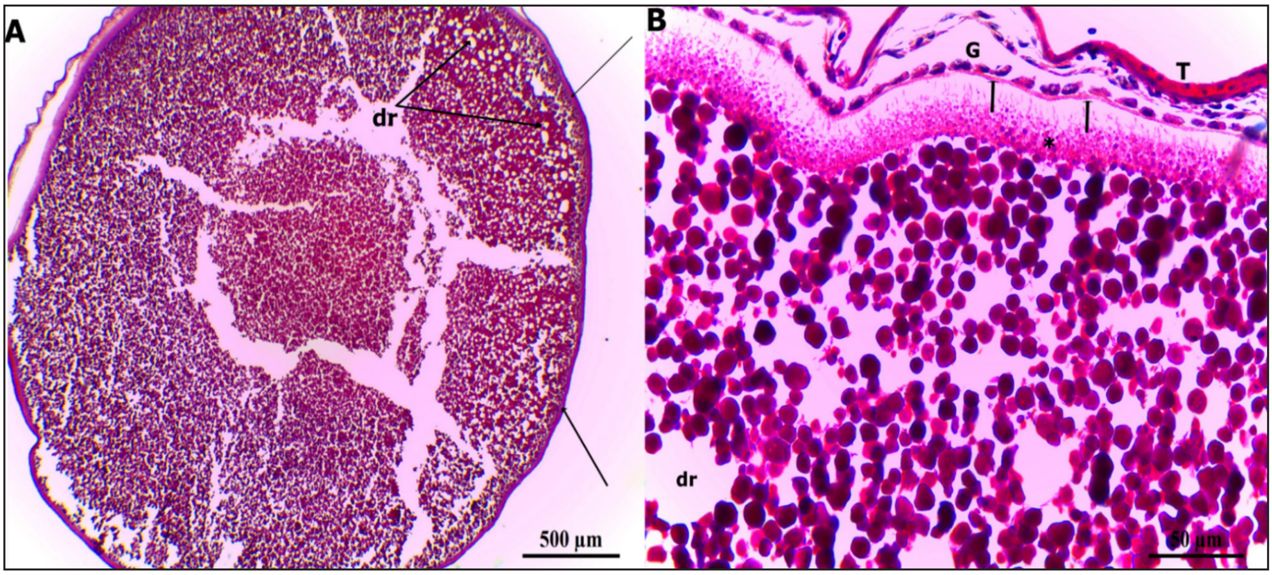

Figure 6. Ovarian follicle of *G. geckoides* from Catimbau National Park on vitellogenic stage. (A) Small and intermediate lipid droplets (dr) and defined yolkmembrane (arrows) (Bar = 500 µm). (B) Detail of the Figure A. The yolk membrane (arrows) exhibits a hyaline band and a zonaradiata. Abundant small yolk platelets (*) in the periphery of the ooplasm merge as they move toward the central area of oocyte. The granulosa is reduced to small and cubical cells. Granulosa (G), and theca (T) (Bar = 50 µm).

Table 3. Influence of abiotic and biotic factors analyzed under the reproductive cellular structures modifications.

Celular structures (Females)	**Biotic factors**				**Celular structures (Females)**	**Abiotic factors**			
	Estimate	**Std. Error**	**z-value**	**p-value**		**Estimate**	**Std. Error**	**z-value**	**p-value**
Oocyte diameter					**Oocyte diameter**				
(Intercept)	8.672	4.518	1.920	**0.050**	(Intercept)	5.009	1.087	4.605	< **0.001**
Precipitation	-0.032	0.018	-1.759	0.078	K1	-21.320	11.933	-1.787	0.074
Temperature	-0.051	0.125	-0.408	0.683	Gonadal Volume	0.010	0.021	0.469	0.638
Humidity	-0.005	0.019	-0.270	0.787	Body Temperature	0.107	0.045	2.376	**0.017**
Granulosa diameter					**Granulosa diameter**				
(Intercept)	4.972	3.027	1.642	**0.028**	(Intercept)	3.504	1.920	1.824	**0.05**
Precipitation	-0.001	0.001	-0.786	0.432	K1	-1.499	9.912	-0.146	0.883
Temperature	-0.015	0.062	-0.247	0.805	Gonadal Volume	0.227	0.278	0.815	0.415
Humidity	-0.044	0.132	-0.335	0.738	Body Temperature	0.015	0.063	0.24	0.81
Germinal bed diameter					**Germinal bed diameter**				
(Intercept)	-9.843	3.780	-2.604	< **0.001**	(Intercept)	-1.589	1.836	-0.866	**0.021**
Precipitation	0.003	0.001	2.613	< **0.001**	K1	22.667	9.204	2.463	**0.013**
Temperature	0.432	0.126	3.427	< **0.001**	Gonadal Volume	0.728	0.265	2.738	< **0.001**
Humidity	0.174	0.059	2.950	< **0.001**	Body Temperature	0.151	0.060	2.491	**0.012**

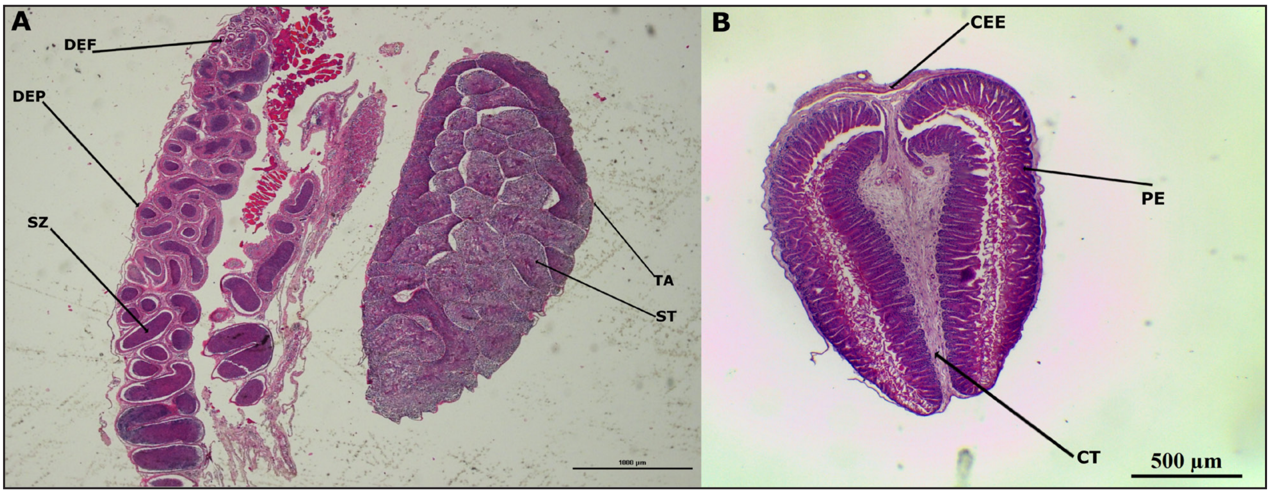

Figure 7. Panoramic view of testis and anterior testicular ducts (A) and hemipenis (B) of *G. geckoides* sexually matures individuals from Catimbau National Park. The tests displayed seminiferous tubules with active spermatogenesis, with seminiferous tubules (ST) surrounded by tunica albugínea (TA), also containg reproductive cells in all maturation stages. The ductuliefferentes (DEF) have not spermatozoa (Sz), and the ductus epididymis (DEP) is full of spermatozoa. Hemipenis displayed connective tissue (CT) in central region, surrounded by squamousepithelium (PE) and stratifiedcolumnar epithelium (CEE). Scale bars: A = 1 mm; B = 0.5 mm.

occurs more frequently during the rainy season. Eggs are normally laid at the end of the rainy season (Ferreira *et al.,* 2002; Mamou*et al.,* 2017).

An endogenous mechanism appears to be triggered in *G. geckoides* after individuals reach sexual maturity. This can make them sensitive to environmental variations and affect their body indices and gonadal development in different ways. According to our results, RFB accumulation differed between sexes. Females experienced a constant reduction in RFB until the end of the rainy season, whereas males accumulated RFB until the end of this period, with the reduction in females associated with the gestation process. These results indicate that lipid reserves in females increases as precipitation increases and the air temperature decreases, while increasing humidity is associated with the reduction of gonadal reserves.

In contrast, RWL and RGS showed synchronized activity, with both indices showing a reduction

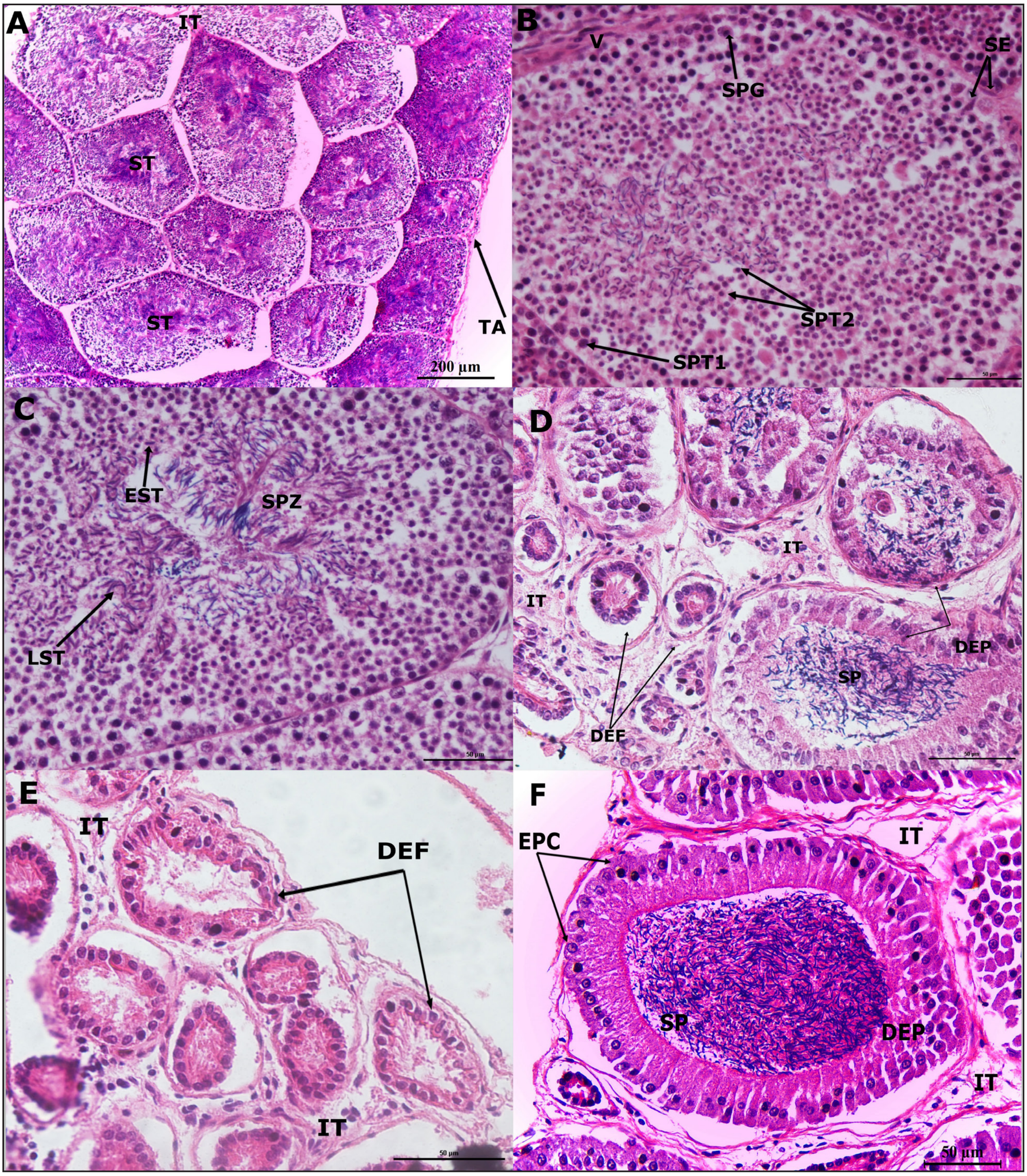

Figure 8. Tests structures of *G. geckoides* from Catimbau National Park. All the seminiferous tubules (ST) display constant spermatogenesis, envolved by tunica albuginea (TA), variating the maturation activity from a tubule to another (A). Seminiferous tubule under spermiogenesis process, with the presence of blood vessels (v) and Sertoli cells (SE) on the periphery, and abundance of spermatogonia (SPG), primary spermatocytes (SPT1) and secondary spermatocytes (SPT2) (B). Seminiferous tubule under espermiation process, with high abundance of early (EST) and late spermatids (LST), as well as sperms (SPZ) (C). Ductuliefferentes (DEF) and ductus epididymis (DEP), surrounded by interstitial tissue (IT) during spermatogenesis process (D). The ductuliefferentes have not spermatozoa, and they are surrounded by interstitial tissue (IT) (E). Theepididymal duct is lined by a columnar epithelium (EPC), and its lumencontains spermatozoa (SP), .The interstitial tissue (IT)is observed around the epididymal duct. The cuboidal epithelium of the ductuliefferentes possesses large stereocilia (arrowhead) in the apical region (F). Scale bars: A = 0.2 mm; B,C,D,E,F = 50 µm.

Table 4. ANOVA's tests for differences throughout the annual cycle (months) and across the phases of the spermatogenic cycle in selected structures, as well as reproductive cell stages, of the testis and anterior seminiferous tubes of *G. geckoides*, for Catimbau National Park, Pernambuco, Brazil northeastern.the reproductive cellular structures modifications.

Analyzed pattern	Model	F	P
Seasonality			
	Seminiferous tubule diameter	4.54	< 0.001
	Seminiferous epithelium height	10.94	< 0.001
	Ductuli efferentes diameter	11.40	< 0.001
	Ductus epididymis diameter	13.09	< 0.001
	Ductus epididymis heigth	21.98	< 0.001
	Primary spermatocytes	9.41	< 0.001
	Secondary spermatocytes	12.02	< 0.001
	Sperms	18.94	< 0.001
Annual cycle			
	Seminiferous tubule diameter	2.15	< 0.01
	Seminiferous epithelium height	4.04	< 0.001
	Ductuli efferentes diameter	12.48	< 0.001
	Ductus epididymis diameter	3.44	< 0.001
	Ductus epididymis heigth	10.89	< 0.001
	Primary spermatocytes	5.77	< 0.001
	Secondary spermatocytes	5.62	< 0.001
	Sperms	10.88	< 0.001

until the beginning of the rainy period in females and males. However, females showed substantially greater variation in body indices than males.Such sexual differences in energy reserves are likely because of the high energy costs faced by females during vitellogenesis and gestation process (Ramirez-Bautista yVitt, 1997; Schaffner, 1998). In males, abiotic factors influenced gonad volume only, which increased with increasing temperature, humidity, and precipitation, indicating a constant increase in gonad volume. Variation in gonad volume indicates that males in *G. geckoides* show variation in mating activity during each period, although the production of sperm occurs throughout the year without gonadal quiescence. Finally, reduced RGS levels indicate reduced mating activity during periods of abundant rainfall, potentially because most females in the population are gestating (Galdino*et al.,* 2003; Ramirez-Bautista *et al.,* 2009).

High humidity increased hatching success and brood survival (Derickson, 1976; Norval*et al.,* 2019). In a population of *G. geckoides* from the Catimbau National Park, females had a fixed single egg brood in their oviducts (Vitt, 1986; Alcântara*et al.,*2019). In geckos, low investment in egg number is compensated for by the production of more than one brood per year, with females potentially investing similar amounts of their energy reserves in consecutive gestations (Guedes *et al.,* 2020; Oitaven *et al.,* 2022). The dominance of gravid females during periods of high precipitation and minor variation in air temperature suggests that this combination of abiotic conditions is favorable for gestation and egg deposition. This is potentially because increased rainfall and more stable temperatures reduce the risk of egg desiccation and brood mortality (Colli *et al.,* 2003; Norval *et al.,* 2019). Consequently, energy reserves are significantly reduced as the rainy season progresses, especially in reproductively active females.

The alternations between lipid reserves and gonad conditions in females, and the fact that some females simultaneously had ovaries at different stages, that is, pre-vitellogenic and vitellogenic, provide evidence that throughout the annual cycle of *G. geckoides*, more than one vitellogenesis process may take place simultaneously in some individuals. This process causes a marked reduction in lipid reserves, followed by the appearance of yolk follicles and oocytes. The process is characterized by an increase in lipid and hepatic reserves.An increase in liver volume correlates with an increase in lipids in this organ, which are then used in the second vitellogenesis process (Aun and Martori, 2010; Paz *et al.,* 2019). In the Caatinga, these stages coincide with late periods of the dry and rainy seasons. This is likely to be an adaptive reproductive strategy, because producing multiple broods maximizes the probability that some broods will have favorable environmental conditions for their development and survival. This ultimately increases their reproductive success (Vitt, 1986; Colli *et al.,* 2003). In contrast, the lack of variation in the energy reserves of males suggest that sperm production and copulation do not impose high energy costs to males (Guillete and Casas-Andreu, 1980; Ramírez-Bautista *et al.,* 2006),

Table 5. Summary results of GLM models between the selected structures, as well as reproductive cells of the testis and seminiferous tubes of *G. geckoides* and abiotic factors (Temperature, precipitation and humidity), such as biotic factors (body temperature, growth rate and gonadal volume) in Catimbau National Park, Pernambuco, Northeastern Brazil.

Model Analyzed	Abiotic factors					Biotic factors				
	Effect	Estimate	Std. Error	t	P	Effect	Estimate	Std. Error	t	P
Seminiferous tubule diameter	Intercept			1.62	0.1	Intercept			2.66	< 0.001
	Precipitance	0.22	0.15	1.45	0.14	Body temperature	-0.11	2.67	-0.04	0.94
	Temperature	0.7	5.98	0.11	0.9	K1	125.17	153.89	0.81	0.41
	Humidity	0.41	0.88	0.47	0.63	Gonadal Volume	8.00	1.82	4.39	< 0.001
	Overall model: F = 0. 87; r = 0.01, P = 0.45					Overall model: F = 7. 13; r = 0.08, P< 0.001				
Seminiferous epithelium height	Intercept			2.49	< 0.01	Intercept			3.38	< 0.001
	Precipitance	0.15	0.07	2.1	0.03	Body temperature	-5.00	1.31	-3.81	< 0.001
	Temperature	-2.11	2.86	-0.73	0.46	K1	-39.94	75.06	-0.53	0.59
	Humidity	0.05	0.42	0.13	0.89	Gonadal Volume	-0.51	0.89	-0.57	0.56
	Overall model: F = 2. 05; r = 0.02, P< 0.01					Overall model: F = 5. 09; r = 0.06, P< 0.001				
Ductuli efferentes diameter	Intercept			5.02	< 0.001	Intercept			2.99	< 0.001
	Precipitance	-0.01	0.04	-0.18	0.85	Body temperature	-3.4	0.93	-3.66	< 0.001
	Temperature	-8.27	1.87	-4.4	< 0.001	K1	4.55	52.79	-0.08	0.93
	Humidity	-0.15	0.27	-0.57	0.56	Gonadal Volume	-0.74	0.63	-1.17	0.24
	Overall model: F = 15.98; r = 0.18, P < 0.001					Overall model: F = 5. 38; r = 0.06, P< 0.001				
Ductus epididymis diameter	Intercept			-0.14	0.88	Intercept			1.37	0.17
	Precipitance	0.04	0.13	0.36	0.71	Body temperature	-5.76	2.44	-2.35	< 0.01
	Temperature	6.62	5.26	1.25	0.2	K1	-132.28	139.52	-0.94	0.34
	Humidity	1.55	0.78	1.98	0.04	Gonadal Volume	1.90	1.65	1.15	0.25
	Overall model: F = 1.54; r = 0.02, P = 0.20					Overall model: F = 2.36; r = 0.03, P = 0.07				

Ductus epididymis height	Intercept			1.01	0.31	Intercept			0.20	0.83
	Precipitance	0.07	0.06	1.16	0.24	Body temperature	-3.67	1.12	-3.27	< 0.001
	Temperature	0.42	2.45	0.17	0.86	K1	-168.57	64.79	-2.6	< 0.001
	Humidity	0.36	0.36	0.99	0.32	Gonadal Volume	0.28	0.75	0.37	0.70
	Overall model: F = 1.19; r = 0.01, P = 0.31					Overall model: F = 4.48; r = 0.05, P< 0.001				
Primary Spermatocytes	Intercept			-1.2	0.22	Intercept			1.03	0.30
	Precipitance	-0.15	0.14	-1.17	0.24	Body temperature	0.38	0.08	4.78	< 0.001
	Temperature	0.55	0.19	2.91	< 0.01	K1	5.50	5.54	0.99	0.32
	Humidity	0.01	0.02	0.47	0.63	Gonadal Volume	-0.24	0.06	-3.89	< 0.001
	Overall model: F = 6.00; r = 0.06, P < 0.001					Overall model: F = 13.85; r = 0.13, P < 0.001				
Secondary Spermatocytes	Intercept			2.34	< 0.01	Intercept			2.24	0.02
	Precipitance	0.17	0.17	0.99	0.32	Body temperature	0.24	0.13	1.88	0.06
	Temperature	-0.18	0.3	-0.61	0.54	K1	8.21	9.09	0.90	0.36
	Humidity	-0.04	0.04	-0.97	0.33	Gonadal Volume	-0.26	0.10	-2.62	< 0.001
	Overall model: F = 0.64; r = 0.07, P = 0.58					Overall model: F = 3.91; r = 0.04, P < 0.001				
Espermatozoa	Intercept			6.83	< 0.001	Intercept			1.55	0.12
	Precipitance	0.02	0.01	1.9	0.05	Body temperature	-0.56	0.27	-2.06	0.03
	Temperature	-3.05	0.57	-5.33	< 0.001	K1	-10.05	18.50	-0.54	0.58
	Humidity	-0.32	0.08	-3.99	< 0.001	Gonadal Volume	0.38	0.20	1.90	0.05
	Overall model: F = 10.26; r = 0.10, P < 0.001					Overall model: F = 2.94; r = 0.03, P = 0.03				

maintaining constant levels in their energy reserves (Sexton and Brown, 1977; Galdino *et al.,* 2003; Ramirez-Bautista *et al.,* 2006; Oitaven *et al.,* 2022).

The population of *G. geckoides* analyzed displayed continuous and acyclic activity in their reproductive patterns. Despite the marked seasonality in the Caatinga area, temperatures tended to be adequate, allowing these cycles to be maintained for females and males. This study represents the first examination of energy cycles and reproductive strategies in *G. geckoides* in the Caatinga area, describing the cycles of different body indices and gonads, while also testing the effects of environmental factors and morphological traits. Despite seasonal variations in energy reserves, *G. geckoides* are constantly active throughout the year and reproduces more than once in a single year. The energy required to sustain continuous activity and related vital processes, that is, maintenance and reproduction, in *G. geckoides* is dependent on diet. This indicates that prey availability seems to be sufficient at the Caatinga domain.

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Supplemental online material legends

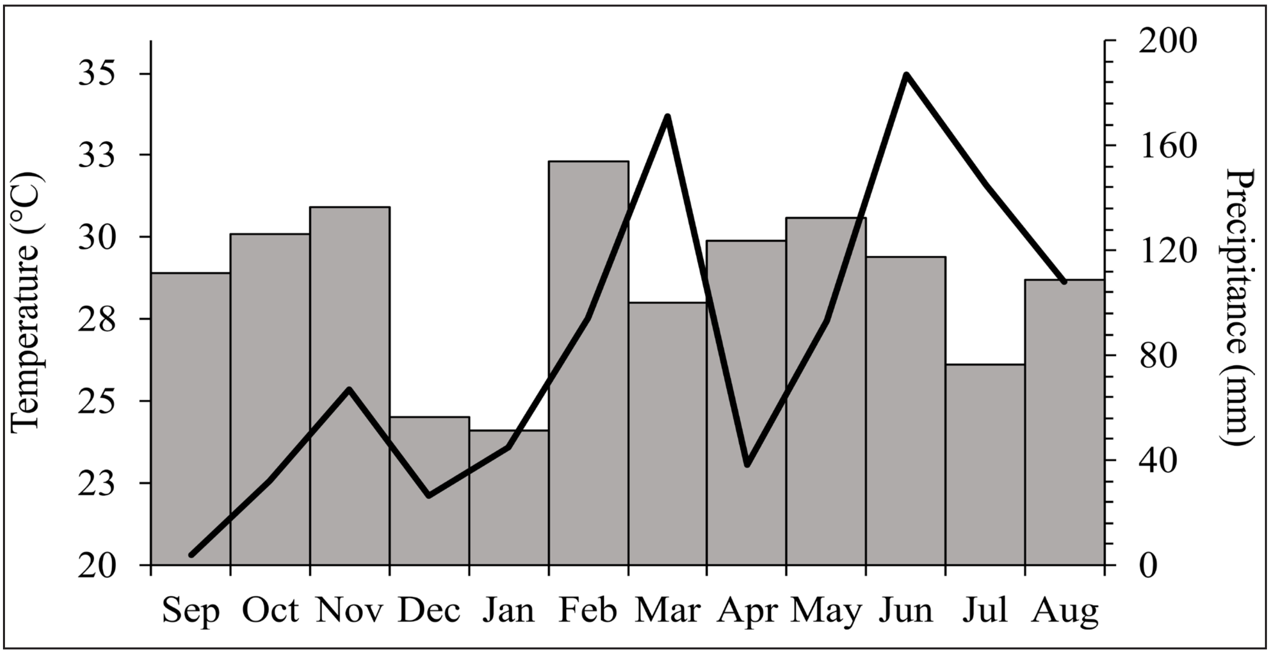
Supplementary material 1. Climatological variations at Catimbau National Park, regarding data recorded by the municipalities of Buíque, Pernambuco state, Northeastern Brazil, and Picuí, Paraiba state, Northeastern Brazil, from September 2018 to September 2021. Total rainfall, between 0.5 to 188(mm–line) and average monthly temperature, between 24 to 33(°C–bars).Climatological variations recorded by Pernambuco Water and Climate Agency database (APAC), with many meteorological, and information recorded from 2018 to 2019 according to many municipalities of Pernambuco state, Northeastern Brazil.

Supplementary material 2. Summary of the total numbers of female and male *G. geckoides* collected by month and year in Catimbau National Park, municipality of Buíque, Pernambuco state, and Picuí municipality, Paraíba state, Brazil. The quantity of asterisks (*) denotes how many gravid females were found in that month.

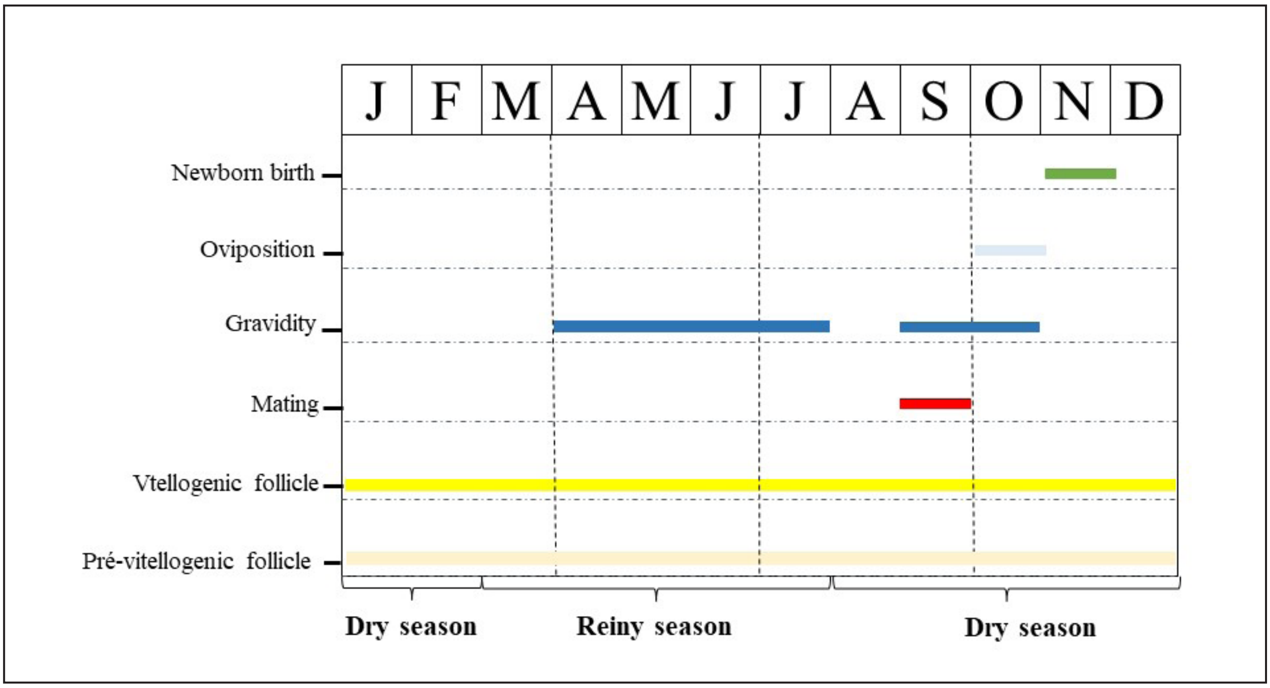
Supplementary material 3. Correlation analysis comparing body structures (SVL and Weight), as independent variables, and body reserves (lipids, hepatic and gonadal), as dependent variables, for both female and male of *G. geckoides* Spix, 1825 individuals from Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, from September 2018 to September 2021.

Supplementary material 4. Summary of results of the Generalized Linear Model (GLM) fitted to test the influence of body indices and gonad volume, according to seasonal variations (precipitation levels), on the appearance of individuals of *G. geckoides*Spix, 1825 in Catimbau National Park, municipality of Buíque, Pernambuco, and Picuí municipality, Paraíba, Brazil, from September 2018 to September 2021. Body indices: liposomal ratio (RFB), hepatosomatic ratio (RWL), gonadosomatic ratio (RGS).

Supplementary material 5. Reproductive cycle from female of *G. geckoides* at Catimbau National Park, including follicles maturation, mating, gestation, as well as eggs laying and hatchling.



Sampling month	2018 – 2021		Monthly total
	Females	Males	
September	03	03	06
October	02	02	04
November	06	02	08
December	03	03	06
January	03	03	06
February	03	08	11
March	04	06	10
April	03*	11	14
May	04**	05	09
June	04**	04	08
July	03*	08	11
August	06*	09	15
Total			**108**



Predictors	Low precipitation levels (n = 66)			High precipitation levels (n = 63)		
	Estimate ± SE	z-value	p	Estimate ± SE	z-value	p
Intercept	3.305 ± 4.718	0.701	0.484	-3.446 ± 4.709	-0.732	0.464
RFB	-0.001 ± 0.060	-0.022	0.982	0.004 ± 0.060	0.083	0.934
RWL	-0.115 ± 0.317	-0.363	0.717	0.136 ± 0.321	0.426	0.670
RGS	0.446 ± 0.478	0.933	0.351	-0.533 ± 0.499	-1.069	0.285
Gonadal volume	-0.028 ± 0.060	-0.467	0.640	0.024 ± 0.060	0.398	0.691

Trabajo

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Cytogenetic study in the Brazilian semiarid lizard *Tropidurus hispidus* (Squamata, Tropiduridae)

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ABSTRACT
Different classes of repetitive DNA sequences are found in eukaryotes, often composing substantial portions of the genome, associated with their important role in the structural and functional genome organization. In this work, we mapped repetitive DNA sequences (18S rDNA, microsatellites and telomeric motifs) in the karyotype of *Tropidurus hispidus,* a species of lizard from the Brazilian semiarid region. We found a diploid number of 2n = 36 (6 pairs of biarmed macrochromosomes and 12 pairs of microchromosomes). The 18S rDNA clusters were localized at the subterminal region of the long arm of pair 2. The telomeric probes produced signals at terminal, interstitial and centromeric positions of some chromosome pairs, which might indicate the occurrence of chromosomal rearrangements via chromosome fusions. Microsatellite sequences were found in at least two distinct patterns - clustered in the telomeric/pericentromeric regions or observed as scattered signals in the chromosomes. This study represents an initial step to explore the evolutionary dynamics of repetitive sequences in the *Tropidurus* genus and considering the scarcity of data concerning the chromosomal mapping of repetitive sequences in Tropiduridae species, it reinforces the importance of integrating other methodologies, including the isolation and physical mapping of different repetitive DNA sequences, contributing to understanding the patterns of karyotypic evolution in lizards.

Key words: Reptile chromosomes; Microsatellite; 18S rDNA; Telomeric repeats.

Introduction

Tropidurus Wied, 1825 is a genus belonging to the family Tropiduridae, comprising 28 species found in open landscapes in tropical and subtropical South America (Carvalho, 2013; Uetz *et al.*, 2022). Among them, *Tropidurus hispidus* (Spix, 1825) stands out for having a wide distribution, with a continuous and uniform presence in the Caatinga biome and the coastal areas of northeastern Brazil (Carvalho, 2013; Carvalho *et al.*, 2016; Uetz *et al.*, 2022).

Cytogenetic data available for *T. hispidus* show a diploid number of 2n = 36 (Kasahara *et al.*, 1987, 1996), with a typical karyotype of Squamata lizards, exhibiting macrochromosomes (metacentric and submetacentric) and microchromosomes (Veronese

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et al., 2003; Santos *et al.*, 2008; Carvalho *et al.*, 2015; Da Silva *et al.*, 2020a). However, the amount and distribution of constitutive heterochromatin in this species, detected by C-banding, show variation between populations, which could be related to different evolutionary processes such as sex chromosomes differentiation. (Kasahara *et al.*, 1983, 1987, 1996).

An important approach to advancing the cytogenetic knowledge of organisms is to characterize their genome content and organization. In this sense, considering the common occurrence of repetitive DNA sequences in eukaryotic genomes and the wide heterogeneity regarding their distribution and content, the chromosomal mapping of these sequences can provide important pieces of information concerning their role in the evolutionary process (Biscotti *et al.*, 2015; Garrido-Ramos, 2017).

Different classes of repetitive DNA sequences are found in eukaryotes, often composing substantial portions of the genome (Charlesworth *et al.*, 1994; Plohl *et al.*, 2012; López-Flores and Garrido-Ramos, 2012; Garrido-Ramos, 2017). These sequences can be found dispersed (DNA transposons and retrotransposons) or organized *in tandem* (multigenic families, including rDNA and other genes encoding proteins; satellites, and microsatellites) (for review, see López-Flores and Garrido-Ramos, 2012; Biscotti *et al.*, 2015; Garrido-Ramos, 2017).

Repetitive DNA sequences, mainly satellite DNAs, have already been reported at telomeric and centromeric heterochromatic regions, euchromatic regions, as well as at supernumerary and sex chromosomes of plants, insects, fish, amphibians, birds and lizards (Vittorazzi *et al.*, 2011; Carvalho *et al.*, 2016; Gatto *et al.*, 2016, 2019; Milani *et al.*, 2017; Palacios-Gimenez *et al.*, 2017; Silva *et al.*, 2017; Kretschmer *et al.*, 2018; Ruiz-Ruano *et al.*, 2019; Utsunomia *et al.*, 2019; Crepaldi and Parise-Maltempi, 2020; Da Silva *et al.*, 2020b; Ferretti *et al.*, 2020; Cholak *et al.*, 2020). These sequences play important roles in genome organization and often serve as hotspots of genome rearrangements and evolutionary innovations (Garrido-Ramos, 2017).

The available data concerning the distribution of repetitive sequences in lizards are still limited considering the group's diversity known so far. However, the information gathered up to now includes rDNAs, telomeric sequences, transposable elements, COT1, and satDNAs, which helped to understand their genomic dynamics and organization, as well as evolutionary processes they are involved (e.g Bertolotto *et al.*, 2001; Pellegrino *et al.*, 2009; Chaiprasertsri *et al.*, 2013; Rojo *et al.*, 2014; Rovatsos *et al.*, 2015; Carvalho *et al.*, 2015, 2016; Giovannotti *et al.*, 2018, 2020; Da Silva *et al.*, 2020a).

In this sense, considering the scarcity of data concerning the chromosomal mapping of repetitive sequences in Tropiduridae, we analyzed the lizard *T. hispidus* from the Brazilian semiarid, aiming to map some repetitive elements in the chromosomes of this species, adding valuable information to the cytogenetic knowledge of Brazilian reptiles.

Materials y methods

A sample of 10 specimens of *T. hispidus* (6 males and 4 females) was collected at the Picos, state of Piauí, Brazil (6°54'22.9"S 41°33'49.8"W), under a governmental license (number 47710-1/2015) issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). All laboratory procedures were performed at the Biology Laboratory, at the Instituto Federal de Educação, Ciência e Tecnologia do Piauí (IFPI), campus Picos.

Cell suspensions were obtained according to Bertollo *et al.* (1978), using bone marrow extraction from individuals previously treated with 1% colchicine injected intraperitoneally for 24 hours, followed by hypotonic treatment and fixation. Chromosome suspensions were dropped on slides and stained with 10% Giemsa to determine the chromosomal number and morphology.

For fluorescence *in situ* hybridization (FISH) experiments, 18S rDNA probes were obtained from *Hoplias malabaricus* (Pisces: Erythrinidae) genomic DNA (Cioffi *et al.*, 2009). The polymerase chain reaction (PCR) was carried out with primers 18SF (5' CCGAGGACCTCACTAAACCA 3') and 18SR (5' CCGCTTTGGTGACTCTTGAT 3') following Cioffi *et al.* (2009). The telomeric sequence probe was obtained by PCR without DNA templates, using the primers $(TTAGGG)_5$ and $(CCCTAA)_5$ according to Ijdo *et al.* (1991). PCR products of the 18S rDNA were labeled with digoxigenin-11-dUTP by nick translation (Dig-Nick Translation mix; Roche®) following the manufacturer's recommendations and used in FISH experiments according to Pinkel *et al.* (1986). The detection was made using Anti-Digoxigenin Rhodamine (Roche).

FISH experiments with microsatellite probes followed the protocol of Kubat *et al.* (2008), using the oligonucleotides $(CAA)_n$, $(CA)_n$, $(CAC)_n$, $(GAG)_n$, $(GA)_n$, and $(GAA)_n$ directly labeled with Cy5-fluorochrome at the 5' end during synthesis (Sigma-Aldrich). Chromosomes were counterstained with DAPI (2 mg/mL) in VectaShield® mounting medium (Vector).

At least ten metaphases were analyzed for each experiment. Giemsa-stained metaphases were photographed at a Nikon Eclipse microscope coupled with a Thiachron camera and processed using AMscope 3.7® software. The chromosomes were ordered in decreasing size, and the morphology of the chromosomes was determined based on the arm ratio, following Guerra (1986). FISH images were captured using a Zeiss-Axiophot microscope coupled to a fluorescence system and were processed employing the Axiovision software (Zeiss). The metaphase images were later optimized for brightness, contrast and mounting using the Adobe Photoshop CS6 program and Corel Draw X7 software.

Results

Males and females of *T. hispidus* presented 2n = 36, with 12 biarmed macrochromosomes (M) and 24 microchromosomes (m) without observable heteromorphic or sex-related chromosome pair (Fig. 1). No secondary constrictions were observed in conventional staining. Telomeric probes detected the telomeres of chromosomes and, in addition, produced conspicuous signals located at interstitial and centromeric positions of pairs 2 and 4 (Fig. 2 - A). The 18S rDNA probe hybridized at the distal end of the long arm of pair 2, which coincides with the nucleolus organizer region (NOR) (Fig. 2 - B).

Microsatellite probes revealed at least two distinct sequences of repeat motifs: telomeric/pericentromeric regions and scattered signals in the chromosomes. Therefore, $(CA)_n$, $(CAA)_n$, $(GA)_n$, $(CAC)_n$, and $(GAG)_n$ were found in a relatively uniform dispersed distribution in all chromosomes (Fig. 3). Although scattered signals for these arrays were generally observed in *T. hispidus* cells, some of the signals formed band-like patterns in distinct chromosomes and positions, depending on the sequence of the probe. For the motif $(GAA)_n$, signals occurred mainly at telomeric regions of macro and microchromosomes.

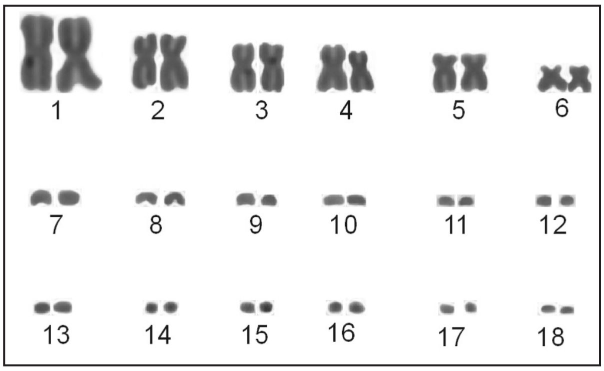

Figure 1. Giemsa-stained karyotype of a female *Tropidurus hispidus* (2n=36, 12M+24m). Pairs 1-6 indicate macrochromosomes (M), while pairs 7-18, microchromosomes (m).

Discussion

In a broad context, chromosomal studies, and particularly those that use *in situ* hybridization, have been able to locate different repetitive DNA sequences in chromosomes and show the distribution of the various classes of this type of intriguing sequences (Biscotti *et al.*, 2015). In this sense, here we mapped repetitive sequences (18S rDNA, microsatellites, telomeric motifs) in the karyotype of *T. hispidus*. In addition, we performed an analysis of the karyotype of *T. hispidus* in conventional staining in Giemsa, to support the repetitive sequence mapping data.

The specimens of *T. hispidus* we investigated in this work showed 12 biarmed macrochromosomes and 24 microchromosomes, as well as found by Kasahara *et al.* (1996) for *T. hispidus* individuals from other localities. In their study, Kasahara and colleagues identified a dot-like Y chromosome in male individuals, thus recognizing an XX:XY sexual differentiation mechanism in *T. hispidus* (Kasahara *et al.*, 1996). For the population analyzed in this work, this differentiated chromosome was not observed, probably due to the quality of the cell material observed and the absence of meiotic analysis.

Microsatellite and satellite DNA accumulation may be associated with various genomic aspects, including differentiation of sex chromosomes, with a significant role in the suppression of recombination, degeneration, and heterochromatinization (Pokorná *et al.*, 2011; Matsubara *et al.*, 2006, 2015; Palacios-Gimenez *et al.*, 2017; Ferretti *et al.*, 2020; Crepaldi and Parise-Maltempi, 2020; Zattera *et al.*, 2020). Among lizards, one example of accumulation of repetitive DNA associated with sex chromosome differentiation is provided by the lacertid *Eremias velox*, in which the sex chromosomes are homomorphic, and the W chromosome is highly heterochromatic (Pokorná *et al.*, 2011). Regarding our data, the analyzed microsatellites were distributed

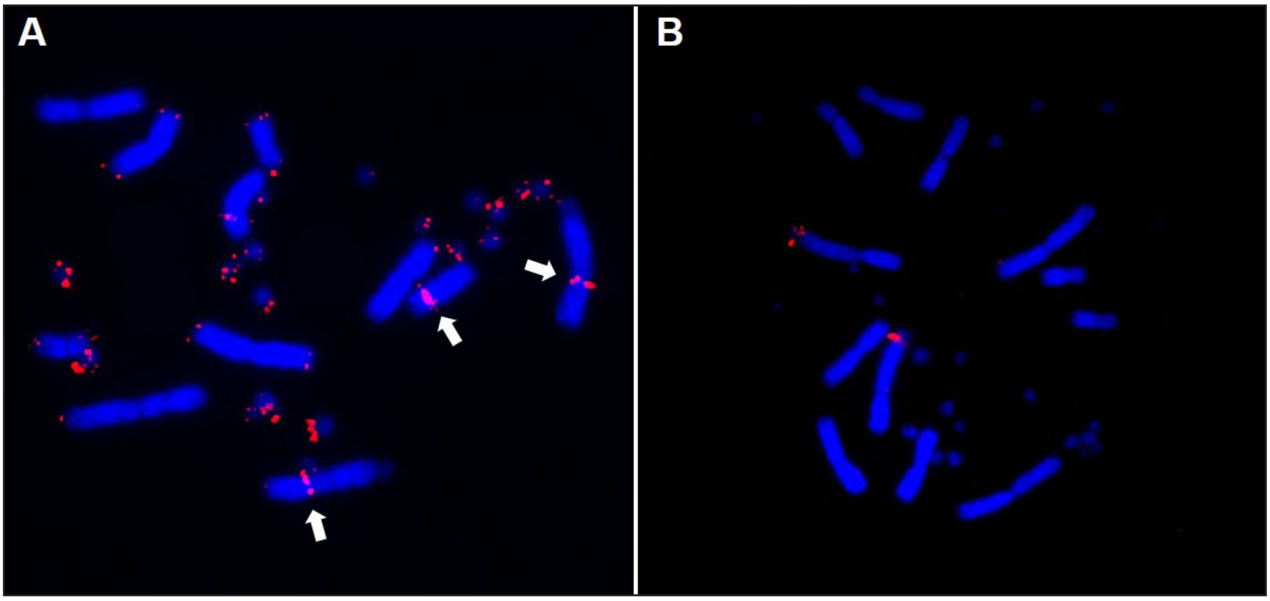

Figure 2. Metaphase chromosomes of *Tropidurus hispidus* hybridized to a telomeric sequence probe (A) and a 18S rDNA probe (B). Arrows in A indicate interstitial telomeric sequences (ITSs).

in different regions of several chromosomes of *T. hispidus*. In addition, male and female individuals analyzed herein did not exhibit differences regarding the distribution of the microsatellite sequences.

In the karyotype of *T. hispidus*, the motifs $(CA)_n$, $(CAA)_n$, $(GA)_n$, $(CAC)_n$, and $(GAG)_n$ were extensively dispersed along the chromosomes, whereas the motif $(GAA)_n$ was accumulated at telomeric and pericentromeric regions of macrochromosomes and some microchromosomes. Similarly, telomeric and pericentromeric blocks of $(GAA)_n$ were previously observed in the lizards *Eremias velox* (Pokorná *et al.*, 2011), *Salvator merianae* (Da Silva *et al.*, 2020a), and *Cyrtodactylus jarujini* (Thongnetr *et al.*, 2021). It has been suggested that repetitive sequences accumulated specifically in the terminal/subterminal region are related to a fundamental role in the chromosomal stabilization and functioning, a common phenomenon in the karyotypes of animals (Oliveira *et al.*, 2017; Cioffi *et al.*, 2011; Xu *et al.*, 2013; Poltronieri *et al.*, 2014; Ruiz-Ruano *et al.*, 2015; Ernetti *et al.*, 2019; Zattera *et al.*, 2020).

Telomeres are regions of repetitive DNA motifs and associated proteins which play a crucial role in maintaining chromosome structure as a single unit, preventing fusions or degeneration, and loss of genetic information during replication events (Blackburn, 2001). Interestingly, despite this remarkable stability, motifs $(TTAGGG)_n$ can also be found at non-terminal positions of chromosomes as interstitial telomeric sequences (ITSs) (Meyne *et al.*, 1990; Lin and Yan, 2008). In addition to usual signals at chromosomal ends, the telomeric probe revealed the presence of ITS at centromeric and pericentromeric regions of chromosome pairs 2 and 4 of *T. hispidus* analyzed herein, possibly due to the occurrence of chromosomal rearrangements, as already described for reptiles (e.g. Pellegrino *et al.*, 2009; Rojo *et al.*, 2014; Rovatsos *et al.*, 2015; Da Silva *et al.*, 2020a; Clemente *et al.*, 2020). This feature is quite interesting and would require further research through more refined genomic analyses to clarify whether the $(TTAGGG)_n$ motif at the centromeres of Tropiduridae lizards has a non-telomeric origin.

In conclusion, it is remarkable that the organization and composition of repetitive sequences in reptiles need clarification not only through physical mapping, which can reveal some aspects of the distribution of these sequences and support their importance in the genome organization and function, but also by refined analyses and studies involving genomic sequencing. To assist in the evolutionary and taxonomic understanding of this group, classical and molecular cytogenetic techniques were used to find potential molecular markers for this group of organisms. Thus, our current data reinforce the importance of increasing the number of chromosomal analyses in the Tropiduridae family, contributing to the understanding of genomic organization and karyotypic evolution in lizards.

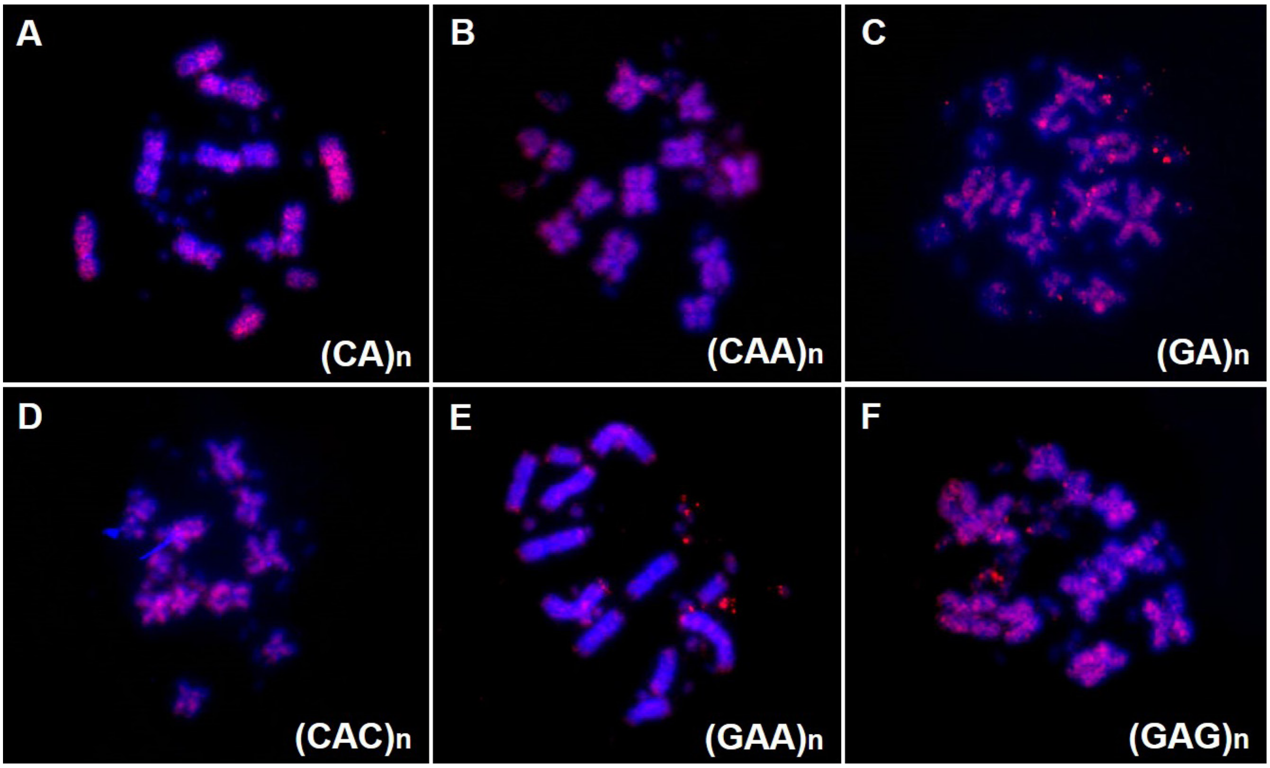

Figure 3. Metaphase chromosomes of *Tropidurus hispidus* after fluorescent *in situ* hybridization with probes for microsatellite motifs.

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Nota

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Functional morphology of the tongue of snake *Bothrops jararaca* (Reptilia: Squamata)

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ABSTRACT
The tongue has a series of functions that can be related to feeding, such as transporting food to the back of the mouth, sensory function and capturing prey. The tongue of the reptiles has great morphological and functional variations between orders. This study aimed to described the morphological characteristics of the tongue of the snake *Bothrops jararaca*, relating them to the habitat and eating habits of this species. This work used five adult animals which were collected in the municipality of Viçosa, in the Zona da Mata Mineira region. The animals were euthanized, with anesthetic overdose, for the removal of the tongue, which was used for histological processing and scanning electron microscopy. The tongue had stratified epithelium with an extensive degree of keratinization and pigmentation. It was shown to be strongly muscular, innervated and vascularized. Keratinization and pigmentation is an important protection mechanism, due to the protrability of this organ, which is exposed to the environment to capture odors and locate prey. Mucous secretion was present as an important tool for lubrication and protection of the lingual lining. The tongue of the *B. jararaca* has no papillae and taste buds, being its function strictly olfactory, in association with the vomeronasal organ.

Key Words: Histology; Microscopy; Buccal cavity; Viperidae.

Bothrops jararaca (Wied-Neuwied, 1824), popularly known as jararaca, is a species of snake of the family Viperidae found in Brazil, Paraguay and northern Argentina (Warrell, 2004). It inhabits forests but can also be found in degraded areas (Campbell and Lamar, 2004). It is a poisonous species with mainly nocturnal habits (Sazima, 1992; Warrell, 2004) and ambush behavior, which can also use the active search strategy, depending on the size of the prey (Greene, 1997). It feeds mainly on small vertebrates, but adult and young individuals have different feeding habits. The young animals prey mainly on frog amphibians, while the adults, prey on rodents (Sazima, 1992).

Generally, the main function of the tongue is to contribute to feeding, participating in the capture of prey, ingestion and swallowing of food, tasting and, in some reptiles, it can play a role in olfaction in association with the vomeronasal organ (Schwenk, 2000; Iwasaki, 2002). In snakes, there is a growing evidence that the vomeronasal organ and, indirectly, tongue flicking, is involved in many activities including tailing, recognizing, striking, and swallowing prey, predator recognition, courtship, maternal care, aggregation, exploration, and habitat selection (Burghardt, 1980; Teshera and Clark, 2021).

The tongue of reptiles has morphological and functional variations between orders, reflecting differences in feeding, habitat and habits (Iwasaki, 2002; Koca *et al*., 2007). Thus, this study aimed to describ the morphological characteristics of the tongue of *Bothrops jararaca*, through macro and microscopic analysis.

For this work, five adult animals of *Bothrops jararaca* species were used. The animals were collected in the municipality of Viçosa, in the Zona da Mata Mineira region (IBAMA license: 10504-1). The entire procedure was conducted in accordance with the "Ethical Principles for the Use of Laboratory Animals" (Brazilian College of Animal Experimentation - COBEA, 1991), which was approved by the Ethics Committee for the Use of Animals (CEUA) of Federal University of Viçosa (protocol 27/2016). The animals were euthanized with an intraperitoneally

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injected overdose of pentobarbital. After euthanasia, the animals were measured and weighed. Tongue fragments were collected and fixed in Carson's formalin (Carson *et al.* 1973) for 24 hours.

The fragments were analyzed for anatomical description, with the use of a stereoscopic microscope (Olympus SZ40). The tongue was divided into three regions for histological analysis: anterior (apex), middle (body) and posterior (root) thirds. Then, fragments of these regions were dehydrated in a growing ethyl series, included in glycol-methacrylate resin and sectioned by a rotating microtome (RM2055, Leica), which provided 3 µm thick semi-serial sections. The sections were stained with toluidine blue in 1% sodium borate, for histological description, and subjected to the following histochemical methods: Alcian blue (AB) pH 2.5 and pH 0.5, aiming to detect acidic mucins and mucin sulfated acids, respectively (Bancroft and Stevens, 1996); periodic acid of Schiff (PAS) for neutral mucins (Pearse, 1968); Sudan black for lipids (McManus and Mowry, 1960) and Ponceau's xylidine (PX) (Kiernan, 1990) for the identification of general proteins. The observation and photographic record of the sections were performed in a light microscope coupled with a digital camera (Olympus BX53). Fragments from the three regions were also used for scanning electron microscope analysis. They were placed in stubs, taken to the carbon evaporator (Quorum Q150 T) and then metallized with gold by the Balzers Union FDU 010 Modular Equipment, composed of Metallizer ("Sputter coating attachment") SCA 010. Next, the material was analyzed using a scanning electron microscope (Leo, 1430VP).

Among the results of this work, it was observed that the *Bothrops jararaca*' tongue is surrounded by the buccal floor, which is a tubular structure housing the tongue (Fig. 1A). The tongue of this species is long, elongated and narrow, tapering at the apical end, which is bifurcated (Fig. 1A). Pigmentation is observed throughout the tongue (Fig. 1B), especially on the dorsum, whose texture is almost smooth, with small transverse grooves arranged dorsolaterally (Fig. 1C). In the venter, a deep central groove is observed, starting from the bifurcation and arranged throughout the tongue (Fig. 1B).

The protractile and bifurcated tongue of the *Bothrops jararaca* allows the capture of chemical substances from the environment and potential prey, taking these substances to the vomeronasal organ. Also known as the Jacobson organ, it is a small tubular structure with auxiliary olfactorial function, located in the posterior-inferior portion of the cartilaginous nasal septum, close to the union with the vomer (Burghardt, 1970). Some studies indicate the occurrence of a co-evolutionary process between the tongue and the vomeronasal organ in the irradiation of squamates (Cooper, 1994; Cooper, 1997a; Filoramo and Schwenk, 2009). This process is related to the shape and elongation of the tongue and presence of bifurcation (Cooper, 1994; Cooper, 1995; Cooper, 1997b). Parker *et al.* (2008) suggested that the lingual bifurcation in snakes serves to increase area of chemosensory detection.

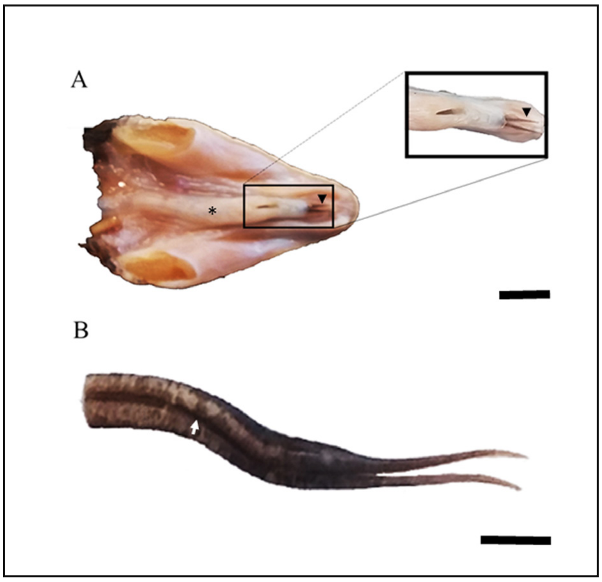

Figure 1. Anatomy of the tongue of *Bothrops jararaca*. A) Buccal floor forming a tubular structure (asterisk) that surrounds the tongue, whose apex is bifurcated (insert, arrow head). B) Ventral face of the tongue with deep central groove (arrow). Scale: 1cm

Histologically, the tongue of the snake *B. jararaca* has a stratified squamous epithelium widely keratinized and pigmented throughout its length (Fig. 2A). This characteristic may be related to the "flicking" pattern (when the tongue protrudes out of the mouth) of the tongue of this species, due to external stimuli, such as the presence of prey or predators, also involving behavioral and seasonal aspects (Gove, 1979; Graves and Halpern, 1990; Bels *et al.*, 1994). The "flicking" of a snake's tongue is a form of adsorption of odorant in the air (Halpern *et al.*, 1986). In other words, it has a sensory function, capturing odorous molecules for the vomeronasal organ. Therefore, the tongue is strongly exposed to environmental aggressions, while keratin and pigment protect and provide resistance against da-

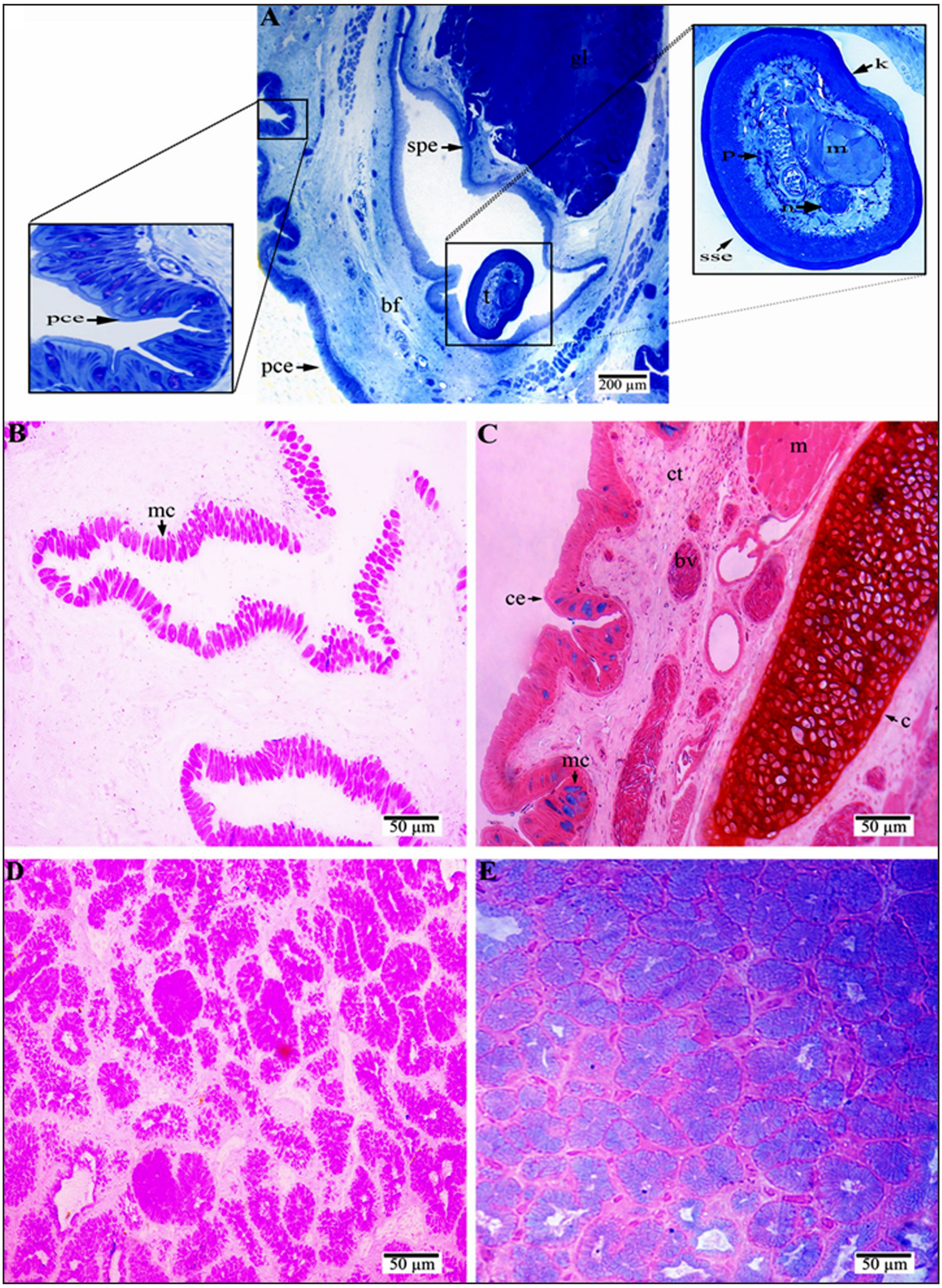

Figure 2. Light microscopy photos of the tongue and buccal floor of *Bothrops jararaca*. A) Buccal floor (bf) of pseudostratified ciliated epithelium (pce) on the external surface and stratified polyhedral epithelium (spe) on the internal surface, facing the cavity where the tongue (t) is located, which has a squamous stratified epithelium (sse) with keratin (k) and pigmentation (p). Anterior region; toluidine blue staining. B) Buccal floor, showing the pseudostratified ciliated epithelium and PAS-positive mucous cells (mc). Anterior region; periodic acid Schiff (PAS) staining. C) Buccal floor, showing the pseudostratified ciliated epithelium with AB-positive mucous cells (mc). Posterior region; Alcian blue (AB) and safranin staining. D) Branched tubular mucous gland of the buccal floor, with PAS-positive secretion. Middle region; periodic acid Schiff (PAS) staining. E) Branched tubular mucous gland with AB-positive secretion. Middle region; Alcian blue (AB) and safranin staining. bv: blood vessel; c: cartilage; ct: connective tissue; gl: gland; m: muscle (skeletal striated); n: nerve.

mage (Stern, 1980; Avery, 1987). *Bothrops jararaca* inhabits dry environments with high temperatures, thus, keratinization is extremely important to protect the protractile tongue of these animals.

In *Bothrops. jararaca,* the tongue is highly muscular, filled with skeletal striated muscle, which is arranged in a unidirectional and compacted manner, permeated by blood vessels and nerve bundles; and showed no lingual glands and no adipose tissue (Fig. 2A). This disposition of the muscle tissue, unidirectional and juxtaposed, may imply more targeted movements of the tongue, since the tongue of this species is not related to the admission of food, but to the capture of odorous molecules. The muscular tissue allows the sliding and stretching of the tongue, in the "tongue-flicking" behavior (El-sayyad, 2011). According to Smith *et al.* (1990), the tongue of snakes is composed of a few muscle groups, which are responsible for its flicking movement, and, despite general similarities, distinct patterns of intrinsic tongue musculature characterize each infraorder of snakes.

Through histochemical methods, it was observed that the oral floor is lined with pseudostratified ciliated epithelium (Fig. 2A) with PAS-AB-positive mucous cells (Fig. 2B and C), and filled with loose connective tissue with blood vessels, muscle bundles and a well-developed compound gland, whose duct opens in the cavity where the tongue is located (Fig. 2A). This cavity is lined with stratified polyhedral epithelium. The compound gland can be classified as branched tubular mucosa, with PAS-AB-positive and PX-negative secretion (Fig. 2 D and E), characteristic of sublingual salivary gland. Mucous secretion is important due to its lubricating action, prevention of damage to the epithelium, aid in swallowing and defense against harmful bacterial colonizations (Arellano *et al.*, 1999; Radaelli *et al.*, 2000; Domeneghini *et al.*, 2005). Neutral mucus is denser than acidic mucus and therefore has a greater capacity to form protective barriers (Beamish *et al.*, 1972). The protection and lubrication provided by mucous secretion are crucial in view of the protractability of the tongue, especially in the snake *B. jararaca*, whose tongue moves within the tubular structure of the oral floor. No serous cells were observed in the glands of the studied specie. Therefore, there is no secretion of enzymes or other proteins, which differs from the observed in some species of reptiles, such as the American chameleon *Anolis carolinensis*, whose lingual epithelium presents cells with mucous and serous granules (Rabinowitz and Tandler, 1986).

According to scanning microscopy, on the dorsum of the tongue of *B. jararaca*, small longitudinal grooves are observed, in addition to transverse grooves on the dorsolateral edges (Fig. 3A). There are porous undulations along the dorsum and venter of the tongue (Fig. 3B), and the epithelial surface has a flattened but irregular appearance (Fig. 3C). The snake *B. jararaca* showed no lingual papillae and the tongue is almost smooth, which is certainly related to the fact that the tongue of this species, as well as for other snakes, are not important for the direct admission of food, but exclusively used in cooperative olfaction, together with the vomeronasal organ (McDowell, 1972; Gillingham and Clark, 1981). In snakes, the vomeronasal organ facilitates prey identification, courtship, and aggregation (Teshera and Clark, 2021). According to the review made by these authors, all viperids that hunt large prey inject venom to kill it, and this reliance on venom is associated with a suite of related physiological and behavioral traits that has facilitated the evolutionary success of this group. One of these traits is the process termed strike-induced chemosensory searching. This specialized form of chemosensory searching is a critical aspect of the overall phenotype that allows viperids to kill and ingest prey items that are not only well defended with teeth and claws, but also can be larger than the snake itself. The vomeronasal organ and the tongue comprise the accessory olfactory system, which is one of three major chemosensory systems possessed by squamates, the other two being gustation and the main olfactory system. The prioritization of the accessory olfactory system over the main olfactory system occurs in snakes and some lizards.

Corroborating the histological findings, in scanning electron microscopy the buccal floor had a velvety surface on its external face (facing the oral cavity) (Fig. 3D), certainly due to the presence of ciliated cells. On the internal face (facing the cavity where the tongue is housed), its surface was fragmented (Fig. 3E), due to the presence of polyhedral cells.

This study aimed to describe the morphological structure of the tongue of *B. jararaca*. The tongue of this species is protractile and bifid, with great exposure to the environment for the identification of odors and the location of prey, requiring protection mechanisms, such as stratified pigmented keratinized epithelium and mucus secretion. This structure

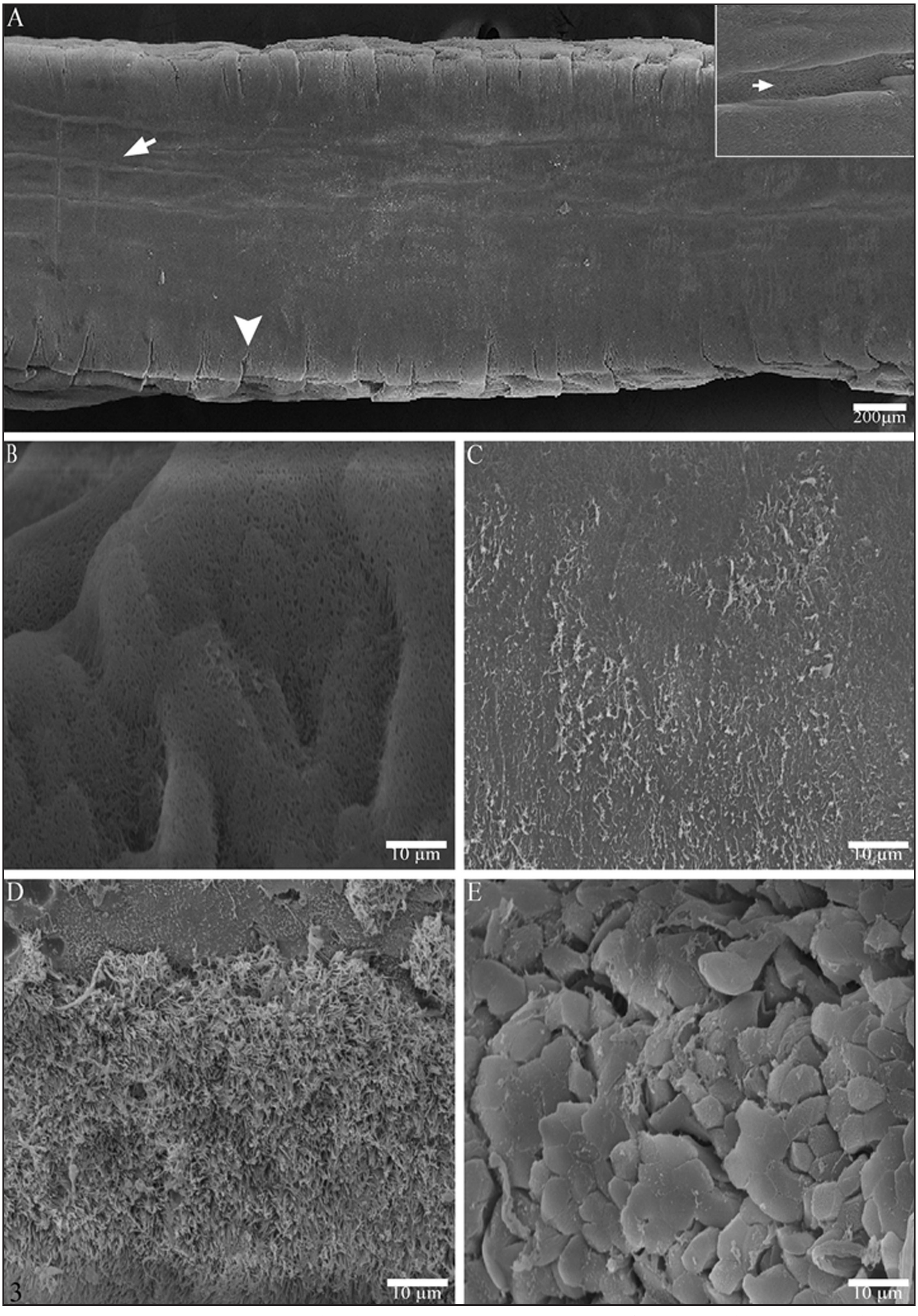

Figure 3. Scanning microscopy photos of *Bothrops jararaca* tongue. A) Dorsum of the tongue showing the small longitudinal grooves (arrow) and transverse grooves (arrow head). B) Undulations with a porous aspect on the lingual surface. C) Flattened epithelium of the lingual surface. D) Surface with velvety appearance, due to the presence of ciliated cells. E) Surface with a fragmented aspect, due to the presence of polyhedral cells.

has a strictly sensory function, with participation in the sense of smell, presenting a smooth surface devoid of papillae. The absence of taste buds on the tongue of this species reveals that this organ has no role in tasting. Thus, there is a clear relationship between the morphology of the tongue and the feeding behavior of this species of reptiles, and in the case of the *Bothrops jararaca* with an important olfactory sensory function for capturing prey.

Acknowledgments

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Nota

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Lizards *Polychrus acutirostris* (Sauria: Polychrotidae) and *Ameiva ameiva* (Sauria: Teiidae) as possible dispersers of the toxic plant *Lantana camara* (Verbenaceae)

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ABSTRACT
Several species of lizard consume considerable amounts of plant material, and some can be seed dispersers. Between April and May 2021, five lizards were found that had consumed fruit of *Lantana camara*, a female specimen of *Polychrus acutirostris* and four specimens (three males and one female) of *Ameiva ameiva*. *Lantana camara* (Verbenaceae) is a plant naturalized in Brazil found in all regions, including areas of Caatinga, Carrasco, and Cerrado. It has fruit resistant to the digestion of its consumers. In ruminants, its consumption is associated with toxicity, with hepatotoxic and photosensitization effects. Our data present, for the first time, the consumption of these fruit by these two lizard's species and suggest active feeding of *L. camara* as an additional feed resource, indicating that these lizards could be potential dispersers of *L. camara* seeds.

Key Words: Sauria; Carrasco; Diet; Sements, Fruit.

The diet of various lizard species comprises various groups of arthropods and a considerable amount of plant material (Cooper and Vitt, 2002), including fruits and seeds (Castro and Galetti, 2004). These animals can play an important (although still vague) role as seed dispersers (Valido and Olesen, 2019) or pollinators (Gomes *et al*., 2014), a role widely associated with birds and mammals (Santos *et al*., 2019).

The lizard, *Polychrus acutirostris* (Fig. 1D) Spix, 1825; (Polychrotidae), is known as "calango-cego," "papa-vento," and/or "Lagarto-preguiça" owing to its lethargic movement. It occurs in regions of Brazil such as the Cerrado and Caatinga, with diurnal, arboreal, and foraging habits of the sit-and-wait type (Vanzolini *et al*., 1980; Garda *et al*., 2012). The species, *Ameiva ameiva* (Fig. 1C) (Linnaeus, 1758; Teiidae), known as "calango verde" or "tijubina," occurs in different ecosystems, from the semi-arid regions of northeast Brazil to the humid forests of the Amazon Basin, and occurs in anthropized areas (Vitt and Colli, 1994). It presents a diurnal, terrestrial, and foraging feeding habit. The adults of this species are more abundant in the Caatinga in rainy periods (Sales *et al*., 2011; Freitas *et al*., 2012). Both species have an omnivorous diet, comprising small arthropods and plant material (seeds, flowers, and fruits). The ingestion of plant material by *P. acutirostris* can be considered passive, ingesting when consuming another component of the diet (Vitt, 1995; Cooper and Vitt, 2002; Garda *et al*., 2012).

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Belonging to the Verbenaceae family, the species, *Lantana camara* (Fig.1B), is a naturalized and non-endemic plant of Brazil, found in all regions, including areas of the Caatinga, Carrasco, and Cerrado (Silva and Salimena, 2015). They have fruit that change from green to dark red when ripe, and the seeds are resistant to the digestion of their consumers (Ghisalberti, 2000). In ruminants, their consumption is associated with toxicity with hepatotoxic and photosensitization effects (D'Oliveira *et al.*, 2018).

Between the months of April and May 2021 (the rainy season), an active search was carried out in an area of Carrasco in the Araripe Plateau, Crato, Ceará, Brazil (7°17´29.95″ S, 39°34´17.54″ W). Five lizards that had consumed fruit (it was possible to see the remains of the digested fruits) of *Lantana camara* were found: one female specimen of *P. acutirostris* (mass = 21g; snout vent length (SVL) = 118.8 mm) and four specimens (three males and one female) of *A. ameiva* (m = 60 g, 65.5 g, 64 g, and 92 g, SVL = 134 mm, 136.6 mm, 130.6 mm, and 139 mm, respectively). Before dissecting, the lizards were euthanized with 2% Lidocaine, fixed with 10% formalin, and preserved in 70% alcohol. The specimens were deposited in the Herpetological Collection of the Universidade Federal do Cariri (CHERP-UFCA). During the dietary analysis, we found that *A. ameiva* had ingested between two and seven *L. camara* fruit/seeds. Fourteen fruit/seeds had been consumed by *P. acutirostris* (Fig. 1A). This is the first record of consumption of this plant species by the Polychrotidae family. In Teiidae, there is a record of *L. camara* being consumed by the lizard, *Salvator merianae* (Diniz *et al.*, 2021).

In comparison with birds and mammals, lizards are not recognized as seed dispersers (Cooper and Vitt, 2002). The accumulation of new studies on the diet and behavior of animals indicates the opposite (Castro and Galetti, 2004; Fonseca *et al.*, 2012; Passos *et al.*, 2013; Gomes *et al.*, 2016). Although the consumption of plant material by *P. acutirostris* and *A. ameiva* is reported to occur accidentally, the considerable number of fruit and seeds (fourteen in a single individual) that we found suggests active feeding of the fruit, which could be an additional feed resource. This point of view may be suggested

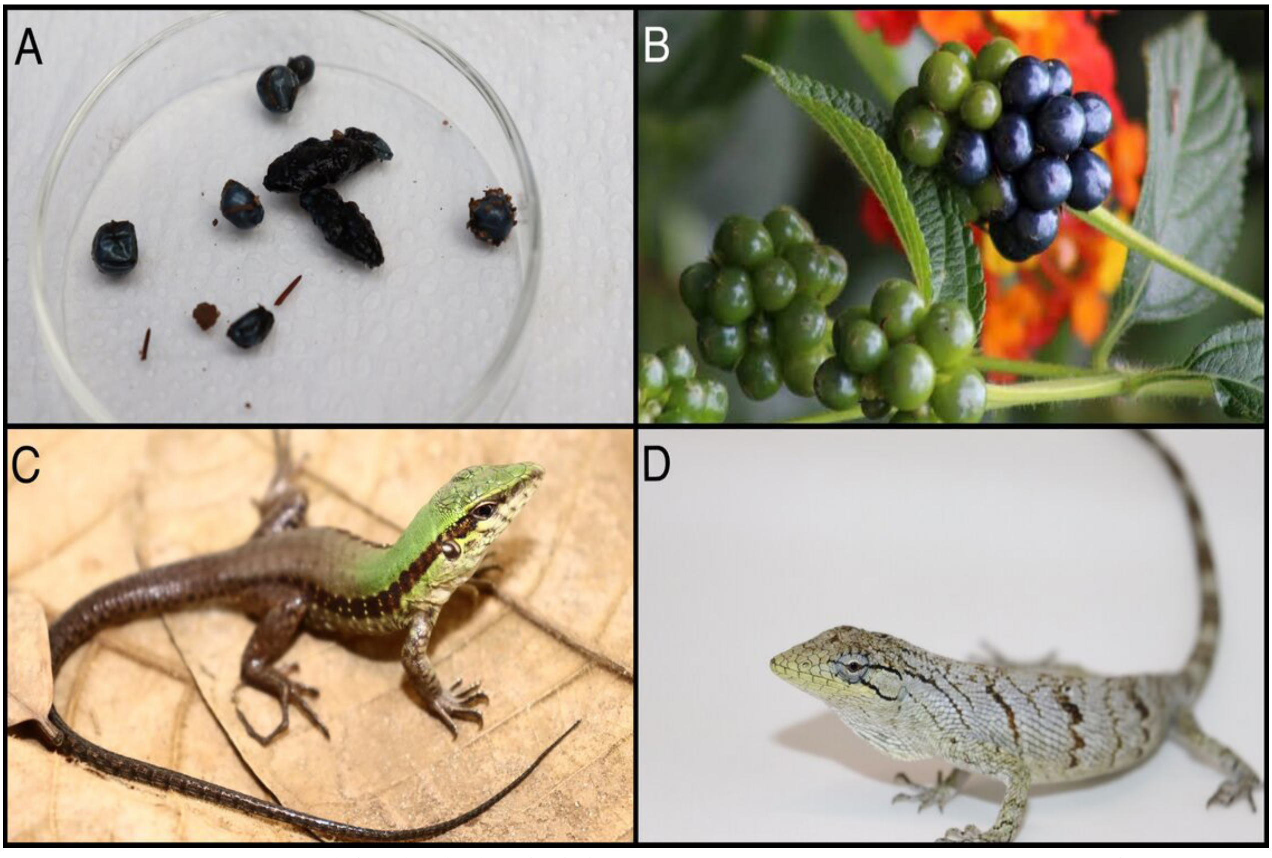

Figure 1. A= *Lantana camara* seeds found in the diet of *Polychrus acutirostris*; B= *L. camara* in natura; C= Specimen of *Ameiva ameiva* do Carrasco in the Araripe Plateau, northeastern Brazil; D=Specimen of *Polychrus acutirostris* in the Araripe Plateau, northeastern Brazil; Photos: ACD= Samuel Ribeiro; B= Jefferson T. Souza.

due to the fact that the remains of the diet included only larvae of lepidopterans, beetles and termites that are not seed carriers. In this way, these lizards are potential dispersers of these seeds, which sugests that additional studies should investigate the reproductive success that occurs with seeds that pass through the gastrointestinal tract of these lizards

The toxicity of *L. camara* in ruminants has been reported in cases of excessive ingestion by hungry animals, in which the alkaloids present in the leaves and green fruit produce neurological and hepatic symptoms (Sharma *et al.*, 2007). However, the possible toxicity to the two lizard species should be investigated by the composition of diets with the toxic plant, *L. camara*. The present study addresses questions of feeding behavior to elucidate the natural history of the above mentioned Polychrotidae and Teiidae lizards.

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Nota

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New records of malformations in Amazonian anurans

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ABSTRACT
Reports on morphological malformations in amphibians have been extensively documented worldwide. However, in the Amazon Forest records are recent and still a horizon to be explored, considering that they are potential cues of serious environmental problems. Here, we report five individuals of different species with limb morphological malformations (*Callimedusa tomopterna*, *Trachycephalus cunauaru*, *Dendropsophus marmoratus*, *Ceratophrys cornuta* and *Rhinella proboscidea*), collected between February 2020 and April 2022 during fieldwork expeditions at localities of Brazilian Amazonia, and a rare case of Siamese twins in a specimen of *Synapturanus ajuricaba* previously deposited at the Herpetological Collection of Instituto Nacional de Pesquisas da Amazônia. We provide radiographs and discuss about the affected bone structures. These records provide the first malformation cases known for adult specimens in the abovementioned species, and also in the family Phyllomedusidae.

Key Words: Morphological abnormalities; Radiography.

Records of morphological anomalies in amphibians have appeared in the literature for over 450 years (Gesner, 1554), but significantly increased over the last decades (Ouellet, 2000; Lannoo, 2008; Johnson *et al*., 2010; Henle *et al*., 2017). The occurrence of deformations in amphibians can be caused by many factors [*e.g.*, parasitic infestation (Johnson *et al*., 1999; Kiesecker, 2002), UV-B radiation (Blaustein *et al*., 1997; Ankley *et al*., 2000), microbial diseases (Sessions and Ruth, 1990), chemical and agribusiness pollution (Oullet *et al*., 1997; Taylor *et al.,* 2005; Lannoo, 2008; Koleska and Jablonski, 2016), and other disorders]. Malformations were considered the consequence of serious environmental problems, which highlights the importance of their reports (Johnson *et al*., 2003), especially when they occur in preserved natural environments (e.g., Mônico *et al*., 2019).

A variety of reports of morphological malformations in amphibians already documented in the literature involved different families, concerning species with varied habitat use and distributed in all continents (Henle *et al*., 2017). In the Amazon Forest however, these records are quite recent (Carvalho *et al*., 2008; Peloso, 2016; Ramalho *et al*., 2017; Sousa and Costa-Campos, 2016; Santos *et al*., 2017; Sousa and Costa-Campos, 2017; Oliveira-Souza *et al*., 2020; Souza *et al.,* 2021; Sodré *et al*., 2022), and appear as an unexplored topic.

Here, we report six individuals from different families with morphological malformations, five of them collected between February 2020 and April 2022 during fieldwork expeditions in localities of Brazilian Amazonia, and an additional specimen previously deposited at Herpetological Collection of Instituto Nacional Pesquisas da Amazônia (INPA-H), Manaus, Amazonas state, Brazil. The classification of the deformities follows Meteyer (2000) and Lanoo (2008). Collected specimens were also deposited in the INPA-H. Radiographs images were taken on a Nikon XT V 160 Electronics X-ray Inspection.

The first individual was an adult male of *Callimedusa tomopterna* (Cope, 1868) collected on 13 February 2020 at Reserva Florestal Adolpho Ducke (RFAD; 2°55’44.1”S, 59°58’34.2”W; WGS 84; 104 m elevation), Manaus municipality, Amazonas state.

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The individual (INPA-H 42556; SVL = 43.3 mm) presented ectromelia with ectrodactyly in the left hindlimb (Fig. 1A), characterized by shortening of the tibiale-fibulare, metatarsal bones fused, and absent phalanges (Figs. 1B, 1C). This nocturnal anuran is arboreal, and belongs to the Phyllomedusidae family, being widely distributed in the Amazon Forest (Frost, 2022). Its reproduction takes place in forest environments, the clutches are deposited in leaf nests over ponds, and when hatching, tadpoles fall into the water where they develop until metamorphosis (Lima *et al.*, 2012). We found this individual in the vegetation about 4 m above the ground level. Despite it lacks toes and adhesive discs, an important structure for treefrogs (Smith *et al.*, 2006), the malformation didn't seem to have hindered its natural growth and development. When captured and handled, the treefrog did not demonstrate any unusual behavior resulting from its malformation.

The second specimen was an adult male of *Trachycephalus cunauaru* Gordo *et al.*, 2013. We collected the specimen (INPA-H 43890; SVL = 65.3 mm) on 15 December 2020 in Iranduba municipality, Amazonas state (3°08'49.7"S, 60°13'40.3"W; WGS 84; 82 m elevation). This individual showed ectrodactyly in the left forelimb (Fig. 2A), characterized by the absence of fingers II, III and IV (Figs. 2B, 2C). *Trachycephalus cunauaru* is nocturnal, inhabits the canopy of "*terra firme*" forests and seasonally flooded forests and lay floating egg clutches in tree hollows filled with water, where tadpoles complete their development (Gordo *et al.*, 2013). We found

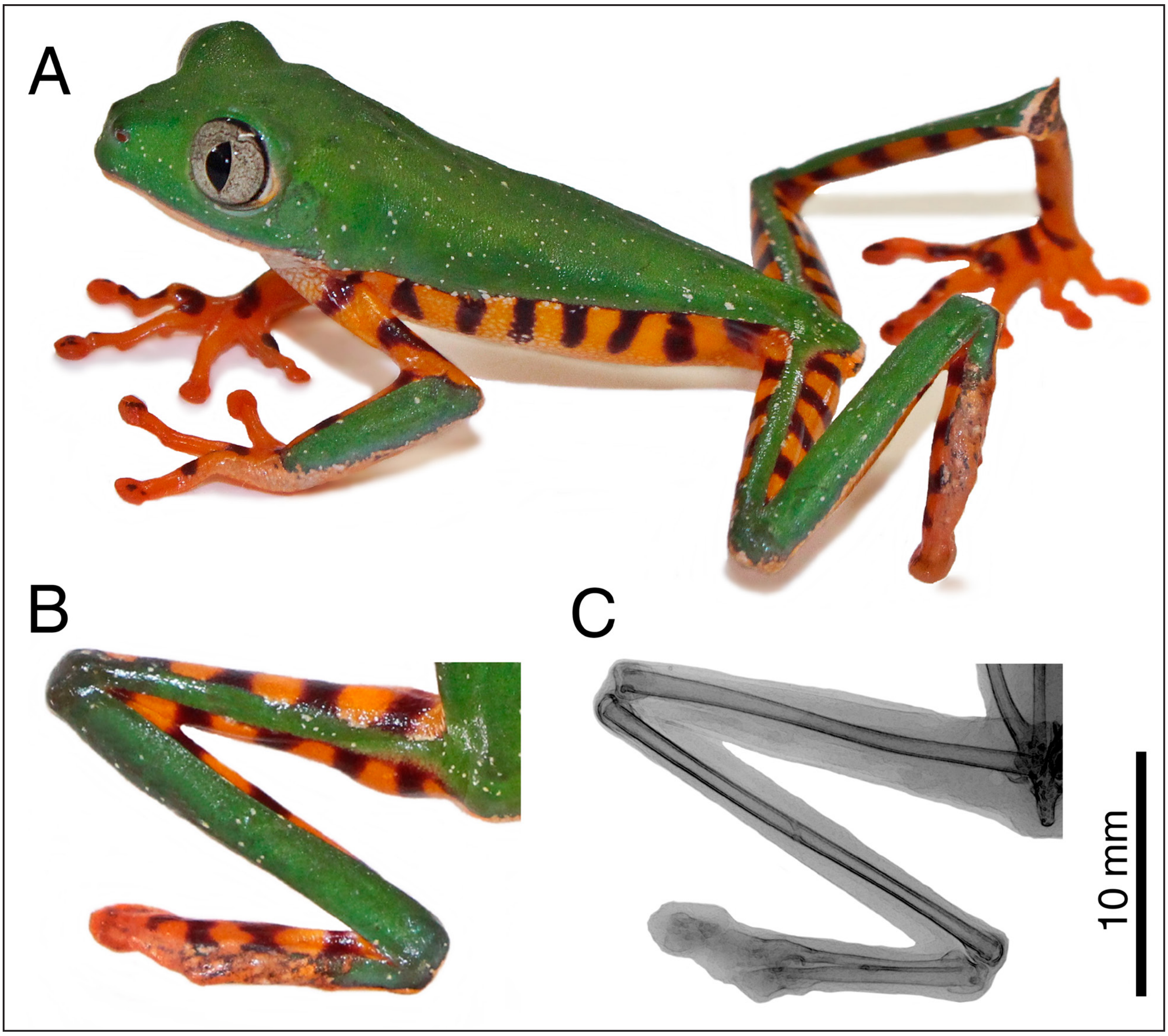

Figure 1. Adult male of *Callimedusa tomopterna* with ectromelia and ectrodactyly in the left hindlimb. (A) Natural posture of specimen alive; (B) Left hindlimb malformed; (C) Radiography.

the individual in a cavity with water, 70 cm above the ground, calling along with another adult male. When captured and handled, the frog did not show any unusual behavior generated by the malformation, despite the absence of three toes and the respective adhesive discs.

The third individual was an adult male of *Dendropsophus marmoratus* (Laurenti, 1768) (INPA-H 43886; SVL= 31.4 mm) collected on 17 December 2020 in Iranduba municipality, Amazonas state (3°08’49.7”S, 60°13’40.3”W; WGS 84; 77 m elevation). The individual showed syndactyly in the left forelimb (Fig. 3A), a malformation characterized by partial or complete fusion of one or more fingers. The radiography showed in this case indicated that all the fingers and phalanges were fused and united by the skin, without adhesive discs on fingers II, III and IV (Figs. 3B, 3C). We found the individual on the ground, calling next to a water pond. This species is nocturnal and inhabits the vegetation of humid areas on “*terra firme*” forests and primary alluvial forests (Morato *et al*., 2014). We could not observe whether the malformation was causing any unusual behavior, as we were aware of the malformation after the specimen was euthanized.

Figure 2. Adult male of *Trachycephalus cunauaru* with ectrodactyly in the left forelimb. (A) Natural posture of specimen alive; (B) Left forelimb malformed; (C) Radiography.

The fourth individual was a juvenile of *Ceratophrys cornuta* (Linnaeus, 1758) collected on 07 December 2021 in Vitória do Xingu municipality, Pará state (3°30’18.2”S, 51°53’07.4”W; WGS 84; 129 m elevation). The individual (INPA-H 44019, SVL= 56.5 mm) has ectrodactyly with brachydactyly in the right hindlimb (Figs. 4A, 4B), malformations characterized by absence of toes and phalanges, respectively (Figs. 4C, 4D). Even with the radiography, it was not possible to determine which fingers are absent or which are shortened (phalanges absent), and is only evident the absence of two fingers. The species is nocturnal, terrestrial and its reproduction occurs in ‘*várzea*’ wetlands forest ponds (Lima *et al.,* 2012). We observed the individual on the forest floor, and certainly the absence of spade like interdigital membranes must affect the burrowing capacity (Fabrezi *et al.,* 2019). The same malformations (i.e. ectrodactyly and brachydactyly) were already reported on its congener *C. cranwelli* (Medina *et al*., 2013).

The fifth individual was a *Rhinella proboscidea* juvenile (Spix, 1824) (unvouchered; SVL= 29.8 mm) collected on 03 May 2022 at Cachoeira da Suçuarana, Balbina Village, Presidente Figueiredo municipality, Amazonas state (1°54’29.5”S, 59°24’23.2”W). The individual has syndactyly with brachydactyly in the right forelimb (Figs. 5A, 5B), with fingers II, III and IV completely fused and shortened (absence of phalanges; Fig. 5C). The species is diurnal, inhabits

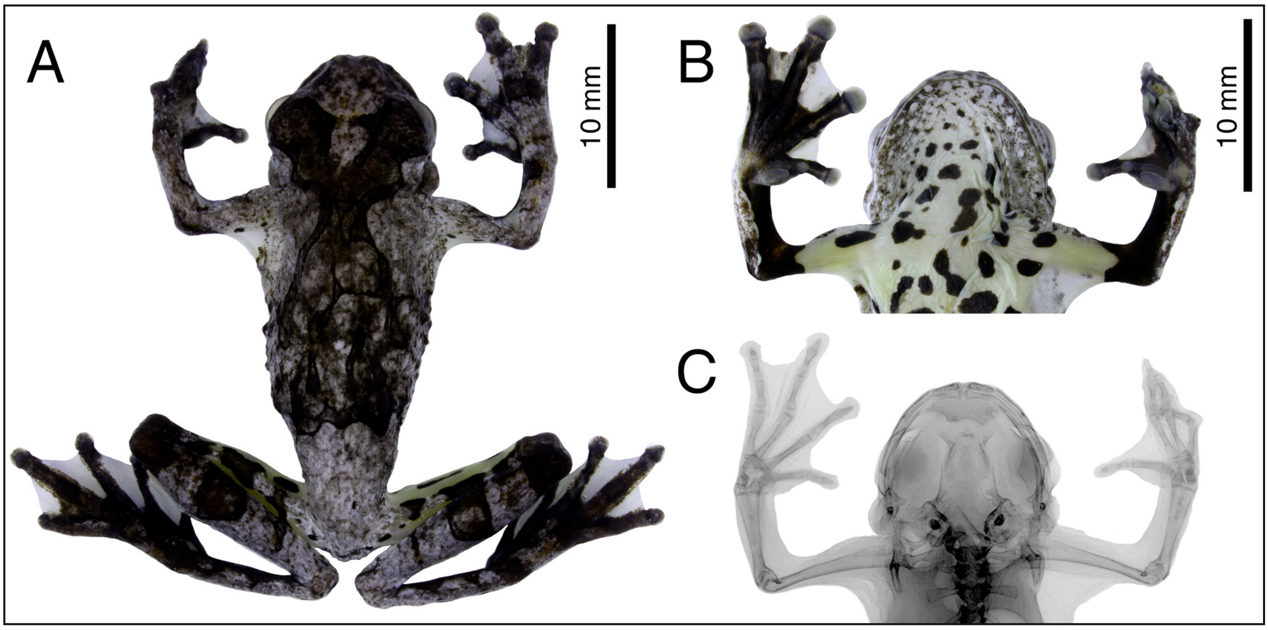

Figure 3. Adult male of *Dendropsophus marmoratus* with syndactyly in the left forelimb. (A) Dorsal view of specimen; (B) Forelimbs in ventral view; (C) Radiography.

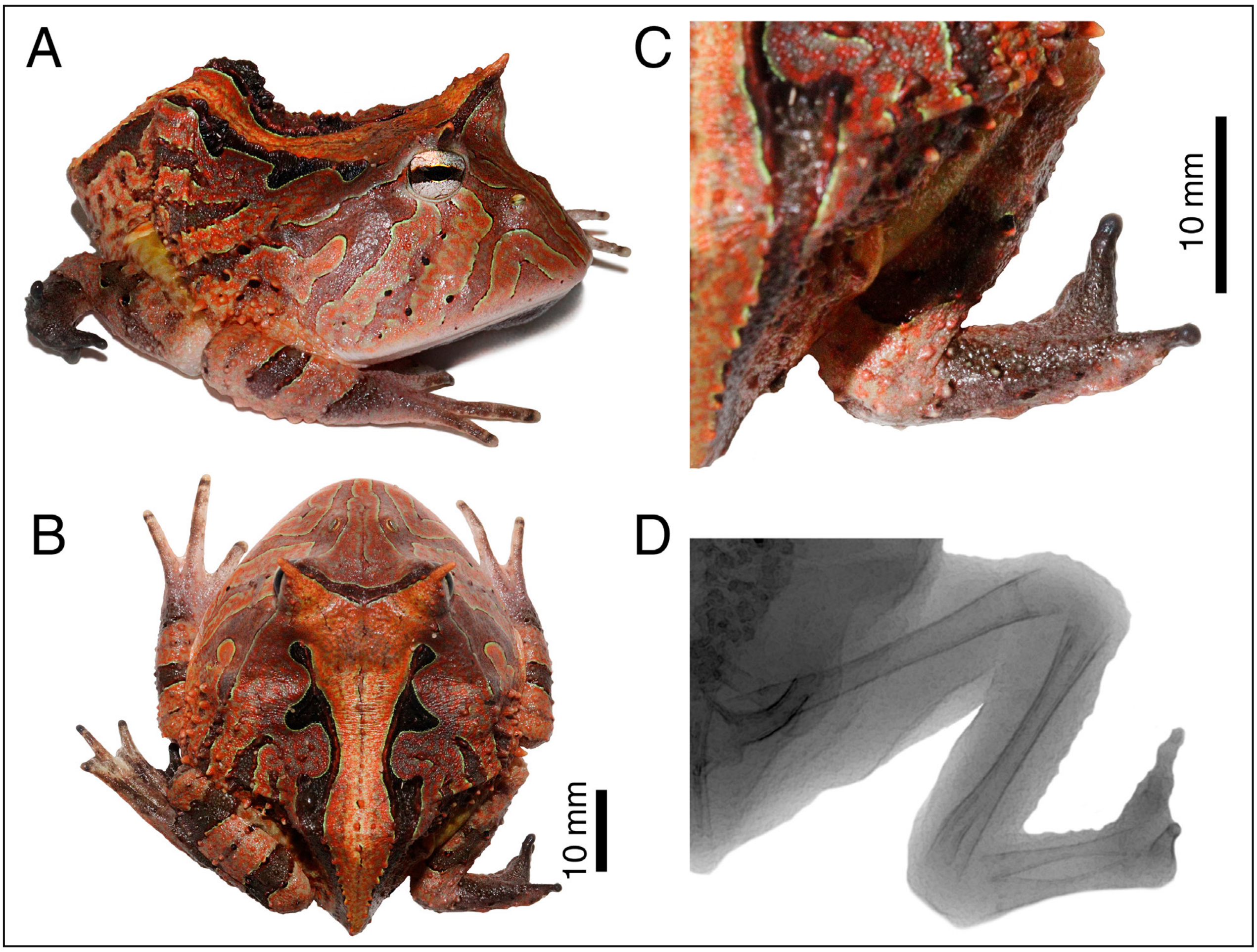

Figure 4. Juvenile male *Ceratophrys cornuta* with ectrodactyly and brachydactyly in the right hindlimb. (A) Natural posture of specimen alive; (B) Dorsal view of specimen; (C) Right hindlimb malformed; (D) Radiography.

the forest floor and breeds in ponds or seeps near the headwaters of streams (Lima *et al*., 2012). Souza *et al.* (2021) reported one case of anophthalmy (absence of an eye) for this species in Manaus region.

Finally, a juvenile specimen of *Synapturanus ajuricaba* Fouquet *et al*., 2021 (INPA-H 15837; SVL= 6.8 mm of left and 6.9 mm of right) collected in April 2004 at Reserva Florestal Adolpho Ducke by Marcelo Menin. The species is fossorial, with diurnal and nocturnal calling activity, and its reproduction occurs in burrows out of the water (Lima *et al*., 2012). The specimen is, an extremely rare case of Siamese twins (Figs. 6A, 6B), that has two heads, two vertebral columns, two pairs of forelimbs, but only one pair of hindlimbs. Among the few cases that have been recorded for the world, most of them derived from individuals born in captivity (Henle *et al.*, 2017), which makes this record even more relevant.

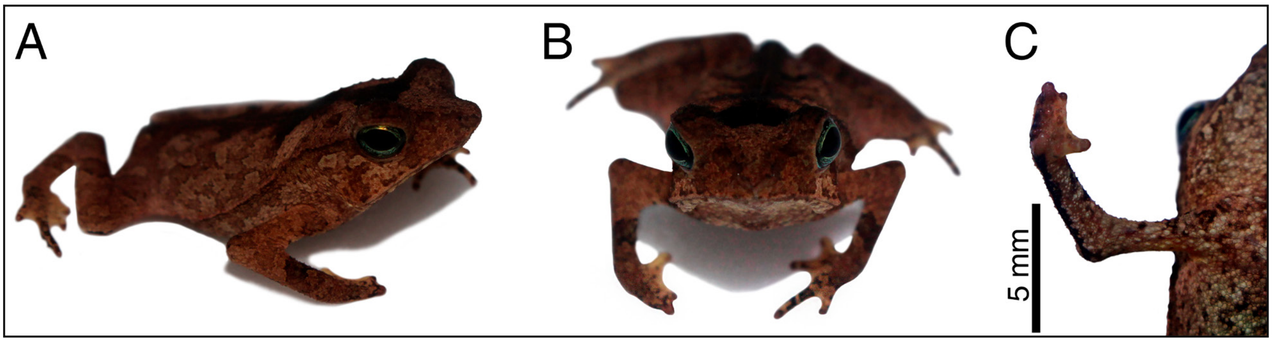

Figure 5. Juvenile of *Rhinella proboscidea* with syndactyly and brachydactyly in the right forelimb. (A) Dorsal view of individual alive; (B) Frontal view; (C) Right forelimb in ventral view.

The most common reports of the malformation in anuran species are from taxa that mostly depend on water for their development. The aquatic environments are an important vehicle for many of the main factors that can cause these anomalies (Henle *et al*., 2017). In our study, the development of five out of the six species is related to this environment, but the most impressive and severe case occurred in *S. ajuricaba*, a species that does not rely at all on water bodies for reproduction. This finding suggests that among our records different causes should have taken place for the induction of malformations.

Reports of anuran malformations in the Amazon Forest are alarming growing. Even when mining activities in the Amazon is associated to the release of many potentially teratogenic substances (Britson and Threlkeld, 1998; Prati *et al*., 2002), none of our records were close to mining areas. The use of mercury is common in the Brazilian for gold mining (Biller, 1994; Wasserman *et al*., 2007; Ramos *et al*., 2020), and its eventual link with anuran malformations as of other mining by-products should be carefully studied.

Our records represent the first known malformation cases on adults of the six studied species, and also for the family Phyllomedusidae and the genus *Synapturanus*.

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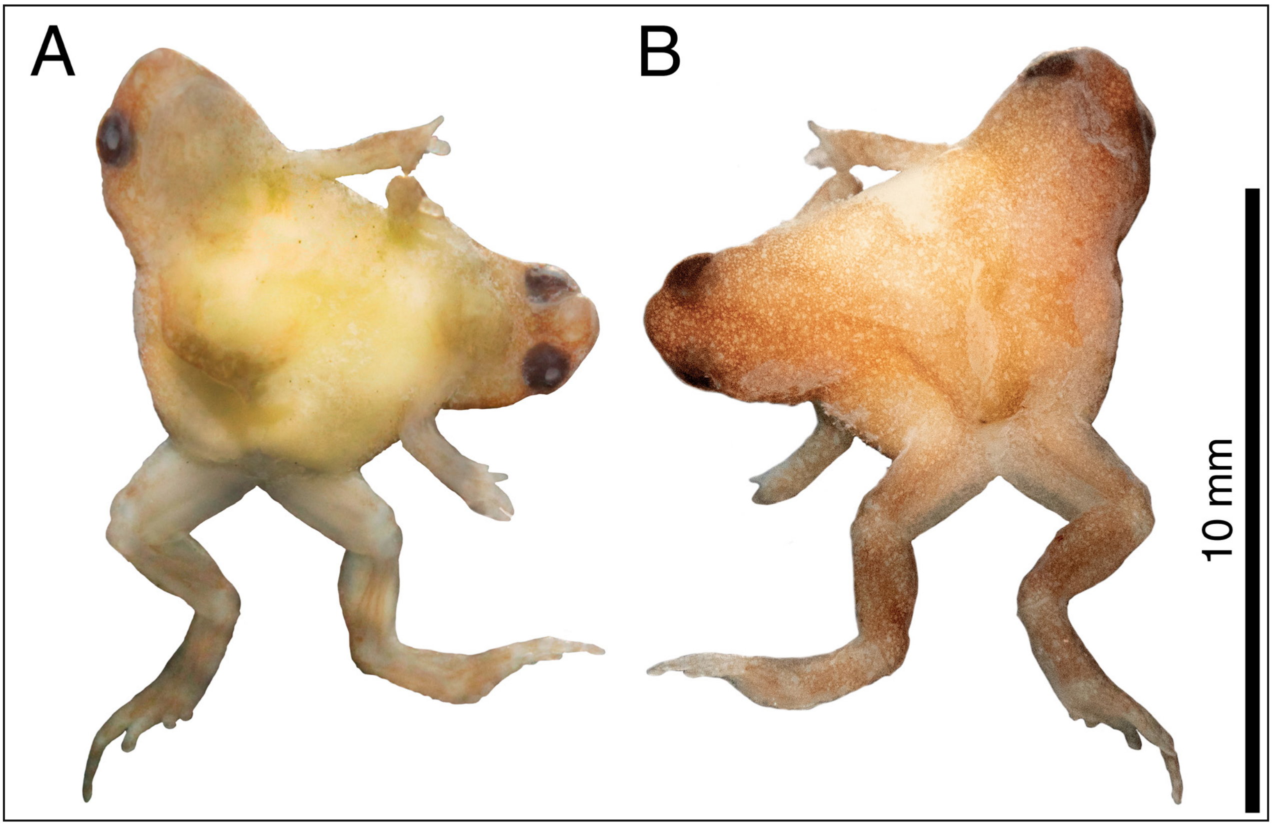

Figure 6. Juvenile Siamese twins of *Synapturanus ajuricaba*. (A) Dorsal view; (B) Ventral view.

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Novedad zoogeográfica

Cuad. herpetol. 37 (1): 85-88 (*2023*)

Primer registro documentado de *Phalotris cuyanus* (Cei, 1984) (Serpentes, Dipsadidae) en la provincia de La Rioja, Argentina

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Localidad— Aimogasta, Departamento Arauco (28°33’15.72”S; 66°47’59.14”O; 882 m s. n. m.), La Rioja, Argentina (Fig. 2). El ejemplar muestra las características determinantes de la especie: Macho de 485 mm de Longitud Total, 442 mm Longitud Hocico-Cloaca, 43 mm Longitud Cola, y 17,5 g de peso, 259 escamas ventrales, superando las medidas reportadas por Leynaud et al (2005); coleccionado en una finca de olivos el 7 de diciembre de 2018. Depositado en la colección de Zoología del Centro Regional de Investigaciones Científicas y Transferencia Tecnológica de La Rioja (CRILAR): CRILAR-HP 18 (Fig. 1).

Comentarios- Phalotris cuyanus o “falsa coral cuyana, coralina o collareja”, al igual que los demás integrantes del Género *Phalotris*, suelen ser de difícil detección por sus hábitos fosoriales y generalmente están poco representados en colecciones (Lema *et al*., 2005; Leynaud *et al*., 2005). Además, la falta de datos sobre su comportamiento, reproducción y dieta, hace que la especie se encuentre catalogada como “Insuficientemente Conocida” para la Argentina (Arzamendia, 2012).

P. cuyanus es considerada una especie endémica de la provincia biogeográfica del Monte (Leynaud *et al*., 2005, Jansen y Kohler, 2008, Scrocchi y Giraudo,

Figura 1. *Phalotris cuyanus*. Aimogasta, Departamento Arauco, La Rioja, Argentina. CRILAR-HP 18.

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2012), cuya distribución comprende las provincias de Mendoza, San Luis y San Juan (Quiroga, 2018; Williams 2020) y junto con *Phalotris punctatus*, son las dos especies más australes del grupo de *Phalotris tricolor* y las más cercanas geográficamente entre sí (Leynaud *et al*., 2005). Sin embargo, según los trabajos recientes de Videla et al. (2018) y Williams *et al*. (2020), la distribución de *P. punctatus* tiene su límite sur en la Provincia de Catamarca y *P. cuyanus* su límite norte en la Provincia de San Juan por lo que no conocíamos cual especie se encontraba en La Rioja.

Este nuevo registro representa una extensión en la distribución conocida de *P. cuyanus* de aproximadamente 370 km al norte en relación con la localidad más cercana conocida (Caucete, San Juan) (Martins y De Lema, 2017; Videla *et al*., 2018) (Fig. 2). Si bien hemos recibido comentarios de otros ejemplares observados cerca de la Ciudad de La Rioja Capital, no poseemos un registro fotográfico fehaciente de los mismos, por lo que no se incluyen estas localidades en esta nota.

Nuestro conocimiento de la distribución geográfica de algunas serpientes en el noroeste de Argentina ha crecido notablemente en las últimas décadas (Giraudo *et al*., 2012; Williams *et al*., 2021). No obstante, para las especies que habitan en la provincia de La Rioja, la información disponible sigue siendo insuficiente.

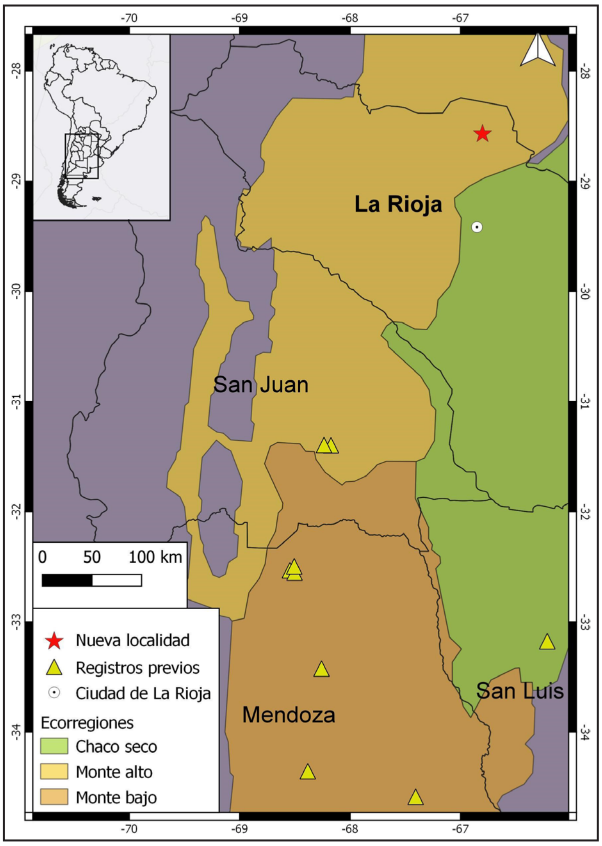

Figura 2. Localidades de *Phalotris cuyanus* previamente reportadas por Martins & De Lema (2017), y el nuevo registro en Aimogasta, La Rioja, Argentina.

Agradecimientos

Agradecemos a la Lic. Magda Brizuela por recuperar este ejemplar y acercarlo al instituto. A María Rivarola y el MV. Manuel Luque por colaborar con la identificación. A la Dra. Amelia Chemisquy por colaborar con la revisión de esta nota.

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Novedad zoogeográfica

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New record of an alien snake *Pantherophis guttatus* (Squamata: Colubridae) in the state of Pernambuco, Brazil

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Locality. - Municipality of São Lourenço da Mata (7 °57'55.4"S 35°03'52.2"W) (29°47'38.5"S, 67°59'05.8"W), in the Area de Proteção Ambiental Aldeia Beberibe. Data: May 3, 2020. Sighted and photographed by the Interdisciplinary Amphibian and Reptile Laboratory Team. Images deposited in the Herpetological Collection of the Interdisciplinary Laboratory of Amphibians and Reptiles (LIAR 123).

Comment.- The Convention on Biological Biodiversity (CBD) defines exotic species as "those which occur outside past or present natural ranges". Invasive alien species, therefore, are defined as "species whose introduction and/or dispersion threaten biological diversity". These can be introduced intentionally and unintentionally, through introduction and dispersion pathways and vectors (Sampaio and Schmidt, 2013; Davis, 2009).

Biological invasions are considered one of the main threats to biodiversity, resulting in native population declines, environmental, economic and human health risks, as well as ecosystemic risks (GISP, 2007; Leão *et al.*, 2011; Vasconcellos, 2001). Among invasive vertebrates, reptiles are not very representative in Brazilian lists, in terms of the number of taxa recorded in natural environments, with lizard and testudines representatives both reported in protected areas (Instituto Hórus, 2021). Concerning snakes, few records are available of those introductions (Fonseca *et al*, 2014; Assis *et al.*, 2018), however a well-established list of exotic pets is available on social networks (Magalhães and São-Pedro, 2012; Evangelista, 2015; Alves *et al.*, 2019) is noted for this group, comprising an imminent risk of introduction (Eterovic and Duarte, 2002; Fonseca *et al.*, 2017).

The snake *Pantherophis guttatus* (Linnaeus, 1766), popularly known as the corn snake, it is a semi-arboreal snake belonging to the Colubridae family, small in size (about 1800 mm in total length), distributed throughout North America, inhabiting forested, open and urban environments preying on small mammals, birds, amphibians and reptiles (Fisher and Csurhes, 2009; Hammerson 2007). It is sold as a pet in many countries and, according to Magalhães and São-Pedro (2012), pet escapes and abandonments are the main cause of this species introduction in natural environments. In Brazil, it has been registered so far by Fonseca *et al.* (2014) in a Conservation Unit and in an urban area located in a residential complex, both in the Atlantic Rainforest domain in the state of Bahia. The aim of this study was, therefore, to document another *P. guttatus* record, the first in a natural environment in the state of Pernambuco.

On May 3rd 2021 one corn snake individuals was rescued by the Environmental Military Police - CIPOMA team, in an open natural area within a Sustainable Use Conservation Unit located in the Atlantic Rainforest domain, the Area de Proteção Ambiental Aldeia Beberibe, an environmental protection area in the municipality of São Lourenço da Mata (7 °57'55.4"S 35°03'52.2"W) (Fig. 1). The specimen was measured with a measuring tape, weighed using a digital scale, sexed and later sent to a containment box awaiting disposal, to be carried out by the Centro de Triagem e Reabilitação de Animais Silvestres - CETRAS Tangará. The specimen was a male, its total length was 65 cm, 54 cm snout vent length and 11 cm tail length, weighing 36 g (Fig. 1).

Pantherophis guttatus as an exotic traded species constitutes a crime according to Brazilian law (Fonseca *et al.*, 2017). The records of corn snakes

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Figure 1. A and B – Map indicating the *Pantherophis guttatus* record at the Aldeia Beberibe Conservation Unit; C – Specimen screening (biometry); D and E – Full body image of the animal.

reported here may have originated from the illegal trade practices of intentionally released pets or them escaping captivity, corroborating Alves *et al* (2019). According to Assis *et al.* (2018) this species holds generalists ecological habits, this way the lack of control in its creation and comercy of these exotic species may result in an invasive exotic species in the atlantic rain forest in a short extent of time.

In general, *P. guttatus* records in natural environment are scarce, although the unnatural presence of the species in the Brazilian territory through the pet market has been previously reported and constitutes a threat (Alves *et al.*, 2019). As it feeds on prey similar to native species, this species can pose a threat to the local avifauna (Kraus and Carvalho 2001; Hayes *et al.*, 2004), and other snake species with regard to *Cryptosporidium* parasitism (Xiao *et al.*, 2004). According to Fonseca *et al.* (2017), as species *Pantherophis guttatus*, *Python regius and Python bivittatus* were identified as species exhibiting greater probability of establishment and dispersion, considering species distribution model results and specific attribute evaluations. The ecological niche modeling has been widely used in the attempt to foresee potential invasion sites and is an important tool to conservational biology.

In Brazil, the issue of biological invasions still requires further research and is not widely known by most Conservation Units (CUs) managers. CUs are protected territorial spaces which aim to maintain biodiversity, natural and cultural resources through legal and institutional mechanisms (Brito, 2008) and the introduction of exotic species should be the target of attention and management to "monitor", "prevent", "control" or eradicate exotic species that threaten ecosystems and their biota (CBD, Article 80).

Risk analysis protocols are some of the available strategies for screening potential invaders, which can also be applied to assess the risk of introduced species and limit or prohibit their importation or trade (Instituto Horus, 2021). Detecting potential invaders before they are introduced is necessary to prevent the spread and impact that invasive alien species can have on natural ecosystems (Rosa *et al.*, 2018).

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Novedad zoogeográfica

Cuad. herpetol. 37 (1): 92-96 (*2023*)

Nuevos registros de *Amphisbaena fuliginosa* Linnaeus, 1758 (Squamata: Amphisbaenidae) para la Amazonia Boliviana y primer registro para el departamento del Beni

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Localidad.— Estado Plurinacional de Bolivia, Departamento del Beni, Provincia Vaca Diez, Municipio de Riberalta, calle Tipa. Lat. -11.012784, Long. -66.067789, 143 m s.n.m. Fecha: 29 de octubre de 2019. Colectado por Alina Monje Justiniano y Vincent A. Vos. Depositado en la colección de herpetología del Centro de Investigación de Recursos Acuáticos de la Universidad Autónoma del Beni José Ballivián (CIRAH-951). El espécimen CIRAH-951 (Fig. 1A-B) mide 22.2 cm de longitud hocico cloaca (LHC) y 3.2 cm de longitud de la cola (LC), presenta 207 anillos del cuerpo, 26 anillos en cola, 20/20 segmentos en el anillo medio del cuerpo, 7 poros preanales. Tiene un patrón de color de fondo blanco; marcas dorsales negras, transversales, generalmente sin cruzar el vientre. Estado Plurinacional de Bolivia, Departamento del Beni, Provincia Vaca Diez, Municipio de Riberalta, barrio Los Tajibos, calle Cuta. Lat. -11.024988, Long. -66.047035, 144 m s.n.m. Fecha: 24 de septiembre de 2022. Colectado por Ruben D. Layme. Depositado en la colección de herpetología del Centro de Investigación de Recursos Acuáticos de la Universidad Autónoma del Beni José Ballivián (CIRAH-1000). El espécimen CIRAH-1000 (Fig. 1C-D) mide 22.6 cm de LHC y 4 cm de LC, presenta 211 anillos del cuerpo, 26 anillos en cola, 20/22 segmentos en el anillo medio del cuerpo, 7 poros preanales. Tiene un patrón de color de fondo blanco; marcas dorsales negras, transversales, generalmente sin cruzar el vientre. Estado Plurinacional de Bolivia, Departamento del Beni, Provincia Vaca Diez, Municipio de Riberalta, carretera a la comunidad de Santa María. Lat. -11.107513, Long. -65.947656, 177 m s.n.m. Fecha: 28 de septiembre de 2022. Colectado por Vincent A. Vos. Depositado en la colección de herpetología del Centro de Investigación de Recursos Acuáticos de la Universidad Autónoma del Beni José Ballivián (CIRAH-1001). El espécimen CIRAH-1001 (Fig. 1E-F) mide 28.8 cm de LHC y 1.4 cm de LC, presenta 199 anillos del cuerpo, 6 anillos en cola, 22/24 segmentos en el anillo medio del cuerpo, 8 poros preanales. Tiene un patrón de color de fondo blanco; marcas (algo manchado) dorsales negras, transversales, varias de las cuales cruzan al vientre. Los dos primeros especímenes fueron encontrados muertos en el área urbana de la dicha ciudad, uno de ellos fue encontrado en el patio de una vivienda particular y el otro en la calle Cuta, mientras el tercero también hallado muerto en la carretera rural hacia la comunidad de Santa María (aproximadamente a 12 km al sur este de la ciudad), corresponde a una zona boscosa degradada con cultivos.

Comentarios.— En Bolivia, la familia Amphisbaenidae está compuesta por 13 especies, distribuidas particularmente en tierras bajas del bosque Amazónico, Cerrado, bosque Chiquitano y Yungas (Fugler, 1989; Dirksen y De la Riva, 1999; Embert, 2007); cuatro de estas especies (*Amphisbaena cegei*, *A. darwinii*, *A. silvestrii* and *A. slateri*) presentan distribución algo restringida en el territorio boliviano, mientras el resto aparentemente tienen amplia distribución, entre ellas *Amphisbaena fuliginosa* (Embert, 2007). El rango de distribución de ésta última es amplia en el Neotrópico (Murphy *et al.*, 2010; Roberto *et al.*, 2021), abarca gran parte de la región Amazónica y parte del Cerrado (Chalkidis *et al.*, 2002; Vanzolini, 2002a, b; Lemos y Facure, 2007; Nogueira-Costa *et al.*, 2013; Costa-Campos *et al.*, 2014; Roberto *et al.*, 2014; van der Hoek y Jarrín-V, 2017; Roberto *et al.*, 2021). Es considerada una especie politípica, que agrupa a cinco subespecies (Vanzolini, 1951; Vanzolini, 2002a; Roberto *et al.*, 2021).

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Figura 1. Especímenes de *Amphisbaena fuliginosa* de la ciudad de Riberalta, Beni, Bolivia. A-B: vista dorsal y ventral del espécimen CIRAH-951; C-D: vista dorsal y ventral del espécimen CIRAH-1000; E-F: vista dorsal y ventral del espécimen CIRAH-1001.

El diseño y coloración de *Amphisbaena fuliginosa*, así como algunas características merísticas de la especie varía de una región a otra en Sudamérica (Vanzolini, 1951; Vanzolini, 2002a; Roberto *et al.*, 2021). Predominantemente de hábitos fosoriales y ocasionalmente sale a la superficie (Vanzolini, 2002a, b; Lemos y Facure, 2007; Nogueira-Costa *et al.*, 2013). Se alimentan principalmente de hormigas (Formicidae) y termitas (Isoptera) (Esteves *et al.*, 2011).

Amphisbaena fuliginosa fue reportada por primera vez en Bolivia para el departamento de La Paz por Boettger (1891) y Vanzolini (2002a), posteriormente para Chuquisaca por Rhodes (1963), Santa Cruz por De la Riva (1993), Montero *et al.* (1995) y Scrocchi y Gonzáles (1996) y Cochabamba por Dirksen y De la Riva (1999), pero aun no reportada para el departamento del Beni, a pesar de ser en el segundo departamento más grande de Bolivia. Asimismo, solamente cuatro (*Amphisbaena alba*, *A. angustifrons*, *A. bolivica* y *A. camura*) de las 13 especies, están reportadas para el departamento del

Beni (Dirksen y De la Riva, 1999).

Los especímenes de *Amphisbaena fuliginosa* representan un aporte importante para el mejor conocimiento sobre la distribución de la especie en Bolivia (Fig. 2). Sin embargo, comparando los caracteres merísticos de los tres especímenes del presente estudio con los descritos por Vanzolini (2002a) para las diferentes subespecies de *A. fuliginosa*, resulta que dos de estas (CIRAH-951, 1000) coinciden con *A. fuliginosa bassleri* y una (CIRAH-1001) con *A. fuliginosa wiedi* (excepto en los anillos de la cola) y no así con el patrón de color descritos por este autor. Además, constituyen los primeros registros para el departamento del Beni (Riberalta), ciudad ubicada a orillas del río Beni en la Amazonia boliviana y una de las regiones escasamente estudiadas, pero altamente diversa.

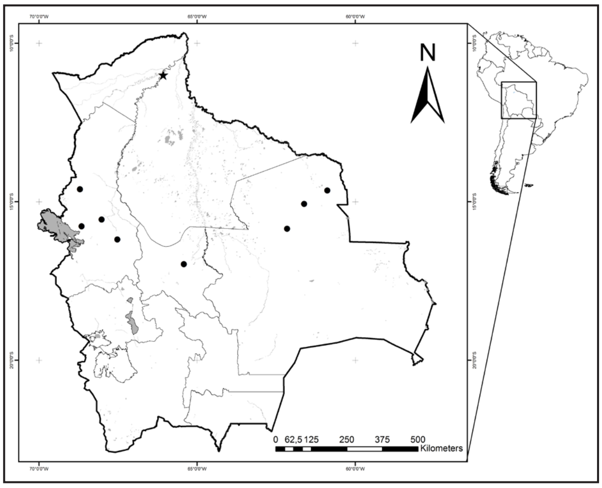

Figura 2. Distribución de *Amphisbaena fuliginosa* en Bolivia. Nuevos registros para el departamento del Beni (estrellas) y registros previos de la especie para Bolivia (puntos negros) basado en Dirksen y De la Riva (1999) y Vanzolini (2002a). En el mapa, consideramos la localidad de Charobamba mencionado por Vanzolini (2002a) y no el mencionado por Dirksen y De la Riva (1999).

Las "cutuchis" como comúnmente se las conoce a las diferentes especies de amphisbenidos en Bolivia, son confundidas generalmente con serpientes o gusanos por su forma serpentiforme, incluso existen actitudes erróneas, creencias y mitos sobre estos organismos que es común entre la poblacion del oriente boliviano, creando una fama equivocada como especie peligrosa, razón por la cual son comúnmente matadas (Rivas *et al.*, 2022). En consecuencia, diversas organizaciones e instituciones (museos, centros de investigación y otros) están encarando campañas de difusión y concienciación, a través de talleres informativos y educativos (presencial y virtual), distribución de guías de campo entre otro material de difusión, con la finalidad de evitar estas acciones de la gente sobre estos reptiles.

Agradecimientos

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Novedad zoogeográfica

Cuad. herpetol. 37 (1): 97-98 (*2023*)

Primer registro de *Amphisbaena bolivica* (Squamata: Amphisbaenidae) en San Luis, Argentina

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Localidad.— República Argentina. Provincia de San Luis, Departamento Ayacucho, localidad de Lujan, Reserva Natural Estricta Quebrada de las Higueritas (32°23'43.8"S; 65°55'02.7"W). Fecha de colección: enero de 2021. Colector: Samuel Olivieri. El ejemplar fue depositado en la colección CH-UNSL (Amphisbaena bolivica-CH-UNSL 0622).

Comentarios.— El género *Amphisbaena* incluye una gran cantidad de especies, y se distribuye ampliamente por América del Sur y Centroamérica (Vanzolini, 2002). En Argentina se reconocen 10 especies (Avila *et al.* 2013), una de las cuales es *A. bolivica*, destacándose por su gran tamaño. Esta especie se distribuye en el centro-norte de Argentina, y ha sido registrada en las provincias de Catamarca, Chaco, Córdoba, Formosa, Jujuy, La Rioja, Salta, Santa Fe, Santiago del Estero, Tucumán (Montero, 1996; Abdala *et al.*, 2012) y recientemente se ha registrado por primera vez en San Juan (Gómez Alés *et al.* 2019). Su estado de conservación es considerado como No Amenazada por Abdala *et al.* (2012) y como Preocupación Menor según la IUCN (Arzamendia *et al.* 2016).

Un ejemplar, identificado como *Amphisbaena bolivica* Mertens, 1929, fue colectado al norte de la provincia de San Luis, en la Reserva Natural Estricta Quebrada de las Higueritas (Fig. 1). Esta reserva posee categoría 1 según la ley provincial N° IX-0309-2004, otorgándole el término de "reserva natural estricta" ya que tiene como objetivo preservar áreas significativas por la excepcionalidad de sus ecosistemas, de sus comunidades naturales o de sus especies de flora y fauna y donde la interferencia humana directa se reduce al mínimo. La reserva en cuestión, conserva la ecorregión de Chaco Serrano, la cual se distingue por la presencia de jarilla hembra (*Larrea divaricata*), tala (*Celtis ehrenenbergiana*), algarrobo chileno (*Prosopis chilensis*), algarrobo negro (*Prosopis nigra*) y brea (*Parkinsonia praecox*) (Cabrera, 1976; Oyarzabal *et al.*, 2018). El ejemplar fue encontrado muerto en la calzada asfáltica que cruza por el interior de la reserva natural, y presentaba el extremo caudal aplastado, se desconocen las causas de su muerte, pero se presume que pudo ser por un vehículo.

Montero (1996) y Vanzolini (2002) distinguen a *A. bolivica* de otras especies similares gracias a que presenta coloración dorsal marrón- marrón sepia, mientras que la región ventral es blanquecina y la cabeza blanca, característica distintiva de los ejemplares de esta especie encontrados en Argentina. El individuo hallado en San Luis presenta dichas características de coloración (Fig. 2), y posee 214 anillos en el cuerpo y 56 escamas por anillo en la zona media del cuerpo, coincidiendo con los valores

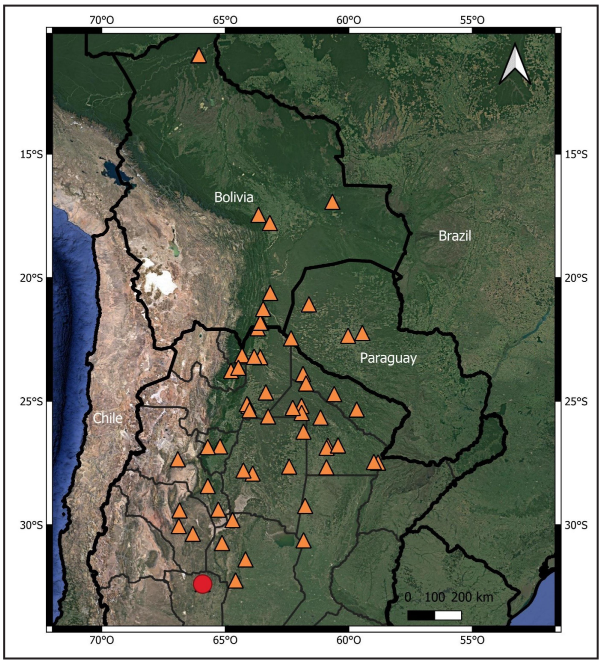

Figura 1. Mapa del sur de Sudamérica, donde se indican los registros previos de *Amphisbaena bolivica* en Argentina, Bolivia y Paraguay (triángulos naranjas). Se indica el nuevo registro en el norte de San Luis (círculo rojo).

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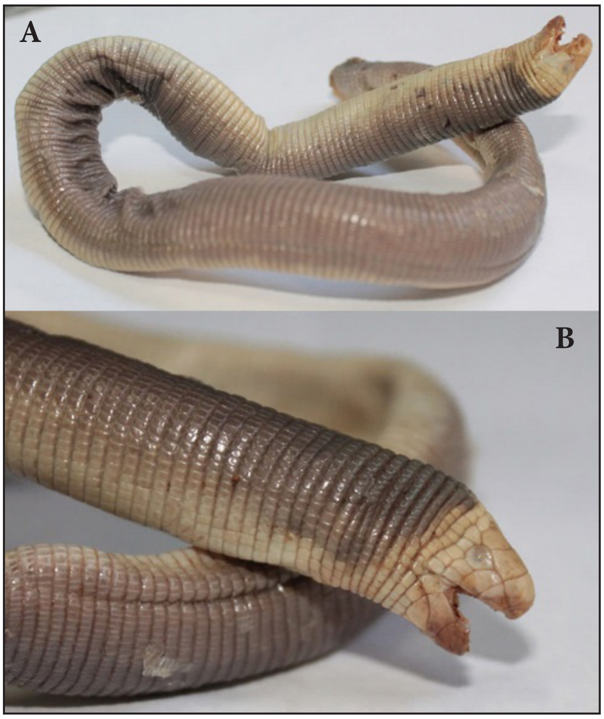

Figura 2. Fotografías del ejemplar de *Amphisbaena bolivica* encontrado en la Reserva Natural Estricta Quebrada de las Higueritas, San Luis, Argentina. A) Imagen del cuerpo completo, donde se observa a diferencia de coloración entre dorso y vientre. B) Detalle de la cabeza de coloración blanca, característica de la especie en Argentina.

indicados para la especie (Vanzoini, 2002; Montero *et al*., 2019). No fue posible contar la cantidad de anillos en la zona posterior del cuerpo, ya que dicha parte del ejemplar se encuentra dañada.

Este nuevo registro permite aumentar a 12 las provincias en las cuales se encuentra la especie, indicando, además, su presencia en un área protegida. Con *A. bolivica* se contabilizan, hasta la fecha, cuatro especies del género *Amphisbaena* en la provincia de San Luis (Abdala *et al*. 2012). Dado que los trabajos de prospección de fauna son escasos. Es necesario incrementar los esfuerzos de muestreo enfocados en este grupo en la provincia, principalmente dentro de áreas protegidas.

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Novedad zoogeográfica

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Nuevos registros de serpientes en el Chaco biogeográfico de Argentina

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Drymarchon corais corais (Boie, 1827).

Localidad 1: Argentina, Provincia de Formosa: Departamento Bermejo: (1) Atropellado sobre Ruta Nacional 81 (24°27'31.76"S; 60°57'24.87"O, WGS84, 147 m s.n.m.), 12/1997, colectores: Roberto Aguirre y Eduardo F. Schaefer, depositado en la Colección Herpetológica "Lic. Blanca Beatriz Álvarez" de la Universidad Nacional del Nordeste (UNNEC 13739).

Localidad 2: Argentina, Provincia de Formosa: Departamento Bermejo: Fotografiado atropellado en Ruta Nacional 81 (24°30'51.26"S; 60°52'27.52"O, WGS84, 146 m s.n.m.), 23/12/2016, observadores: José L. Acosta, Cynthya E. González, Marta I. Duré, Cynthya E. González, Soledad A. Palomas, E.F. Schaefer (UNNEC-A 00046).

Localidad 3: Argentina, Provincia de Formosa: Departamento Matacos: Atropellado sobre Ruta Provincial 39 (24°7'28.52"S; 61°56'54.53"O, WGS84, 184 m s.n.m.), 27/07/2017, colectores: J.L. Acosta, C.E. González, M.I. Duré, S. Palomas, E.F. Schaefer (UNNEC 13801).

Localidad 4: Argentina, Provincia de Formosa: Departamento Matacos: Ruta Provincial 9 (24°16'46.87"S; 61°51'36.62"O, WGS84, 182 m s.n.m.), 30/01/2019, colectores: J.L. Acosta, C.E. González, S.A. Palomas, E.F. Schaefer, José J. Valdés (UNNEC 13613).

Localidad 5: Argentina, Provincia de Formosa: Departamento Matacos: Observado y fotografiado dentro de la Reserva Natural Formosa (24°14'5.63"S; 61°49'51.59"O, WGS84, 180 m s.n.m.), 11/03/2015, observadores: Mónica Pereira, Ezequiel Navarro, Ariel R. González (UNNEC-A 00047).

Localidad 6: Argentina, Provincia de Salta, Departamento de San Martín: Dragones (23°15'2.95"S; 63°20'29.96"O), 01/2017, colector: Agustín Scanferla, depositado en la Colección Herpetológica del Instituto de Bio y Geociencias de Salta (IBIGEO 6655).

Comentarios: El género *Drymarchon* se distribuye desde el sur de los Estados Unidos hasta Argentina (Peters y Orejas Miranda, 1970). La subespecie *D. c. corais*, se encuentra en Venezuela, Brasil, Bolivia y Paraguay hasta el norte de Argentina, donde sus registros se limitan a la región chaqueña de las provincias de Salta, Chaco y Formosa (Peters y Orejas Miranda, 1970; Williams y Franccini, 1991; Bergna *et al.*, 1992; Giraudo y Scrocchi, 2002; Giraudo *et al.*, 2012).

Además de los 6 ejemplares verificados que presentamos de *D. c. corais,* de los cuales 4 fueron colectados y 2 son registros fotográficos, obtuvimos 5 observaciones adicionales de ejemplares no fotografiados: 2 dentro de la Reserva Natural Formosa (RNF) (24°18'38.38"S; 61°48'39.32"O; 24°18'38.59"S; 61°48'45.18"O), 2 en cercanías de la

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RNF (24°20'2.84"S; 61°42'2.65"O; 24°13'40.83"S; 61°56'29.70"O) y 1 en el Aeropuerto de Las Lomitas (24°43'46.27"S; 60°33'3.07"O); todos de la Provincia de Formosa en el distrito Chaco Seco (Fig. 1).

Giraudo y Scrocchi (2002) notaron que *D. c. corais* fue mencionada para Formosa por Bergna *et al.* (1992) sobre la base del ejemplar UNNEC 175 de la localidad de La Rinconada, no obstante en un trabajo posterior sobre serpientes de Formosa, Chaco y Corrientes basado en la colección UNNEC no se menciona a la especie (Alvarez *et al.*, 1996). Revisamos el ejemplar UNNEC 175 y corresponde a la especie *Erythrolamprus albertguentheri*, por ende, los nuevos registros presentados constituyen las primeras citas con material de referencia para la Provincia de Formosa. Por su parte, Williams y Franccini (1991), mencionan a esta especie para la provincia del Chaco, sin hacer comentarios respecto a localidades o material de referencia. Además, Giraudo y Scrocchi (2002) citan esta especie en Salta por primera vez en base a dos localidades: un individuo (FML 07960) de alrededores de Santa Victoria Este (Departamento Rivadavia) y otro (FML 07961) a 10 km de Hickman (Departamento General San Martín), ambos depositados en Colección Herpetológica de la Fundación Miguel Lillo (Tucumán) que fueron revisados por los autores del presente trabajo. Considerando que revisamos el material disponible de la especie en Argentina, los nuevos registros aquí aportados, constituyen los más australes conocidos para la especie. No encontramos material de referencia para la Provincia del Chaco, por lo que consideramos necesario confirmar su presencia con ejemplares testigo. Teniendo en cuenta que *D. c. corais* fue categorizada como Amenazada en Argentina (Giraudo *et al.*, 2012), destacamos su registro en la RNF como única área protegida donde la especie ha sido confirmada. Se observó que presenta hábitos semiarborícolas (Fig. 2) y se comprobó que predó dos serpientes arborícolas adultas, un ejemplar de *Chironius maculoventris* registrado en el sistema digestivo del individuo UNNEC 13801 y otro de *Philodryas baroni* regurgitado por uno de los ejemplares de la RNF (UNNEC-A 00047). En coincidencia con Scrocchi y Kretzschmar (2012) la expansión de cultivos intensivos que generan deforestación y fragmentación del bosque chaqueño, afectaría directamente a esta especie semiarborícola, hecho agravado por su gran tamaño (3 mts. aproximadamente), coloración y comportamiento (Fig. 3), sufriendo además atropellamientos y persecución humana (Fig. 4). Consolidar y aumentar la superficie de áreas protegidas en la región (por ejemplo Reserva de Biósfera Riacho Teuquito) e implementar los corredores propuestos para el Gran Chaco, junto con campañas educativas y de difusión, podrían contribuir a la conservación de las poblaciones de esta especie Amenazada en Argentina.

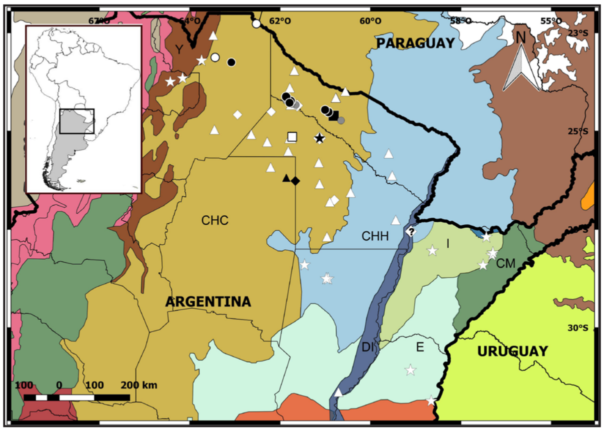

Figura 1. Registros colectados y fotografiados (círculos negros), en colecciones (círculos blancos) y observados (círculos grises) de *Drymarchon corais corais*. Registro colectado (cuadrado negro) y registro bibliográfico (cuadrado blanco) de *Pseudoboa nigra*. Registro colectado (rombo negro) y registros bibliográficos (rombos blancos) de *Psomophis genimaculatus*. Se indica con "?" un registro de MACN sin número para Corrientes (Scrocchi y Giraudo, 1997). Registro colectado (triángulo negro) y registros bibliográficos (triángulos blancos) de *Philodryas erlandi*. Registro colectado (estrella negra) y registros bibliográficos (estrellas blancas) de *Tantilla melanocephala*.

Pseudoboa nigra (Duméril, Bibron & Duméril, 1854).
Localidad: Argentina, Provincia de Formosa: Departamento Patiño: 18 km en dirección noroeste de Las Lomitas por Ruta Nacional 81 (24°35'27.75" S; 60°45'51.12"O, WGS84, 140 m s.n.m.), 08/05/2017, colectores: Eduardo Gabriel Etchepare, S.A. Palomas, E.F. Schaefer (UNNEC 13738).
Comentarios: El género *Pseudoboa* comprende seis especies de serpientes que se distribuyen en gran parte de América del Sur, al este de Los Andes desde Chaco (Argentina) y Rio Grande do Sul (Brasil), hasta el oeste de los Andes en Colombia, y en el oeste de Panamá; además, habitan las islas Tobago y Granada (Bailey, 1970; Cei, 1993; Giraudo, 2001, Etchepare *et al.*, 2015). La especie *Pseudoboa nigra* había sido excluida de las listas de serpientes de Argentina por no contar con material de referencia (Giraudo y Scrocchi, 2002) y posteriormente Etchepare *et al.* (2015) confirman su presencia en Fuerte Esperanza, Departamento General Güemes, Provincia del

Chaco. En esta contribución se adiciona por primera vez *P. nigra* (Fig. 5) en la Provincia de Formosa. Este ejemplar juvenil cubre un vacío en el conocimiento de su distribución entre las poblaciones de Argentina y Paraguay (Fig. 1).

Psomophis genimaculatus (Boettger, 1885).
Localidad: Argentina, Provincia de Santiago del Estero: Departamento Copo: Pampa de los Guanacos (26°16'7.35"S; 61°42'44.54"O), 03/27/2017, colectores: Dionel Aguiar, E.G. Etchepare (UNNEC 13407).
Comentarios: El género sudamericano *Psomophis* comprende tres especies, *P. joberti* (Sauvage, 1884), *P. obtusus* (Cope, 1863) y *P. genimaculatus* (Boettger, 1885), las dos últimas presentes en Argentina (Giraudo *et al.*, 2012). En este país, los registros de *Psomophis genimaculatus* son escasos, incluyendo las provincias del Chaco, Corrientes y Formosa (Lions y Alvarez, 1997; Scrocchi y Giraudo, 1997; Nenda 2007; Acosta *et al.*, 2010). En este trabajo, presentamos el primer registro para la provincia de Santiago del Estero sobre la picada del límite interprovincial entre las provincias de Santiago del Estero y Chaco. Esta especie considerada Insuficientemente Conocida en Argentina (Nenda, 2012), presenta pocos registros en áreas protegidas, encontrándose hasta el momento sólo en la Reserva Natural Formosa (Scrocchi y Giraudo, 1997). El hallazgo del individuo reportado (Fig. 6) se produjo a 33 km del Parque Nacional Copo, en un área transicional de bosques degradados de quebracho colorado (*Schinopsis lorentzii* y *S. quebracho-colorado*) y blanco (*Aspidosperma quebracho-blanco*) con cultivos de soja y algodón (Fig. 1).

Philodryas erlandi (Koslowsky, 1889).
Localidad: Argentina, Provincia de Santiago del Estero: Departamento Copo: Ruta Nacional 16 entre las localidades de Pampa de los Guanacos y Los Pirpintos (26°11'11.11"S; 61°56'33.58"O), 03/27/2017, colectores: D. Aguiar, E.G. Etchepare. (UNNEC 13408).
Comentarios: Según la última revisión de la especie *Philodryas erlandi,* su distribución incluye la biorregión del Chaco de Argentina, Bolivia y Paraguay (Cacciali *et.al*, 2016). En Argentina, se distribuye en las provincias de Chaco, Formosa, Salta y Santa Fe (Lavilla *et al.*, 1995; Álvarez *et al.*, 1996, 2009;

Figura 2. Ejemplar de *Drymarchon corais corais* (UNNEC-A 00047), exhibiendo sus hábitos semiarborícolas, fotografiado por los Guardaparques Mónica Pereira, Ezequiel Navarro, Javier A. Juarez, Daniel Ramirez y Ariel R. González, dentro de la Reserva Natural Formosa, Formosa.

Figura 3. Ejemplar de *Drymarchon corais corais* (UNNEC 13613) colectado en la Ruta Provincial 9, Formosa.

Figura 4. Ejemplar de *Drymarchon corais corais* (UNNEC 13801) atropellado sobre Ruta Provincial 39, Formosa.

Figura 5. Ejemplar de *Pseudoboa nigra* (UNNEC 13738) colectado en Ruta Nacional 81, Formosa.

Arzamendia y Giraudo, 2002; Kacoliris *et al.*, 2006; Giraudo *et al.*, 2012). Fue citada para la provincia de Santiago del Estero por Álvarez *et al.* (2002), pero no considerada en la lista de especies en esta provincia en trabajos posteriores (Giraudo *et al.*, 2012; Cacciali *et al.*, 2016). Dada su amplia distribución, actualmente no hay preocupaciones con respecto al estado de conservación de la especie dentro de su rango (Motte *et al.*, 2009; Giraudo *et al.*, 2012). En la presente contribución, se revisó y constato la identidad del ejemplar UNNEC 4580 publicado por Álvarez *et al.* (2002), proveniente de Ea. Taboada, Departamento Copo (25°55’6.61”S; 62°20’29.11”O), y reportamos un ejemplar más de *P. erlandi* para la provincia que confirma su presencia en Santiago del Estero (Fig. 1, 7).

Tantilla melanocephala (Linnaeus, 1758)
Localidad: Argentina, Provincia del Chaco, Departamento General Güemes, Paraje La Armonía (25°10´56.7″S; 61°05´47.1″O), 31/10/22, colectores: D. Aguiar, E.G. Etchepare, E.F. Schaefer (UNNEC 13818).
Comentarios: Tantilla es un género diverso de colúbridos ampliamente distribuido en América, desde el sur de Estados Unidos hasta el norte de Argentina (Peters y Orejas-Miranda 1970). En el caso de la especie *T. melanocephala* se encuentra presente en Centro y Sudamérica, siendo su límite austral el norte de Argentina y Uruguay (Peters y Orejas-Miranda 1970; Cunha y Nascimento, 1993; Cacciali *et al.*, 2016b; Nogueira *et al.*, 2019). En Argentina, su distribución es relativamente poco conocida y está confirmada para las provincias de Corrientes (Giraudo, 2001; Montanelli y Álvarez, 1997; Schaefer y Álvarez, 2000), Entre Ríos (Vuoto, 1998; Etchepare, 2005), Santa Fe (Nogueira *et al.*, 2019), Jujuy (Ramos *et al.*, 2013; Burgos Gallardo *et al.*, 2020), Buenos Aires y Salta (Wilson y Mena, 1980). En la provincia del Chaco, trabajos que analizan distribuciones generales de serpientes incluyen la especie sin indicar material de referencia (Giraudo, 2001; Giraudo y Scrocchi, 2002; Jowers *et al.*, 2020), al igual que Koslowsky (1898) que menciona su presencia para el “Gran Chaco”, área que incluía las provincias de Chaco y Formosa en esa época. El ejemplar reportado, que confirma la presencia para la provincia del Chaco, fue encontrado en los límites de una selva riparia con abundancia de algarrobos (*Neltuma nigra*) y un quebrachal de quebracho blanco (*Aspidosperma quebracho-blanco)*, ambos degradados con avance de vinal (*Neltuma ruscifolia*).

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Figura 6. Ejemplar de *Psomophis genimaculatus* (UNNEC 13407) Pampa de los Guanacos, Santiago del Estero.

Figura 7. Ejemplar de *Philodryas erlandi* (UNNEC 13408) colectado en la Ruta Nacional 16, entre Pampa de los Guanacos y Los Pirpintos, Santiago del Estero.

Figura 8. Ejemplar de *Tantilla melanocephala* (UNNEC 13.818) colectado en Paraje La Armonía, Chaco.

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