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Trabajo

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Dos nuevas especies del grupo *Pristimantis boulengeri* (Anura: Strabomantidae) de la cuenca alta del río Napo, Ecuador

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Pristimantis omarrhynchus: urn:lsid:zoobank.org:act:886B9E45-BCA0-4796-8AB9-C81AFF2FCD81
Pristimantis miltongallardoi: urn:lsid:zoobank.org:act:3BEEB8E8-9970-4CEE-938E-EF72C0158037

ABSTRACT
Through the combination of morphological and phylogenetic evidence, we describe two species of *Pristimantis* from the upper basin of the Napo River. Both species have well-defined dorsolateral folds, a conical tubercle on the eyelid, a papilla on the tip of the snout, weakly expanded discs, and small size (female SVL < 28.2 mm). *Pristimantis omarrhynchus* sp. nov. differs from its sister species, *Pristimantis miltongallardoi* sp. nov., by the absence of iridophores on the belly, subacuminate snout in dorsal view, and narrow digits. Our phylogeny and morphological evidence, are conclusive in assigning them to the *Pristimantis boulengeri* species group, closely related to *P. boulengeri*, *P. cryptopictus*, *P. dorspictus*, and *P. brevifrons*. The new species are the first reported for the *P. boulengeri* group in Ecuador and the Amazon basin. We also comment on the correct identity of GenBank sequences previously assigned to *P. thymelensis* and *P. myersi*.

Key words: Andes; Phylogenetics; Call; *Pristimantis omarrhynchus* sp. nov.; *Pristimantis miltongallardoi* sp. nov.; Systematics; Terrarana.

RESUMEN
A través de la combinación de evidencia morfológica y filogenética describimos dos especies de *Pristimantis* de la cuenca alta del río Napo. Las dos especies presentan pliegues dorsolaterales bien definidos, un tubérculo cónico en el párpado, una papila en la punta del hocico, discos poco dilatados y tamaño pequeño (hembras LRC < 28,2 mm). *Pristimantis omarrhynchus* sp. nov. se diferencia de su especie hermana, *Pristimantis miltongallardoi* sp. nov., por la ausencia de iridóforos en el vientre, hocico subacuminado en vista dorsal y dígitos estrechos. Nuestra filogenia, en combinación con la evidencia morfológica, son concluyentes para asignarlas al grupo de especies *Pristimantis boulengeri* y cercanamente relacionadas a *P. boulengeri*, *P. cryptopictus*, *P. dorspictus* y *P. brevifrons*. Las nuevas especies son las primeras reportadas para el grupo *P. boulengeri* en el Ecuador y la cuenca amazónica. También comentamos la identidad correcta de secuencias GenBank previamente asignadas a *P. thymelensis* y *P. myersi*.

Palabras clave: Andes; Canto; Filogenia; *Pristimantis omarrhynchus* sp. nov.; *Pristimantis miltongallardoi* sp. nov.; Sistemática; Terrarana.

Introducción

La región noroccidental de la cuenca del río Amazonas se caracteriza por que sus sistemas tributarios nacen del ramal oriental de los Andes de Ecuador (Lynch, 1979; Lynch y Duellman, 1980; Vasconcelos *et al.*, 2019). Particularmente la cuenca alta del río Napo, integra bosques montanos de los Andes con los bosques tropicales Amazónicos que presentan singular importancia por su alto nivel de vulnerabi-

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lidad frente a las dinámicas de cambios de cobertura y uso de la tierra (Cuesta *et al*., 2009).

En estos biomas se concentra una gran diversidad y endemismo de fauna anura (Lynch y Duellman, 1973, 1980; Guayasamin y Funk, 2009; Morales-Mite y Yánez-Muñoz, 2013; Morales-Mite *et al*., 2013), resaltando en sus comunidades, la abundancia y redundancia de la diversidad de ranas de desarrollo directo del género *Pristimantis* (Lynch y Duellman, 1980; Flores y Vigle, 1994; Zimmerman y Simberloff, 1996; Guayasamin y Funk, 2009; Bejarano-Muñoz, *et al*., 2015).

Específicamente en las subcuencas de los ríos Coca y Aguarico, se encuentran biomas piemontanos y montano bajos entre los 600 y 2200 msnm, que acoge un importante punto caliente de diversidad de ranas terrestres *Pristimantis*, con más de 24 especies reportadas hasta el momento de las cuales el 38% se encuentran amenazadas y casi la mitad de las especies (46%) son endémicas de estos sistemas montañosos (Lynch y Duellman, 1980; Morales y Yánez-Muñoz, 2013; Bejarano-Muñoz *et al*., 2015; Ron *et al*., 2020).

Nuestros análisis y revisión de colecciones generadas en la zona durante los últimos seis años a través de diferentes iniciativas institucionales y expediciones en el área han determinado a partir de inferencia filogenética el hallazgo de dos especies no descritas del grupo de especies *Pristimantis boulengeri* (*sensu* González-Durán *et al*., 2017) para Ecuador. El grupo *P. boulengeri* está compuesto por nueve especies endémicas de Colombia, distribuidas en las cordilleras Occidental y Central entre los 1140 m a 3200 m de elevación. Cinco sinapomorfías han sido sugeridas para el grupo: (1) dedo III con un tubérculo subarticular distal doble; (2) tubérculo subarticular distal doble del dedo IV; (3) peritoneo parietal cubierto con iridóforos; (4) saco vocal extendido; (5) hocico con papila pequeña (González-Durán *et al*., 2017). En este manuscrito describimos dos nuevas especies para Ecuador; y adicionalmente describimos el canto de una de ellas.

Materiales y métodos

Extracción, amplificación y secuenciación de ADN

Para la extracción del ADN empleamos el protocolo de tiocianato de guanidina (Esselstyn *et al*., 2008), las muestras fueron obtenidas de tejido de hígado o músculo preservado en etanol al 95%. El ADN extraído fue cuantificado en un NanoDrop (Thermo Scientific) y diluido en alícuotas a una concentración de 20 ng/µl. Los procesos de amplificación del gen mitocondrial 16S ARNr (16S) y del gen nuclear activador de la recombinación (RAG-1) fueron llevados a cabo bajo protocolos estandarizados de la reacción en cadena de la polimerasa (PCR). Los cebadores empleados fueron 16L19 y 16H36E para 16S y RAG1FF2 y RAG1FR2 para RAG-1 (Heinicke *et al*., 2007). Realizamos la extracción y amplificación de ADN en el Laboratorio de Biología Molecular del Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ). Purificamos los productos amplificados con la herramienta ExoSap y los enviamos a la empresa Macrogen (Macrogen Inc., Seúl, Corea) para su posterior secuenciación.

Las secuencias generadas se ensamblaron y editaron manualmente en el programa GeneiousPro 5.4.6 (Biomatters Ltd.). En el proceso de edición se cortaron ambos extremos de las secuencias para evitar pares de bases de baja calidad. Los códigos de acceso a GenBank asignados para las muestras generadas en este estudio son presentados en el Apéndice I. Además de las secuencias obtenidas *de novo*, nuestro muestreo genético incluyó información de los genes mitocondriales 12S ARNr (12S) y citocromo oxidasa I (COI) para varias especies del género *Pristimantis* disponibles en la base de datos del portal GenBank (https://www.ncbi.nlm.nih.gov/genbank/). La matriz concatenada está disponible en el repositorio Zenodo (Zenodo.org), DOI: zenodo.6407122.

Análisis filogenéticos

Las nuevas secuencias se compararon con las secuencias de la base de datos de GenBank mediante la herramienta BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) con la finalidad de corroborar su identidad genérica y determinar especies afines que permitan evaluar la posición filogenética de las nuevas especies. La búsqueda mostró que las dos especies nuevas están estrechamente relacionadas con especies del grupo *Pristimantis boulengeri*. Por lo tanto, incluimos en la matriz secuencias disponibles del grupo *P. boulengeri* (*sensu* González-Durán *et al*., 2017, Patiño-Ocampo *et al*., 2022) y de sus clados más cercanos como son los grupos de especies de *P. leptolophus*, *P. myersi* y *P. devillei*. Finalmente, incluimos especies de otros clados representativos del género *Pristimantis* para posicionar filogenéticamente nuestro grupo interno dentro de *Pristimantis*. Utilizamos secuencias de los géneros *Niceforonia* y

Strabomantis como grupo externo.

Las secuencias de GenBank utilizadas corresponden a información publicada previamente en: Arteaga y Guayasamin, 2011; Arteaga *et al*., 2013, 2016; Barrio-Amorós *et al*., 2013; Chávez y Catenazzi 2016; Crawford *et al*., 2013; Darst y Cannatella, 2004; De Oliveira *et al*., 2017; Elmer *et al*., 2007; Faivovich *et al*., 2005; Fouquet *et al*., 2012; García-R *et al*., 2012; González-Durán *et al*., 2017; Guayasamin *et al*., 2015, 2017; Hedges *et al*., 2008; Heinicke *et al*., 2007, 2009, 2015; Hutter y Guayasamin, 2015; Jablonski *et al*., 2017; Kok *et al*., 2012, 2018; Lehr *et al*., 2012, 2017, Lehr y Von May 2017; Ortega-Andrade y Venegas, 2014; Pinto-Sánchez *et al*., 2012; Rivera-Correa y Daza, 2016; Rivera-Prieto *et al*., 2014; Shepack *et al*., 2016; Székely *et al*., 2016; Von May *et al*., 2017; Zhang *et al*., 2013; Patiño-Ocampo *et al*., 2022.

Las secuencias fueron alineadas en el programa GeneiousPro 5.4.6 mediante el algoritmo MUSCLE (Robert, 2004) y una posterior revisión y corrección manual de la matriz en el programa Mesquite v2.75 (Maddison y Maddison, 2011). Los loci codificantes (RAG-1 y COI) se tradujeron en aminoácidos para corroborar la ausencia de codones de terminación. En total la matriz combinada de ADN tuvo 3984 pares de bases. Mediante el programa PartitionFinder v1.1.1 (Lanfear *et al*., 2012) se estimaron simultáneamente los modelos de evolución de caracteres y el mejor esquema de partición para nuestros datos. La matriz fue dividida en seis particiones a priori para el análisis: una para 12S y para 16S, una para la posición 1 y 2 del codón de RAG-1, una para la posición 3 del codón de RAG-1 y una partición por cada posición del codón de COI.

Inferimos el árbol filogenético óptimo bajo un enfoque de máxima verosimilitud (ML) utilizando el programa IQ-TREE v1.6.12 (Nguyen *et al*., 2015). Estimamos el soporte de los nodos mediante dos metodologías: (1) 200 réplicas de bootstrap no paramétrico (comando -b 200 en IQ-TREE) y (2) 1000 réplicas de la prueba de aproximación de cociente de verosimilitud similar a la metodología de Shimodaira–Hasegawa ([SH]-aLRT) (Guindon *et al*., 2010) (-alrt 1000). El resto de los parámetros del programa se mantuvieron en sus valores por defecto. Por último, se analizaron las distancias genéticas *p*-no corregidas estimadas a partir del gen 16S para las especies nuevas y las especies relacionadas, utilizando el programa Mesquite v2.75 (Maddison y Maddison 2011). El rango del tamaño de las secuencias comparadas entre individuos fue de 728 pb a 1158 pb.

Características morfológicas

La descripción de las especies sigue el formato estandarizado de Lynch y Duellman (1997); las definiciones diagnósticas de los caracteres son las propuestas por Duellman y Lehr (2009); además la determinación del carácter tubérculo hiperdistal subarticular sigue la propuesta de Ospina-Sarria y Duellman (2019), y Ron *et al*., (2020); la clasificación sistemática de la familia sigue a Heinicke *et al*., (2017) y a nivel de grupo a González-Durán *et al*., (2017). Los especímenes de la serie tipo fueron fotografiados en la noche y 12 horas después de su captura, para luego sacrificarlos en una solución de benzocaína, fijarlos en formalina al 10% y preservarlos en etanol al 70%. El sexo y la determinación del estado adulto de los especímenes se determinaron por características sexuales secundarias (almohadillas nupciales, saco vocal y hendiduras vocales) y por la inspección directa de las gónadas a través de incisiones ventro-laterales. Se tomó las siguientes medidas siguiendo a Duellman y Lehr (2009): ON: distancia órbita-narina (desde el margen anterior de la órbita hasta el margen posterior de la narina); LC: longitud cefálica (desde el margen posterior de la mandíbula hasta el extremo del rostro); AC: ancho cefálico (entre las comisuras de la boca); DIO: distancia interorbital (tomada en el ancho de la base del cerebro entre las órbitas); EN: distancia internarinal (en línea recta entre los bordes internos de las narinas); LRC: longitud rostro-cloacal (distancia desde la punta de la cabeza hasta la cloaca); LT: longitud de la tibia (distancia desde la rodilla hasta el borde distal de la tibia); LP: longitud del pie (desde el margen proximal del tubérculo metatarsal interno hasta la punta del dedo IV); LM: longitud de la mano (desde la base del tubérculo tenar hasta la punta del dedo III); DT: diámetro horizontal del tímpano. Las medidas fueron tomadas con un calibre electrónico (precisión ± 0.01 mm) y redondeados al 0.1 mm más cercano. Los patrones de coloración en vida fueron tomados de las notas de campo y fotografías digitales a color. Las localidades, sus coordenadas y elevaciones fueron determinadas con base a las notas de campo de los colectores tomadas con un GPS. Los especímenes examinados (Apéndice II) están depositados en la colección herpetológica del Instituto Nacional de Biodiversidad, Quito, Ecuador (DH-MECN), el Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ) y la colección de anfibios del Instituto de Ciencias Naturales (ICN), Universidad Nacional de Colombia.

Análisis acústico

Las grabaciones fueron obtenidas el 13 de diciembre del 2014 por Jorge Brito M., correspondientes al ejemplar DHMECN 11483 y se encuentran disponibles en los sitios web Neocanto (https://bioweb.bio/neocanto) y Anfibios del Ecuador (https://bioweb.bio/faunaweb/amphibiaweb). Analizamos cinco cantos de anuncio del individuo DHMECN11483. Las grabaciones se hicieron con una grabadora digital Olympus WS-802, a una frecuencia de muestreo de 44.1 kHz y 16 bits de resolución en formato PCM-wav. Los análisis se realizaron en Raven Pro 1.6.3 para Mac OS (Bioacoustics Research Program, 2014). Las variables temporales fueron medidas en el oscilograma; para facilitar la medición, se atenuó en 12 dB las frecuencias de grabación < 2000 Hz (frecuencias que excluyen por completo al canto de anuncio). Las variables de frecuencia fueron medidas en un espectro de poder obtenido con una ventana de Hann al 50% de superposición y 512 muestras; la resolución temporal fue de 5,8 ms y la resolución espectral de 10,8 Hz. Las figuras de los oscilogramas y espectrogramas se obtuvieron con el programa Raven Pro 1.6.3. La terminología de las variables acústicas se basa en la definición centrada en el canto de Köhler *et al*., (2017).

Los parámetros que se analizaron fueron: (1) Frecuencia dominante: frecuencia de mayor energía medida a lo largo de todo el canto; (2) Frecuencia de la segunda armónica: frecuencia más alta correspondiente a 2x de la frecuencia dominante (primera armónica); (3) Duración del canto: tiempo desde el inicio hasta el final de un canto; (4) Intervalos entre cantos: tiempo desde el final de un canto al inicio del siguiente; (5) cantos/serie de cantos: número de cantos en una serie de cantos consecutivos. Debido a que cada canto tiene una nota, la duración del canto equivale a la duración de la nota.

Resultados

Relaciones Filogenéticas

Nuestra filogenia, es congruente con trabajos previos (Rivera-Correa y Daza, 2016; Rivera-Correa *et al*., 2017; González Durán *et al*., 2017; Patiño-Ocampo *et al*., 2022) que determinan la monofilia del grupo de especies *Pristimantis boulengeri* y lo asocian estrechamente como clado hermano del grupo de especies *P. leptolophus*. El clado formado por ambos grupos tiene alto soporte (bootstrap = 94; Fig. 1).

El análisis filogenético determinó que las nuevas especies se posicionan con un alto soporte (bootstrap = 99) en el clado conformado por el grupo de especies de *Pristimantis boulengeri* (*sensu* González Durán *et al*., 2017) (Fig. 1). Son parte de un clado que contiene a *P. brevifrons* + *P. angustilineatus* + *P. urani* + *P. boulengeri* + *P. cryptopictus* + *P. dorsopictus* con un alto soporte (bootstrap = 95; Fig. 1). Dentro del grupo de especies de *P. boulengeri*, el subclado formado por *P. quantus* + *P. myops* es hermano de un clado conformado por todas las demás especies. Las nuevas especies son hermanas entre sí con bajo soporte. Distancias genéticas de las nuevas especies con sus especies más cercanamente relacionadas son presentadas en la Figura 1B.

En nuestra filogenia, los especímenes correspondientes a las secuencias AY326009 y JX564889, previamente reportados como "*P. myersi*" por Guayasamin *et al*., (2018), son genéticamente muy similares (distancia *p* no corregida para 16S = 0,1%) a los especímenes *P. ocreatus* KU 208508 (EF493682) y QCAZ 13664, además fueron colectados en la localidad tipo de *P. ocreatus*. Basados en esta evidencia concluimos que la identificación de "*P. myersi*" para las secuencias GenBank de Guayasamin *et al*., (2018) son incorrectas. Las mismas secuencias (AY326009 y JX564889), fueron identificadas incorrectamente como "*P. thymelensis*" por Zhang *et al*., (2013), Darst y Cannatella (2004) y trabajos subsecuentes (por ejemplo, Padial *et al*., 2014).

Posición filogenética

Asignamos las nuevas especies al grupo de especies *Pristimantis boulengeri* (*sensu* González-Durán *et al*., 2017) con base a sus posiciones filogenéticas y a cinco características morfológicas propuestas como sinapomorfías para el grupo (González Durán *et al* 2017): (1) tubérculo subarticular distal doble en el dedo III de la mano, (2) tubérculo subarticular distal doble en el dedo IV de la mano, (3) peritoneo parietal cubierto con iridóforos; (4) saco vocal extendido; y (5) hocico con papila. Las distancias genéticas *p* no corregidas (16S) de las dos especies con respecto a las demás especies del grupo es > 3,3%; la distancia genética entre las dos nuevas especies es 6,4% (Fig. 1B).

Pristimantis omarrhynchus sp. nov.

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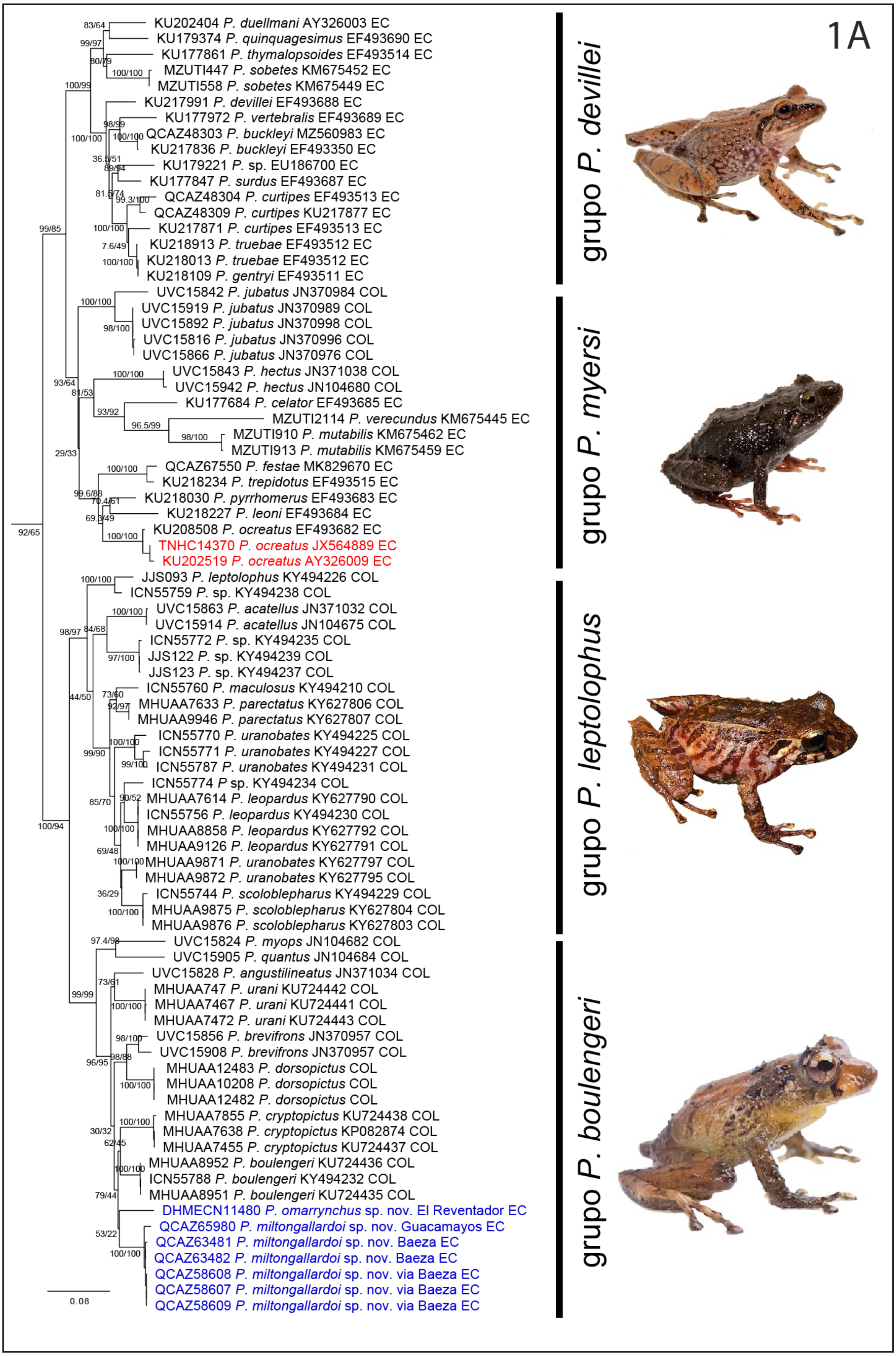
1A
83/64
KU202404 P. duellmani AY326003 EC
99/97
KU179374 P. quinquagesimus EF493690 EC
80/79
KU177861 P. thymalopsoides EF493514 EC
100/100
MZUTI447 P. sobetes KM675452 EC
100/99
MZUTI558 P. sobetes KM675449 EC
KU217991 P. devillei EF493688 EC
98/99
KU177972 P. vertebralis EF493689 EC
100/100
100/100
QCAZ48303 P. buckleyi MZ560983 EC
KU217836 P. buckleyi EF493350 EC
36.5/51
KU179221 P. sp. EU186700 EC
89/94
KU177847 P. surdus EF493687 EC
81.5/74
99.3/100
QCAZ48304 P. curtipes EF493513 EC
QCAZ48309 P. curtipes KU217877 EC
99/85
100/100
KU217871 P. curtipes EF493513 EC
7.6/49
KU218913 P. truebae EF493512 EC
100/100
KU218013 P. truebae EF493512 EC
KU218109 P. gentryi EF493511 EC
UVC15842 P. jubatus JN370984 COL
100/100
UVC15919 P. jubatus JN370989 COL
98/100
UVC15892 P. jubatus JN370998 COL
UVC15816 P. jubatus JN370996 COL
UVC15866 P. jubatus JN370976 COL
100/100
UVC15843 P. hectus JN371038 COL
93/64
UVC15942 P. hectus JN104680 COL
81/53
KU177684 P. celator EF493685 EC
93/92
MZUTI2114 P. verecundus KM675445 EC
96.5/99
98/100
MZUTI910 P. mutabilis KM675462 EC
29/33
MZUTI913 P. mutabilis KM675459 EC
100/100
QCAZ67550 P. festae MK829670 EC
KU218234 P. trepidotus EF493515 EC
99.6/88
70.4/61
KU218030 P. pyrrhomerus EF493683 EC
69.3/49
KU218227 P. leoni EF493684 EC
92/65
100/100
KU208508 P. ocreatus EF493682 EC
TNHC14370 P. ocreatus JX564889 EC
KU202519 P. ocreatus AY326009 EC
100/100
JJS093 P. leptolophus KY494226 COL
ICN55759 P. sp. KY494238 COL
100/100
UVC15863 P. acatellus JN371032 COL
98/97
84/68
UVC15914 P. acatellus JN104675 COL
ICN55772 P. sp. KY494235 COL
97/100
JJS122 P. sp. KY494239 COL
JJS123 P. sp. KY494237 COL
ICN55760 P. maculosus KY494210 COL
44/50
73/60
92/97
MHUAA7633 P. parectatus KY627806 COL
MHUAA9946 P. parectatus KY627807 COL
100/100
ICN55770 P. uranobates KY494225 COL
99/90
ICN55771 P. uranobates KY494227 COL
99/100
ICN55787 P. uranobates KY494231 COL
ICN55774 P sp. KY494234 COL
85/70
90/52
MHUAA7614 P. leopardus KY627790 COL
ICN55756 P. leopardus KY494230 COL
100/94
100/100
MHUAA8858 P. leopardus KY627792 COL
MHUAA9126 P. leopardus KY627791 COL
69/48
100/100
MHUAA9871 P. uranobates KY627797 COL
MHUAA9872 P. uranobates KY627795 COL
36/29
ICN55744 P. scoloblepharus KY494229 COL
100/100
MHUAA9875 P. scoloblepharus KY627804 COL
MHUAA9876 P. scoloblepharus KY627803 COL
97.4/98
UVC15824 P. myops JN104682 COL
UVC15905 P. quantus JN104684 COL
UVC15828 P. angustilineatus JN371034 COL
73/61
99/99
MHUAA747 P. urani KU724442 COL
100/100
MHUAA7467 P. urani KU724441 COL
MHUAA7472 P. urani KU724443 COL
98/100
UVC15856 P. brevifrons JN370957 COL
UVC15908 P. brevifrons JN370957 COL
96/95
98/88
MHUAA12483 P. dorsopictus COL
100/100
MHUAA10208 P. dorsopictus COL
MHUAA12482 P. dorsopictus COL
100/100
MHUAA7855 P. cryptopictus KU724438 COL
30/32
MHUAA7638 P. cryptopictus KP082874 COL
62/45
MHUAA7455 P. cryptopictus KU724437 COL
100/100
MHUAA8952 P. boulengeri KU724436 COL
ICN55788 P. boulengeri KY494232 COL
79/44
MHUAA8951 P. boulengeri KU724435 COL
DHMECN11480 P. omarrynchus sp. nov. El Reventador EC
QCAZ65980 P. miltongallardoi sp. nov. Guacamayos EC
53/22
QCAZ63481 P. miltongallardoi sp. nov. Baeza EC
100/100
QCAZ63482 P. miltongallardoi sp. nov. Baeza EC
QCAZ58608 P. miltongallardoi sp. nov. via Baeza EC
QCAZ58607 P. miltongallardoi sp. nov. via Baeza EC
0.08
QCAZ58609 P. miltongallardoi sp. nov. via Baeza EC
grupo P. devillei
grupo P. myersi
grupo P. leptolophus
grupo P. boulengeri

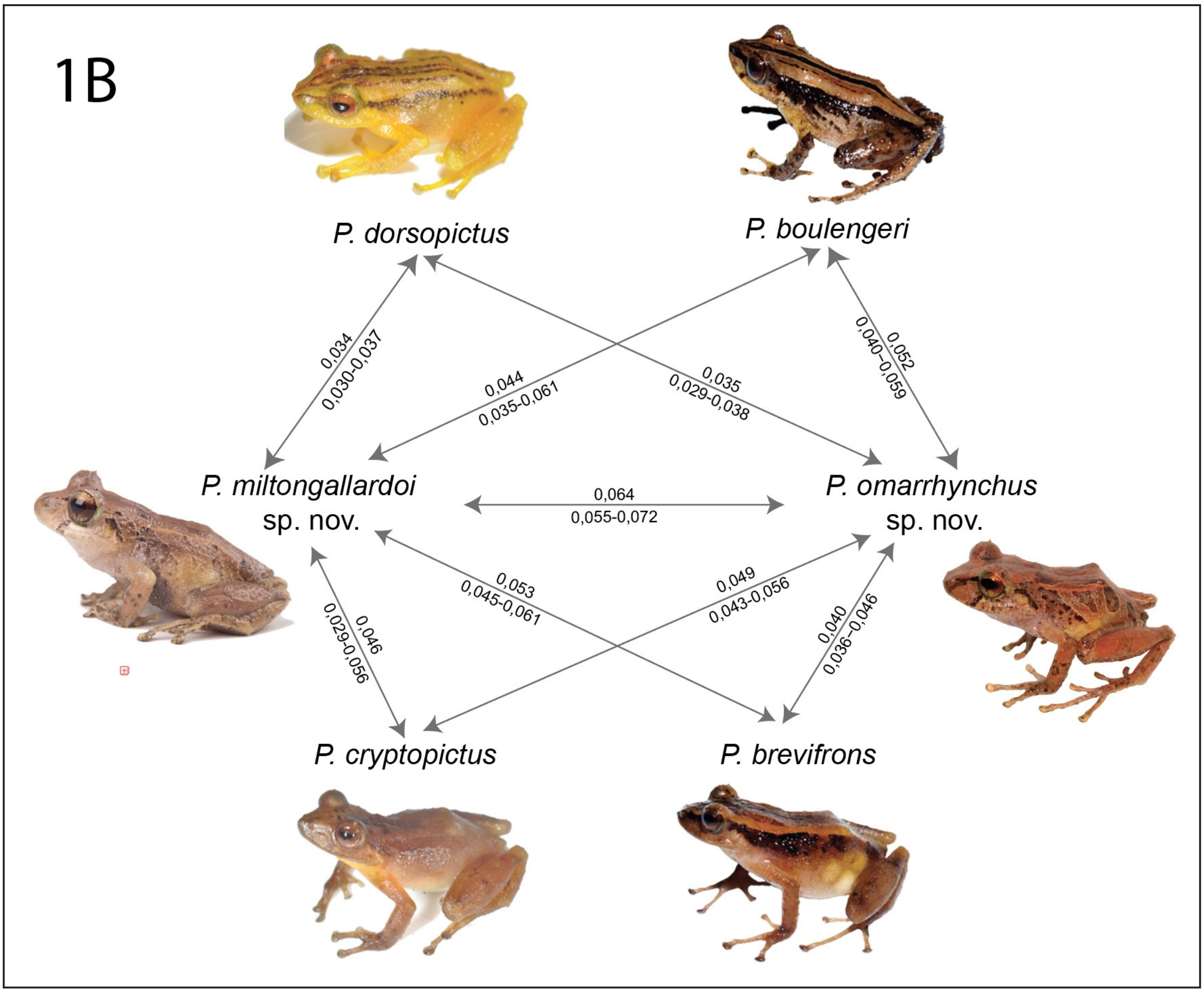

Figura 1. (B) Distancias genéticas *p*-no corregidas (16S) entre las nuevas especies y sus especies más cercanamente relacionadas (en base a 1A). El valor sobre la flecha corresponde al promedio de la distancia genética; bajo la flecha se muestra el rango. Fotografías: Patricia Bejarano, Santiago Ron y Mauricio Rivera-Correa, iNaturalist.

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Pristimantis sp. 1 Bejarano-Muñoz *et al*., (2015)
Holotipo (Fig. 2–4, 6, 7)
Hembra adulta DHMECN 11480 (tejido QCAZ 77345), colectada en la Reserva Cayambe-Coca, El Reventador (pueblo junto a la vía Quito-Nueva Loja); (0.086141 S; 77.599214 O, 1890 msnm), cantón Gonzalo Pizarro, parroquia El Reventador, provincia de Sucumbíos, República del Ecuador, el 13 de diciembre de 2014 por Patricia Bejarano-Muñoz, María Pérez, Mario H. Yánez-Muñoz, Jorge Brito M. y Glenda Pozo Z.

Paratipos
Hembra adulta DHMECN 11484, machos DHMECN 11476, 11482, 11485, 11487–8, 11491–2, con los mismos datos del holotipo. Hembra QCAZ

←**Figura 1.** (A) Relaciones filogenéticas del grupo de especies de *Pristimantis boulengeri* y clados cercanamente relacionados. Árbol de máxima verosimilitud obtenido en base a una matriz de secuencias de ADN de un total de 3984 pares de bases (genes 12S, 16S, COI y RAG-1) y con un muestreo de 97 individuos, incluyendo especies de los grupos de *P. leptolophus*, *P. devillei* y *P. myersi*. Los valores de soporte se muestran como porcentajes junto a las ramas, SH-aLRT antes de la barra oblicua y bootstrap no paramétrico después. Los grupos externos no se muestran. El número del museo, código de acceso GenBank, nombre de la especie y país se presentan para cada muestra. Las abreviaturas son: **COL** Colombia y **EC** Ecuador. Los especímenes mostrados en rojo tienen identificaciones corregidas con respecto a identificaciones de estudios previos y el GenBank. El número de campo del espécimen KU 202519 fue corregido con respecto a la base de datos GenBank usando como referencia la base de datos de la colección de la Universidad de Kansas (disponible en https://collections.biodiversity.ku.edu/KUHerps/).

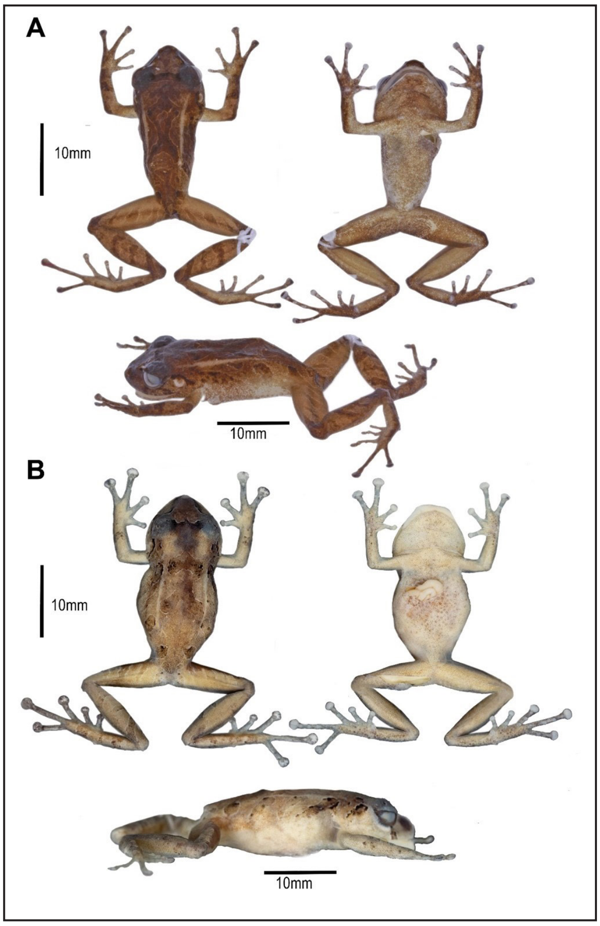

Figura 2. Aspecto dorsal, ventral y lateral en preservado (A) *Pristimantis omarrhynchus* sp. nov. Holotipo, hembra adulta (DHMECN 11480); (B) *Pristimantis miltongallardoi* sp. nov. Holotipo, hembra adulta (QCAZ 65980). Fotografías: M. H. Yánez-Muñoz y Santiago Ron.

10836, colectado el 5 de abril de 1996 por Andrew Gluesenkamp, en el Volcán Reventador (0.104389 S; 77.58833 O, 1700 msnm), cantón Gonzalo Pizarro, parroquia El Reventador, provincia de Sucumbíos, República del Ecuador.

Etimología

El epíteto específico es un patronímico que resulta de la combinación del nombre personal "*Omar*" y el término latín "*rhyncus=nariz*" y que hace alusión al sobrenombre bajo el cual los compañeros de aulas conocían al célebre herpetólogo ecuatoriano Omar Torres-Carvajal, curador de reptiles del Museo de Zoología QCAZ cuya notable y sobresaliente contribución al estudio de los reptiles neotropicales es digna de reconocimiento.

Nombre común sugerido: Cutín de Omar Torres
Nombre común en inglés sugerido: Omar´s Rain Frog

Diagnosis

Pristimantis omarrhynchus caracterizada por: (1) piel del dorso lisa con algunos tubérculos redondeados esparcidos en los flancos y pelvis con dos tubérculos subcónicos redondeados escapulares; vientre areolado; pliegues dorsolaterales continuos desde la parte posterior del párpado hasta la parte media del dorso en la cintura pélvica; pliegue discoidal presente; (2) anillo y membrana timpánica prominente, redondeada 43–62% del diámetro del ojo, margen superior del anillo timpánico cubierto por el pliegue supratimpánico; con tubérculos postrictales subcónicos pequeños; (3) hocico subacuminado en vista dorsal y redondeado en vista lateral, con papila en la punta del hocico; (4) párpado superior con un tubérculo cónico prominente rodeado por algunos tubérculos redondeados pequeños; párpado más estrecho que la distancia interorbital; tubérculo interorbital pequeño; cresta craneal ausente; (5) procesos vomerinos presentes oblicuos de contorno con 2 a 4 dientes; (6) machos con hendiduras vocales, con saco vocal subgular y almohadillas nupciales pequeñas y oblicuas, no queratinizadas de color blanco; (7) dedo I de la mano más pequeño que el dedo II, discos ligeramente expandidos en los dedos II-III-IV; dedos III y IV con tubérculo subarticular distal doble; (8) dedos sin rebordes cutáneos laterales; (9) tubérculos ulnares presentes; (10) talón con un tubérculo grande, pequeños tubérculos en el borde externo de la pierna, rodilla y tarso; pliegue tarsal interno ausente; (11) tubérculo metatarsal interno oval, más de tres veces que el tubérculo metatarsal externo pequeño subcónico, pocos tubérculos supernumerarios bajos; (12) dedos del pie sin rebordes cutáneos laterales o indistintos; almohadillas ligeramente más grandes que el ancho de los dedos, sin membranas interdigitales; dedo V más largo que el dedo III y alcanza al tubérculo subarticular del dedo IV; tubérculos hiperdistales presentes en todos los dedos; (13) coloración dorsal desde café claro verdoso hasta café oscuro rojizo, con una marca en forma)(café oscura; pliegues dorsolaterales crema; superficie de las extremidades, superficies ocultas de los muslos y flancos con barras café separadas por barras cremas; vientre y garganta cafés a crema amarillentos; superficies ocultas de las axilas e ingles cremas amarillentas a rojizas; superficies anteriores de los muslos y tibia cafés; iris plateado con retículos dorados verdoso y línea media café cobriza; banda cantal y timpánica café oscura a negra; superficies posteriores de los muslos con barras desde café oscuras a claro (amarillentas en vida); (14) LRC en hembras 23,5–27,3 mm (promedio = 25,26 ± 1,9 *n* = 3); machos 12,1–20 mm (promedio = 16,7 ± 2,4 *n* = 15).

Comparación con especies similares

Pristimantis omarrhynchus se diferencia de otros congéneres de *Pristimantis* en los Andes Norte de Sudamérica, entre la vertiente oriental de Ecuador y los Andes occidentales y centrales de Colombia, por la presencia de pliegues dorsolaterales bien definidos, marcas dorsales generalmente en forma de)(café oscuro, tubérculos escapulares redondeados, hocico subacuminado en vista dorsal, vientre sin iridóforos, un tubérculo cónico en el párpado, papila en la punta del hocico, dígitos poco dilatados y pequeño tamaño corporal (hembras LRC < 28,2 mm). Su especie hermana (Fig 1), presenta iridóforos en el vientre y hocico en vista dorsal redondeado (sin iridóforos y hocico subacuminado en vista dorsal en *P. omarrhynchus*). A diferencia de otros miembros del grupo de especies de *Pristimantis boulengeri* (*sensu* González Durán *et al.,* 2017), la nueva especie presenta pliegues dorsolaterales (ausentes en la mayoría de especies del grupo excepto *P. baiotis* y *P. quantus*), marcas en forma de)(café oscuro (marcas oscuras irregulares en *P. boulengeri*, *P. brevifrons*, *P. cryptopictus*, *P. quantus* y *P. urani*, diseño de rayas en *P. angustilineatus* y *P. dorsopictus*, manchas en forma de chevrones en *P. quantus*), tubérculos escapulares redondeados (ausente en las demás especies) un tubérculo cónico en el párpado (ausente en *P. angustilineatus*, *P. myops* y *P. urani*; bajo, no cónico en *P. boulengeri*), tubérculos en el talón y tarso (ausente en *P. angustilineatus*, *P. brevifrons*, *P. cryptopictus* y *P. urani*; bajos, no cónicos, en *P. boulengeri*) Tabla 3. Otras diferencias con las especies del grupo son presentadas en el Apéndice III.

Descripción del holotipo

Hembra adulta, cabeza ligeramente más ancha que larga; hocico redondeado en vistas dorsal y lateral; distancia ojo narina 11.3% de la LRC, con un tubérculo pequeño y aplanado en la punta del hocico; canto rostral recto con la región loreal ligeramente cóncava; narinas pequeñas dirigidas lateralmente; área interorbital plana con un tubérculo subcónico, más ancha que el párpado superior, el cual equivale al 82% la distancia interorbital; cresta craneal ausente; párpado superior con un tubérculo cónico rodeado de pequeñas verrugas elevadas; membrana timpánica diferenciada de la piel que la rodea, anillo timpánico evidente y redondeado, con el margen superior cubierto 1/3 por el pliegue supratimpánico anterodorsal, tímpano visible dorsalmente, diámetro del tímpano 51% del diámetro del ojo, pliegue supratimpánico cubierto por tubérculos postrictales subcónicos; coanas grandes y oblicuas de contorno, no cubiertas por el piso palatal del maxilar; procesos de los odontóforos vomerinos presentes, de contorno oblicuo con 2–3 dientes; lengua ligeramente más ancha que larga, de forma acorazonada, 3/4 adherida al piso de la boca.

Textura del dorso lisa, flancos y pelvis finamente granular, vientre areolado, pliegues dorsolaterales que se extienden desde la parte posterior del ojo hasta la pelvis, pliegue discoidal conspicuo, se extiende desde la mitad de los flancos hasta la región ventral; cloaca rodeada por varias verrugas aplanadas. Brazos esbeltos con tubérculos ulnares bajos; dedos sin rebordes cutáneos laterales, tres tubérculos palmares pequeños redondeados, tubérculo tenar ovalado; tubérculos subarticulares redondos, con tubérculos supernumerarios, dedos estrechos con almohadillas definidas por surcos circunmarginales, almohadilla del dedo I no expandida. Dedo III y IV de la mano con tubérculo subarticular distal doble.

Extremidades posteriores esbeltas, longitud de la tibia 53% de la LRC, tubérculos subcónicos en el borde externo de la rodilla y tarso; pliegue tarsal interno ausente; talón con un tubérculo cónico y varios subcónicos; dedos del pie estrechos, con débiles rebordes cutáneos laterales, sin membranas interdigitales; tubérculos subarticulares redondos y prominentes; tubérculo metatarsal interno ovalado, cuatro veces el tamaño del externo que es subcónico; tubérculos supernumerarios presentes, bajos; discos ligeramente expandidos en todos los dedos; dedo V mayor al III y alcanza la base del tubérculo subarticular distal del dedo IV.

En vida, presenta el dorso café con marcas en forma de)(café oscuro a negro rodeada de café claro, cabeza con marcas café oscuro en fondo café claro, banda interorbital, párpados, banda cantal y supratimpánica café oscura a negra, superficies dorsales café con barras café oscuras; pliegues dorsolaterales café claro rojizo; manos y pies café rojizo con manchas café oscuras a negras, dígitos crema amarillentos; vientre, pecho y garganta café con pequeñas y tenues marcas negras, superficies ocultas de las extremidades color café cremoso; iris finamente reticulado dorado verdoso con una línea media café cobrizo (Fig. 4, 8, 12).

Tabla 1. Variación morfométrica (en mm) de las series tipo de *Pristimantis miltongallardoi* sp. nov. y *Pristimantis omarrhynchus* sp. nov. Abreviaturas: ON = distancia órbita-narina; LC = longitud cefálica; DIO = distancia interorbital; EN = distancia internarinal; LRC = longitud rostro-cloacal; LT = longitud de la tibia; LP = longitud del pie; LM = longitud de la mano; DT = diámetro horizontal del tímpano.

	Pristimantis omarrhynchus				***Pristimantis miltongallardoi***			
	Hembras		Machos		Hembras		Machos	
	Rango (media ± DE) n = 3		Rango (media ± DE) n = 15		Rango (media ± DE) n = 10		Rango (media ± DE) n = 4	
LRC	23,5–27,3	(25,2 ± 1,9)	12,1–19,9	(16,7 ± 2,3)	22,3–28,2	(25,2 ± 1,6)	17–18,7	(17,7 ± 0,7)
LC	9,1–9,4	(9,3 ± 0,2)	4,8–7,1	(5,9 ± 0,7)	8,9–10,04	(9,5 ± 0,5)	6,4–7,3	(6,8 ± 0,3)
AC	9,2–10,4	(9,6 ± 0,6)	3,9–7,8	(6,3 ± 1,0)	9,1–11	(9,9 ± 0,5)	6,6–7,2	(6,8 ± 0,2)
DIO	2,8–3,2	(3,1 ± 0,2)	1,3–2,3	(1,9 ± 0,3)	2–3,2	(2,8 ± 0,3)	2–2,4	(2,2 ± 0,2)
ON	2,4–2,9	(2,7 ± 0,3)	1,04–6,1	(1,8 ± 1,2)	2,2–3,5	(2,8 ± 0,4)	1,6–2,2	(1,8 ± 0,3)
EN	2,4–2,7	(2,5 ± 0,2)	1,1–6,8	(2,3 ± 1,7)	1,09–2,5	(2,2 ± 0,4)	1,1–1,8	(1,5 ± 0,3)
DT	1,4–1,7	(1,5 ± 0,1)	0,6–5,9	(1,4 ± 1,3)	1,02–1,7	(1,4 ± 0,2)	0,8–1,1	(0,9 ± 0,1)
Tibia	12,4–14,6	(13,3 ± 1,1)	6,7–16,1	(9,7 ± 2,0)	12,4–14	(13,1 ± 0,5)	9,1–10,4	(9,7 ± 0,5)
Pie	11,7–13	(12,2 ± 0,7)	5,04–13,9	(8,3 ± 1,9)	10,7–13,6	(12,6 ± 0,8)	7,9–9,7	(8,8 ± 0,7)
Mano	7,08–8,1	(7,5 ± 3,1)	3,1–11,4	(5,5 ± 2,0)	7,03–9,3	(8,2 ± 0,8)	5,1–6,03	(5,5 ± 04)

En preservado, presenta coloración dorsal café oscura a café claro cremoso, con una marca en el dorso en forma de)(y manchas irregulares desde la cabeza hasta la cloaca café oscuro; banda supratimpánica café oscura desde la parte posterior del ojo hasta la inserción del brazo; pliegues dorsolaterales café claros; flancos con barras café oscuras a negras con interespacios café claros a cremas, marcas blancas en la punta de los tubérculos; extremidades anteriores con marcas café oscuras a negras, dedos café claros con algunas marcas negras en los dedos III y IV; superficies dorsales de los muslos y extremidades posteriores con barras cafés oscuro separado por café claro; ingles, axilas, vientre, garganta y superficies ocultas de las extremidades café claro a crema; superficies palmares y plantares café claro (Figs. 2, 3, 6, 7).

Medidas del holotipo (mm)

Longitud rostro cloaca LRC = 23,5; longitud de la tibia LT = 12,4; longitud del pie LP = 11,7; longitud de la cabeza LC = 9,1; ancho de la cabeza AC = 9,4; distancia interorbital DIO = 3,2; distancia internarinal EN = 2,4; distancia ojo narina ON = 2,8; diámetro horizontal del tímpano DT = 1,7.

Variación

La variación morfométrica más notable, es que las hembras en promedio son 1,5 veces más grandes que los machos (Tabla 1). La coloración en preservado de la serie tipo presenta dorso café oscuro, café claro o crema, pliegues dorsolaterales claros; región interorbital, banda cantal-supratimpánica café oscura o negra; barras de las extremidades y flancos café oscuro separado por barras cremas o poco definidas en algunos individuos, superficies anteriores de los muslos y piernas café claras; dígitos crema. Observamos tres morfos de coloración en hembras, las marcas café oscuro en el dorso son variables incluyendo formas de X, ^, y)((Fig. 6). Los machos presentaron cinco morfos de coloración desde tonalidades café oscuro rojizo a café claro o crema grisáceo, con antebrazos y muslos amarillentos o blanquecinos, vientres y gargantas homogéneamente crema inmaculado hasta café (Fig. 6); uno de los morfos es disruptivo de la serie tipo (DHMECN 11482), con la banda rostral-dorsolateral completa y sin diseños dorsales (Fig. 6H).

La coloración en vida de la serie tipo varía desde dorso café rojizo hasta naranja claro amarillento sin diseño dorsal (DHMECN 11485) (Fig. 4G–H) o generalmente con una marca en forma)(café oscura. Otros individuos presentaron una barra mediodorsal café oscura (QCAZ 77383, Fig. 4E) o una mancha cefálica crema (QCAZ 77384, Fig. 4F); pliegues dorsolaterales naranja crema, superficie de las extremidades, superficies ocultas de los muslos y flancos con barras café oscuras separadas por barras

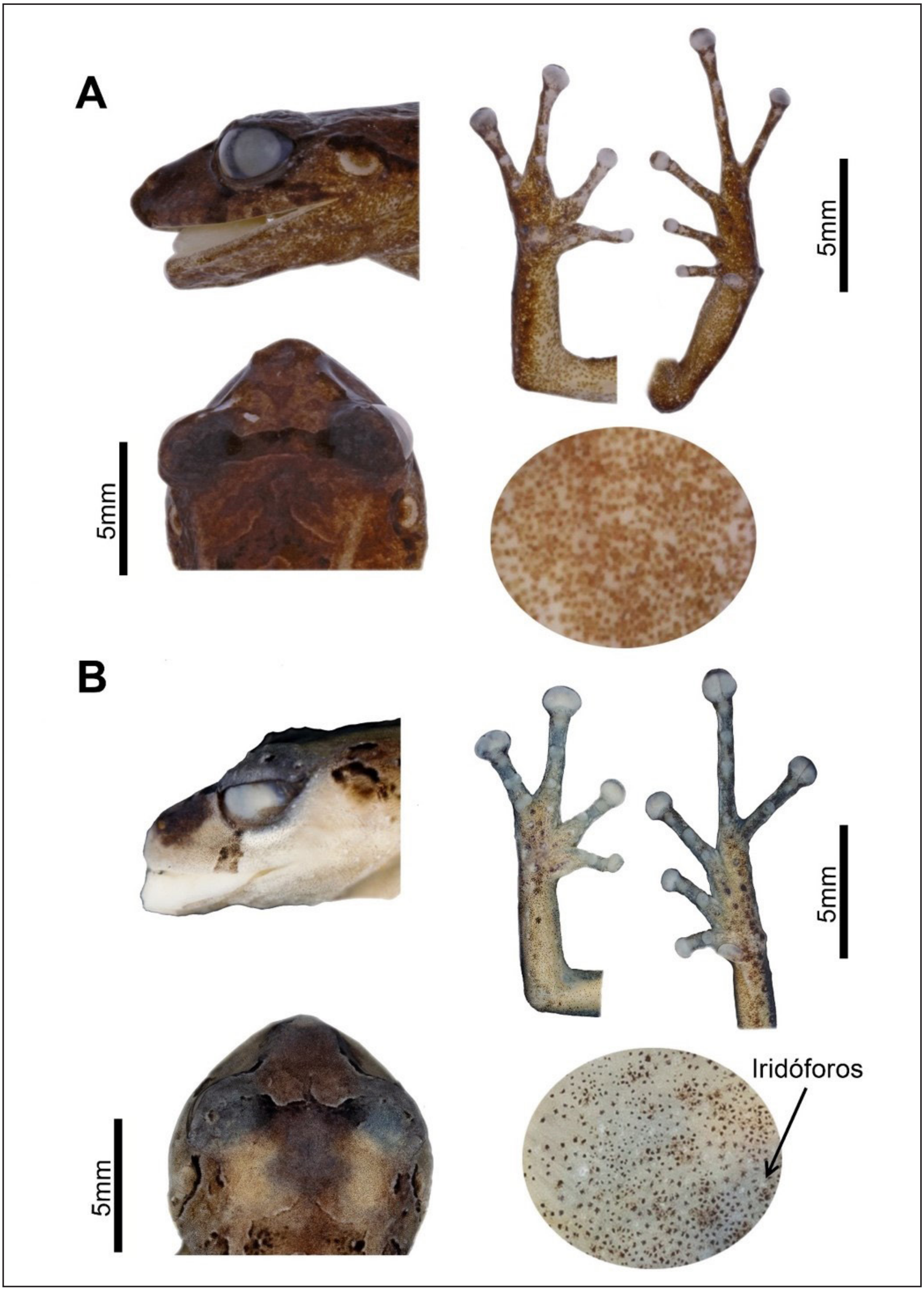

Figura 3. Detalle de la cabeza, patas y vientre en preservado (A) *Pristimantis omarrhynchus* sp. nov. Holotipo, hembra adulta (DHMECN 11480); (B) *Pristimantis miltongallardoi* sp. nov. Holotipo, hembra adulta (QCAZ 65980). Fotografías: M. H. Yánez-Muñoz y Santiago Ron.

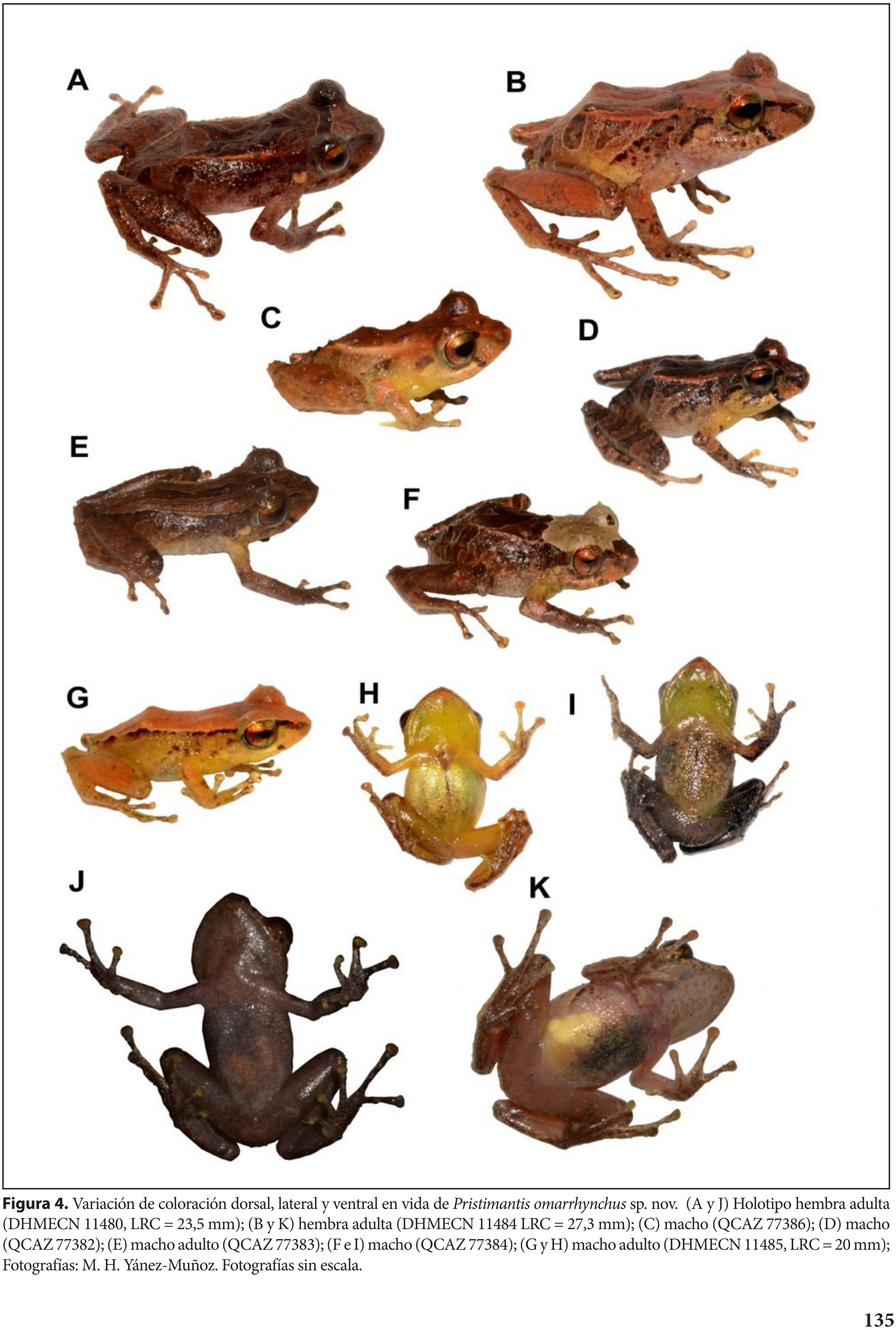

Figura 4. Variación de coloración dorsal, lateral y ventral en vida de *Pristimantis omarrhynchus* sp. nov. (A y J) Holotipo hembra adulta (DHMECN 11480, LRC = 23,5 mm); (B y K) hembra adulta (DHMECN 11484 LRC = 27,3 mm); (C) macho (QCAZ 77386); (D) macho (QCAZ 77382); (E) macho adulto (QCAZ 77383); (F e I) macho (QCAZ 77384); (G y H) macho adulto (DHMECN 11485, LRC = 20 mm); Fotografías: M. H. Yánez-Muñoz. Fotografías sin escala.

cremas (Fig. 4); vientre y garganta cafés a crema amarillentos (Fig. 4H–K); superficies ocultas de las axilas e ingles crema amarillentas a rojizas en hembras y machos; superficies anteriores de los muslos y piernas cafés; iris reticulado plateado verdoso con una línea media café cobrizo; banda interorbital, cantal y timpánica café oscuro a negra; superficies posteriores de los muslos con barras café oscuro a claro amarillento (Fig. 4).

Pristimantis omarrhynchus tiene plasticidad fenotípica, en vida, en la prominencia de sus tubérculos. En el caso del holotipo DHMECN 11480 y el paratipo DHMECN 11483, hubo una reducción en el tamaño de tubérculos a las 12 horas de haber sido colectados: el holotipo redujo el tamaño de sus tubérculos del párpado (Fig. 8G y 12A) y el paratipo redujo su papila en el hocico (Fig. 12B). Además, los tubérculos cónicos en párpados y talón, así como papila, fueron más desarrollados en machos vocalizadores registrados en septiembre de 2017 (Fig. 4 D–F) vs. machos sin actividad vocal registrados en diciembre de 2014 (Fig. 4C, G).

Descripción del canto

El canto de *Pristimantis omarrhynchus* (DHMECN 11483) es un clic que se emite en series de cinco o seis repeticiones (Fig. 9; Tabla 2). Su frecuencia dominante promedio es 4,04 kHz (DE = 0,06) y corresponde a la primera armónica; una segunda armónica tenue tiene 7,97 kHz (DE = 0,11). Los cantos tienen una sola nota; su duración promedio de 66 ms (DE = 11,9). El intervalo entre cantos es muy corto (promedio 9,2 ms, DE = 2,9) y las series de cantos se emiten a intervalos irregulares (rango 7,2–71,5 s). En la misma grabación hay un canto similar, pero de menor amplitud que probablemente corresponda a otro macho (no colectado) que cantaba antífonamente. Esos cantos se diferencian por tener de 3 a 4 cantos por serie.

El canto de *P. omarrhynchus* es similar a cantos de otras especies del grupo *P. boulengeri*. El canto de *P. boulengeri* también consiste en un clic emitido en series de 1 a 9 repeticiones (Ríos-Soto y Ospina 2018). Similarmente, el canto de *P. dorsopictus* y *P. cryptopictus* es un clic, con la diferencia de que no es emitido en series sino de modo individual (Patiño-Ocampo *et al.,* 2022). El canto de otras especies del grupo es desconocido.

Distribución e historia natural

Pristimantis omarrhynchus se conoce para dos localidades de las estribaciones orientales de los Andes de Ecuador en las provincias de Napo y Sucumbíos en los tributarios Quijos y Coca (Fig. 10; 11). Fue registrada en bosque maduro y secundario en las faldas del volcán Reventador entre los 1700 y 1800 msnm, en los bosques siempreverde montano y montano bajo de la cordillera oriental de los Andes norte de Ecuador (tipos de vegetación MAE 2013) y en Bosque Montano Oriental (Ron *et al.*, 2022). Estás áreas corresponden a los límites del piso zoogeográfico Subtropical y piso Templado (Albuja *et al.,* 2012). Los especímenes tipo fueron colectados en vegetación arbustiva hasta 1,5 m del suelo aproximadamente, en su mayoría dentro del bosque maduro y algunos ejemplares en los claros, bordes de bosque y áreas intervenidas (Figs. 11-12). Las hembras grávidas (DHMECN 11480, 11484, QCAZ 10836) fueron colectadas en diciembre de 2014 y abril 1996, machos vocalizadores fueron registrados en septiembre de 2017, sugiriendo una reproducción continua a lo largo del año. La serie tipo colectada en los alrededores del volcán Reventador fue registrada durante actividad volcánica, antes y después de la caída de ceniza.

Pristimantis miltongallardoi **sp. nov.**

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Pristimantis cf. *petersi* Guayasamin y Funk (2009)
Pristimantis sp. 2 Funk *et al.*, (2003)

Holotipo (Figs. 2, 3, 5, 7)
Hembra adulta QCAZ 65980, Reserva Ecológica Antisana, Cordillera de los Guacamayos, Sector la Virgen (sendero Jumandi); (0.64119 S; 77.8375 W, 1927 msnm), cantón Quijos, parroquia Cosanga, provincia de Napo, República del Ecuador, colectado el 26 de noviembre de 2016 por Santiago Ron, Francisca Hervas, Gustavo Pazmiño, Javier Pinto, Jhael Ortega.

Paratipos

Todos los paratipos fueron colectados en la provincia de Napo, República del Ecuador. QCAZ 65979, 65980–1, 65988 con los mismos datos del holotipo; Estación Científica Yanayacu (0.5988 S; 77.8895 W, 2100 msnm), cantón Quijos, parroquia Cosanga, hembra QCAZ 22377 colectada el 2 de junio de 1989 por Luis Coloma, QCAZ 23122 colectado el 20 de febrero de 2003 por Diego Almeida, QCAZ 33038

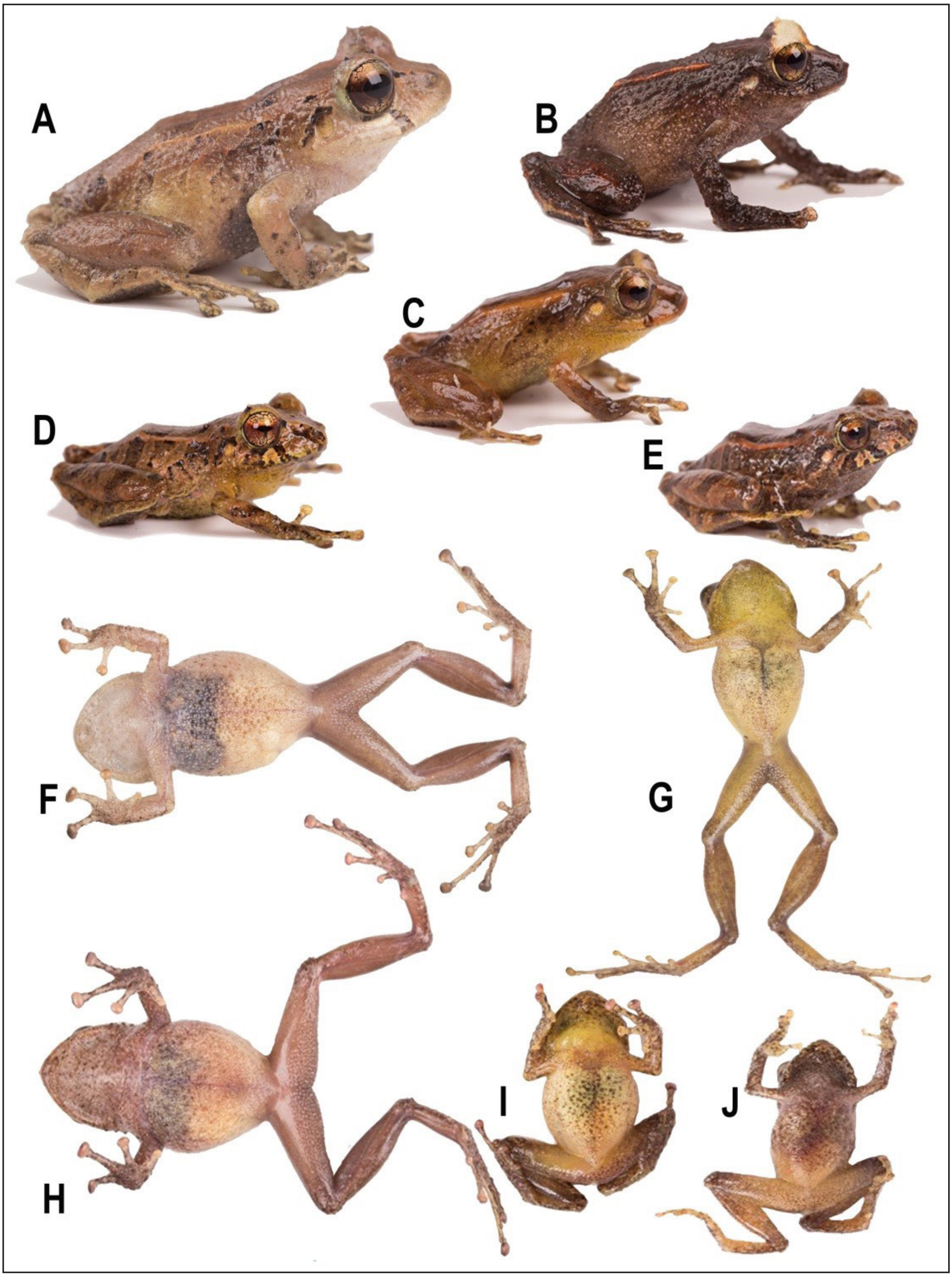

Figura 5. Variación de coloración lateral y ventral en vida de *Pristimantis miltongallardoi* sp. nov. (A y F) Holotipo hembra (QCAZ65980 LRC = 27mm); (B, H) hembra (QCAZ 65981, LRC = 25 mm); (C, G) macho (QCAZ 65979, LRC = 17 mm); (D, I) macho (QCAZ 63482); (E, J) juvenil (QCAZ 63481). Fotografías: Santiago Ron. Fotografías sin escala.

Tabla 2. Estadísticas descriptivas de las variables acústicas del canto de *Pristimantis omarrhynchus* (macho DHMECN 11483). Las abreviaciones son kHz = kilohertzios, ms = milisegundos, DE = desviación estándar.

Variable	*P. omarrhynchus* sp. nov.	
	Rango (media ± DE)	*n*
Frecuencia Dominante (kHz)	3,90–4,16 (4,05 ± 0,06)	25
Segunda Armónica (kHz)	7,90–8,17 (7,97 ± 0,11)	5
Duración de la serie de cantos (ms)	341,1–380,2 (362,6 ± 17,2)	5
Intervalos entre cantos (ms)	4,95–14,4 (9,19 ± 2,88)	20
Duración de los cantos (ms)	55–98 (66,15 ± 11,99)	25
Cantos/serie	5–6	5

colectado el 7 de julio de 2005 por Carolina Proaño; Cordillera de los Guacamayos, entre la Virgen y el Río Urcusiqui (0.6502 S; 77.8264 W 1800 msnm), cantón Quijos, parroquia Cosanga, hembras QCAZ 50075–076, machos QCAZ 50082–083, colectados el 31 de marzo de 1998 por Felipe Campos.

Etimología

El epíteto específico es un patronímico que resulta de la combinación del nombre y apellido de un admirado científico miembro de la Academia de Ciencias de América Latina, Milton H. Gallardo, Ph.D. en Biología y profesor de Genética y Evolución en la Facultad de Ciencias, Universidad Austral de Chile. Fue parte del programa Prometeo en Ecuador y desde esta posición impulsó la gestión del Museo Ecuatoriano de Ciencias Naturales y apoyó su transición al actual Instituto Nacional de Biodiversidad. Su obra "*Evolución: el curso de la vida*" es uno de sus legados más importantes de su carrera para las nuevas generaciones Latinoamericanas.

Nombre común sugerido: Cutín de Milton H. Gallardo

Nombre común en inglés sugerido: Milton´s Rain Frog

Diagnosis

Pristimantis miltongallardoi caracterizada por: (1) piel del dorso lisa con algunos tubérculos redondeados esparcidos en los flancos y pelvis con dos tubérculos subcónicos redondeados escapulares; vientre areolado; pliegues dorsolaterales continuos desde la parte posterior del párpado hasta la parte media del dorso en la cintura pélvica; pliegue discoidal presente; (2) anillo y membrana timpánica prominente, redondeada 38–52% del diámetro del ojo, margen superior del anillo timpánico cubierto por el pliegue supratimpánico; con tubérculos postrictales subcónicos pequeños; (3) hocico redondeado en vistas dorsal y lateral, con papila en la punta del hocico; (4) párpado superior con un tubérculo cónico prominente rodeado por algunos tubérculos redondeados pequeños; párpado más estrecho que la distancia interorbital; tubérculo interorbital pequeño; cresta craneal ausente; (5) procesos vomerinos presentes oblicuos de contorno con 2 a 4 dientes; (6) machos con hendiduras vocales, con saco vocal subgular y almohadillas nupciales pequeñas y oblicuas, no queratinizadas de color blanquecino; (7) dedo I de la mano más pequeño que el dedo II, discos ligeramente expandidos en los dedos II-III-IV; dedos III y IV con tubérculo subarticular distal doble; (8) dedos sin rebordes cutáneos laterales; (9) tubérculos ulnares presentes; (10) talón con un tubérculo grande, pequeños tubérculos en el borde externo de la pierna, rodilla y tarso; pliegue tarsal interno ausente; (11) tubérculo metatarsal interno oval, más de tres veces que el tubérculo metatarsal externo pequeño subcónico, pocos tubérculos supernumerarios bajos; (12) dedos del pie sin rebordes cutáneos laterales o indistintos; almohadillas ligeramente más grandes que los dedos, sin membranas interdigitales; dedo V más largo que el dedo III y alcanza al tubérculo subarticular del dedo IV; tubérculo hiperdistal presente en todos los dedos; (13) coloración dorsal desde café claro verdoso hasta café oscuro naranja o rojizo, con dos marcas en forma ^ café oscura; pliegues dorsolaterales desde crema hasta anaranjado; superficie de las extremidades, superficies ocultas de los muslos y flancos con barras café separadas por barras cremas; vientre y garganta cafés a crema amarillentos, peritoneo con iridóforos; superficies ocultas de las axilas e ingles cremas amarillentas a rojizas; superficies anteriores de los muslos y piernas cafés; iris con retículos dorado verdoso y con una línea media café cobriza; banda cantal y timpánica café oscura a negra; superficies posteriores de los muslos con barras desde café oscuras a claro (amarillentas en vida); (14) LRC en hembras 22,3–28,2 mm (promedio = 25,2 ± 1,6 *n* = 10); machos 17–18,7 mm (promedio = 17,7 ± 0,7 *n* = 4).

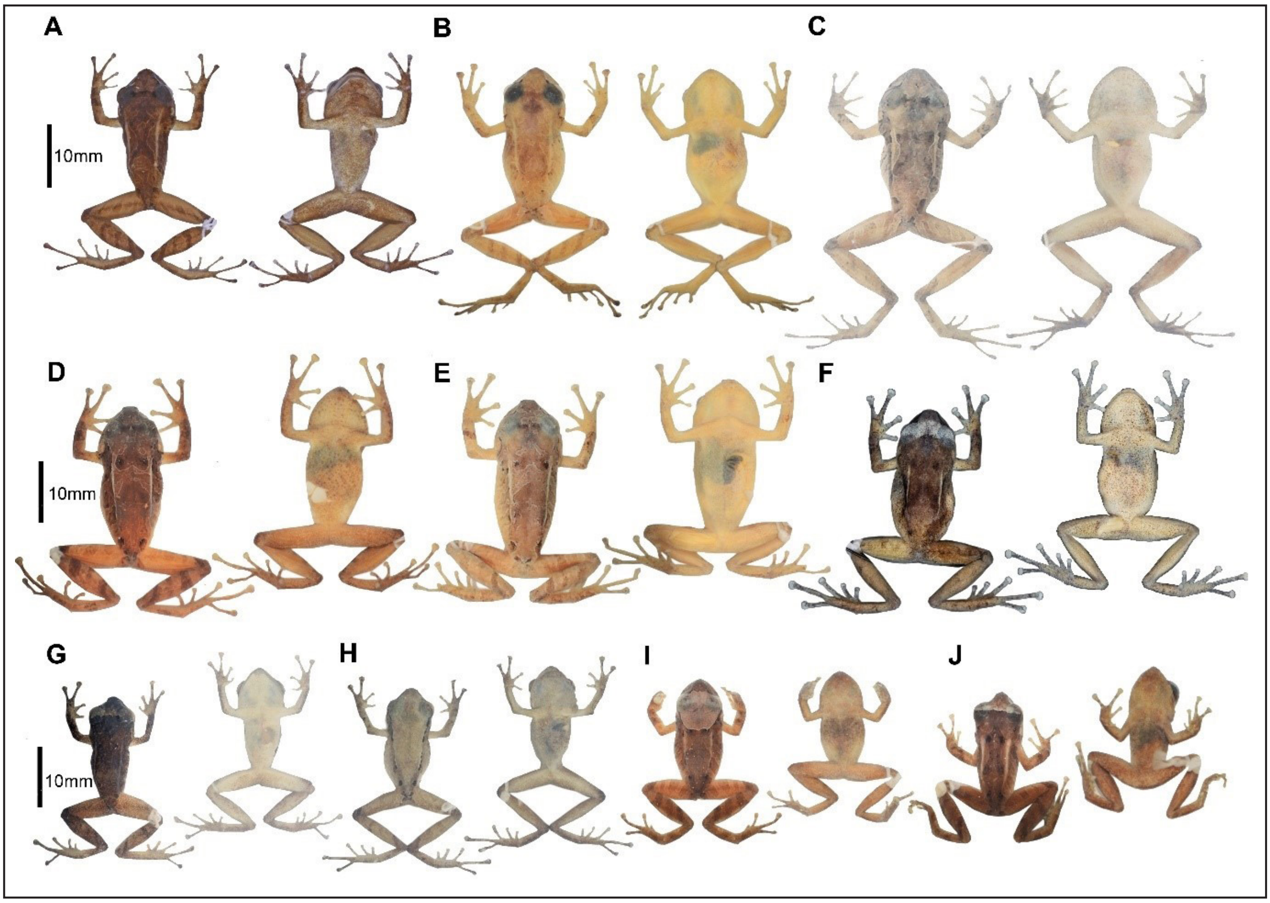

Figura 6. Variación de coloración en preservado del dorso y vientre. *Pristimantis omarrhynchus* sp. nov. (A) Holotipo, hembra adulta (DHMECN 11480); (B) hembra adulta (QCAZA 10836); (C) Paratipo hembra (DHMECN 11484); *Pristimantis miltongallardoi* sp. nov. (D) hembra (QCAZA 22377); (E) hembra (QCAZA 33038); (F) hembra (QCAZA 56981). *Pristimantis omarrhynchus* sp. nov. (G) Paratipo macho (DHMECN 11488). (H) Paratipo macho (DHMECN 11482); *Pristimantis miltongallardoi* sp. nov. (I) macho adulto (QCAZA 50082); (J) macho adulto (QCAZA 50083); Fotografías: M. H. Yánez-Muñoz y Santiago Ron.

Comparación con especies similares

Pristimantis miltongallardoi se diferencia de sus congéneres del grupo *P. boulengeri*, por la presencia de pliegues dorsolaterales bien definidos, marcas dorsales en forma de ^ café oscuro, tubérculos escapulares redondeados, un tubérculo cónico en el párpado, hocico redondeado en vista dorsal, papila en la punta del hocico, vientre con iridóforos, dígitos dilatados y pequeño tamaño corporal (hembras LRC < 28,2 mm). Se diferencia de su especie hermana, *Pristimantis omarrhynchus* sp. nov. por la presencia de iridóforos en el peritoneo, hocico redondeado en vista dorsal y los dígitos dilatados (sin iridóforos, subacuminado y dedos estrechos en *P. omarrhynchus*). Se diferencia de otros miembros del grupo *Pristimantis boulengeri* (*sensu* González Durán *et al.*, 2017), por presentar pliegues dorsolaterales (ausentes en la mayoría de las especies del grupo, excepto *P. baiotis* y *P. quantus*), marcas dorsales en forma de ^ café oscuro (marcar oscuras irregulares en *P. boulengeri*, *P. brevifrons*, *P. cryptopictus*, *P. quantus* y *P. urani*, diseño de rayas en *P. angustilineatus* y *P. dorsopictus*, marcas en forma de chevrones en *P. quantus*), con tubérculos escapulares redondeados (ausente en los demás miembros a excepción de *P. omarrhynchus*), un tubérculo cónico en el párpado rodeado por algunos más pequeños (ausente en *P. angustilineatus*, *P. myops* y *P. urani*; bajo, no cónico en *P. boulengeri*), tubérculos en el talón y tarso (ausente en *P. angustilineatus*, *P. brevifrons*, *P. cryptopictus* y *P. urani*; bajos, no cónicos, en *P. boulengeri*) Tabla 3. Otras diferencias con las especies del grupo son presentadas en el Apéndice III.

Descripción del holotipo

Hembra adulta, cabeza ligeramente más ancha que larga; hocico redondeado en vistas dorsal y lateral; distancia ojo narina 11% de la LRC, con un tubér-

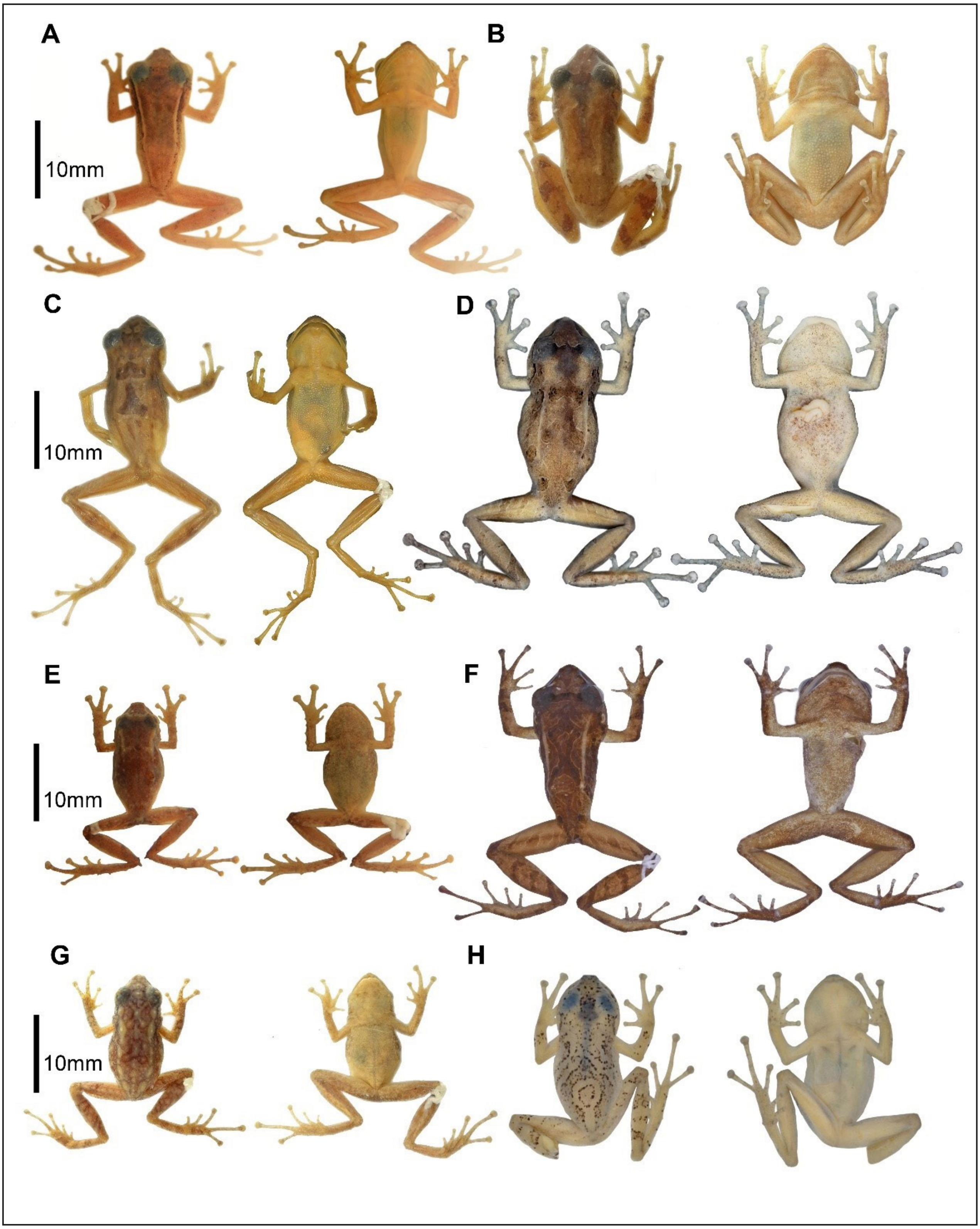

Figura 7. Vista dorsal y ventral de algunas especies del grupo *Pristimantis boulengeri* en preservado. (A) *Pristimantis angustilineatus*, Holotipo macho (ICN 39598, LRC = 19,8 mm); (B) *Pristimantis boulengeri* (ICN 34035, LRC=24,3 mm); (C) *Pristimantis brevifrons*, Holotipo (UMMZ 166572, LRC=22,3 mm); (D) *Pristimantis miltongallardoi* sp. nov., Holotipo (QCAZ 65980 LRC= 27 mm); (E) *Pristimantis myops*, Holotipo (ICN 39684, LRC= 15,6 mm); (F) *Pristimantis omarrhynchus* sp. nov., Holotipo (DHMECN 11480, LRC= 23,5); (G) *Pristimantis quantus*, Holotipo (ICN 29340, LRC= 15,9); (H) *Pristimantis urani*, Holotipo (MHUA-A 7471, LRC= 23,4). Fotografías: M. H. Yánez-Muñoz (A, B, E, F, G); The University of Michigan Museum of Zoology, Division of Reptiles and Amphibians (C); Santiago Ron. (D); Mauricio Rivera-Correa. (H).

culo pequeño y aplanado en la punta del hocico; canto rostral recto con la región loreal ligeramente cóncava; narinas pequeñas dirigidas lateralmente; área interorbital plana con un tubérculo subcónico, más ancha que el párpado superior, el cual equivale al 78% la distancia interorbital; cresta craneal ausente; párpado superior con un tubérculo cónico rodeado de pequeñas verrugas elevadas; membrana timpánica diferenciada de la piel que la rodea, anillo timpánico evidente y redondeado, con el margen superior cubierto 1/3 por el pliegue supratimpánico anterodorsalmente, tímpano visible dorsalmente, diámetro del tímpano 28% del diámetro del ojo, pliegue supratimpánico cubierto por tubérculos postrictales subcónicos; coanas grandes y oblicuas de contorno, no cubiertas por el piso palatal del maxilar; procesos de los odontóforos vomerinos presentes, de contorno oblicuo con 2–3 dientes; lengua ligeramente más ancha que larga, de forma acorazonada, ¾ adherida al piso de la boca.

Textura del dorso lisa, flancos y pelvis finamente granular, vientre areolado, pliegues dorsolaterales que se extienden desde la parte posterior del ojo hasta la pelvis, pliegue discoidal conspicuo, se extiende desde la mitad de los flancos hasta la región ventral; cloaca rodeada por varias verrugas aplanadas. Brazos esbeltos con tubérculos ulnares bajos; dedos sin rebordes cutáneos laterales, tres tubérculos palmares pequeños redondeados, tubérculo tenar ovalado; tubérculos subarticulares redondos, con tubérculos supernumerarios, dedos estrechos con almohadillas definidas por surcos circunmarginales, almohadilla del dedo I no expandida. Dedo III y IV de la mano con tubérculo subarticular distal doble.

Extremidades posteriores esbeltas, longitud de la tibia 46% de la LRC, tubérculos subcónicos en el borde externo de la rodilla y tarso; pliegue tarsal interno ausente; talón con un tubérculo cónico y varios subcónicos; dedos del pie estrechos, con débiles rebordes cutáneos laterales, sin membranas interdigitales; tubérculos subarticulares redondos y prominentes; tubérculo metatarsal interno ovalado, 4 veces el tamaño del externo que es subcónico; tubérculos supernumerarios presentes, bajos; discos ligeramente expandidos en todos los dedos; dedo V mayor al III y alcanza la base del tubérculo subarticular distal del dedo IV.

En vida, presenta el dorso café con marcas en forma de ^ café oscuro a negro rodeada de café claro, cabeza con marcas café oscuro en fondo café claro, banda interorbital, párpados, banda cantal y supratimpánica café oscura a negra, superficies dorsales café con barras café oscuras; pliegues dorsolaterales café claro naranja; manos y pies café rojizo con manchas café oscuras a negras, dígitos crema amarillentos; vientre, pecho y garganta café con pequeñas y tenues marcas negras, peritoneo con iridóforos, superficies ocultas de las extremidades color café cremoso; iris finamente reticulado dorado verdoso con una línea media café cobrizo (Fig. 5, 8).

En preservado, presenta coloración dorsal café oscura a café claro cremoso, con una marca en el dorso en forma de ^ y manchas irregulares desde la cabeza hasta la cloaca café oscuro; banda supratimpánica café oscura desde la parte posterior del ojo hasta la inserción del brazo; pliegues dorsolaterales café claros; flancos con barras café oscuras a negras con interespacios café claros a cremas, marcas blancas en la punta de los tubérculos; extremidades anteriores con marcas café oscuras a negras, dedos café claros con algunas marcas negras en los dedos III y IV; superficies dorsales de los muslos y extremidades posteriores con barras cafés oscuro separado por café claro; ingles, axilas, vientre, garganta y superficies ocultas de las extremidades café claro a crema; superficies palmares y plantares café claro (Figs. 2–3).

Medidas del holotipo (mm)

Longitud rostro cloaca LRC = 27; longitud de la tibia LT = 12,39; longitud del pie LP = 12,8; longitud de la cabeza LC = 10,04; ancho de la cabeza AC = 10,25; distancia interorbital DIO = 2,56; distancia internarinal EN = 2,07; distancia ojo narina ON = 2,97; diámetro horizontal del tímpano DT = 1,02.

Variación

Las hembras tienen un tamaño promedio de 1,5 veces más grandes que los machos (Tabla 1). La coloración en preservado de la serie tipo presenta el dorso desde café oscuro, café claro o crema, pliegues dorsolaterales claros; región interorbital, banda cantal-supratimpánica café oscura o negra; barras de las extremidades y flancos café oscuro separado por barras cremas o poco definidas en algunos individuos, superficies anteriores de los muslos y piernas café claras; dígitos crema. Observamos tres morfos de coloración en hembras, desde café oscuro homogéneo a marcas variables café oscuro a claro en el dorso incluyendo formas de X y ^ y barra inter orbital (Fig. 6 D, E, F). Los machos presentaron cuatro morfos de coloración desde tonalidades café

Tabla 3. Principales caracteres diagnósticos de las especies del grupo *Pristimantis boulengeri.*

Especie	**LRC**	**Pliegue dorso lateral**	**Tubérculos en el párpado**	**Tubérculos en el talón**	**Tubérculos en el borde externo del tarso**	**Tubérculos escapulares**	**Patrón coloración dorsal**
P. miltongallardoi	♂17,7 (17,0–18,7; n = 5) ♀25,2 (22,3–28,2; n = 10)	Presente	Un tubérculo cónico prominente rodeado por algunos pequeños	Un tubérculo grande	Presentes, pequeños	Presentes, redondeados	Dorso con marcas en forma “^” café oscuro
P. omarrhynchus	♂16,7 (12,1–20,0; n = 15) ♀25,2 (23,5–27,3; n = 3)	Presente	Un tubérculo cónico prominente rodeado por algunos tubérculos bajos	Un tubérculo grande	Presentes, pequeños	Presentes, redondeados	Dorso con marcas en forma “)(“café oscuro
P. angustilineatus	♂18,1 (15,8–20,4; n = 61) ♀22,6 (20,8–24,8; n = 18)	Ausente	Sin tubérculos	Ausentes	Ausente	Ausente	Dorso con rayas finas dorsolaterales blancas delimitadas de negro por debajo
P. baiotis	♂18,1–18,5 ♀21,5	Presente	Tubérculos cónicos	Subcónico	Presentes	Ausente	Dorso sin diseño específico
P. boulengeri	♂22,1 (18,6–25,6; n = 87) ♀30,0 (27,3–33,8; n = 17)	Ausente	Bajos, no cónicos	Tubérculos no cónicos	Ausente	Ausente	Dorso con marcas café y barra interorbital oscura
P. brevifrons	♂17,4 (15,1–19,7; n = 3) ♀22,8 (21,2–25; n = 5)	Ausente	Bajos	Ausente	Presente	Ausente	Dorso con marcas marrón oscuro
P. cryptopictus	♂24,08 (20,6–27,2; n = 30)	Ausente	Un tubérculo redondeado rodeado por algunos pequeños	Ausente	Presente	Ausente	Dorso con o sin puntos o manchas irregulares en el cuerpo, sin franjas o líneas longitudinales

P. dorsopictus	♂(21,31,6; n = 7) ♀(19,0–22,0; n = 8)	Ausente	Granular	Presentes, pequeños	Presentes, pequeños	Ausente	Dorso con bandas longitudinales o manchas en forma de V de color café oscuro
P. myops	♂11,9 (10,9–13,6; n = 34) ♀15,5 (14,6–17,2; n = 38)	Ausente, interorbital presente, pliegue dérmico occipital en forma de W	Ausente, pliegue interorbital y sacral	Presentes, pequeños	Presentes, subcónicos	Ausente	Dorso con marcas dispersas oscuras
P. quantus	♂11,9 (11,6–14,5; n = 4) ♀25,3 (14,4–16,7; n = 9)	Presente poco definidos	Tubérculo cónico	Pequeños subcónicos	Pequeños subcónicos	Ausente	Dorso con verrugas y marcas oscuras verdosas en forma de “^”
P. urani	♂18,9 (18,7–19,1; n = 2) ♀22,5 (21–23,4; n = 4)	Ausente	Sin tubérculos	Ausentes	Ausentes	Ausente	Dorso con muchas manchas y marcas marrón oscuro dispersas

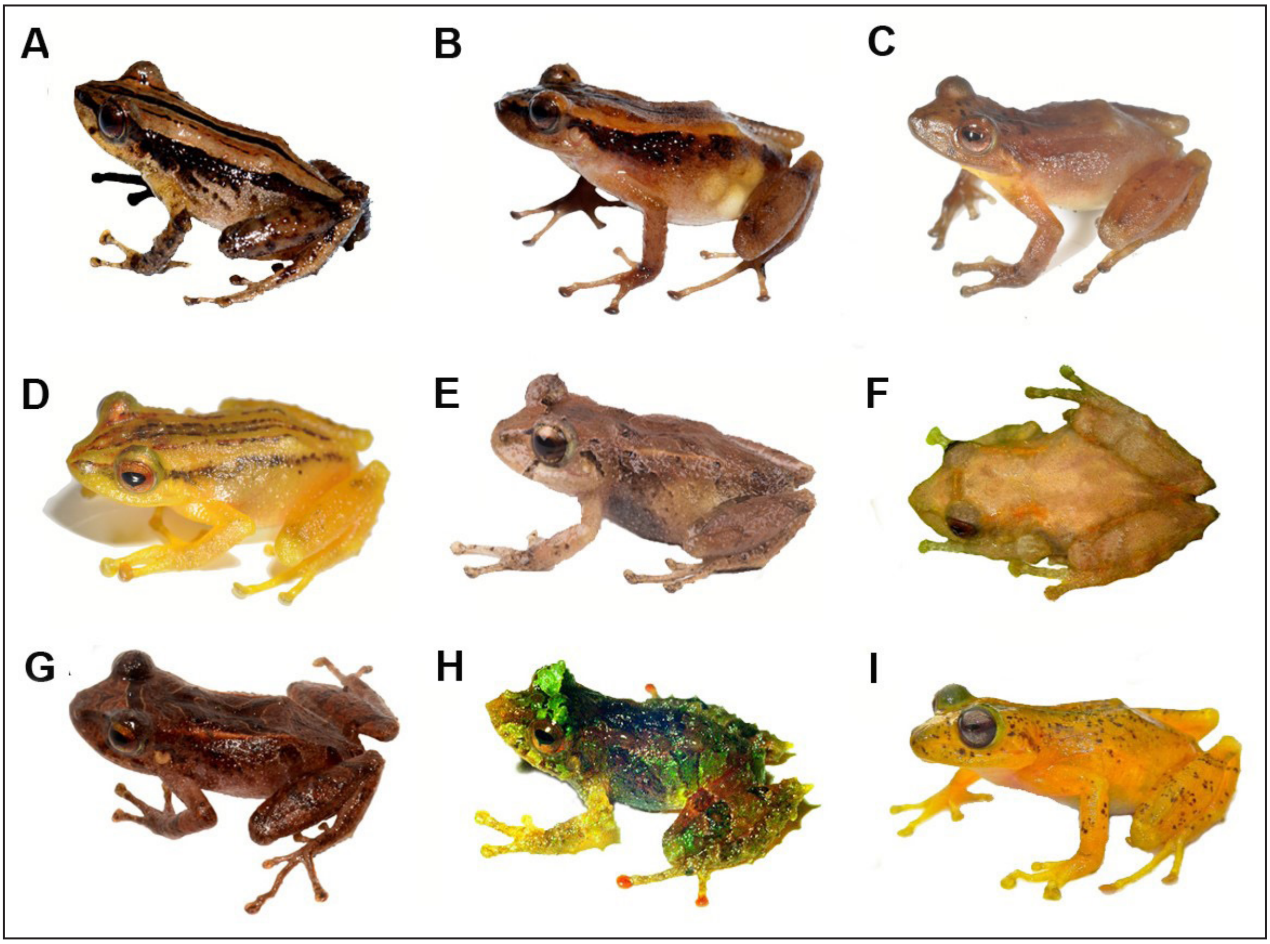

Figura 8. Variación de coloración en vida de algunas especies del grupo *Pristimantis boulengeri*. (A) *Pristimantis boulengeri* (MAR 2760); (B) *Pristimantis brevifrons* (MAR 2305); (C) *Pristimantis cryptopictus* (MHUA A12475); (D) *Pristimantis dorsopictus* (MHUA A12492); (E) *Pristimantis miltongallardoi* sp. nov., Holotipo (QCAZ 65980); (F) *Pristimantis myops* (iNaturalist); (G) *Pristimantis omarrhynchus* sp. nov., Holotipo (DHMECN 11480); (H) *Pristimantis quantus* (ICN 29315); (I) *Pristimantis urani*, Holotipo (MHUA-A 7471). Fotografías: M. Rada (A, B); Mauricio Rivera-Correa (C, D); S. Ron (E); J. J. Ospina-Sarria INaturalist (F); M. H. Yánez-Muñoz (G); J. D. Lynch (H); F. Duarte (I). Fotografías sin escala.

oscuro a crema grisáceo, con antebrazos y muslos amarillentos o blanquecinos, vientres y gargantas homogéneamente crema inmaculado hasta café; los machos QCAZ 50082 y 50083 presentan mancha cefálica o banda interorbital crema (Fig. 6I y 6J).

La coloración en vida de la serie tipo varía desde dorso café rojizo hasta naranja claro amarillento sin diseño dorsal (QCAZ 65981) (Fig. 5B) con marcas en forma ^ en el dorso (Fig. 5A, C–E), pliegues dorsolaterales naranja crema, superficie de las extremidades, superficies ocultas de los muslos y flancos con barras café oscuras separadas por barras cremas (Fig. 5 A–G); vientre y garganta cafés a crema amarillentos (Fig. 5 F–J); superficies ocultas de las axilas e ingles crema amarillentas a rojizas en hembras y machos; superficies anteriores de los muslos y piernas cafés; iris reticulado dorado verdoso con una línea media café cobrizo; banda interorbital, cantal y timpánica café oscuro a negra; superficies posteriores de los muslos con barras café oscuro a claro amarillento (Fig. 5).

Distribución e historia natural

Pristimantis miltongallardoi se conoce solo para la provincia de Napo en tres localidades de las estribaciones orientales de los Andes en los drenajes de la subcuenca del río Cosanga (Fig. 10; 11). Ha sido registrada en bosque secundario, claros, bordes de bosques y áreas intervenidas, algunos individuos fueron encontrados en la vía a Baeza a los 1840 msnm; en Cosanga, Estación Científica Yanayacu es una especie abundante que se encuentra en bosque primario en vegetación baja (Guayasamin y Funk, 2009), en un intervalo altitudinal entre 1800–2137 msnm y al sur de la cordillera de los Guacamayos a 1927 msnm. Habita los bosques siempreverde mon-

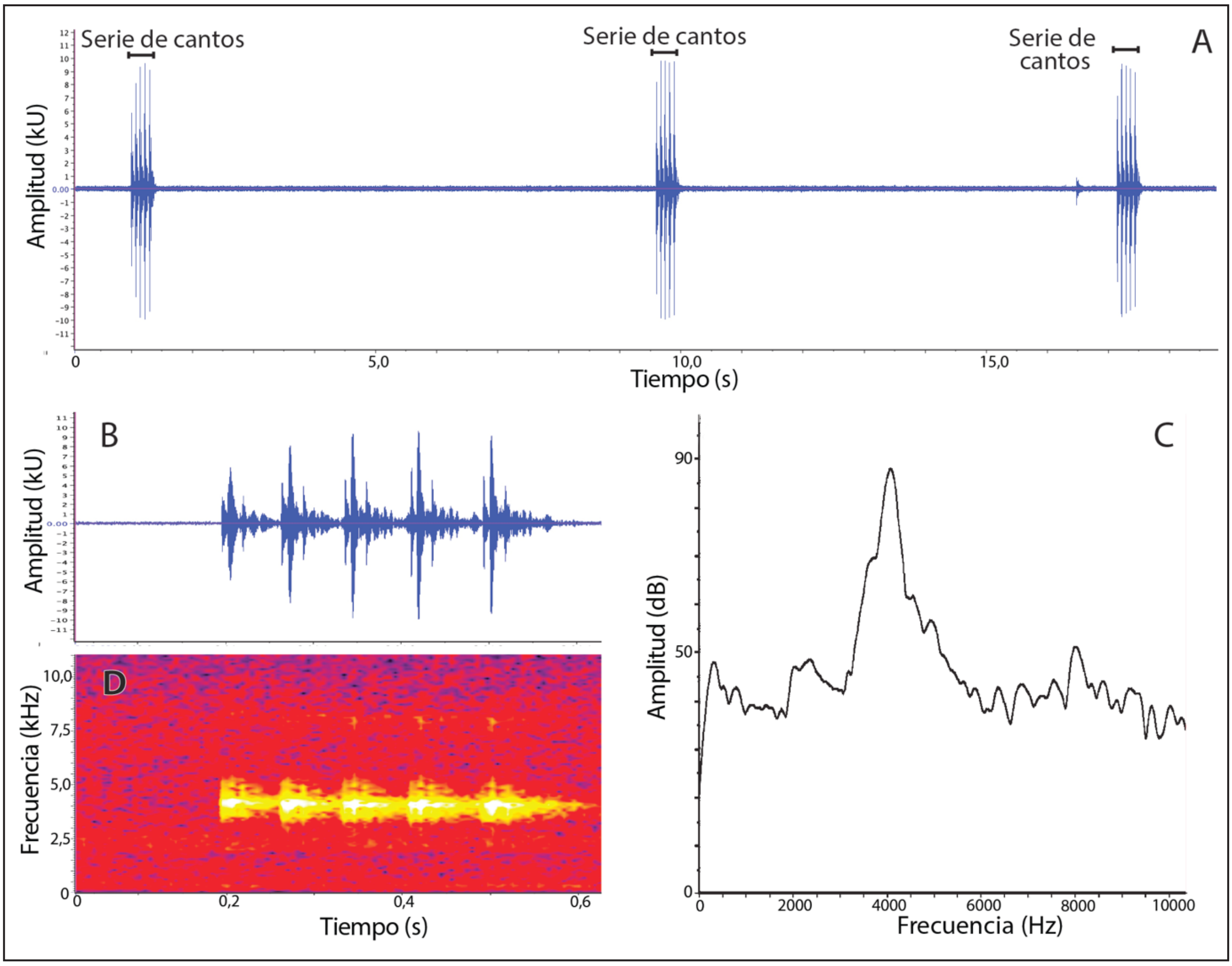

Figura 9. Canto de *Pristimantis omarrhynchus* sp. nov. A y B son oscilogramas, C es un espectro de poder y D es un espectrograma. (A) Tres series de cantos consecutivas; (B) y (D) serie de cinco cantos; (C) Espectro de poder de un canto. Macho DHMECN 11483, LRC = 17,6 mm, laderas del volcán Reventador, Provincia Sucumbíos, Ecuador.

tano de la cordillera oriental de los Andes norte de Ecuador (MAE, 2013) y Bosque Montano Oriental acorde con Ron *et al.*, 2022. Corresponde a los límites altitudinales de los pisos zoogeográficos Subtropical y Templado (Albuja *et al.*, 2012) (Fig. 10).

Discusión

Hasta el presente trabajo, el grupo de especies *Pristimantis boulengeri* era endémico de los Andes de Colombia (González-Durán *et al.*, 2017; Patiño-Ocampo *et al.*, 2022). *Pristimantis omarrhynchus* y *P. miltongallardoi* corresponden a las primeras especies del grupo para los Andes de Ecuador y para la cuenca amazónica. Las nuevas especies presentan las sinapomorfías del grupo propuestas por González-Durán *et al.*, (2017): (1) dedo III con un tubérculo subarticular distal doble; (2) tubérculo subarticular distal doble del dedo IV; (3) peritoneo parietal cubierto con iridóforos (desconocido en *P. myops* y *P. quantus*); (4) saco vocal extendido; y (5) hocico con papila pequeña. Con la adición de las dos nuevas especies, el grupo *Pristimantis boulengeri* ahora incluye 11 especies: *P. angustilineatus* (Lynch, 1998); *P. baiotis* (Lynch, 1998); *P. boulengeri* (Lynch, 1981); *P. brevifrons* (Lynch, 1981); *P. cryptopictus* Patiño-Ocampo *et al.*, (2022); *P. dorsopictus* (Rivero y Serna, 1988); *P. miltongallardoi* (presente estudio); *P. myops* (Lynch, 1998); *P. omarrhynchus* (presente estudio); *P. quantus* (Lynch, 1998), *P. urani* Rivera-Correa y Daza 2016. *Pristimantis angustilineatus*, *P. baiotis*, *P. myops*, *P. quantus* y *P. urani* se encuentran distribuidas en la cordillera Occidental de Colombia, desde los 1500 m a 2500 m de elevación; *P. dorsopictus* y *P. cryptopictus* son de la cordillera Central entre los 1800 m a 3100 m. *Pristimantis boulengeri*

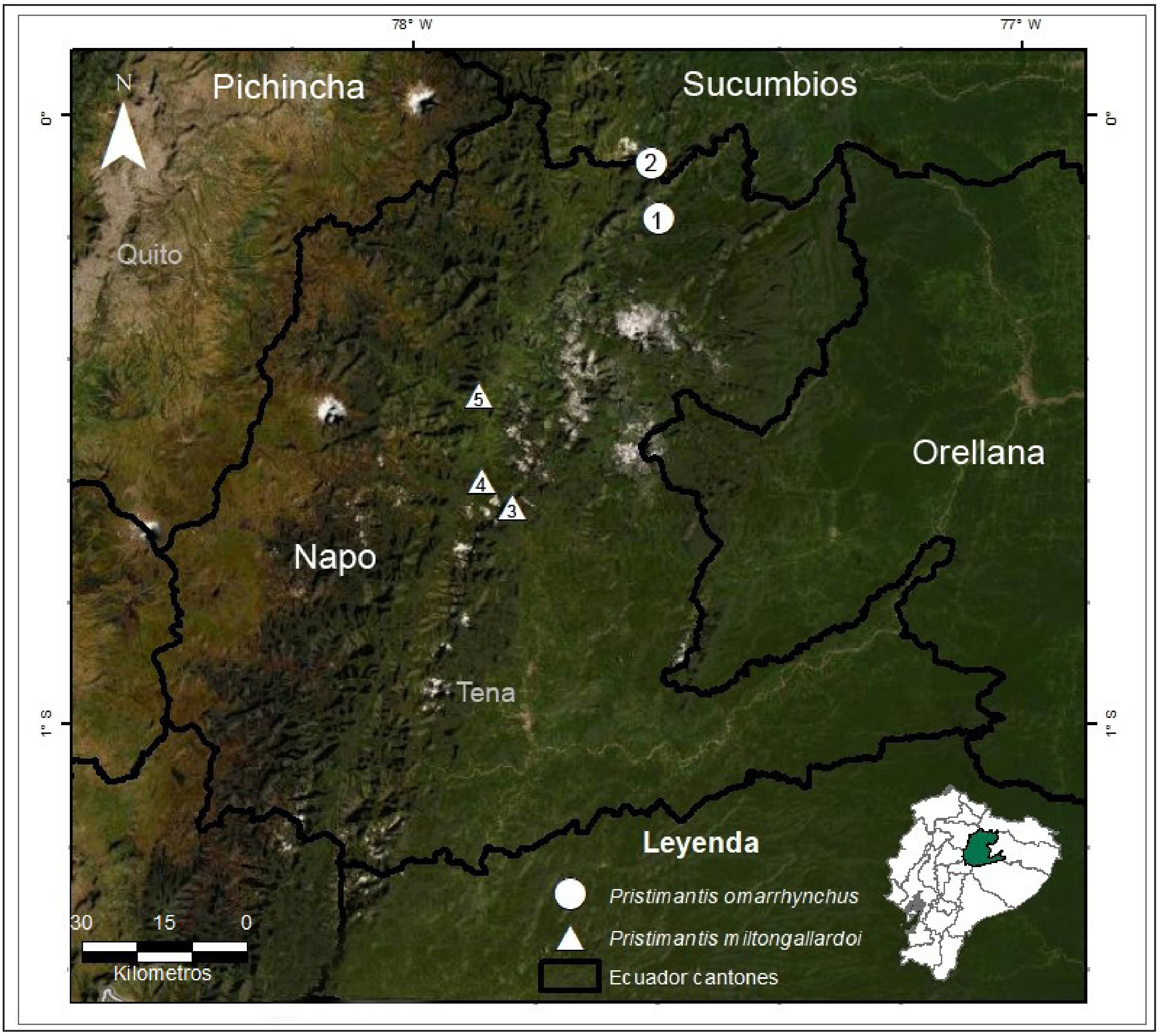

Figura 10. Mapa de distribución de *Pristimantis omarrhynchus* sp. nov. (1) Localidad tipo, laderas del volcán Reventador; Río Azuela, Reserva Ecológica Cayambe-Coca; (2) Sector embalse compensador, Hidroeléctrica Coca Codo Sinclair. *Pristimantis miltongallardoi* sp. nov. (3) Localidad tipo, Cordillera de los Guacamayos; (4) Estación Científica Yanayacu; (5) Sector San Isidro antigua Vía Baeza-Cosanga.

y *P. brevifrons* se encuentran en ambas cordilleras entre los 1140 m a 3200 m (Lynch, 1981, 1998; Rivero y Serna, 1988; Rivera-Correa y Daza, 2016; Patiño-Ocampo *et al.*, 2022).

Recientes estudios (Ospina-Sarria y Duellman, 2019; Ron *et al.*, 2020) han identificado que los tubérculos hiperdistales también están presentes en otros grupos de *Pristimantis* (*e.g.*, *P. leptolophus* y *P. lacrimosus*). Ese carácter al igual que la papila en la punta del hocico y saco vocal extendido, ha sido registrado en especies de *Pristimantis* del subgénero *Huicundomantis* o de los grupos de especies *P. lacrimosus* y *P. unistrigatus* (Lynch, 1979; Lynch y Duellman, 1980, 1997; Páez y Ron *et al.*, 2022; Ron *et al.*, 2020). Por otro lado, el peritoneo con iridóforos ha sido observado por inspección directa en *P. eremitus* (grupo de especies de *P. lacrimosus*), *P. calcarulatus* (grupo de especies *P. unistrigatus*) y *P. galdi* (grupo de especies de *P. lacrimosus*) (Obs. Pers. MYM). Se requiere un análisis comprehensivo de la distribución de estos caracteres para determinar si son homólogos u homoplásicos entre los clados de *Pristimantis* que los presentan.

En *Pristimantis omarrhynchus* sp. nov. hay plasticidad fenotípica en rasgos taxonómicamente importantes y que se asumen fijos a nivel de especie (Lynch y Duellman, 1997; Duellman y Lehr, 2009), por ejemplo, los tubérculos cónicos sobre el párpado,

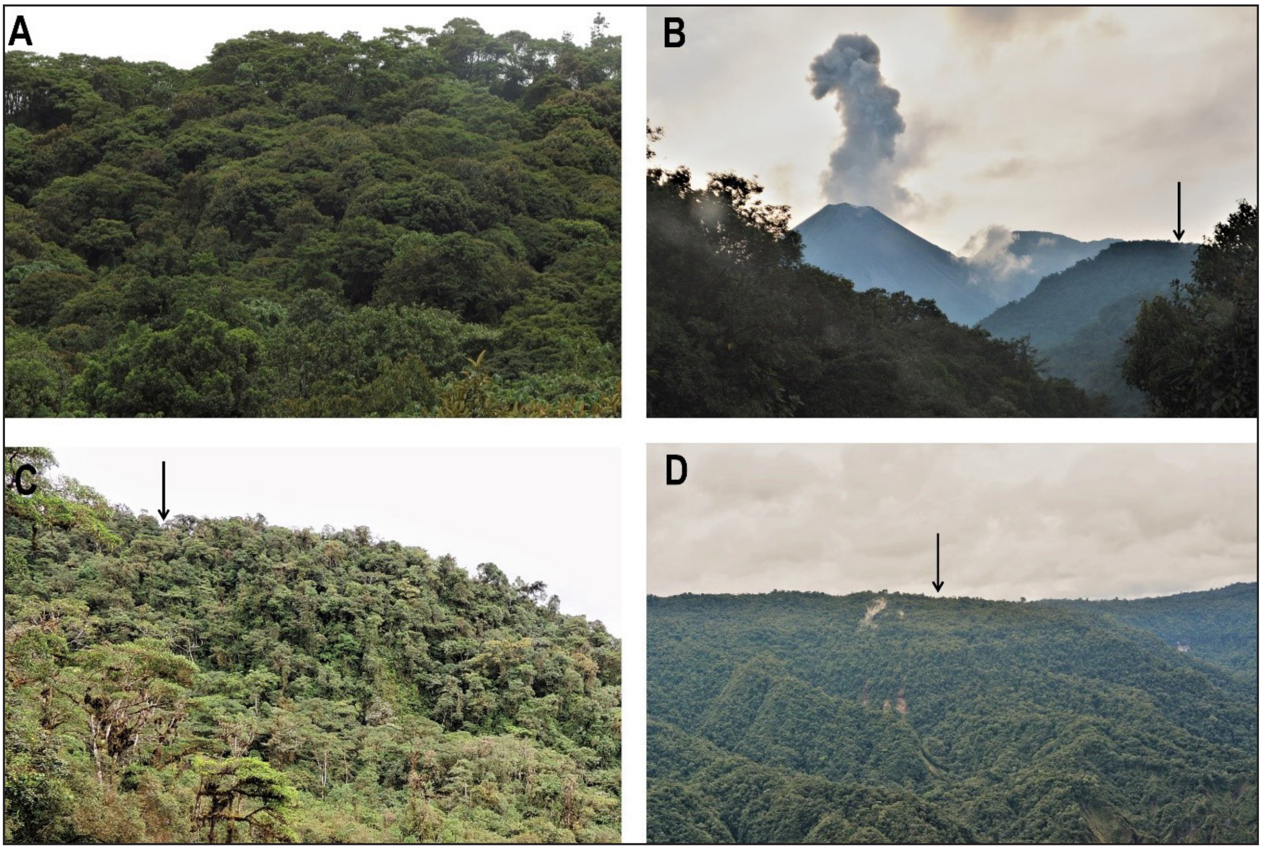

Figura 11. Paisaje general de las áreas de colección de *Pristimantis omarrhynchus* sp. nov. y *Pristimantis miltongallardoi* sp. nov. A) Estación Científica Yanayacu, B) Ladera del Volcán Reventador; C) Reserva Ecológica Cayambe Coca; D) Sector embalse compensador Hidroeléctrica Coca Codo Sinclair. Flechas indican áreas de colección. Fotografías: M.H. Yánez-Muñoz.

talón o papila, variaron en pocas horas en el mismo individuo. La plasticidad fenotípica de la tuberculación de la piel ha sido poco documentada y solo se conoce en *P. mutabilis* y *P. sobetes* (Guayasamin *et al.*, 2015). Por lo tanto, es importante tenerla en cuenta para fotografiar y documentar los especímenes antes y después de la manipulación, con ello evaluar si la plasticidad es más frecuente de lo hasta ahora reportado en el género *Pristimantis*.

En la filogenia identificamos secuencias con determinación taxonómica incorrecta para los especímenes KU 202519 y TNHC-GDC14370, siendo inicialmente identificadas como *P. thymelensis* (Hedges *et al.*, 2008), y posteriormente como *P. myersi* (Guayasamin *et al.*, 2018). No obstante, dichas secuencias son casi idénticas a *P. ocreatus* (KU 208508 y QCAZ 13664), ambos especímenes de la localidad tipo de esta última especie, lo que indica un error en la determinación.

En nuestro análisis y en filogenias previas (e.g., González-Durán *et al.*, 2017; Rivera-Correa *et al.*, 2017, Rivera-Correa y Daza, 2016; Jetz y Pyron, 2018), los grupos de especies *P. boulengeri*, *P. leptolophus*, *P. myersi* y *P. devillei* forman un clado con alto soporte (Fig. 1A). Este clado podría tener una equivalencia taxonómica de subgénero para el cuál estaría disponible el nombre *Trachyphrynus* (Goin y Cochran, 1963).

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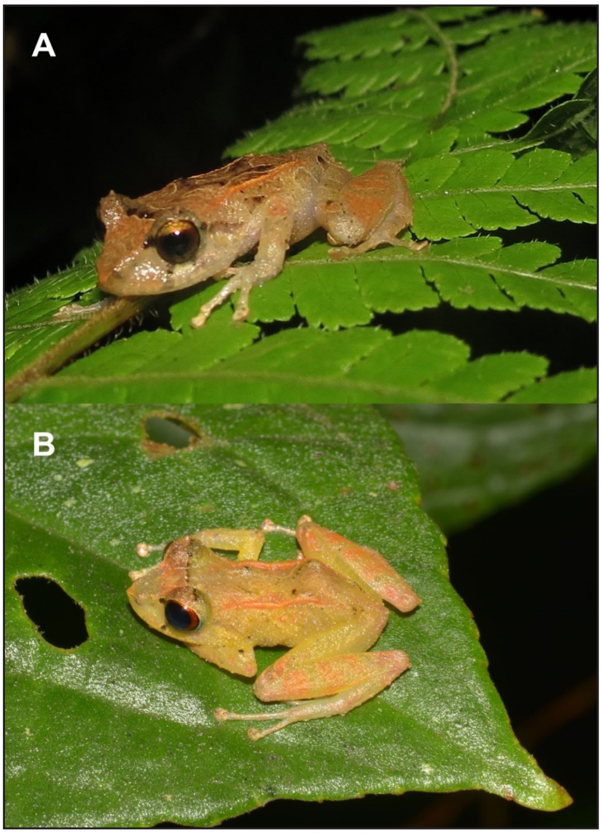

Figura 12. *Pristimantis omarrhynchus* sp. nov. *in situ.* (A) Holotipo, hembra adulta (DHMECN 11480, LRC = 23,5 mm) registrada a 0.80 cm del suelo en hoja de helecho; (B) Paratipo, macho adulto (DHMECN11483, LRC = 17,6 mm) registrado a 1 m. del suelo en el ápice de una hoja cantando. Fotografías: Patricia Bejarano-Muñoz (A); M.H. Yánez-Muñoz (B).

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Apéndice I. Números de acceso para las secuencias de ADN generadas para el análisis filogenético. Todas las localidades están en Ecuador. Las secuencias corresponden al gen 16S a no ser que se especifique algo distinto.

Taxon	Voucher	Localidad	Coordenadas	Altitud (m)	No. accesión GenBank
Pristimantis omarrhynchus sp. nov.	DHMECN 11480 / QCAZ77345	El Reventador, Provincia Sucumbíos	0.086141 S 77.599214 O	1890	OM339541
Pristimantis miltongallardoi sp. nov.	QCAZ 58607	San Isidro, ~12 km SE Cuyuja, Provincia Napo	0.449579 S 77.95183 O	1994	MW504199
Pristimantis miltongallardoi sp. nov.	QCAZ 58608	San Isidro, ~12 km SE Cuyuja, Provincia Napo	0.449579 S 77.95183 O	1994	MW504201
Pristimantis miltongallardoi sp. nov.	QCAZ 58609	Reserva Yanayacu, Provincia Napo	0.5899 S 77.8755 O	2058	MW504200
Pristimantis miltongallardoi sp. nov.	QCAZ 63481	Baeza antigua sendero al Río Papallacta, Provincia Napo	0.459500 S 77.89239 O	1843	MW504202
Pristimantis miltongallardoi sp. nov.	QCAZ 63482	Baeza antigua sendero al Río Papallacta, Provincia Napo	0.459500 S77.89239 O	1843	MW504203

Apéndice II. Material examinado y referido.

Pristimantis omarrhynchus sp. nov. (Ecuador): Provincia Sucumbíos: El Reventador, Río Azuela: QCAZ 77382–391: 1727–2000 msnm.

Pristimantis miltongallardoi sp. nov. (Ecuador): Provincia Napo: Reserva Ecológica Antisana, Cordillera de los Guacamayos, Sector la Virgen (sendero Jumandi): QCAZ 65982, 65984–987, 65990–992, 65995: 1927 msnm. Provincia Napo: Cosanga, Estación Científica Yanayacu: QCAZ 18936–977, 18992, 19506–508, 19009–016, 19058–062, 19498–505, 19509, 22372–376, 39822–825: 2100 msnm. Provincia Napo: Cuyuja, antigua vía Napo Baeza, sendero que baja de la carretera Baeza-Papallacta al río Papallacta: QCAZ 63481–487: 1843 msnm. Provincia Napo; sector San Isidro aproximadamente a 12 km SE de Cuyuja: QCAZ 58607–609: *Pristimantis omarrhynchus* sp. nov. (Ecuador): Provincia Sucumbíos: El Reventador, Río Azuela: QCAZ 77382–391: 1727–2000 msnm.

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Pristimantis angustilineatus (Colombia): Departamento Valle del Cauca, municipio El Cairo, vereda Las Amarillas, sitio El Boquerón, 19.85 km del cementerio de El Cairo: ICN 39598, Holotipo: 2140–2150 msnm.

Pristimantis boulengeri (Colombia): Departamento de Quindío: ICN 34035.

Pristimantis myops (Colombia): Departamento Valle del Cauca, municipio El Cairo, vereda Las Amarillas, El Boquerón (límite con Depto. Choco), 19.6 km del cementerio de El Cairo: ICN 39684, Holotipo: a 2130 msnm.

Pristimantis quantus (Colombia): Departamento de Valle del Cauca, Municipio El Cairo, vereda Las Amarillas, El Boquerón (límite con Depto. Choco): ICN 29340, Holotipo: 2100–2250 msnm.

Pristimantis baiotis (Colombia): Departamento de Antioquia, municipio de Urrao, Parque Natural Nacional Las Orquídeas, vereda río Calles, Quebrada Las Canoas: ICN 19170, Holotipo: 1780-1870 msnm.

Apéndice III. Comparación de caracteres utilizados entre especies del grupo de especies de *Pristimantis boulengeri*.

Especie	LRC	Pliegue dorsolateral	Tímpano	Hocico en vista Dorsal	Hocico en vista lateral
P. miltongallardoi	♂ 17,7(17,0–18,7; n = 5) ♀ 25,2(22,3–28,2; n = 10)	Presente	Presente	Redondeado	Redondeado
P. omarrhynchus sp nov.	♂ 16,7(12,1–20,0; n = 15) ♀ 25,2(23,5–27,3; n = 3)	Presente	Presente	Subacuminado con papila en la punta	Redondeado
P. angustilineatus	♂ 18,1(15,8–20,4; n = 61) ♀ 22,6(20,8–24,8; n = 18)	Ausente	Presente	Subacuminado con papila en la punta	Agudamente redondeado
P. baiotis	♂ 18,1–18,5 ♀ 21,5	Presente	Presente	Acuminado	Protuberante
P. boulengeri	♂ 22,1(18,6–25,6; n = 87) ♀ 30,0(27,3–33,8; n = 17)	Ausente	Presente	Subacuminado con papila en la punta	Agudamente redondeado
P. brevifrons	♂ 17,4(15,1–19,7; n = 3) ♀ 22,8(21,2–25; n = 5)	Ausente	Presente	Subacuminado con papila en la punta	Protuberante
P. crytopictus	♂ 24,08(20,6–27,2; n = 30)	Ausente	Presente	Acuminado con papila en la punta	
P. dorsopictus	♂ (21,31,6; n = 7) ♀ (19,0–22,0; n = 8)	Ausente	Presente	Redondeado con protuberancia en la punta	Redondeado, corto
P. myops	♂ 11,9(10,9–13,6; n = 34) ♀ 15,5(14,6–17,2; n = 38)	Ausente	Presente	Ovoide	Redondeado, corto
P. quantus	♂ 11,9(11,6–14,5; n =4) ♀ 25,3(14,4–16,7; n =9)	Presente poco definidos	Presente	Subacuminado con papila en la punta	Agudamente redondeado
P. urani	♂ 18,9(18,7–19,1; n = 2) ♀ 22,5(21–23,4; n =4)	Ausente	Presente	Redondeado	Truncado

Apéndice III. Continuación

Especie	Tubérculos en el párpado	Vomerinos	Hendiduras	Almohadillas nupciales	Reborde cutáneos mano
P. miltongallardoi sp nov.	Un tubérculo cónico prominente rodeado por algunos pequeños	Oblicuos, bajos 2 a 4	Presente	Presentes	Ausentes
P. omarrhynchus sp nov.	Un tubérculo cónico prominente rodeado por algunos tubérculos bajos	Oblicuos, bajos 2 a 4	Presente	Presentes	Ausentes

P. angustilineatus	Sin tubérculos	Medianos, bajos de 2 a 4	Presentes	Presente	Ausente
P. baiotis	Tubérculos cónicos	Oblicuos, bajos	Presentes	Presente	Presente
P. bounlengeri	Bajos, no cónicos	Oblicuos de 2 a 5	Presentes	Presentes	Ausente
P. brevifrons	Bajos	Ausentes o pequeños	Presentes	Ausentes	Presente
P. crypopictus	Un tubérculo redondeado rodeado por algunos pequeños	Pequeños, oblicuos de 3 a 5	Presentes	Presente	Ausente
P. dorsopictus	Granular	Pequeños	Presentes	Ausentes	Ausente
P. myops	Ausente, pliegue interorbital y sacral	Ausentes	Ausentes	Ausentes	Presente
P. quantus	Tubérculo cónico	Ausentes	Presentes	Ausentes	Presentes
P. urani	Sin tubérculos	Ausentes	Presentes	Presente	Presentes

Apéndice III. Continuación

Especie	**Tubérculo metatarsal externo**	**Tubérculos supernumerarios**	**Reborde cutáneos del pie**	**Coloración**
P. miltongallardoi sp. nov.	Pequeño subcónico	Presentes, bajos	Ausente	Dorso café claro verdoso hasta café oscuro naranja o rojo, marcas en forma “^” café oscuro, flancos y superficies ocultas de muslos y extremidades con barras café separado por barras cremas, vientre y garganta café a crema amarillenta.
P. omarrhynchus sp. nov.	Pequeño subcónico	Presentes, bajos	Ausente	Dorso café claro verdoso hasta café oscuro rojizo, marcas en forma “) (“ café oscuro, flancos y superficies ocultas de muslos y extremidades con barras café separado por barras cremas, vientre y garganta café a crema amarillento.
P. angustilineatus	Redondeado	Indistintos	Estrechos	Dorso amarillo bronceado con rayas finas dorsolaterales blancas delimitadas de negro por debajo.
P. baiotis	Oval	Presentes	Presentes	Dorso café, pliegues dorsolaterales crema, ingles incoloras, vientre crema con puntos negros.

P. boulengeri	Oval	Numerosos	Estrechos	Dorso café pálido con marcas café, barra interorbital oscura, vientre crema, superficies posteriores de los muslos café pálido
P. brevifrons	Subcónico	Presentes	Presentes	Dorso café pálido amarillento con marcas marrón oscuro, superficies ocultas de las ingles café, vientre inmaculado.
P. cryptopictus	Cónico	Presentes, bajos	Ausente	Dorso amarillo claro, naranja o café oscuro, con o sin puntos o manchas irregulares en el cuerpo, sin franjas o líneas longitudinales, vientre crema.
P. dorsopictus	Alargado	Presentes, bajos	Ausente	Dorso amarillo verdoso con pintas grises, flancos rojizos, región inguinal a veces con puntos blancos, vientre inmaculado.
P. myops	Redondeado	Presente, indistintos	Presente	Dorso café con marcas dispersas oscuras, ingles crema pigmentadas de amarillo, muslos salmón o naranjas, vientre fuertemente moteado de café o negro.
P. quantus	Cónico	Pequeños y bajos	Ausente	Dorso café con marcas oscuras verdosas, garganta amarilla, puntos en los flancos amarillentos o rojizos, vientre crema reticulado café.
P. urani	Redondeado	Pequeños y bajos	Ausente	Dorso verde amarillento con marcas marrón oscuro, vientre crema.

Trabajo

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Parasitic helminths in *Boana pulchella* (Duméril & Bibron, 1841) (Anura: Hylidae) and their relation with host diet, body size, and habitat

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ABSTRACT
We analyzed the diet and helminthological fauna of the frog *Boana pulchella* from the extreme south of Brazil. A total of 100 males were collected from two wetland areas in the state of Rio Grande do Sul: Ilha dos Marinheiros (n = 50) in the Rio Grande municipality, and the UFPel/Embrapa (n = 50) in the Capão do Leão municipality. *Boana pulchella* food items and helminths found in different organs were identified and quantified. We analyzed the relationship between helminth assemblage and host diet, body size, and sampling sites. *Boana pulchella* presented a generalist diet, composed mainly of terrestrial insects, with diet richness higher at Ilha dos Marinheiros. Helminth fauna was composed of Nematoda, Cestoda, Digenea, and Acanthocephala, with no difference in helminth richness and abundance between sampling sites. However, the abundance of helminths presented a significant correlation with the volume of items found in the gastrointestinal contents of the anurans from UFPel/Embrapa-CL. Although for some helminth taxa there were significant differences in prevalence and mean intensity of infection among host size classes, the GLM (generalized linear model) between helminth abundance and anuran SVL (snout-vent length) was not significant. *Oxyascaris oxyascaris*, Cosmocercinae gen. spp., *Ochoterenella* sp., Diplostomidae gen. spp., *Pseudoacanthocephalus* sp., and *Centrorhynchus* sp. were the main taxa constituting the helminth assemblage associated to *B. pulchella* males at the sampling sites. The occurrence of helminths at larval and adult stages suggests that *B. pulchella* may occupy different trophic levels in the biological cycles of those helminths. This helminth parasitic fauna associated with *B. pulchella* is mainly composed of taxa with heteroxenous cycles involving several intermediate and paratenic hosts, which agrees with the observations of a typical generalist anuran diet in this species.

Key words: Anurans; Diet; Helminths; Nematoda; Cestoda; Digenea; Acanthocephala; Swamps; Brazil.

Introduction

Parasitic infections can be influenced by several factors related to the host including diet, body size or mass, age, behavior, hormones, as well as immunity and genetic diversity (Wegner *et al.*, 2003; Blanchet *et al.*, 2009). The complex life cycle of several helminth species may involve prey-predator interactions, and consequently, the analysis of host diet composition may provide hints of which helminth groups it can harbors (Brooks & Hoberg, 2000). Some studies on amphibian parasites suggest that they may become infected by gastrointestinal helminths through the ingestion of arthropods acting as intermediate or

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paratenic hosts, which are the main items of the diet in most species (Duré *et al.*, 2004; Van *et al.*, 2006; Akani *et al.*, 2011; Klaion, 2011). Anuran body size is a relevant feature for parasitological studies, as it was observed that larger hosts usually present less nematode load, maybe due to enhanced resistance and/or more efficient physical protection mechanisms (Santos *et al.*, 2013).

Parasitological studies can provide indirect information about the environment since parasites can be viewed as indicators of environmental impacts (Sures, 2004). According to Mackenzie (2007), landscape alterations caused by anthropic actions can induce changes in parasite transmission, increasing or decreasing parasitism depending on the magnitude of the impact as well as the life history of the parasites and hosts. For example, eutrophication of aquatic environments can result in increased parasite populations (Spalding *et al.*, 1993; Coyner *et al.*, 2002).

Amphibians are important environmental regulators, contributing to the decomposition of organic matter and nutrient cycling, in addition to serving as bioindicators of pollution (Hocking & Babbit, 2014). Host and helminth community responses to environmental impacts may vary depending on the type and intensity of stressors, the life cycle of the parasite, and time of exposure to the stressors (Marcogliese, 2004). For amphibians, a global decline of many species and/or populations has been recorded in the past decades (Kelehear *et al.*, 2017; Guerrero & Yanez-Muñoz, 2018), which enhances the need for basic biological baseline information to be gathered.

Brazil harbors the highest anuran diversity worldwide with 1,155 known species distributed across 20 families (Frost, 2022). *Boana pulchella* (Duméril & Bibron, 1841) (Hylidae) is a frog commonly found in southern Brazil, inhabiting vegetation near water bodies in open areas, and is also present in Argentina, Uruguay, and Paraguay (Maneyro *et al.*, 2017; Frost, 2022). Its diet is composed mainly of insects such as dipterans and coleopterans, as well as spiders (Maneyro & Rosa, 2004; Solé & Pelz, 2007; Rosa *et al.*, 2011; Antoniazzi *et al.*, 2013). Studies on the interactions between endo/ectoparasites and *B. pulchella* are limited, with records of Monogenea (Vaucher, 1987), Cestoda larvae (Borteiro *et al.*, 2015) and intradermal mite larvae (Silveira *et al.*, 2019). Additionally, Draghi *et al.* (2020) studied the helmintofauna of this species from two agroecosystem areas in Argentina. The goals of our study were to identify the parasitic helminth assemblage of *B. pulchella* in southern Brazil, and analyze its relationships with host diet, size, and sampling site.

Materials and methods

2.1 Study area

Fieldwork was carried out in two areas in the extreme south of Brazil, the municipalities of Capão do Leão (CL) (31º48'23” S and 52º25'07” W) and Rio Grande (RG) (32°00'00” S and 52°09'00” W). Both are in the coastal plains of the state of Rio Grande do Sul (Fig. 1). The regional climate is classified as humid subtropical with average temperatures ranging from 14.6°C in winter to 22°C in summer. Annual rainfall varies from 1,150 to 1,450 mm, and precipitation occurs all year round (Seeliger *et al.*, 1998).

Sampling in the CL was carried out in an area of Universidade Federal de Pelotas, and Empresa Brasileira de Pesquisa Agropecuária (UFPel/Embrapa-CL) (Fig. 1A). This area is characterized by temporary ponds with a predominance of herbaceous vegetation as well as aquatic macrophyte species (*e.g. Eichornia crassipes* and *Salvina herzogii*). The vegetation cover is mostly of native grasslands and there are patches of *Eucalyptus* spp. trees, as those bordering the studied pond. Sampling in the RG was carried out in Ilha dos Marinheiros (IM-RG) (Fig. 1B), a continental island located in the estuarine region of Lagoa dos Patos. The study site is characterized by the presence of ephemeral and permanent ponds which are formed on sandy terrain, with partially exposed dunes where grass and shrub vegetation are predominant. Macrophytes like those in CL can be found near the water bodies in this area.

2.2 Collection and morphometric characterization of anurans

Field trips were carried out from August to October 2016, and from March to July 2017, since no individuals were found during summer. Sampling at each site was made every thirty days, during the first hours after sunset. We searched for *B. pulchella* for up to 3 hours or until we had captured 10 individuals, detected mostly by their advertisement call. We collected a total of 100 male specimens (n = 50 at CL, and n = 50 at RG), that once captured were stored in plastic containers and euthanized in

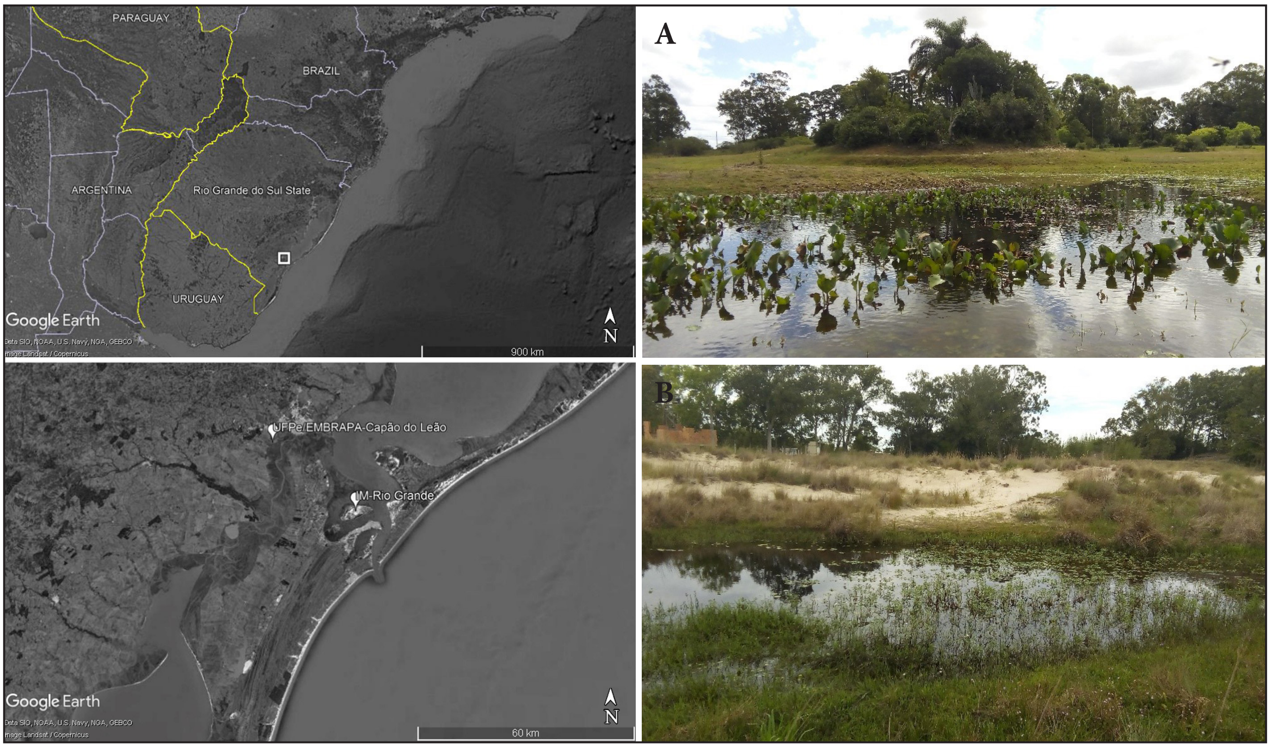

Figure 1. Collection sites of *Boana pulchella* (Duméril & Bribon, 1841) (Hylidae) in Universidade Federal de Pelotas, and Empresa Brasileira de Pesquisa Agropecuária (UFPel/Embrapa-CL), municipality of Capão do Leão (**A**) and Ilha dos Marinheiros (IM-RG), municipality of Rio Grande (**B**), Rio Grande do Sul State, southern of Brazil.

agreement with Resolution No. 1000 of the Federal Council of Veterinary Medicine (CFMV, 2012). The frogs were weighed, measured (snout-vent length), and refrigerated or frozen before necropsy. The study was licensed and approved by the Instituto Chico Mendes for Biodiversity Conservation (ICMBio no 43658-1), and approved by the Animal Ethics and Experimentation Commission (CEEA/UFPel no 6387 - 2016).

2.3 Qualitative and quantitative analysis of the diet

Host diet was investigated by analyzing the stomach and intestinal contents of 82 specimens (n = 50 from IM-RG, n = 32 from UFPel/Embrapa-CL). Food items were identified to the lowest taxonomic level using a stereomicroscope, based on Needham & Needham (1978) and Mugnai *et al.* (2010). The volume of each item, presented in ml, was estimated by measuring length, width, and height (Huckembeck *et al.*, 2014).

Frequency of occurrence (FO) and volume percentage (V) of food items were calculated respectively, as the percentage of digestive tracts in which the food item was found, and the relative volume (%) of a given item to the total volume of all food items found in the digestive tract. The alimentary index (AI) of each item was calculated as: $AI = FO \times V/\Sigma(FO \times V) \times 100$, where $\Sigma(FO \times V)$ is the sum of the products of all food items (Kawakami & Vazzoler, 1980).

Feeding strategy and the importance of items in the diet of *B. pulchella* males were analyzed using a graphic method by Costello (1990). This method allowed us to identify the food ecology of predators through the relationship between the volume percentage (V) of a specific prey and its frequency of occurrence (FO). Food items were classified into broad groups, that is, Gastropoda, Arachnida, Crustacea, Entognatha, Insecta, and vegetable remains.

To compare the richness of the diet between the sampling sites, we used the rarefaction curve through incidence-type (Chao & Jost, 2012). This analysis was performed using the Inext package, in the R program (Hsieh *et al.*, 2016).

2.4 Collection, preparation, identification of helminths and infection parameters

All frogs were dissected searching for helminths, with particular attention to the oral cavity, esophagus, stomach, intestines, bladder, kidneys, testicles, cloaca, lungs, heart, liver, and celomic cavity.

Helminths were fixed in AFA (70° ethanol, 37% formaldehyde, and acetic acid) for 24 hours and subsequently preserved in 70° ethanol. Some specimens of Digenea, Cestoda, and Acanthocephala were stained with Langeron's carmine or Delafield's hematoxylin and mounted in Canada balsam. Nematodes were clarified in Amann's lactophenol on semi-permanent slides for taxonomic identification (Amato & Amato, 2010).

Initial parasite identification was done according to Kiewiadomska (2002), Jones (2005) and Tkach (2008) for Digenea; Petrochenko (1971) for Acantocephala; Schmidt (1986) and Khalil *et al.* (1994) for Cestoda; and Chabaud (2009) and Anderson & Bain (2009) for Nematoda. Specific identification followed Bacher & Vaucher (1985), Freitas (1958) and Travassos (1920) for Nematoda, and Travassos *et al.* (1969) for Digenea. Voucher specimens were deposited in the Coleção de Helmintos do Laboratório de Parasitologia de Animais Silvestres (714 – 757 CHLAPASIL/UFPel), Rio Grande do Sul, Brazil, and Coleção Helmintológica do Instituto Oswaldo Cruz (39321, 39322, 39746a, 39746b, 39746c, 39747 CHIOC), Rio de Janeiro, Brazil. Prevalence (P), mean intensity of infection (MII), and mean abundance (MA) were estimated according to Bush *et al.* (1997).

2.5 Analysis of helminth assemblage relative to host diet, size, and sampling sites

Spearman correlation tests were performed to assess whether diet composition and volume are related to helminth abundance, using the Past software (Hammer *et al.*, 2001) and STATA v. 15.

Boana pulchella individuals were grouped into two size classes based on snout-vent length (SVL). Class I was composed of 46 individuals with SVL = 27-37 mm (33.02 ± 2.70), and Class II was composed of 54 individuals with SVL = 38-49 mm (41.55 ± 2.36). The relationships of helminth assemblage composition with host size classes and sampling sites were assessed using P and MII values tested through chi-square (X^2) and t-test ($p \leq 0.05$), respectively. The analyses were performed in the Quantitative Parasitology 3.0 software (Reiczigel *et al.*, 2019). The chi-square and t-test were done for cases in which helminths prevalence was ≥10% (Bush *et al.*, 1990).

A generalized linear model (GLM) analysis with a Poisson distribution and log link function was used to assess whether the abundance of parasites varied according to host body size and sampling sites, using the Stata software (StataCorp, 2007). Finally, a rarefaction curve was employed to compare helminth richness between sites (Chao & Jost, 2012; Hsieh *et al.*, 2016).

Results

3.1 Diet

Substantial gastrointestinal content was found in seventy anurans (85.4%). Animal and plant items, and also some of anthropic origin were identified (Table 1). The sampled males of *B. pulchella* displayed a generalist feeding strategy, mainly preying upon Insecta (Fig. 2). Coleoptera and Lepidoptera stood out among the insects with the highest alimentary indices (AI) being 20.9% and 5.1%. Diptera was present in 14.6% of anurans with an AI of 1.8%, and unidentified insects showed an AI of 26.4%. Araneae was the most frequent group among the Arachnida food items (14.6%), displaying the highest alimentary index (10.1%). Plant remains were recorded with a frequency of 35.4% and an AI of 28% (Table 1).

Through the rarefaction curve it was observed that the diet richness was higher in the IM-RG population because their confidence intervals did not overlap (Fig. 3). Coleoptera was the group with the highest FO (32%) and AI (28.2%) among prey items. Diptera and Isopoda were also significant food items. Araneae was also important, with a FO of 18% and AI of 8.6%. Plant remains were found in 30% of the anurans, with an AI of 24.1% (Table 1).

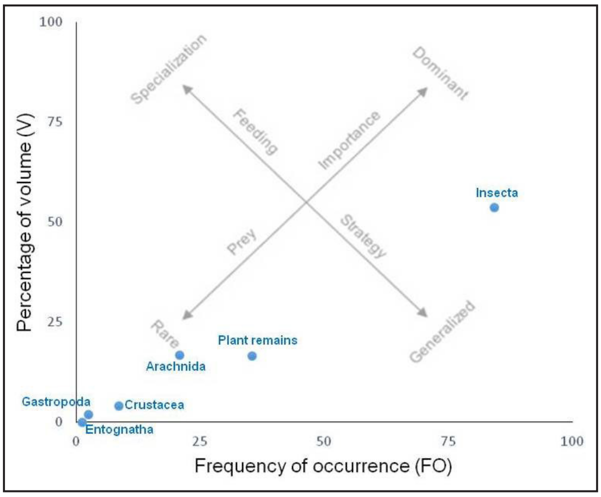

Figure 2. Graphic representation of Costello (1990) of the frequency of occurrence (FO) and percentage of volume (V) of the groups of food items found in the diet of *Boana pulchella* (Anura: Hylidae) males in southern Brazil.

Table 1. Frequency of occurrence (FO), volume (V) and alimentary index (AI) for the items found in the gastrointestinal contents of *Boana pulchella* (Anura: Hylidae) males from two sampling sites in southern Brazil. The values are presented as percentage.

ITEMS	TOTAL SAMPLE (n=82)			SAMPLING SITES					
				IM-RG (n=50)			UFPel/Embrapa-CL (n=32)		
	FO	V	AI	FO	V	AI	FO	V	AI
GASTROPODA	2.4	1.9	0.2	2	2.6	0.2	3.1	0.6	0.1
ARACHNIDA									
Acari	4.9	0.1	0.02	8	0.1	0.04	-	-	-
Araneae	14.6	14.4	10.1	18	10.9	8.6	9.4	20	9.8
Opiliones	1.2	2.6	0.2	2	4.3	0.4	-	-	-
CRUSTACEA									
Isopoda	8.5	4.2	1.7	14	6.8	4.2	-	-	-
ENTOGNATHA									
Collembola	1.2	0.03	<0.00	2	0.04	<0.00	-	-	-
INSECTA									
Hymenoptera	2.4	0.1	0.02	4	0.2	0.04	-	-	-
Hemiptera	4.9	3.1	0.7	2	0.4	0.03	9.4	7.4	3.6
Coleoptera	23.2	18.2	20.9	32	20.1	28.2	18.8	15.2	14.8
Diptera	14.6	2.6	1.8	24	4.2	4.4	-	-	-
Lepidoptera (larvae)	9.8	10.8	5.1	8	7.5	2.6	12.5	16.2	10.5
Unidentified insects	29.3	18.8	26.4	32	16.9	23.7	25	21.9	28.5
Unidentified arthropods	9.8	4.3	2	12	6.3	3.3	6.3	1.2	0.4
Plant remains	35.4	16.5	28	30	18.3	24.1	43.6	13.7	31.1
Anthropic material (synthetic)	1.2	0.2	0.01	2	0.4	0.03	-	-	-
Unidentified items	3.7	2.1	0.4	4	1.0	0.2	6.3	3.9	1.3

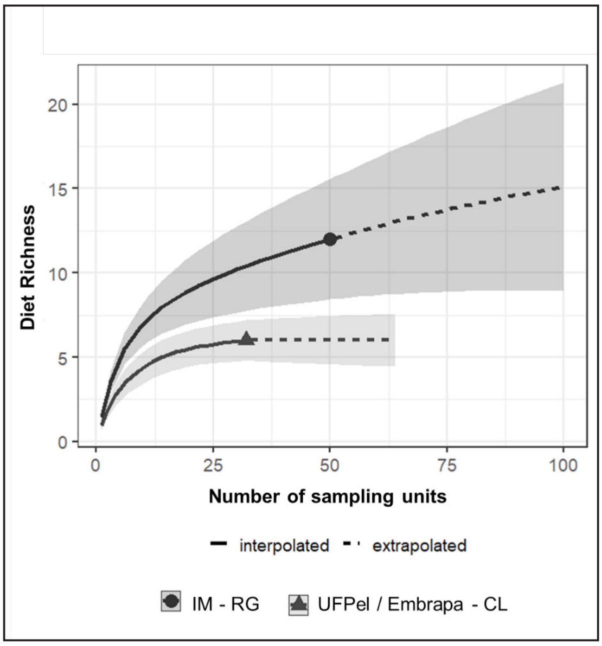

Figure 3. Rarefaction curve of the diet richness of *Boana pulchella* (Anura: Hylidae) males in two sampling sites (IM-RG, and UFPel/Embrapa-CL) in southern Brazil

The diet of UFPel/Embrapa-CL anurans included plant remains with the highest FO (43.6%) and AI (31.1%). Coleoptera at this site (FO of 18.8% and AI of 14.8%) and Lepidoptera (FO of 12.5% and AI of 10.5%) were the most important items of animal origin. Araneae was found with low FO (9.4%) and AI (9.8%), however it displayed a relatively high V (20%) (Table 1).

3.2 Helminth assemblage

Eighty-seven anurans (87%) presented helminths in the following sites of infection: lungs, liver, kidneys, stomach, intestines, and free or encysted in the celomatic cavity. Helminths belonging to Nematoda, Cestoda, Digenea, and Acanthocephala were identified, represented by an adult and larval forms (Table 2).

Nematoda was the taxonomic group with the highest richness in the parasitic assemblage of *B. pulchella*. Cosmocercinae gen. spp. had the highest prevalence (P) and mean intensity of infection (MII)

Table 2. Helminths of *Boana pulchella* (Anura: Hylidae) males in southern Brazil, and their respective sites of infestation and parasitological parameters (prevalence – P, as %, mean intensity of infection - MII, mean abundance – MA, and range - R).

HELMINTHS	SITES OF INFESTATION	P	MII	MA	R
NEMATODA					
Oxyascaris oxyascaris	Intestine	18	1.50	0.27	1-3
Falcaustra sp.	Intestine	1	1.00	0.01	1
Cosmocercinae gen. spp.	Intestine	33	5.88	1.94	1-31
Rhabdias sp.	Lung	2	1.00	0.02	1
Ochoterenella sp.	Intestine and coelomic cavity	10	3.80	0.38	1-12
Physaloptera sp. (larva)	Stomach	1	1.00	0.01	1
Acuariinae gen. sp. (larvae)	Liver, stomach, intestine and coelomic cavity	8	1.38	0.11	1-4
Unidentified larva	Intestine	1	1.00	0.01	1
CESTODA					
Cyclophyllidea gen. sp.	Intestine	1	1.00	0.01	1
Unidentified larva	Coelomic cavity	1	1.00	0.01	1
DIGENEA					
Haematoloechus ozorioi	Lung	1	1.00	0.01	1
Catadiscus sp.	Intestine	5	1.60	0.08	1-2
Diplostomidae gen. spp. (metacercariae)	Kidney	32	4.19	1.34	1-21
Crassiphialinae gen. sp. (metacercaria)	Coelomic cavity	1	1.00	0.01	1
ACANTHOCEPHALA					
Pseudoacanthocephalus sp.	Intestine	27	2.22	0.60	1-7
Centrorhynchus sp. (cystacanths)	Stomach, intestine and coelomic cavity	59	6.68	3.94	1-41

(33%; 5.88 helminths/host), followed by *Oxyacaris oxyascaris* Travassos, 1920 (18%; 1.50 helminths/host) and *Ochoterenella* sp. (10%; 3.80 helminths/host). *Rhabdias* sp. and *Falcaustra* sp. were found with P of 2% and 1%, respectively. Acuariinae stood out among the larval forms with a prevalence of 8% and an MII of 1.38 larvae/host, contrasting with the other taxa, which displayed an MII of 1 larva/host (Table 2).

Regarding Digenea, metacercariae of Diplostomidae parasitized the kidney of 32% of anurans with MII of 4.19 helminth/host, followed by *Catadiscus* sp. whose prevalence was 5% and MII of 1.60 helminth/host, while metacercaria of Crassiphialinae gen. sp. and *Haematoloechus ozorioi* Freitas & Lent, 1939 were found in 1% of anurans with MII of 1 helminth/host (Table 2). Acanthocephala and Cestoda were represented by two taxa each (Table 2). *Centrorhynchus* sp. (cystacanths) infected 59% of anurans with MII of 6.68 helminths/host, while *Pseudoacanthocephalus* sp. was found with a prevalence of 27% and MII of 2.22 helminths/host. Cestoda was represented by a specimen of Cyclophyllidea gen. sp. and an unidentified larva (Table 2).

3.3 *Boana pulchella* helminth assemblage: aspects related to diet and host size, and sampling sites

The abundance of helminths presented a significant correlation only with the volume of items found in the gastrointestinal contents of the anurans from UFPel/Embrapa-CL ($r^2 = 0.50$, $p < 0.01$).

Analyses on helminth assemblage and host size revealed that *O. oxyascaris* and Cosmocercinae were significantly more prevalent in the anurans belonging to Class I, with P of 26.1% (X^2, p = 0.026) and 39.1% (X^2, p = 0.022) respectively. Acuarinae larvae and *Pseudoacanthocephalus* sp. had higher prevalences in Class II hosts, of 13% (X^2, p = 0.047) and 35.2% (X^2, p = 0.046) respectively (Table 3). Only the Cosmocercinae showed significant differences in the mean intensity of infection between the two size classes with greater MII for Class I (9.50 helminths/host) (t-test, p = 0.0275) (Table 3). No significant differences were found in P and MII between host size classes infected by Digeneans belonging to Diplostomidae and *Centrorhynchus* sp. cystacanths (Table 3). Infections by the other helminths could not be compared as they were not detected in both classes. It is interesting to highlight that *Ochoterene-*

Table 3. Prevalence (P, as %), mean intensity of infestation (MII), mean abundance (MA) and range (R) of parasitic helminths of *Boana pulchella* (Anura: Hylidae) males in relation to the size classes (snout-vent length, SVL) in southern of Brazil.

HELMINTHS	SVL							
	Class I (27- 37mm) (n=46)				Class II (38 - 49mm) (n=54)			
	P	MII	MA	R	P	MII	MA	R
NEMATODA								
Oxyascaris oxyascaris	26.1^{a}	1.50	0.41	1-3	9.3^{a}	1.60	0.19	1-4
Falcaustra sp.	2.2	1.00	0.02	1	-	-	-	-
Cosmocercinae gen. spp.	39.1^{b}	9.50^{bb}	3.83	1-31	18.5^{b}	1.80^{bb}	0.33	1-5
Rhabdias sp.	-	-	0	-	3.7	1.00	0.04	1
Ochoterenella sp.	-	-	0	-	18.5	3.80	0.70	1-12
Physaloptera sp. (larva)	-	-	0	-	1.9	1.00	0.02	1
Acuariinae gen. sp. (larvae)	2.2^{c}	1.00	0.02	1	13^{c}	1.43	0.19	1-4
Unidentified larva	2.2	1.00	0.02	1	-	-	0	-
CESTODA								
Ciclophyllidea gen. sp.	2.2	1.00	0.02	1	-	-	0	-
Unidentified larva	-	-	0	-	1.9	1.00	0.02	1
DIGENEA								
Haematoloechus ozorioi	2.2	1.00	0.02	1	-	-	0	-
Catadiscus sp.	10.9	1.60	0.17	1-2	-	-	0	-
Diplostomidae gen. spp. (metacercariae)	37	5.12	1.89	1-14	27.8	3.20	0.89	1-21
Crassiphialinae gen. sp. (metacercaria)	-	-	0	-	1.9	1.00	0.02	1
ACANTHOCEPHALA								
Pseudoacanthocephalus sp.	17.4^{d}	1.63	0.28	1-2	35.2^{d}	2.47	0.87	1-7
Centrorhynchus sp. (cystacanths)	56.5	6.73	3.80	1-41	61.1	6.64	4.06	1-39
Centrorhynchus sp. (cystacanths)	56.5	6.73	3.80	1-41	61.1	6.64	4.06	1-39

a - X^2, p = 0.026; b - X^2, p = 0.022; c - X^2, p = 0.047; d - X^2, p = 0.046; bb - t-test, p = 0.0275

lla sp. was found only in Class II anurans, with a P of 18.5% and an MII of 3.8 helminths/host. Besides, *Catadiscus* sp. was present in only 10.9% of Class I hosts, with an MII of 1.60 (Table 3). Although there were significant differences in the prevalence and mean intensity of infection for taxa among host size classes, the GLM between helminth abundance and anuran SVL was not significant (z = -1.55, p = 0.120).

The richness of helminths was not different between the sampling sites (Fig. 4). However, there was a significant difference in the prevalence of *O. oxyascaris*, Cosmocercinae gen. spp. and *Ochoterenella* sp. between the two sampled sites. A higher prevalence of the first two taxa was observed in anurans from IM-RG (28%; X^2, p = 0.009, and 52%; X^2, p < 0.000, respectively), while *Ochoterenella* sp. was found with a higher prevalence (18%; X^2, p < 0.008) in anurans from UFPel/Embrapa-CL. The mean intensity of Cosmocercinae gen. spp. was significantly higher in anurans from IM-RG (7.08 helminth/host; t-test, p = 0.0205) (Table 4). *Catadiscus* sp. occurred only in IM-RG parasitizing 10% of the hosts with an MII of 1.6 helminths/host. In general, although there is a variation in the prevalence of some taxa between sampled sites, the abundance of helminths did not vary significantly between the two localities (z = 1.76, p = 0.079).

Discussion

The study of the helminth fauna of *B. pulchella* in southern Brazil showed a greater number of taxa than a previous study in Buenos Aires province, Argentina, by Draghi *et al.* (2020). The prevalence of parasitism found by these authors was also lower as they recorded 35% of 150 hosts parasitized by at least one helminth species. Those differences may be associated with the characteristics of the sampling

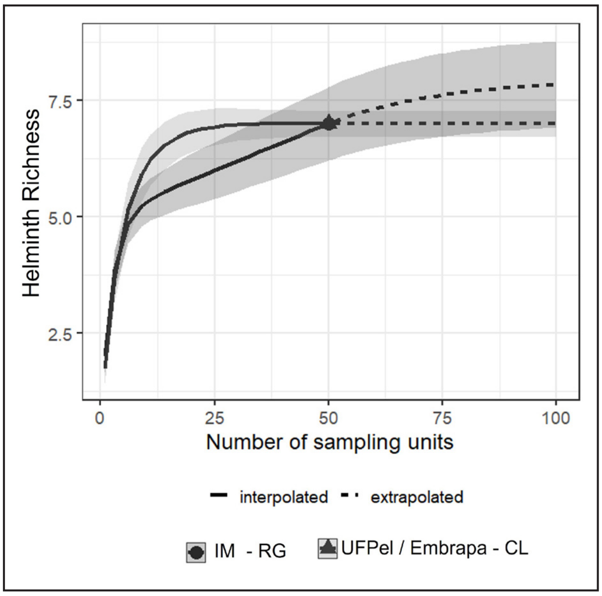

Figure 4. Rarefaction curve of the parasitic helminth richness of *Boana pulchella* (Anura: Hylidae) males in two sampling sites (IM-RG, and UFPel/Embrapa-CL) in southern Brazil.

sites, since Draghi *et al.* (2020) sampled cultivated and pasture areas.

Most parasitic helminths of *B. pulchella* have heteroxenous cycles that involve animals of several groups as intermediate and paratenic hosts, including anurans (Table 5). The generalist diet of *B. pulchella* observed in this study (insects, crustaceans, mollusks, and vegetation) is similar to previous ones (Maneyro & Rosa, 2004; Solé & Pelz, 2007; Rosa *et al.*, 2011; Antoniazzi *et al.*, 2013), and may provide the link between free-living forms of helminths and anurans. In this sense, studies on the diet and helminth fauna of anurans provide complementary information to improve our understanding of host-parasite relationships.

Some of the observed parasitic infections may be considered occasional since the prevalence of most helminths was less than 10%, and the intensity of infection of 11 taxa ranged from 1 to 7 helminths. The low rates of *O. oxyascaris*, *Falcaustra* sp., *Physaloptera* sp., Acuariinae gen. sp., *H. ozorioi*, and Cestoda may be related to the low consumption of intermediate and paratenic hosts that probably do not constitute prey commonly ingested by *B. pulchella* due to availability or preference bias for certain items. On the other hand, the infection rates of acanthocephalans may be related to the ingestion of arthropods, which were well represented in the diet of this species at both sampling sites. According to the literature, other taxa that compose the helminth parasitic assemblage of *B. pulchella* (Cosmocercinae gen. spp., *Rhabdias* sp., *Catadiscus* sp. and Diplostomidae gen. spp.) can infect the anuran through the ingestion of immature forms during the tadpole phase or through active penetration of the skin (Table 5).

The infections by metacercariae of Diplostomidae and by cystacanths of *Centrorhynchus* sp. suggest that *B. pulchella* acts as an intermediate host for digeneans and as a paratenic host for the acanthocephalans since the above mentioned larval forms were found with expressive P% and MII values.This anuran act as a trophic bridge between the hosts at the lower level (invertebrates) and the hosts at the top of the trophic chain (birds and mammals, for example). The latter may acquire infections by ingesting anurans, thus ensuring the continuity of the parasitic cycle (Table 5). *Centrorhynchus* species, for example, were reported in natural predators of anurans, such as *Rupornis magnirostris* (Gmelin, 1788) (Accipitriformes) (Machado, 1940; Moura *et al.*, 2017), and *Guira guira* (Gmelin, 1788) (Cuculiformes) (Lunaschi & Drago, 2010). On the other hand, a study carried out in agroecosystems in Argentina reported neither cystacanths nor metacercariae associated with *B. pulchella* (Draghi *et al.*, 2020). The different land use in the sampling sites of Brazil (present study) and Argentina (Draghi *et al.*, 2020) may have influenced the parasitic infections in *B. pulchella*, since in the firstwe had no crops or livestock, as in Argentina. The sampling sites of the present study consist of temporary ponds with similar vegetation, to which anurans were closely associated, which may have contributed to the similarity in the richness and abundance of parasitic helminths.

It is important to highlight that parasite-host relationships can be influenced by several other factors. Intrinsic characteristics related to hosts (e.g. maturity, gender, reproductive behaviour, size, diet) (Poulin, 1996; Von Zuben, 1997; Wilson *et al.*, 2002; Klein, 2004) and parasites (e.g. size, quantity, fecundity rate, reproductive modes, dispersal ability) (Crofton, 1971; Von Zuben, 1997; Wilson et al, 2002; Khokhlova et al, 2010), as well as extrinsic factors (e.g. temperature, humidity, anthropogenic changes) (Marcogliese, 2005; Hudson *et al.* 2006; Koprivnikar *et al.*, 2012), can act on the complex network of parasite-host-environment interactions.

Similarly to the present study, Draghi *et al.* (2020) found no relationship between host body

Table 4. Prevalence (P, as %), mean intensity of infestation (MII), mean abundance (MA) and range (R) of parasitic helminths of *Boana pulchella* (Anura: Hylidae) males in two sampling sites from southern Brazil.

HELMINTHS	**SAMPLING SITES**							
	IM-RG (n=50)				**UFPel/Embrapa-CL (n=50)**			
	P	**MII**	**MA**	**R**	**P**	**MII**	**MA**	**R**
NEMATODA								
Oxyascaris oxyascaris	28[a]	1.43	0.40	1-3	8[a]	1.75	0.14	1-3
Falcaustra sp.	2	1.00	0.02	1	-	-	0	-
Cosmocercinae gen. spp.	52[b]	7.08[bb]	3.68	1-31	14[b]	1.43[bb]	0.20	1-3
Rhabdias sp.	4	1.00	0.04	1	-	-	0	-
Ochoterenella sp.	2[c]	1.00	0.04	1	18[c]	4.11	0.74	1-12
Physaloptera sp. (larva)	2	1.00	0.02	1	-	-	0	-
Acuariinae gen. sp. (larvae)	2	1.00	0.02	1	14	1.43	0.20	1-4
Unidentified larva	2	1.00	0.02	1	-	-	0	-
CESTODA								
Ciclophyllidea gen. sp.	2	1.00	0.02	1	-	-	0	-
Unidentified larva	-	-	0	-	2	1.00	0.02	1
DIGENEA								
Haematoloechus ozorioi	2	1.00	0.02	1	-	-	0	-
Catadiscus sp.	10	1.60	0.16	1-2	-	-	0	-
Diplostomidae gen. spp. (metacercariae)	36	5.17	1.86	1-14	28	3.00	0.82	1-21
Crassiphialinae gen. sp. (metacercaria)	-	-	0	-	2	1	0.02	1
ACANTHOCEPHALA								
Pseudoacanthocephalus sp.	22	2.09	0.46	1-5	32	2.31	0.74	1-7
Centrorhynchus sp. (cystacanths)	58	6.69	3.88	1-41	60	6.67	4.00	1-39

a - X^2, p = 0.009; b - X^2, p = < 0.000; c - X^2, p = 0.008; bb - t-test, p = 0.0205

Table 5. Helminth parasites of *Boana pulchella* (Anura: Hylidae) males in southern Brazil and their respective hosts and possible means of infestation, considering the information available for life cycles of similar species or species of the same family.

Helminths	**Definitive Host**	**Intermediate host (IH)/ paratenic host (PH)**	**Possible means of infestation in anurans**	**References**
Nematoda				
Oxyascaris oxyascaris	Amphibians	Direct cycle – Invertebrates (PH)	Ingestion of PH	Anderson (2000)
Falcaustra sp.	Amphibians, and reptiles	Direct cycle – Freshwater mollusk (PH)	Ingestion of PH	Anderson (2000)
Cosmocercinae	Amphibians	Direct cycle	Ingestion of larvae by tadpoles or skin penetration	Anderson (2000)
Rhabdias sp.	Amphibians, and reptiles	Direct cycle	Penetration of the larvae across the skin	Anderson (2000)
Ochoterenella sp.	Anurans	Mosquito (IH)	Ingestion of IH	Anderson (2000)

Physaloptera sp.	Birds, mammals, reptiles, fish and amphibians	Insects (crickets, cockroaches) (IH)	Ingestion of IH	Anderson (2000)
Acuariinae	Birds, and mammals	Insects (Coleoptera, Orthoptera) (IH)/Amphibians and Reptiles (PH)	Ingestion of IH	Anderson (2000)
Cestoda	Vertebrates	Vertebrates and Invertebrates (IH)	Ingestion of IH	Olsen (1974)
Digenea				
Haematoloechus ozorioi	Anurans	Freshwater mollusk (1st IH), and Odonata (2nd IH)	Ingestion of 2nd IH	Olsen (1974)
Catadiscus sp.	Anurans	Mollusk Planorbidae (IH)	Tadpole ingests metaceraria present in aquatic vegetation	Kehr & Hamann (2003)
Diplostomatidae	Birds, and mammals	Freshwater mollusk (1st IH), fish, amphibians and occasionally mammals (2nd IH)	Penetration of the cercaria in the tadpole	Travassos et al. (1969), Hamann & Gonzáles (2009)
Acanthocephala				
Pseudoacanthocephalus sp.	Amphibians and reptiles	Aquatic arthropod (IH)	Ingestion of IH	Barger & Nickol (1998)
Centrorhynchus sp.	Birds, and mammals	Crustaceans or Insects (IH), Amphibians and reptiles (PH)	Ingestion of IH	Travassos (1926), Petrochenko (1971), Schmidt (1985), Amato et al. (2003)

1st IH=first intermediate host, 2nd IH=second intermediate host

size and helminth abundance. Host body size has also been considered a good indicator of parasite species richness since larger hosts may provide more space and other resources, possibly widening niche diversity for parasites (Campião *et al.*, 2016). In addition, larger hosts live longer, establishing in less ephemeral habitats than smaller species and may have enhanced exposure to parasites (Poulin, 1997).

According to our findings is possible to conclude that *Boana pulchella* males present a generalist feeding strategy with a diet composed of a wide variety of arthropods, mainly terrestrial insects. Their diverse helminth fauna was composed of larval and adult forms, indicating that at least males of this anuran may act as definitive, intermediate, as well as paratenic hosts. The generalist diet and the association of the species with the vegetation close to aquatic environments are important factors for the persistence and the life cycle of its parasitic helminths. *Oxyascaris oxyascaris*, Cosmocercinae gen. spp., *Ochoterenella* sp., Diplostomidae gen. spp., *Pseudoacanthocephalus* sp., and *Centrorhynchus* sp. are the main taxa of this assemblage.

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Trabajo

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DNA barcoding in Neotropical tadpoles: evaluation of 16S rRNA gene for the identification of anuran larvae from northeastern Brazil

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ABSTRACT
The challenge in studying Neotropical tadpoles is identifying species using only their external morphology. However, the DNA barcode protocol is often implemented to help elucidate taxonomic issues. In fact, the identification of frogs through their unknown tadpoles has already been achieved accurately using this protocol. Despite the successful application of this tool, the efficiency of the 16S rRNA gene as a DNA barcode for Neotropical tadpoles has not been fully assessed. Herein we evaluate the efficacy of the 16S rRNA gene for identifying tadpoles from northeastern Brazil. Samples of 100 tadpole specimens from 12 locations were analyzed. The DNA sequences were individually submitted to a BLAST search and were then aligned with a matrix containing available sequences in the GenBank based on the anurans known to occur in the study area. The 16S rRNA fragment successfully identified the analyzed anuran species. Based on DNA barcoding, 8% of the tadpoles morphologically identified at the species level were incorrect. When an incongruence between morphological and molecular identifications was detected, the morphology of the target morphotype was reexamined, and previously neglected morphological characteristics were identified. DNA barcoding using the 16S rRNA gene facilitated the assessment of tadpole richness in northeastern Brazil. This DNA protocol can be used as a starting point for detecting high levels of genetic divergence, highlighting potential taxa that should be studied from phylogenetic and taxonomic perspectives.

Key words: Amphibia; Mitochondrial Gene; Genetic Divergence; Species Diversity.

Introduction

Anuran amphibians generally have an aquatic larval phase, representing an important trophic component of aquatic environments (Ranvestel *et al.*, 2004; Rossa-Feres *et al.*, 2004; Jordani *et al.*, 2019). During certain periods, these tadpoles are the only evidence of anuran occurrence in some environments. Tadpoles are relatively abundant where they occur, and they are also easy to collect. However, for a long time, tadpoles were neglected by naturalists and researchers, especially in megadiverse assemblages such as tropical regions (Provete *et al.*, 2012; Rossa-Feres *et al.*, 2015). Tadpoles have only recently started to be included more frequently in faunistic inventories and ecological, systematic, and taxonomic studies (Haas, 2003; Larson, 2005; Silva, 2010; Magalhães *et al.*, 2013; Dubeux *et al.*, 2020a). In fact, the tadpoles of a large portion of anuran species are unknown (Altig and McDiarmid, 1999; e.g., Provete *et al.*, 2012; Schulze *et al.*, 2015; Altig *et al.*, 2021). This is an alarming scenario, considering

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that amphibians are the most threatened vertebrate group with known declining or extinct populations (IUCN, 2022). Additionally, many species will likely become extinct without formal descriptions or identification of their tadpoles (Crawford *et al.*, 2010). In Brazil, the country with the highest worldwide anuran diversity (1,144 species; Segalla *et al.*, 2021), the tadpoles of about half of identified species are unknown (Provete *et al.*, 2012).

One significant challenge when studying tadpoles is species identification. When using only external morphology, species identification can be hampered by 1) the lack of knowledge of larval diagnostic characteristics (very similar morphologically or non-described tadpoles), 2) the absence of standardization in the nomenclature used for tadpole descriptions, or even 3) the scarcity of identification keys (Provete *et al.*, 2012; Dubeux *et al.*, 2020b). Until recently, the accurate identification of tadpoles was only possible in captivity, where tadpoles were held until they completed their metamorphosis, thereby allowing for the identification of species through their juveniles. However, this introduces the logistical challenge of reproducing environmental characteristics that mirror the natural environment where tadpoles are collected.

Currently, the integration of a molecular approach using the DNA barcode as a protocol has been promising in terms of facilitating tadpole identification (e.g., Vences *et al.*, 2005b; Grosjean *et al.*, 2015; Schulze *et al.*, 2015). Although the original DNA barcoding protocol was used for identifying the information contained in a fragment of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene, in order to accelerate taxonomic descriptions for amphibians in general (Hebert *et al.*, 2003, 2005), the barcode procedure using the mitochondrial 16S rRNA gene has been used in studies worldwide, due to its ease of use and high amplification success rate in different laboratory conditions (Vieites *et al.*, 2009; Vences *et al.*, 2005a, b; Lyra *et al.*, 2017; Koroiva *et al.*, 2020). The information in the 16S rRNA fragment and the analysis of genetic similarity has been confirmed as an accurate method for identifying frog species, including their tadpoles. This approach has already been employed globally and has accurately identified tadpoles from Madagascar, Spain, Bolivia, and Southeast Asia (Thomas *et al.*, 2005; Vences *et al.*, 2005b, b; Grosjean *et al.*, 2015; Schulze *et al.*, 2015).

Despite the efficient application of the DNA barcode tool in refining and accelerating the process of anuran identification, few studies have investigated the efficiency of the 16S rRNA gene as a molecular identification tool in Neotropical tadpoles (e.g., Schulze *et al.*, 2015). Herein we evaluated the effectiveness of the 16S rRNA gene for the identification of specific and generic anuran taxa from northeastern Brazil, using their tadpoles. We assessed the rate of precise morphological identifications and discussed the challenges of the identification of tadpoles in megadiverse assemblages such as Brazilian ecoregions. Additionally, we included some comments on cryptic diversity in the local anurofauna based on genetic divergences and recent phylogenetic hypotheses.

Materials and methods

Sampling and DNA extraction

We used caudal musculature samples from 100 tadpoles obtained from 12 locations in the Caatinga and Atlantic Forest ecoregions in the state of Alagoas, northeastern Brazil (see Appendix I for more information about the locations). All the tadpoles were collected from 2013 to 2018 (licenses: SISBio/ICMBIO 32920, 33507; CEUA 36/2015; SISGEN A6E0CAC). Firstly, we morphologically separated the tadpoles into 51 different morphotypes. Eighteen morphotypes were only identified at the generic level and two at the family level. All tadpoles were morphologically allocated to eight families. Between one and three representatives of each morphotype were preserved in 92% alcohol, and the remaining specimens were preserved in 10% formalin. All specimens were incorporated into the Coleção Herpetológica do Museu de História Natural of Universidade Federal de Alagoas (MHN-UFAL).

The total genomic DNA was extracted from a fragment of tadpole caudal musculature from each morphotype using the Phenol-Chloroform method (Sambrook *et al.*, 1989) or by performing DNA extraction with salts (DNA Precipitation NaCl; Bruford *et al.*, 1992), and stored in 15 µl of autoclaved distilled water.

Polymerase chain reaction and sequencing

A fragment of 550 base pairs (bp) of the 16S rRNA mitochondrial gene from each sample was amplified using the forward primer 16Sar: CGC CTG TTT ATC AAA AAC AT and reverse primer 16Sbr: CCG GTC TGA ACT CAG ATC ACG T (Palumbi *et al.*, 2002) through the polymerase chain reaction

(PCR). Each PCR reaction had a final volume of 25 µl, comprised of 21.4 µl of MixMaster PCR, 0.8 µl of each primer, and 2 µl of DNA (20–100 ng/µl). The thermal cycling conditions followed an initial denaturation of 94° C (1–3 minutes), and 35 cycles of denaturation at 94° C for 45 seconds, pairing at 55° C for 45 seconds and extension at 72° C for one and half minutes. The PCR reactions were stained with syber safe, and electrophoresis in 1% agarose gel was performed to check for the presence of amplicons. The reactions were then visualized on the translumminator with ultraviolet light. The PCR products were purified with isopropanol and sequenced unidirectionally using the Sanger method after the Big Dye® terminator reaction.

Data analyses

The quality of the obtained sequences was checked and edited if necessary, using the software BioEdit Version 7.2.5 (Hall, 2011). Initially, in order to flag potential errors in tissue taxonomic labeling or DNA contamination, we performed Basic Local Alignment Search Tool (BLAST) analyzes on GenBank online platform. This analysis finds regions of local similarity between our samples and the sequences available in the repository database and calculates the statistical significance of the matches. We used the taxonomic identification of the most similar sequences in the database as a starting point to validate the identification of our samples. Each sample in our dataset was analyzed independently. The GenBank sequence with the greatest total similarity and the percentage of similarity is shown in Table 1. When an incongruence between morphological and molecular identifications was detected, the morphology of the target morphotype was reexamined.

We then calculated genetic divergence between our samples and a selection of sequences obtained from the GenBank (selection criteria are presented below) using evolutionary models. This analysis aimed to compare our samples with: (1) conspecific samples of adult specimens collected in the study area or in geographically close areas, in order to associate tadpoles with their adult counterparts; (2) conspecific topotypical samples or samples from areas that are geographically close to the type locality of the nominal taxon, to obtain a preliminary assessment of the taxonomic status of populations in the study area (considering that nominal species with highly divergent populations may present an undescribed diversity) and, (3) sequences with greater total similarity in the BLAST analysis, to validate the taxonomic identification obtained through this analysis.

Our sequences were aligned using the software MAFFT Version 7.310, implementing the iterative refinement method (automatic strategy selection) and default parameters (Katoh and Standley, 2013). They were then combined with a matrix containing sequences available in GenBank (see below for more details; Appendix II) of representatives of anuran species previously registered in the study area (following the anuran list available in Almeida *et al.*, 2016). In the matrix, up to three DNA sequences from different localities for each anuran species were included, prioritizing regions close to the study area and the type locality of the species. When sequences were not available for a certain species (last consultation in July 2020), a closely related representative was included (same genus and/or species group, following the current phylogenetic proposals [e.g., Carcerelli and Caramaschi, 1993; Lourenço *et al.*, 2015; Lyra *et al.*, 2020; specifically, *Crossodactylus dantei* (we used *C. caramaschii*), *Physalaemus caete* (we used *P. signifier*) and *Boana exastis* (we used *B. pardalis*)]. Additionally, we added the sequences with the highest total similarity in the BLAST analysis, even if they had not been previously recorded in the study area (considering the possibility of new records and/or error in the labeling of the GenBank sequences). Following alignment, the tails of sequences imported from GenBank were manually trimmed using the software AliView Version 1.27 (Larsson, 2014) in order to eliminate gene fragments that were not of interest. The FFT-NS-i alignment strategy was selected by MAFFT and the final alignment resulted in a 636 base pair matrix.

To estimate genetic divergences, distance estimates were made from the sequence matrix using the Compute Pairwise Distances function implemented with the Kimura-2-parameters evolutionary model (K2P; Kimura, 1980) using the software MEGA X (Kumar *et al.*, 2018). For a graphical visualization of the groupings, a dendrogram was generated using the Neighbor-Joining method (NJ) implemented with the K2P evolutionary model. The groupings obtained were validated using the bootstrap method (Felsenstein, 1985) with 1,000 pseudoreplicates. Bootstrap values above 97% were considered high and are indicated in the dendrogram. The sequences generated in this study were deposited in GenBank (OP022028 – OP022123; Table 1).

Table 1. Molecular identification of tadpole specimens analyzed. Locations, associated vouchers and GenBank accession numbers of our samples. Genetic divergences [GD; in percentage (%)] calculated using the Compute Pairwise Distances function implemented with the Kimura-2-parameters evolutionary model including our samples and sequences from the GenBank. Sequences with the highest similarity, and their respective percentages, in BLAST analysis.

Species genetic identification	**Locality (municipality)**	**Voucher (MHNUFAL)**	**GenBank access**	**GD closer to study area**	**GD closer to type locality**	**BLAST (GenBank access)**
Aromobatidae						
Allobates olfersioides	Teotônio Vilela	12465-1	OP022079	0	12.18	99.4 (KU495121)
Bufonidae						
Rhinella diptycha	Traipu	13874	OP022111	0	-	99.7(MH004313)
Rhinella diptycha	Arapiraca	13883	OP022116	0	-	100 (GU178784)
Rhinella diptycha	Arapiraca	13885	OP022117	0	-	99.7(MH004313)
Rhinella diptycha	Traipu	13897	OP022121	0	-	99.7 (DQ415572)
Rhinella granulosa	Junqueiro	12462	OP022077	0.51	1.02	99.6 (KP685207)
Rhinella granulosa	Arapiraca	13877	OP022112	0.51	1.02	99.8 (KP685207)
Rhinella granulosa	Batalha	13882	OP022115	0.51	1.02	100 (KP685206)
Rhinella granulosa	Batalha	13887	OP022118	0.51	1.02	99.8 (KP685205)
Rhinella hoogmoedi	Murici	12502	OP022108	1.02	1.54	99.4(KU495521)▪
Hylidae						
Boana albomarginata	Limoeiro de Anadia	13901	OP022122	0	2.57	99.3 (AY549316)
Boana albomarginata	Maceió	12259	OP022070	0	2.57	99.8 (AY549316)
Boana albomarginata	Limoeiro de Anadia	12482	OP022094	0	2.57	99.8 (AY549316)
Boana albomarginata	Coruripe	12474	OP022088	0	2.57	99.8 (AY549316)
Boana albomarginata	Barra de Santo Antônio	12497	OP022104	0	2.57	99.8 (AY549316)
Boana aff. *atlantica* ◊	Maceió	12079-1	OP022051	0.51	2.57	99.8 (MK348503)
Boana aff. *atlantica* ◊	Maceió	12079-2	OP022052	0.51	2.57	100 (MK348503)
Boana faber	Limoeiro de Anadia	12478	OP022092	3.62*	3.62*	97.1 (KY002913)
Boana raniceps	Coruripe	12132	OP022055	0	0.51	100 (KU495288)
Boana semilineata	Maceió	11392-1	OP022028	0*	1.54	99.2 (MH004300)
Boana semilineata	Maceió	11392-2	OP022029	0*	1.54	99.2 (MH004300)
Boana semilineata	Maceió	12080-1	OP022053	0*	1.54	99.2 (MH004300)
Boana semilineata	Maceió	12080-3	OP022054	0*	1.54	99.2 (MH004300)
Boana semilineata	Maceió	12249-1	OP022056	0*	1.54	99.2 (MH004300)
Boana semilineata	Maceió	12249-2	OP022057	0*	1.54	99.2 (MH004300)
Corythomantis greeningi	Traipu	13889	OP022119	0	0.51	100 (MW243395)
Dendropsophus branneri	Coruripe	12470	OP022083	0.51	0.51	99.2 (MT503865)
Dendropsophus branneri	Maceió	12252-1	OP022060	0.51	0.51	99.6 (MT503865)
Dendropsophus branneri	Maceió	12252-2	OP022061	0	0	99.8 (MT503865)
Dendropsophus branneri	Maceió	12252-3	OP022062	0	0	99.8 (MT503865)
Dendropsophus sp.1 (aff. *tapacurensis*)	Barra de Santo Antônio	12491	OP022100	3.11	3.11	94.8 (MW026642)
Dendropsophus sp.2 (aff. *decipiens*)	Satuba	11866-1	OP022044	2.05	2.05	97.7 (MT503952)

Dendropsophus sp.2 (aff. *decipiens*)	Satuba	11866-2	OP022045	2.05	2.05	97.9 (MT503952)
Dendropsophus sp.2 (aff. *decipiens*)	Satuba	11866-3	OP022046	2.05	2.05	97.9 (MT503952)
Dendropsophus sp.2 (aff. *decipiens*)	Satuba	11866-4	OP022047	2.05	2.05	97.9 (MT503952)
Dendropsophus sp.2 (aff. *decipiens*)	Satuba	11866-5	OP022048	2.05	2.05	97.9 (MT503952)
Dendropsophus sp.2 (aff. *decipiens*)	Satuba	12499	OP022106	2.05	2.05	97.7 (MT503952)
Dendropsophus aff. *minutus* ◊	Limoeiro de Anadia	13879	OP022114	0	3.09	100 (MK266721)
Dendropsophus aff. *minutus* ◊	Limoeiro de Anadia	13896	OP022120	0	3.09	100 (MK266721)
Dendropsophus aff. *minutus* ◊	Limoeiro de Anadia	13906	OP022123	0	3.09	100 (MK266721)
Dendropsophus aff. *minutus* ◊	Limoeiro de Anadia	12476	OP022090	0	3.09	100 (MK266721)
Dendropsophus aff. *minutus* ◊	Limoeiro de Anadia	12477	OP022091	0	3.09	100 (MK266721)
Dendropsophus aff. *minutus* ◊	Limoeiro de Anadia	12479	OP022093	0	3.09	100 (KJ833161)
Dendropsophus soaresi	Maceió	12251-1	OP022058	0	0	100 (MT503922)
Dendropsophus soaresi	Maceió	12251-2	OP022059	0	0	100 (MT503922)
Scinax similis ▲	Maceió	12256-1	OP022068	1.02	2.06	99.4 (MH206282)
Scinax similis ▲	Maceió	12256-2	OP022069	1.02	2.06	99.4 (MH206282)
Scinax similis ▲	Maceió	12254	OP022067	0.51	1.53	99.6 (MW114955)
Scinax similis ▲	Maceió	12492	OP022101	1.54	2.59	99.4 (MH206282)
Scinax auratus	Maceió	11472-1	OP022034	5.83	5.83	95.2 (MH004316)
Scinax nebulosus	Maceió	12493	OP022102	0	5.3	100 (EU201095)
Scinax nebulosus	Maceió	11426-3	OP022031	0	5.3	100 (EU201095)
Scinax nebulosus	Maceió	11426-2	OP022030	0.51	5.28	99.7 (EU201095)
Scinax nebulosus	Maceió	11472-2	OP022035	0	5.3	99.7 (EU201095)
Scinax nebulosus	Maceió	11473-1	OP022036	0	5.3	100 (EU201095)
Scinax nebulosus	Maceió	11473-2	OP022037	0	5.3	100 (EU201095)
Scinax nebulosus	Limoeiro de Anadia	13900	OP022109	0	5.3	100 (EU201095)
Scinax x-signatus	Coruripe	12469	OP022082	0	0	100 (MW114963)
Scinax x-signatus	Junqueiro	12458	OP022074	0.51	0.51	99.7 (MW114964)
Scinax x-signatus	Maceió	12253-1	OP022063	1.02	1.02	99.0 (MW114963)
Scinax x-signatus	Maceió	12253-2	OP022064	0	0	99.0 (MW114963)
Scinax x-signatus	Maceió	12253-3	OP022065	1.02	1.02	99.0 (MW114963)
Scinax x-signatus	Maceió	12253-4	OP022066	1.02	1.02	99.0 (MW114963)
Leptodactylidae						
Leptodactylus fuscus	Junqueiro	12456	OP022073	0	-	99.6 (AY911275)
Leptodactylus fuscus	Junqueiro	12460	OP022076	0	-	99.6 (AY911275)
Leptodactylus fuscus	Igaci	12486	OP022097	0	-	99.6 (AY911275)
Leptodactylus fuscus	Teotônio Vilela	12467-2	OP022081	0	-	99.6 (AY911275)

Leptodactylus fuscus	Igaci	12487	OP022098	0	-	99.6 (AY911275)
Leptodactylus fuscus	Maceió	12490	OP022099	0	-	99.6 (AY911275)
Leptodactylus macrosternum	Arapiraca	13873	OP022110	1.02	1.02	99.8 (MH004302)
Leptodactylus macrosternum	Coruripe	12475	OP022089	0	0	100 (MH206305)
Leptodactylus aff. *mystaceus* ◊	Maceió	12500	OP022107	0	5.89	99.8 (MT117857)
Leptodactylus natalensis	Teotônio Vilela	12463	OP022078	0	1.02	99.8 (MH004304)
Leptodactylus troglodytes	Junqueiro	12459	OP022075	0	0	99.3 (KM091620)
Leptodactylus troglodytes	Coruripe	12473	OP022087	0	0	99.6 (MT117853)
Leptodactylus vastus	Maceió	11433	OP022033	0	0	100 (KU495368)
Leptodactylus vastus	Maceió	11867-1	OP022049	0.51	0.51	99.6 (KU495368)
Leptodactylus vastus	Maceió	11867-2	OP022050	0.51	0.51	99.6 (KU495368)
Physalaemus cuvieri	Coruripe	12467	OP022080	0	-	99.7 (KP146012)
Physalaemus cuvieri	Coruripe	12471	OP022084	0	-	99.5 (KP146012)
Physalaemus cuvieri	Barra de Santo Antônio	12494	OP022103	0	-	99.7 (KP146012)
Physalaemus cuvieri	Maceió	12260	OP022071	0	-	100 (KP146012)
Pseudopaludicola aff. *mystacalis* ◊	Maceió	11429	OP022032	0	3.13	100 (KJ147044)
Microhylidae						
Dermatonotus muelleri	Arapiraca	13878	OP022113	0	2.57	99.8 (MH004297)
Elachistocleis cesarii	Coruripe	12472-2	OP022086	1.02*	1.02	98.2 (KM509129)
Elachistocleis cesarii	Coruripe	12472-1	OP022085	1.02*	1.02	98.2 (KM509129)
Odontophrynidae						
Macrogenioglottus alipioi	Maceió	11865-1	OP022041	0	0.51	99.6 (FJ685684)
Macrogenioglottus alipioi	Maceió	11865-2	OP022042	0	0.51	99.6 (FJ685684)
Macrogenioglottus alipioi	Maceió	11865-3	OP022043	0	0.51	99.6 (FJ685684)
Macrogenioglottus alipioi	Maceió	12498	OP022105	0	0.51	99.2 (FJ685684)
Phyllomedusidae						
Hylomantis granulosa	Maceió	11864-1	OP022038	0	0.51	100 (GQ366224)
Hylomantis granulosa	Maceió	11864-2	OP022039	0	0.51	100 (GQ366224)
Hylomantis granulosa	Maceió	11864-3	OP022040	0	0.51	100 (GQ366224)
Pithecopus gonzagai	Junqueiro	12455	OP022072	0	0	100 (KM387490)
Pithecopus gonzagai	Igaci	12483	OP022095	0	0	100 (MW158678)
Pipidae						
Pipa carvalhoi	Igaci	12485	OP022096	0	0	100 (MT261652)

Legend of symbols: - Type location not known or not very specific; * Samples that are more than 1000 km away from the locality of interest (study area or taxon type locality); ◊ Lineage known to be distinct from that from the type locality of the taxon, which was assigned the "aff." (see Results and discussion section for more details); ▲ Species never recorded for the study area; ▪ Identified as Rhinella margaritifera (probably a misidentification).

Results and Discussion

The average length of the obtained sequences was 537 base pairs (440–594bp). The 51 analyzed morphotypes were molecularly designated to 33 taxa, representing 15 genera and eight families (Fig. 1). This represents 48% of anuran species with a registered tadpole phase in the study area (Dubeux *et al.*, 2019). In total, 32% (n = 32) of the specimens were only morphologically identified at a generic level, and only 6% (n = 6) at the family level (Fig. 2). Although incomplete, all these supra-specific identifications have been molecularly corroborated.

For the specimens that were morphologically identified at the specific level, misidentifications were made both at specific (2%, n = 2) and generic

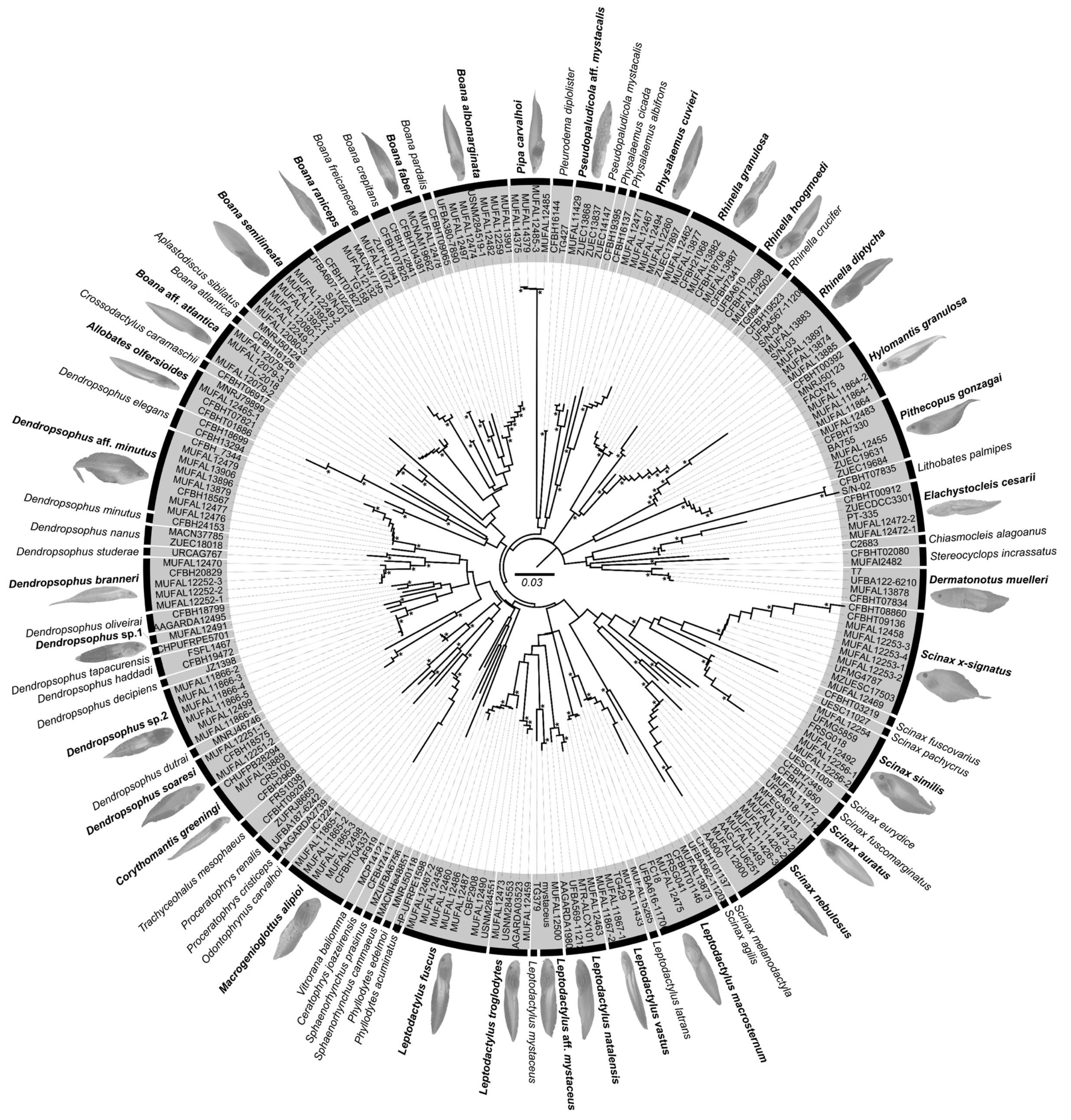

Figure 1. Dendrogram of anurans (tadpoles and adult specimens) that occur in Alagoas state, Brazil. The diagram was built based on 636-bp of DNA sequences of 16S rRNA gene, using the Neighbor-Joining method implemented with the Kimura-2-parameters evolutionary model. Bold species names were sampled in this study. The terminals with acronym MUFAL represent our sequences (except MUFAL 2482, 14375, 14379). *Bootstrap values > 97%.

(6%, n = 6) levels. Additionally, the tadpoles MHN-UFAL 11472-1 and MHN-UFAL 11472-2, both collected on the same day and in the same pond and morphologically identified as having the same morphotype [*Scinax nebulosus* (Spix, 1824)], are actually two distinct species [*S. nebulosus* and *S. auratus* (Wied, 1821)]. This misconception can be explained by the fact that both species are representatives of the *S. rostratus* clade, which are morphologically very similar, presenting the "labial arm" as a larval synapomorphy of the group (Gomes *et al.*, 2014). After revisiting the morphology of these specimens, we could easily differentiate them by the number of labial teeth present in this structure (less than 10 in *S. nebulosus*, more than 15 in *S. auratus*; Dubeux *et al.*, 2020b).

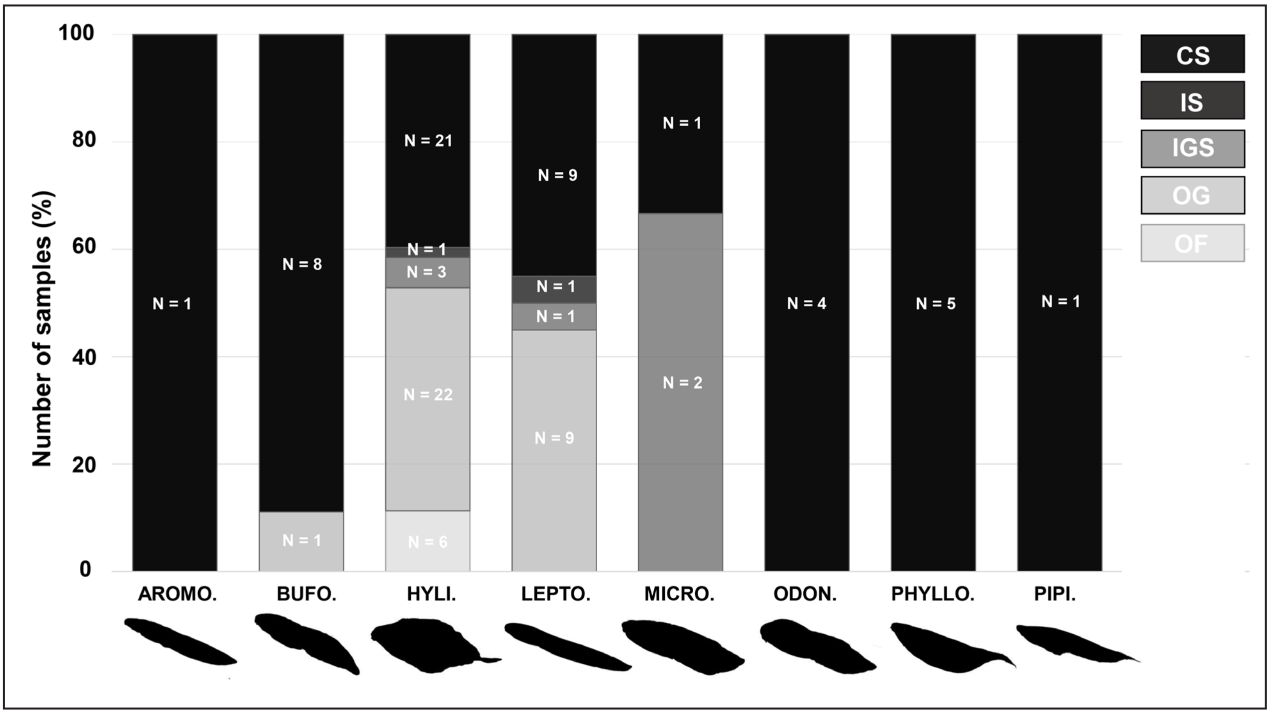

Figure 2. Percentage of morphological identification of tadpoles by family taxonomic level. CS = correct identification at specific level; IS = incorrect identification at specific level, but correct at generic level; IGS = incorrect generic and specific identifications; OG = identification only at generic level; OF = identification only at family level. Abbreviations below bars: AROMO. = Aromobatidae, BUFO. = Bufonidae, HYLI. = Hylidae, LEPTO. = Leptodactylidae, MICRO. = Microhylidae, ODON. = Odontophrynidae, PHYLLO. = Phyllomedusidae, PIPI. = Pipidae.

Additionally, some morphotypes identified as belonging to species recorded in the study area were genetically more similar to closely related taxa that have not been previously recorded in this region. Specifically, MHN-UFAL 1249, morphologically identified as *Dendropsophus oliveirai* (Bokermann, 1963), was genetically more similar to *D. tapacurensis* Oliveira, Magalhães, Teixeira, Moura, Porto, Guimarães, Giaretta & Tinôco, 2021 (GD = 3.11%), which was recently described for the state of Pernambuco (municipality of São Lourenço da Mata, ~150 km away; Oliveira *et al.*, 2021). There is no description for the tadpole of this species, which makes morphological comparisons impossible. The specimens MHN-UFAL 11866(1-5) and MHN-UFAL 12499, morphologically identified as *D. haddadi* (Bastos & Pombal, 1996), were genetically more similar to *D. decipiens* (Lutz, 1925) (GD = 2.05%). Finally, specimens MHN-UFAL 12256(1-2) and MHN-UFAL 12254, morphologically identified as *Scinax eurydice* (Bokermann, 1968), were genetically more similar to *S. similis* (Cochran, 1952) (GD = 1.53 – 2.06%), a species whose type locality is in the state of Rio de Janeiro and whose distribution is only known for the Southeast and Central-west regions of Brazil and Paraguay (Frost, 2022). The tadpoles of these two species present great morphological similarity to each other, as well as to the other representatives of the *S. ruber* clade, and no clear or unambiguous larval diagnostic characters were identified between these species (Rossa-Feres and Nomura, 2006; Dubeux *et al.*, 2020b).

Considering the intraspecific divergence between our sequences and those closest to the study area available in GenBank, the variation between conspecific pairs collected in the state of Alagoas (N = 13 species) ranged from zero (identical haplotypes) to 1.02%. Even when we added pairs of species that do not have adult sequences available for the study area, but that are available for locations up to 500km north of the state of Alagoas (covering the states of Pernambuco and Paraíba; N = 21 species), this range of variation does not change. However, when adding specimens collected up to 500km south of the state of Alagoas to the calculation (covering the states of Sergipe and Bahia; N = 29 species), the variation between conspecific pairs reached 5.83%. These high divergence values can be explained by the fact that the São Francisco River, which forms a southern boundary with the state of Alagoas, is known to be an important geographic barrier for the dispersion of anuran amphibians (e.g., Lima *et al*., 2019; Andrade *et al*., 2020). Additionally, it delimits important biogeographic regions of the Atlantic Forest (Ribeiro *et al*., 2009) and creates zones of endemism (Asfora and Pontes, 2009; Dubeux *et al*., 2020b; França *et al*., 2020). It is expected that intraspecific genetic divergence would be greater in regions south of Alagoas compared to regions north of the state, either because they present lower gene flow or because they already represent independent evolutionary lineages.

All pairs of species had a smaller genetic divergence in relation to the area closer to the study area than to their type locality. However, some populations from the study area were significantly different from those from the type locality of the nominal taxon. Some of these populations are known to represent independent evolutionary lineages or candidates for new species, according to recent phylogenetic studies, for example *Leptodactylus* aff. *mystaceus* (GD = 3.13%; see Silva *et al*., 2020), *Dendropsophus* aff. *minutus* (GD = 3.09%; see Gehara *et al*., 2014), *Boana* aff. *atlantica* (GD = 2.57%; see Lima *et al*., 2019), and *Pseudopaludicola* aff. *mystacalis* (GD = 3.13%; see Fávero *et al*., 2011).

However, there are no recent phylogenetic studies for other species and, based on the high genetic divergence identified here, in addition to other sources of evidence available in the literature, they should be considered as potential taxa for future phylogenetic studies (see below). For example, populations of *Allobates olfersioides* (Lutz, 1925) from Alagoas showed a genetic divergence of 12.18% with samples from localities close to their type locality in the state of Rio de Janeiro. The population of Alagoas, described as *A. alagoanus* (Bokermann, 1967), was synonymized to *A. olfersioides* by Verdade and Rodrigues (2007) based on the morphological similarity of adult individuals. However, recent studies have highlighted the presence of marked differences in the larval morphology (Dubeux *et al*., 2020a, b) and acoustic repertoire (Forti *et al*., 2017) of these two populations.

Another example involves the representatives of the *Dendropsophus decipiens* species group, registered in the state of Alagoas. Although previously associated with *D. oliveirai* (hereafter referred to as *Dendropsophus* sp.1) and *D. haddadi* (hereafter referred to as *Dendropsophus* sp.2), the greater genetic similarity with *D. tapacurensis* and *D. decipiens*, respectively, raises interesting questions about the taxonomic status of these populations. Although genetically more similar (when compared to the sequences available in GenBank) and originating from locations less than 150 km away, the genetic divergence between *Dendropsophus* sp.1 and *D. tapacurensis* was 3.11%, which was higher than the divergence found between formally recognized lineages within this group (Oliveira *et al*., 2021). A similar situation was observed between *Dendropsophus* sp.2 and *D. decipiens*, where the identified divergence was 2.05%. Multiple evolutionary lineages have already been identified in this species complex, including one lineage comprising populations from the state of Alagoas (*D. decipiens* VII; Oliveira *et al*., 2021). Phylogenetic studies with adequate sampling are required in order to assess the taxonomic status of these taxa. The type localities of some species were not included in the matrix due to the lack of geographic specificity in the designation of their locations, specifically: *Rhinella diptycha* (Cope, 1862), *Leptodactylus fuscus* (Schneider, 1799), and *Physalaemus cuvieri* Fitzinger, 1826.

One of the main bottlenecks in the development of research focusing on larval life stages results as a consequence of the difficulty in identifying Neotropical anuran larvae, especially since assemblages often exceed 40 species (e.g., Brandão *et al*., 2004; Vancine *et al.,* 2018) (Rossa-Feres *et al*., 2015; Dubeux *et al*., 2020b). The scarcity of this basic knowledge often becomes a barrier to the advancement of other lines of research involving neotropical tadpoles. In the northern most portion of the Atlantic Forest (north of the São Francisco River), for example, 78 anuran species have been recorded, of which 72 have a larval phase; however, 14% still lack a tadpole description [see Dubeux *et al.* (2020b) for a complete list]. Furthermore, until recently, there was no available identification key for this region (Dubeux *et al*., 2020b). In this study, following sample collection, the tadpoles were identified by different undergraduate students, all with limited experience in this area of research, and the absence of identification keys for tadpoles in the study area or nearby regions (penalties available in 2020), made accurate identification even more difficult. This lack of experience and the scarcity of accessible tools, such as taxonomic keys, may explain, in part, the high rate of incomplete (38%) and incorrect (8%) morphological identification in our data.

With the popularization and low cost of single locus genetic analyzes, the use of molecular techniques for more accurate assessments of biodiversity has become increasingly more common (Fouquet *et al.*, 2007; Vieites *et al.*, 2009; Perl *et al.*, 2014; Grosjean *et al.*, 2015; Schulze *et al.*, 2015; Lyra *et al.*, 2017; Koroiva *et al.*, 2020; Vacher *et al.*, 2020). Several studies describing anuran larvae have used DNA barcoding to confirm the identity of species (e.g., Malkmus and Kosuch, 2000; Moravec *et al.*, 2014; Dubeux *et al.*, 2020c). However, even with the increasing number of sequenced species in recent decades, the under-sampling of species, mainly in regions such as northeastern Brazil, still persists. In the state of Alagoas, for example, 74 anuran species are currently registered (Almeida *et al.*, 2016; Roberto *et al.*, 2017; Lisboa *et al.*, 2019), of which six have no available DNA sequences (*Frostius pernambucensis*, *Crossodactylus dantei*, *Scinax cretatus*, *S. muriciensis*, *S. skuki*, and *Physalaemus caete*). This can hinder accurate assessments of local diversity, with some species remaining genetically unknown.

Despite the lack of available DNA sequences for some species, the 16S rRNA mitochondrial gene was found to be effective in associating tadpole species with their adult counterparts and aided in assessing anuran diversity in the state of Alagoas. Some advantages of using this marker in DNA barcode studies are 1) universal primers for vertebrates and 2) ease of use and high amplification success rate in different laboratory conditions (Vences *et al.*, 2005b). Additionally, for anuran amphibians, the historical use of this gene has resulted in the construction of a database with approximately 75,000 available sequences (GenBank, accessed on April 27th, 2022). Furthermore, anuran amphibian lineages are relatively old entities, and the mitochondrial gene has sufficient variation to unequivocally identify most species (Vences *et al.*, 2005b; Thomas *et al.*, 2005).

The cryptic diversity revealed through DNA barcoding has helped researchers in taxonomic revisions and estimations of local diversity (see below). In some countries anuran diversities have almost doubled when using the DNA barcode method (e.g., Fouquet *et al.*, 2007 for French Guiana and Vieites *et al.*, 2009 and Perl *et al.*, 2014 for Madagascar). In Brazil, recent large-scale studies have revealed diversities that have been neglected for decades (Lyra *et al.*, 2017; Vacher *et al.*, 2020; Koroiva *et al.*, 2020). Considering the genetic lineages documented in previous phylogenetic studies (e.g., Gehara *et al.*, 2014; Lima *et al.*, 2019; Silva *et al.*, 2020; Oliveira *et al.*, 2021) and the potential taxa for taxonomic studies based on the genetic diversity identified here, we suggest that new species should be described/revalidated in the coming years for the study area. Although the methods used here are suitable for associating tadpoles with their adult counterparts, they are not suitable for resolving phylogenetic relationships. These results should be considered as a starting point for the development of integrative studies that aim to investigate and solve taxonomic issues.

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APPENDIX I. Locations with tadpoles sampled in this study, Alagoas state, Brazil.

Ecoregion	Municipality	Coordinates	Number of samples	Number of species
Atlantic Forest	Barra de Santo Antônio	9°23'27.6"S; 35°31'30.8"W	4	3
	Coruripe	10°07'45.0"S; 36°11'09.5"W	11	8
	Junqueiro	9°55'08.0"S; 36°28'46.6"W	7	5
	Maceió	9°33'30.6"S; 35°47'57.5"W	44	16
	Murici	9°12'59.3"S; 35°51'24.2"W	1	1
	Satuba	9°34'51.4"S; 35°50'35.4"W	6	1
	Teotônio Vilela	9°54'16.6"S; 36°22'21.9"W	3	3
Caatinga	Arapiraca	9°44'32.7"S; 36°37'57.2"W	5	4
	Batalha	9°42'49.1"S; 37°06'02.4"W	2	1
	Igaci	9°32'40.0"S; 36°37'53.0"W	4	3
	Limoeiro de Anadia	9°44'16.5"S; 36°27'41.3"W	10	4
	Traipu	9°57'39.8"S; 36°57'34.6"W	3	2

APPENDIX II. Additional 16S rRNA mitochondrial gene fragment sequences of anuran species used in the study.

Species	Voucher	Locality	GenBank
Aromobatidae			
Allobates olfersioides	CFBHT01886	Alagoas: Passo de Camaragibe	KU495122
Allobates olfersioides	MNRJ79899	Rio de Janeiro: Maricá	MF624179
Bufonidae			
Rhinella crucifer	TG094	Bahia: Salvador	KU495499
Rhinella diptycha	CFBH19523	Bahia: Maracás	MW003646
Rhinella granulosa	CFBH7341	Alagoas: Passo de Camaragibe	KP685205
Rhinella granulosa	CFBH21068	Bahia: Caetité	KP685207
Rhinella hoogmoedi	UFBA610	Bahia: Ilhéus	MH538283
Centrolenidae			
Vitreorana baliomma	MCP14121	Bahia: Una	MW366909
Ceratophryidae			
Ceratophrys joazeirensis	CFBH7411	Paraíba: Araruna	KP295617
Hylidae			
Aplastodiscus sibilatus	MLL-2016	Alagoas: Murici	KU184227
Aplastodiscus sibilatus	CFBH32528	Bahia: Ibirapitanga	KU184014
Boana albomarginata	USNM284519	Pernambuco: Caruaru	KF794116
Boana albomarginata	UFBA380/7890	Bahia: Mata de São João	MH004298
Boana atlantica	MUFAL13067	Alagoas: Maceió	MK348506
Boana atlantica	CFBH16126	Bahia: Uruçuca	MK348483

Boana crepitans	CFBHT07825	Alagoas: Campo Alegre	KU495263
Boana crepitans	CFBHT12841	Bahia: Camamu	KU495262
Boana faber	CFBHT04381	Rio de Janeiro: Petrópolis	KU495265
Boana freicanecae	MUFAL11072	Alagoas: Murici	MT823773
Boana freicanecae	ZUFRJ7941	Pernambuco: Jaqueira	MT823774
Boana raniceps	CFBHT07827	Alagoas: Campo Alegre	MW197887
Boana raniceps	MACN37795	Argentina: Santa Fé	KF794140
Boana semilineata	CFBH5424	Rio de Janeiro: Duque de Caxias	AY843779
Corythomantis greeningi	CFBH2968	Alagoas: Piranhas	KF002247
Corythomantis greeningi	CRS100	Bahia: Morro do Chapéu	MW243375
Dendropsophus branneri	CFBH20829	Pernambuco: Bonito	MT503865
Dendropsophus dutrai	MNRJ46746	Sergipe: Indiaroba	MT503923
Dendropsophus elegans	CFBH13294	Sergipe: Itabaiana	MT503966
Dendropsophus elegans	CFBH18699	Bahia: Prado	KY348631
Dendropsophus haddadi	FSFL1467	Bahia: Prado	MT503942
Dendropsophus haddadi	CFBH19472	Espírito Santo: Vitória	MT503941
Dendropsophus minutus	CFBH18567	Alagoas: Campo Alegre	MK266721
Dendropsophus minutus	CFBH24153	Rio de Janeiro: Resende	MT503934
Dendropsophus nanus	ZUEC:18018	Sergipe: Aracaju	MN420278
Dendropsophus nanus	MACN37785	Argentina: Entre Rios	AY549346
Dendropsophus oliveirai	AAGARDA12495	Pernambuco: São Lourenço da Mata	MW026634
Dendropsophus oliveirai	CFBH18799	Bahia: Maracás	MT503956
Dendropsophus soaresi	CFBH18575	Alagoas: Campo Alegre	MT503922
Dendropsophus studerae	URCAG767	Alagoas: Quebrangulo	MT503894
Phyllodytes acuminatus	CHP-UFRPE1598	Pernambuco: Buíque	MN961791
Phyllodytes edelmoi	MNRJ50118	Alagoas: Maceió	MN961798
Phyllodytes gyrinaethes	CFBH44634	Alagoas: Maceió	MN961801
Scinax agilis	UFBA562/11203	Bahia: Mata de São João	MH004314
Scinax auratus	UFBA618/11712	Bahia: Conde	MH004316
Scinax eurydice	UESC11005	Bahia	OK161150
Scinax fuscomarginatus	CFBH7349	Alagoas: Passo de Camaragibe	KJ004153
Scinax fuscomarginatus	CFBHT1950	Minas Gerais: Santana do Riacho	KJ004129
Scinax fuscovarius	CFBHT03219	Minas Gerais: Araxá	KU495554
Scinax melanodactylus	CFBHT01137	Alagoas: Passo de Camaragibe	KU495535
Scinax nebulosus	AAG-UFU6251	Rio Grande do Norte: Nísia Floresta	MK503366
Scinax nebulosus	MPEG:31631	Pará: Belém	MK503362
Scinax pachycrus	UESC11027	Bahia	OK161140
Scinax x-signatus	CFBHT08860	Pernambuco: Fernando de Noronha	KU495578
Scinax x-signatus	CFBHT09136	Bahia: Caetité	KU495576
Scinax x-signatus	CFBHT09136	Bahia: Caetité	KU495576
Sphaenorhynchus cammaeus	MACNHe48851	Alagoas: Quebrangulo	MK266727
Sphaenorhynchus prasinus	MZUFBA6756	Bahia: Mata de São João	MK266752
Trachycephalus mesophaeus	CFBHT09297	Bahia: Aurelino Leal	KU495600
Trachycephalus mesophaeus	FRS1038	São Paulo: Apiaí	MH206468
Leptodactylidae			
Leptodactylus fuscus	USNM284551	Pernambuco	AY911279

Leptodactylus latrans	MUFAL15265	Alagoas: Maceió	MT495901
Leptodactylus macrosternum	CFBHT01146	Alagoas: Passo de Camaragibe	KU495341
Leptodactylus macrosternum	FC18	Bahia: Terra Nova	MT495860
Leptodactylus mystaceus	AAGARDA12501	Pernambuco: São Lourenço da Mata	MT117857
Leptodactylus mystaceus	TG379	Amazonas: São Gabriel da Cachoeira	MT117890
Leptodactylus natalensis	MTR101P28	Alagoas: Maceió	KU495347
Leptodactylus natalensis	AAGARDA1980	Rio Grande do Norte: Parnamirim	MW291330
Leptodactylus troglodytes	USNM284553	Pernambuco	KM091620
Leptodactylus vastus	TG429	Paraíba: Guarabira	KU495368
Physalaemus albifrons	CFBH16137	Ceará: Viçosa do Ceará	KP146010
Physalaemus cicada	CFBH19395	Ceará: Novas Russas	KP146064
Physalaemus cuvieri	ZUEC17897	Pernambuco: Caruaru	KP146012
Pleurodema diplolister	TG427	Paraíba: Caiçara	KU495455
Pleurodema diplolister	CFBH16144	Ceará: Viçosa do Ceara	JQ937185
Pseudopaludicola mystacalis	ZUEC:13837	Maranhão: Barreirinhas	KJ147006
Pseudopaludicola mystacalis	ZUEC:14147	Mato Grosso: Cuiabá	KJ146983
Microhylidae			
Chiasmocleis alagoanus	C2683	Alagoas: Maceió	KC180030
Dermatonotus muelleri	CFBHT07834	Alagoas: Campo Alegre	KU495218
Dermatonotus muelleri	T7	Paraguay	KC179984
Elachistocleis cesarii	ZUEC:DCC3301	Minas Gerais: Serra do Cipó	JN604511
Elachistocleis cesarii	CFBHT00912	São Paulo: Santa Fé do Sul	KU495225
Stereocyclops incrassatus	MUFAL2482	Alagoas: Marechal Deodoro	KC180046
Stereocyclops incrassatus	CFBHT02080	Espírito Santo Linhares	KU495593
Odontophrynidae			
Macrogenioglottus alipioi	CFBHT04337	Bahia: Uruçuca	KU495385
Odontophrynus carvalhoi	JC1224	Bahia: Mucugê	FJ685687
Proceratophrys cristiceps	AAGARDA2739	Ceará: Crato	KX855993
Proceratophrys renalis	ZUFRJ8665	Pernambuco: Caruaru	JN814584
Proceratophrys renalis	UFBA187/6242	Bahia: Cachoeira	MH004311
Phyllomedusidae			
Hylomantis granulosa	MNRJ50123	Alagoas: Murici	GQ366225
Hylomantis granulosa	CFBHT00392	Pernambuco: Jaqueira	KU495255
Pithecopus gonzagai	CFBH7330	Alagoas: Passo de Camaragibe	GQ366330
Pithecopus gonzagai	ZUEC19684	Pernambuco: Limoeiro	MW158582
Pipidae			
Pipa carvalhoi	MUFAL14375	Pernambuco: Buíque	MT261672
Pipa carvalhoi	MUFAL14379	Pernambuco: Buíque	MT261653
Ranidae			
Lithobates palmipes	CFBHT07835	Alagoas: Campo Alegre	KU495377
Lithobates palmipes	01	Guiana	AF467265

Trabajo

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Herpeto-commerce: A look at the illegal online trade of amphibians and reptiles in Brazil

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ABSTRACT
The illegal sale of fauna and flora represents the third-largest illegal trade in the world. Social media has contributed considerably to the increase in this type of trade. We searched for posts announcing the sale of amphibians and reptiles in seven Facebook® groups (three public and four private groups) from 01 January 2019 to 31 July 2020. In total, we found 548 posts made by a total of 201 social network profiles announcing the sale of 1,049 animals. We found 58 herpetofauna species being traded in the network (15 amphibian and 43 reptile species). Most of the sale advertisements originated in Southeast Brazil, predominantly from the state of São Paulo. The most traded species were *Pantherophis guttatus* (N= 467), *Eublepharis macularius* (N= 152), and *Boa constrictor* (N=90). This study presents important data about the illegal herpetofauna trade through Facebook® in Brazil, proving this market is currently fully active. This trade has high growth potential, bringing possible risks to biodiversity and public health. In conclusion, we recommend the implementation of urgent, specific government measures for its regulation and effective inspection.

Key words: Herpetofauna; E-commerce; Animal Trafficking; Pet-trade; Facebook®.

RESUMO
A venda ilegal de fauna e flora representa o terceiro maior comércio ilegal do mundo. As redes sociais têm contribuído consideravelmente para o aumento deste tipo de comércio. Buscamos postagens anunciando a venda de anfíbios e répteis em sete grupos do Facebook® (três grupos públicos e quatro privados) de 01 de janeiro de 2019 a 31 de julho de 2020. No total, encontramos 548 postagens feitas por um total de 201 perfis de redes sociais anunciando a venda de 1.049 animais. Encontramos 58 espécies de herpetofauna sendo comercializadas na rede (15 espécies de anfíbios e 43 espécies de répteis). A maior parte dos anúncios de venda teve origem no Sudeste do Brasil, predominantemente no estado de São Paulo. As espécies mais comercializadas foram *Pantherophis guttatus* (N= 467), *Eublepharis macularius* (N= 152) e *Boa constrictor* (N=90). Este estudo apresenta dados importantes sobre o comércio ilegal de herpetofauna através do Facebook® no Brasil, comprovando que este mercado está atualmente em plena atividade. Esse comércio tem alto potencial de crescimento, trazendo possíveis riscos à biodiversidade e à saúde pública. Em conclusão, recomendamos a implementação de medidas governamentais urgentes e específicas para sua regulamentação e fiscalização efetiva.

Palavras-chave: Herpetofauna; E-commerce; Tráfico de Animais; Comércio Pet; Facebook®.

Introduction

Brazil is the most biodiverse country in the world, with approximately 117 thousand known animal species and around 50 thousand known plant species, showing a high rate of endemic species (Flora Brasileira, 2020; ICMBio, 2020; Charity and Ferreira, 2020). This large number of species makes

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the country a target for intense smuggling of wild species, one of the primary causes of local extinction, along with deforestation, farming activities, and urbanization processes (Hernandez and Carvalho, 2006; Heliodoro, 2009; RENCTAS, 2016). The illegal wildlife trade is responsible for spreading diseases and introducing exotic species, jeopardizing structured communities (Warchol, 2004; Carrete and Tella, 2008; Karesh *et al.*, 2012). This trade is also related to the considerable increase in violence and corruption rates (Warchol, 2004).

In Brazil, the institution responsible for dealing with animal trade is IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis). This institute is also responsible for the supervision and enforcement of the legal purposes, while other agencies have scientific competence on the subject, such as RAN/ICMBio (Centro Nacional de Pesquisa e Conservação de Répteis e Anfíbios), responsible for the herpetofauna (RENCTAS, 2001). The legal definition of the act of illegal trade, described under article 29, section 1, III of Law nº. 9,605/98, which includes in all respects: " Those who sell, exposes for sale, exports or acquires, retains, keeps in captivity or storage, uses or transports eggs, larvae, wild or native species, or in migratory route, as well as products and objects originating from such species, from breeding sites that are not authorized, without proper permit, competent authority or authorization."

Despite the legislation, the wild fauna trade from irregular breedings sites or specimens caught in nature, is still widely practiced in the country (Charity and Ferreira, 2020). The lack of investigation efforts is one of the reasons why this trade still occurs. E-commerce has been neglected, and the trafficking structure seems to benefit from online spaces that are lawless. Animal trafficking seems increasingly interconnected to the online network, which results in higher successes in sales that use these new means and make online supervision difficult (Hernandez and Carvalho, 2006; Siriwat and Nijman, 2018).

Globally, the acquisition of wild animals as pets through the internet has grown over the last several years due to the emergence of websites and social network groups specifically focused on the subject (Jansen *et al.*, 2018; Sy, 2018; Marshall *et al.*, 2020; Strine and Hughes, 2020). In most cases, sale advertisements through social networks have questionable origins (Magalhães and São-Pedro, 2012). Araújo (2014) and Auliya *et al.* (2016a) highlight the sale of amphibians and reptiles (herpetofauna) in this market. The authors also state that these animals are targeted due to the great variety of species, availability of individuals, and fewer care requirements when compared to mammals and birds.

Although the diversity of amphibians and reptiles is high, few species are legally regulated to be traded. According to data published in 2016, out of 10,272 species of reptiles, less than 8% are regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and by the European Wildlife Trade Regulations (EWTR) (Auliya *et al.*, 2016a). For amphibians, less than 3% of recognized species are listed in the three appendices of CITES (Auliya *et al.*, 2016b).

In most cases, the trade of these species jeopardizes their conservation, putting them in endangered or vulnerable statuses. The IUCN Red List of Threatened Species presents more than two thousand species of reptiles as threatened under the category "Biological resource use". This is the third largest threat category for this group, where 769 species are intentionally targeted by collectors for hunting and capture (IUCN, 2022). Over 290 amphibian species from the IUCN Red List are targeted for international pet trade and consumption purposes (Auliya *et al.*, 2016b).

This study aims to shine a light on illegal herpetofauna e-commerce due to the growing popularity of social networks in the last years and their increased use as a platform for worldwide illegal wildlife trading. Here, we collected quali-quantitative data from Brazilian public and private groups on the social network Facebook®, specifically created to sell or exchange herpetofauna individuals throughout the country.

Materials and methods

We searched for Brazilian groups on the social network Facebook® applying the following keywords in Brazilian Portuguese: "anfíbios", "répteis", "animais exóticos", "compra e venda de exóticos", "*pets* exóticos", "répteis e anfíbios" and "répteis e anfíbios venda" (English keywords: "amphibians", "reptiles", "exotic animals", "exotic marketing", "exotic pets", "reptiles and amphibians", and "reptiles and amphibians for sale"). We selected the first 15 groups we found online and got access to seven (four private and three public) that became the object of our research. We verified the number of members of each group, the date they were founded, and number of advertisements (see 'Information from analyzed Facebook® groups' in Appendix S1, Supplementary information).

The research consisted of analyzing all posts between 01 January 2019 and 31 July 2020. We recorded the advertisements related to the sale of amphibian and reptile species, listing the total number of posts and traded animals. The names of the groups and their members will be kept confidential for legal reasons and to avoid higher visibility of the groups, following orientation from the Association of Internet Researchers Committee (Franzke *et al.*, 2020).

We recognized the species mainly through images as well as scientific or popular names mentioned in the posts. We discarded posts that did not have pictures or any other means that would allow us to identify the advertised species correctly. We based the identification of individuals (to species level when possible) on specialized literature. From posts that did not have pictures, we considered the scientific name mentioned and current nomenclature. The nomenclature and taxonomic classification for species of reptiles were based on Uetz *et al.* (2022), and Frost (2021) for amphibians. We classified the species as native from the Brazilian fauna or exotic (non-native), following the lists of Brazilian reptiles (Costa and Bérnils, 2018) and amphibians (Segalla *et al.*, 2021).

We investigated if the species found were included in the appendices of CITES (2021). The threat level for each species was verified in the Brazil Red Book of Threatened Species of Fauna (ICMBio, 2018) and the Red List of the International Union for Conservation of Nature (IUCN, 2022). We analyzed the frequency and location of each advertisement. Therefore, it was possible to understand which Brazilian regions contribute the most to this animal trade.

Results

During the 19 months of sampling, we list a total of 548 posts advertising animals for sale made by 201 Facebook® profiles. In total, 1,049 individuals of herpetofauna were commercialized. We recorded the sale of 58 species (Table 1), belonging to five orders: Anura (37 individuals from 12 species), Caudata (ten individuals from three species), Crocodylia (six individuals from one species), Squamata (Snakes: 815 individuals from 23 species; Lizards: 115 individuals from nine species) and Testudines (66 individuals from ten species) (Fig. 1). Between the groups we analyzed, two of them concentrated the majority of posts, with 218 and 186 on each one, with a total of 798 traded animals (see 'Information from analyzed Facebook® groups ' in Appendix S1, Supplementary information).

Thirteen individuals could not be identified to species level, belonging to the genera *Ceratophrys* sp., *Chelonoidis* sp., and *Pantherophis* sp. We added these species to Table 1; their conservation status was not specified, and their classification as native or exotic was not described for the first two species. The snake genus *Pantherophis* does not occur in Brazil, so we considered it an exotic species.

All analyzed posts refer to traded animals in Brazilian territory. Most animals were being traded in the state of São Paulo (n=506), followed by the state of Rio de Janeiro (n=82) and Distrito Federal (n=42) (Fig. 2). Most of the advertisements are concentrated in the southeast region. We could not verify the trading location of 358 announced individuals due to the information not being described in the posts.

Four announced species (*Caiman latirostris*, *Acrantophis dumerili, Acrantophis madagascariensis*, and *Python molurus*) are listed in Appendix I of CITES (2021), which are species not allowed to be internationally traded due to being endangered. We found twenty-one species in Appendix II, that described species likely to become endangered in the future. In addition, a special license is required for their trade. Only one species (*Crotalus durissus*) is listed in Appendix III, included after a direct request from Honduras. This appendix lists species that need international control, so their exploitation is either restricted or prevented. Half of the species (n=29) do not appear on the CITES list (Table 1).

Concerning the species threat level, only 49 were assessed by IUCN Red List of Threatened Species (2022), four species listed as Vulnerable (VU) (*Podocnemis unifilis*, *Chelonoidis denticulatus*, *Correlophus ciliatus*, *Python bivittatus*), one listed as Critically Endangered (CR) (*Ambystoma mexicanum*), and two listed as Near Threatened (NT) (*Python molurus* and *P. regius*). Regarding the threat level in national scope, out of 28 native Brazilian species announced, only *Ranitomeya ventrimaculata* is not on the list. Twenty-four species are classified as Least Concern (LC), and three other species (*Trachemys dorbigni, Podocnemis expansa*, and *Podocnemis uni-*

Table 1. List of reptile and amphibian species traded in Brazil through Facebook® groups from 01 January 2019 until 31 July 2020.

ORDER/FAMILY/SPECIES	**Nº of Individuals**	**CITES (2021)**	**Conservation Status**		**Native or exotic**
			IUCN Red List (2022)	**Red Book ICMBio (2018)**	
ANURA					
AMPHIBIA					
Bombinatoridae					
Bombina orientalis (Boulenger, 1890)	3	-	LC	-	EX
Brachycephalidae					
Brachycephalus ephippium (Spix, 1824)	5	-	LC	LC	NA
Ceratophryidae					
Ceratophrys aurita (Raddi, 1823)	1	-	LC	LC	NA
Ceratophrys sp. (Wied-Neuwied, 1824)	1	-	-	-	-
Dendrobatidae					
Adelphobates galactonotus (Steindachner, 1864)	6	II	LC	LC	NA
Dendrobates tinctorius (Cuvier, 1797)	2	II	LC	LC	NA
Ranitomeya ventrimaculata (Shreve, 1935)	2	II	LC	-	EX
Hylidae					
Dendropsophus minutus (Peters, 1872)	2	-	LC	LC	NA
Phyllomedusidae					
Pithecopus azureus (Cope, 1862)	7	-	DD	LC	NA
Pithecopus nordestinus (Caramaschi, 2006)	1	-	DD	LC	NA
Pipidae					
Xenopus laevis (Daudin, 1802)	5	-	LC	-	EX
Ranidae					
Lithobates catesbeianus (Shaw, 1802)	2	-	LC	-	EX/invasive species
CAUDATA					
Ambystomatidae					
Ambystoma mexicanum (Shaw & Nodder, 1798)	4	II	CR	-	EX
Salamandridae					
Pleurodeles waltl Michahelles, 1830	4	-	NT	-	EX
Triturus cristatus (Laurenti, 1768)	2	-	LC	-	EX
REPTILIA					
CROCODYLIA					
Alligatoridae					
Caiman latirostris (Daudin, 1802)	6	I	LC	LC	NA
TESTUDINES					
Chelidae					
Hydromedusa tectifera Cope, 1870	1	-	-	LC	NA
Mesoclemmys gibba (Schweigger, 1812)	2	-	-	LC	NA
Mesoclemmys tuberculata (Luederwaldt, 1926)	4	-	-	LC	NA
Emydidae					
Trachemys dorbigni (Duméril & Bibron, 1835)	20	-	-	NT	NA
Geoemydidae					

Rhinoclemmys punctularia (Daudin 1801)	1	-	-	LC	NA
Podocnemididae					
Podocnemis expansa (Schweigger, 1812)	2	II	CD	NT	NA
Podocnemis unifilis Troschel, 1848	2	II	VU	NT	NA
Testudinidae					
Chelonoidis carbonarius (Spix, 1824)	11	II	-	LC	NA
Chelonoidis denticulatus (Linnaeus, 1766)	12	II	VU	LC	NA
Chelonoidis sp.	11	-	-	-	-
SQUAMATA					
Agamidae					
Pogona vitticeps (Ahl, 1926)	33	-	LC	-	EX
Diplodactylidae					
Correlophus ciliatus Guichenot, 1866	6	-	VU	-	EX
Rhacodactylus leachianus (Cuvier, 1829)	1	-	LC	-	EX
Eublepharidae					
Eublepharis macularius (Blyth, 1854)	152	-	LC	-	EX
Iguanidae					
Iguana iguana (Linnaeus, 1758)	49	II	LC	LC	NA
Polychrotidae					
Polychrus acutirostris Spix, 1825	7	-	LC	LC	NA
Polychrus marmoratus (Linnaeus, 1758)	1	-	LC	LC	NA
Teiidae					
Salvator merianae Duméril & Bibron, 1839	16	II	LC	LC	NA
Varanidae					
Varanus exanthematicus (Bosc, 1792)	1	II	LC	-	EX
Boidae					
Acrantophis dumerili Jan, 1860	2	I	LC	-	EX
Acrantophis madagascariensis (Duméril & Bibron, 1844)	2	I	LC	-	EX
Boa constrictor Linnaeus, 1758	90	II	LC	LC	NA
Boa imperator Daudin, 1803	1	II	LC	-	EX
Corallus hortulana (Linnaeus, 1758)	2	II	LC	LC	NA
Epicrates assisi Machado, 1945	5	II	LC	LC	NA
Eryx colubrinus (Linnaeus, 1758)	12	II	LC	-	EX
Eunectes murinus (Linnaeus, 1758)	3	II	LC	LC	NA
Colubridae					
Heterodon nasicus Baird & Girard, 1852	3	-	LC	-	EX
Lampropeltis getula (Linnaeus, 1766)	19	-	LC	-	EX
Lampropeltis californiae (Blainville, 1835)	7	-	LC	-	EX
Lampropeltis polyzona Cope, 1860	6	-	LC	-	EX
Pantherophis guttatus (Linnaeus, 1766)	467	-	LC	-	EX
Pantherophis obsoletus (Say, 1823)	3	-	LC	-	EX
Pantherophis sp.	1	-	-	-	-
Pituophis catenifer (Blainville, 1835)	1	-	LC	-	EX
Xenodon merremii (Wagler, 1824)	1	-	LC	LC	NA
Pythonidae					

Antaresia maculosa (Peters, 1873)	2	II	LC	-	EX
Morelia spilota (Lacépède, 1804)	2	II	LC	-	EX
Python bivittatus Kuhl, 1820	5	II	VU	-	EX
Python molurus (Linnaeus, 1758)	5	I	NT	-	EX
Python regius (Shaw, 1802)	23	II	NT	-	EX
Viperidae					
Crotalus durissus Linnaeus, 1758	2	III	LC	LC	NA
Total	1049				

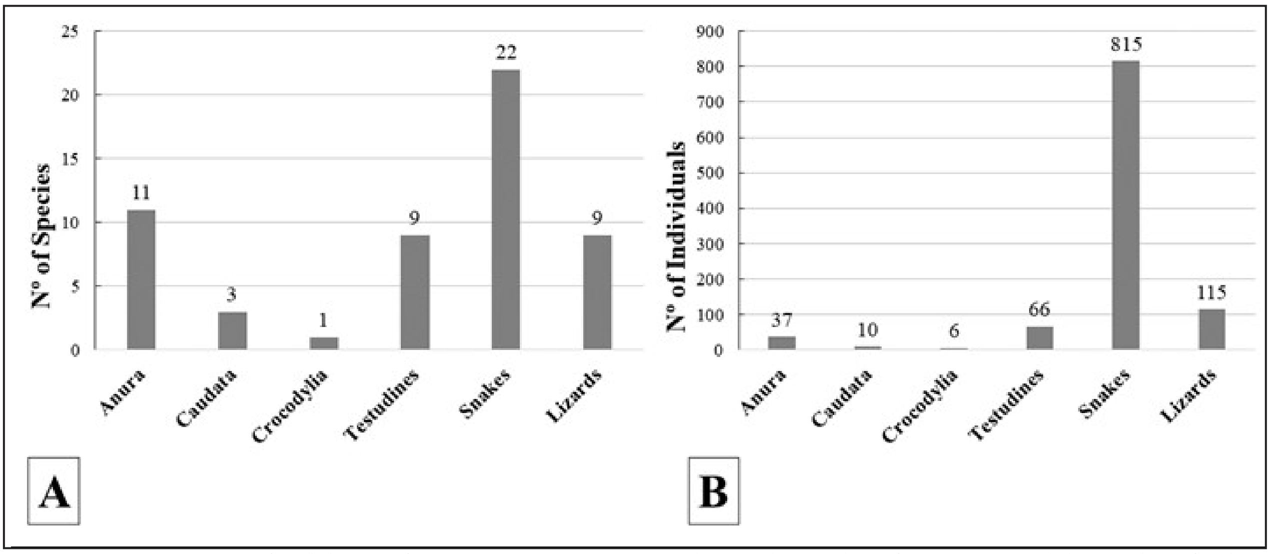

Figure 1. Representation of each herpetofauna taxonomic group, according to the number of species (A) and the number of individuals (B) announced for sale on Brazilian Facebook® groups, from 01 January 2019 until 31 July 2020. Species identified only to genus level were disregarded.

filis) are considered Near Threatened (NT) (ICMBio, 2018). Nearly half of the traded species (n=27; 46%) are exotic, one of which (*Lithobates catesbeianus*) is considered an invasive species in Brazil (Both *et al.*, 2011).

Discussion

Of the 19 sampling months in Facebook® groups, we recorded 548 posts, announcing a total of 1,049 individuals that belong to 58 herpetofauna species. Even though our data present a temporal overlap with those collected by Máximo et al. (2021), our study was more comprehensive, including the analysis of more Facebook® groups and researching not only amphibians but also reptiles. Although our results still represents a small sample of the herpetofauna e-commerce in Brazil, they are enough to prove that this is an active and unregulated market in the country. Due to the lack of information in the posts, it is not possible to confirm if the traded individuals come from illegal breeding sites, illegal imports, or if they were removed from the wild and introduced in the market, as observed by Máximo *et al.* (2021).

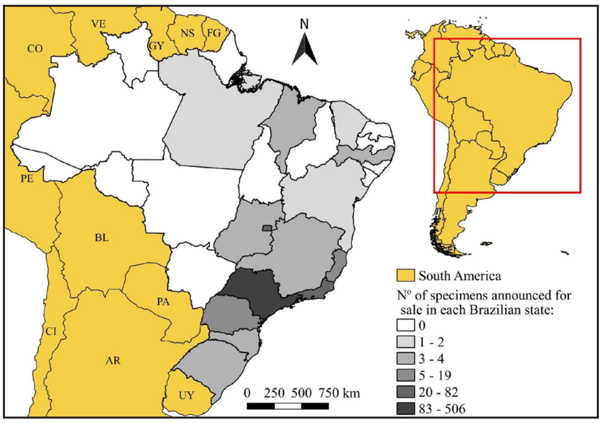

Figure 2. Number of herpetofauna individuals announced for sale on Facebook® from 01 January 2019 until 31 July 2020, in each Brazilian state.

However, the origin of the advertisements strongly suggests that they are mainly illegal trades. Even in advertisements with few potentially legal species, such as *Boa constrictor*, there is no mention of certificates that prove the origin of the animals.

The illegal herpetofauna trade is linked to several demands, from by-products exploitation (skin, meat, carapace, venom, etc.) to the pet market (Carpenter *et al*., 2014; UNODC, 2020). Groups from the social network Facebook® we analyzed were specifically created to promote the trade of amphibians and reptiles as pets. The pet market is among the markets that benefit the most from resources offered by social networks for trading illegal or irregular products (Lavorgna, 2014). For this reason, it is fundamental to understand the consumers' motivations and the characteristics that might make certain animals attractive to this market. Therefore, strategies can be planned to inhibit or regulate such activities. Our data corroborate the demand for large-sized species (e.g. *Ceratophrys* sp., *Lithobates catesbeianus*, snakes of the families Boidae and Pythonidae) or bright-colored animals (e.g., anurans of the families Dendrobatidae and Phyllomedusidae, snakes of the genera *Lampropeltis* sp. and *Panterophis* sp.), as pointed out by previous studies (Van Wilgen *et al*., 2009; Mohanty and Measey, 2019). Usually, the interest in keeping amphibians and reptiles as pets can be motivated by some specific issues, such as the opportunity to observe behaviors rarely seen in the wild (e.g., predation) and the relative ease of captivity maintenance (e.g., small space required, no bathing needed, infrequent feeding.) (Warwick, 2014; Measey *et al*., 2019). However, the false perception of the low captive herpetofauna maintenance results in the mortality of approximately 75% of acquired individuals after one year (Toland *et al*., 2012).

In our study, reptiles correspond to the majority of announced species (n=43; 71%), with a prevalence of snakes (n=22; 38%), showing the preference for these animals among the reptile breeders in Brazil (Alves *et al*., 2019). According to published data in the last report from World Wildlife Crime Report (UNODC, 2020), reptiles are considered the second most trafficked animal globally, behind mammals. Between 2007 and 2017, the main illegally traded living reptiles were tortoises and freshwater turtles (47.4%), followed by snakes (26.7%) and lizards (17.8%) (UNODC, 2020). Brazil has only contributed with information to this report from 2015 to 2016, not providing any data since then. Unfortunately, this leaves a gap in the current knowledge regarding illegal wildlife trading.

One of the main environmental problems resulting from the pet market is the introduction of invasive species (Lockwood *et al*., 2019; Gippet and Bertelsmeier, 2021), which corresponds to one of the main current threats to biodiversity and ecosystems (Simberloff *et al*., 2013; Gallardo *et al*., 2015). Out of the 27 recorded exotic species, some have a high capacity to invade new environments (e.g., *Lithobates catesbeianus*, *Xenopus laevis*, *Panterophis* sp., *Python* sp.) (Kraus, 2009). Although the number of exotic species from the herpetofauna recorded in Brazil is growing (e.g., Eterovic and Duarte, 2002), their impacts on national ecosystems are still practically unknown, with few exceptions, such as the bullfrog (Silva *et al*., 2011; Both *et al*., 2014). However, the invasive herpetofauna might bring several negative consequences to the local biodiversity through predation, competition, hybridization, and disease transmission (Kraus, 2015). It can also bring risks to human health by spreading zoonoses (Mendoza-Roldan *et al*., 2021).

Although amphibian species are traded in a smaller proportion than reptiles, the possible impacts from the illegal trade of this group are not less significant. One of the main threats is related to the transmission of emerging diseases, such as chytridiomycosis, which represents the greatest loss of amphibian biodiversity ever caused by a disease (Scheele *et al*., 2019). Two of the species recorded in this study, the bullfrog (*L. catesbeianus*) and the African clawed frog (*Xenopus laevis*), are possibly the primary species responsible for the global dissemination of the fungus *Batrachochytrium dendrobatidis* (*Bd*) (Kilpatrick *et al*., 2010; O'Hanlon *et al*., 2018). These species are tolerant to chytrid infection and can act as a natural reservoir (James *et al*., 2015). In a recent study, Máximo *et al*. (2021) tested the presence of *Bd* in illegally traded amphibians. The researchers reported that none of the individuals were infected, which might indicate that fungus transmission is low. However, this issue must be further investigated. Besides chytrid, the bullfrog can act as a vector of *Ranavirus* (Santos *et al*., 2020), an emerging virus considered responsible for the mortality of ectothermic vertebrates worldwide (Duffus *et al*., 2015). There is another emerging disease, recently described, caused by the fungus *Batrachochytrium salamandrivorans* (Martel *et al*., 2013). Although it has only been recorded in European countries and

Asia, this disease may be spread to other countries through the global amphibian trade, resulting in another panzootic (Yap *et al.*, 2017).

Regarding online trading in Brazil, we verified that the state of São Paulo is responsible for most advertisements and traded animals, followed by Rio de Janeiro and Distrito Federal. Alves *et al.* (2019) reported the same Federative units as the ones with the most owners of pet reptiles in Brazil. The researchers also indicated the probable existence of trafficking routes for these animals. According to Máximo *et al.*, (2021), the highest concentration of sellers of pet amphibians in Brazil is located in the states of São Paulo and Rio de Janeiro. São Paulo state is considered a key state for understanding and combating this type of activity, due to being the country's primary destination of illegally traded fauna (Charity and Ferreira, 2020). Not accidentally, nearly half of the amphibians and reptiles announced on Facebook®, were for sale in São Paulo, proving the importance of this state in understanding the illegal herpetofauna e-commerce. This fact reinforces that animal trade through social networks strengthens the already existing wildlife trafficking and its distribution networks (Nassaro, 2017; Siriwat and Nijman, 2018, Máximo *et al.,* 2021).

As reported in World Wildlife Crime Report (UNODC, 2020), the trade in digital platforms such as Facebook® and other social networks is dominant in the illegal wildlife trade, especially reptiles. Through e-commerce, anonymous traders with fake profiles are less subject to inspection and reach a much larger audience. We verified that most of the animal trading groups on Facebook® have restricted access, restraining the admittance of new members, consequently jeopardizing inspections. Other factors help explain the success of this clandestine online trade, such as the ease of purchase and lower prices when compared to legal trade. Legally traded animals can cost ten times more money than those illegally sold (Nassaro, 2017). Our data confirm this finding, as we found boa constrictor individuals (*Boa constrictor*) with prices between R$ 300.00 and R$ 1,500.00, while animals of the same species cost from R$ 3,500.00 to R$ 10,000.00 in a legal breeding website (T. Lima, personal communication, 2021). Owning pet reptiles in Brazil is found predominantly among people with medium to high purchasing power due to the high costs associated with purchasing and maintaining these animals (Alves *et al.*, 2019). That makes the low prices in e-commerce an even more important factor in leveraging this market, making this product accessible to a much larger share of the population. This fact reinforces the global phenomenon of gradually replacing the clandestine sale of animals in markets, physical stores, and fairs in favor of illegal online trading (Nijman *et al.*, 2019; Alves *et al.*, 2019; UNODC, 2020).

It is fundamental to know and quantify the number of traded species, emphasizing those classified as threatened, to understand the impact caused by the wildlife trade (Marshall *et al.*, 2020). Based on data from the IUCN Red List of Threatened Species (2022), out of the four species currently listed as vulnerable, only two have information on what causes their threats. *Correlophus ciliatus* is threatened by the categories "Biological resource use", "Natural system modifications" and "Invasive and other problematic species, genes & diseases" and its trade is related to the pet, display animals, and horticulture market. While *Python bivitatus* is mainly threatened by "Agriculture & aquaculture" and "Biological resource use" and its trade is associated with medicine, crafts, pet, clothing and food. Other studies show how the illegal trade of species for several purposes can lead to a considerable decrease in populations, as is the case of *Astrochelys yniphora*, a Madagascar endemic species of tortoise, considered at imminent risk of extinction in 2018, as a consequence of the illegal trade (Mandimbihasina *et al.*, 2018).

The National Biodiversity Policy (Decree Nº 4.339 of 22 August 2002; Brasil, 2002) defines the importance of predicting, preventing, and acting against the origin of processes leading to the decrease or considerable loss of biodiversity. However, there are several flaws in the legislation, and the lack of government investment and attention to this activity in the country is sadly prevalent. The Brazilian legislation features the protection of the native fauna from the illegal trade of vertebrate animals. However, it lacks knowledge of key factors in wildlife trafficking. This interferes with the differentiation between animal traders and pet owners, as reported by Charity and Ferreira (2020) in their study on wildlife trafficking in Brazil.

This problem is also observed through Federal Law nº 9,605/1 998 (Brasil, 1998), where there is no definition for animal trafficking. Therefore, every act against wildlife (killing, chasing, catching, and using wild animals) is considered an "Environmental Crime", and is subject to a fine and penalty of six months to one year in prison. We highlight the Complementary Law nº 140 of 08 December 2011, which establishes that each Brazilian state is responsible for elaborating the assemblage of wild fauna species destined for breeding sites and scientific research (Brasil, 2011). However, to date, only Paraná state has provided this document (Paraná, 2015).

The lack of current documents defining the species likely to be traded possibly stimulates the internal wildlife market, aside from hindering inspections and differentiation of legal from illegal trade. This market is a worrying situation concerning the herpetofauna trade, especially in the Southeast and Midwest of Brazil. Additionally, the flexibility of legislation and mild penalties reinforce the neglect of protecting the wild fauna, and easing illegal activities in the country, especially through social networks, as demonstrated in this study. Since traffickers can easily migrate to other platforms (as observed in other countries) once their illegal activity in a determined social network is detected, governmental regulation of digital media is required (UNODC, 2020).

Aside from highlighting the primary negative aspects of social networks, it is essential to emphasize that they can also be advantageous (Di Minin *et al.*, 2015; Siriwat *et al.*, 2020). Specifically, social networks have become an important place to obtain data that might help us understand the relation of users with issues involving biodiversity, which makes these networks a critical tool for the development of several policies and strategies for conservation (Di Minin *et al.*, 2015; Roberge, 2014; Correia *et al.*, 2021). Initiatives for environmental education and scientific dissemination created with support from social networks are increasingly common, expanding the range of traditional activities, generating information, and stimulating the public interest in protecting biodiversity (Bik and Goldstein, 2013; Roberge, 2014; Irga *et al.*, 2020).

Conclusion

Overall, our results show that herpetofauna e-commerce in Brazil happens with no legal obstacles. This market compromises the integrity of Brazilian reptile and amphibian species since native species proved to be the main target of the trade. Species with a high threat level can be the focus of "animal dealers" due to the profit acquired from them, bringing higher extinction risks to these species. This study presents the data from this form of trade, emphasizing how unrestrained animal trafficking is in Brazilian territory. In conclusion, this study may be used as a tool to combat animal trafficking in Brazil, helping at the same time with the conservation of species that show some level of threat.

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Appendix S1

Information from analyzed Facebook® groups: Availability of groups to users of the social network (Public or private group); Number of participants (information collected on 19 August 2020, may have changed); Date of creation of groups; Number of reptiles and/or amphibians individuals being marketed in each group; Number of publications containing reptiles and/or amphibians being marketed in each group. The asterisk indicates that the group was deleted from the social network during the search period, making it impossible to collect some information.

Groups	Public/Private	Nº of Participants (19/08/2020)	Creation Date	Nº of individuals	Nº of Publication
Group 1	Private	1.831	01/02/2019	399	218
Group 2	Public	892	09/09/2013	106	66
Group 3*	-	-	-	100	60
Group 4	Public	329	29/04/2014	25	23
Group 5	Private	122	15/04/2020	10	7
Group 6	Private	10.915	22/06/2015	399	168
Group 7	Public	6.122	29/05/2013	10	6

Trabajo

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Exploring the morphological diversity of Patagonian clades of *Phymaturus* (Iguania: Liolaemidae). Integrative study and the description of two new species

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Phymaturus maquinchao: urn:lsid:zoobank.org:act:CF1712F1-CE83-4308-8DAA-A82928BB6241

Phymaturus chenqueniyen: urn:lsid:zoobank.org:act:98154EF2-E658-4AC2-878D-CA2534AB4599

ABSTRACT

In the present contribution, we revisited the taxonomy and phylogenetic relationships within the *somuncurensis* and *spurcus* clades of *Phymaturus* lizards. Based on 296 morphological characters and DNA sequences, we evaluated the taxonomic species status of each clade and of two populations sampled in a field trip. Based on this evidence, we describe two new taxa for the genus. We also studied the species recently described for Chubut province, we analyzed their phylogenetic relationships, and compared them with other Patagonian species. Here, we provide data of color in life, squamation, and measurements, and compare in detail the new taxa to other members of their respective clades. We found that the *somuncurensis* clade comprises nine species (plus two other candidate ones) distributed mostly peripherally to the Somuncurá plateau, and present on the margins of it in isolated creeks and small mountain chains. Recent molecular studies arrived to different conclusions about the taxonomic validity of closely related species of the *spurcus* clade of *Phymaturus* lizards. We decided to revisit this group and contribute with a more complete analysis for two reasons: none of these studies revisited carefully the overall morphology and type series, and the only article that revisited this complex of species studied only one color pattern character, without providing voucher information (matching color types-collection specimens-DNA samples-sites). We studied the type series of all species, revisited characters taken from squamation and measurements, revised the color pattern of all terminals, and performed statistical analysis. Our results discovered statistically significant characters, which provide enough morphological support to consider all species of the group as valid in congruence to the multilocus analysis that combined mitochondrial and nuclear data published recently. We also provide discrete color pattern characters that help to adequately differentiate these species.

Key words: Diversity; Lizards; Phylogenetics; Taxonomy; South America

RESUMEN

En la presente contribución, revisamos la taxonomía y las relaciones filogenéticas dentro de los clados *somuncurensis* y *spurcus* de las lagartijas del género *Phymaturus*. Con base en 296 caracteres morfológicos y secuencias de ADN, evaluamos el estado taxonómico de las especies de cada clado y de dos poblaciones muestreadas en un viaje de campo. Con base en este cuerpo de evidencia, describimos dos nuevos taxones. También estudiamos las especies recientemente descritas para la provincia de Chubut, analizamos sus relaciones filogenéticas y las comparamos con otras especies patagónicas. Aquí proporcionamos datos de color en vida, escamación y medidas, y comparamos en detalle los nuevos taxones con otros miembros de sus respectivos clados. El clado *somuncurensis* comprende nueve especies descriptas (más otras dos candidatas)

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distribuidas principalmente en la periferia de la meseta de Somuncurá, presentes en quebradas aisladas y pequeñas cadenas montañosas. Estudios moleculares recientes llegaron a diferentes conclusiones sobre la validez taxonómica de especies estrechamente relacionadas dentro del clado *spurcus* de *Phymaturus*. Decidimos revisar este grupo y contribuir con un análisis más completo por dos razones: ninguno de estos estudios revisó cuidadosamente la morfología general y las series tipos, y el único artículo que revisó este complejo de especies estudió solo un carácter de patrón de color, sin proporcionar información sobre vouchers (coincidencia de tipos de color-especímenes de recolección-muestras de ADN-lugares). Estudiamos la serie tipo de todas las especies, revisamos los caracteres tomados de la escamación y las medidas, revisamos el patrón de color de todos los terminales y realizamos análisis estadísticos. Nuestros resultados descubrieron caracteres estadísticamente significativos, que brindan suficiente apoyo morfológico para considerar válidas todas las especies en congruencia con el análisis multilocus que combinó datos mitocondriales y nucleares publicado hace poco tiempo. También proporcionamos caracteres de patrones de color discretos que ayudan a diferenciar adecuadamente estas especies.

Palabras claves: Diversidad; Reptiles; Filogenética; Taxonomia; America del Sur.

Introduction

The genus *Phymaturus* is known for its extremely endemic species, and often known only from their type locality, despite extensive sampling done over the years by different herpetologists. This distribution pattern is likely caused by the genus habitat, which consists of rocky outcrops with crevices that these animals use as refuge from predators. Unlike its morphologically diverse sister genus, *Liolaemus*, *Phymaturus* has a highly conserved body shape (González Marín *et al.*, 2018), probably due to its restricted saxicolous life-style. Divergence time estimates of the sister genus *Liolaemus* support an Eocene origin, whereas the radiation of the current diversity of *Phymaturus* dates back to the Miocene (Hibbard *et al.*, 2018; Esquerré *et al.*, 2019a). *Phymaturus* lizards are also exclusively herbivorous and viviparous, with biennial reproduction (Boretto *et al.*, 2007; Boretto and Ibargüengoytía, 2009). Due to this morphological conservatism, recognizing new species requires in-depth knowledge of these animal's systematic and diagnostic traits. Furthermore, given the extremely endemic nature of these species, their low population densities, and their biennial pattern of reproduction (Boretto and Ibargüengoytía, 2006; 2009), all *Phymaturus* were considered vulnerable in their latest categorization (Abdala *et al.*, 2012). Therefore, recording the morphological diversity and delineating species within this clade are primary goals for their conservation. Etheridge (1995) divided the genus *Phymaturus* into two species groups, the *patagonicus* and the *palluma* groups, based on morphological characters. In his study, the author proposed apomorphies, but did not present a formal phylogenetic analysis. Etheridge's division (1995) was corroborated later using phylogenetic methods with morphology (Lobo and Quinteros, 2005a; Lobo *et al.*, 2012a) and molecular data (Morando *et al.*, 2013). More recently, two total evidence studies were performed, one for each main species group of the genus (Lobo *et al.*, 2016; Lobo *et al.*, 2018). Knowledge about the diversity of this genus increased exponentially in the last 25 years, from Etheridge (1995), with the recognition of 10 species, to the currently 52 recognized species (Lobo and Barrasso, 2021), with several new species having been described in the last two decades (González Marín *et al.*, 2016a; Scolaro *et al.*, 2016; Troncoso-Palacios *et al.*, 2018; Lobo *et al.*, 2018; Hibbard *et al.*, 2019; Lobo *et al.*, 2019; Lobo *et al.*, 2021; Scolaro *et al.*, 2021, among the most recent ones). New published information in the last years contributed to databases of DNA sequences and morphology for the genus *Phymaturus* (like Troncoso-Palacios *et al.*, 2018; Quipildor *et al.*, 2018a, 2018b; Lobo *et al.*, 2019), providing a very interesting challenge for future research programs.

Within the *patagonicus* group, four clades were recovered in successive phylogenetic analyses (Lobo *et al*., 2012a; Morando *et al*., 2013; Lobo *et al*., 2018): the *indistinctus*, *payuniae*, *somuncurensis*, and *spurcus* clades. In a recent contribution, the composition and phylogenetic relationships within the *payuniae* clade were analyzed and two new species were described, *Phymaturus niger* and *P. robustus* (Lobo *et al*., 2021); however, several populations belonging to the *patagonicus* group remain unrevised.

In the present contribution, we study two clades: the *somuncurensis* and the *spurcus* clades. According to the last analysis (Lobo *et al*., 2018), the *somuncurensis* clade comprises eight species: *P. calcogaster*, *P. camilae*, *P. ceii*, *P. etheridgei*, *P. sinervoi*, *P. somuncurensis*, *P. tenebrosus*, *P. yachanana*, and two candidate species: *P*. sp.22a and *P*. sp22b (Morando *et al*., 2013). Curiously, members of the *somuncurensis* clade are distributed along margins and surrounding the Meseta de Somuncurá, an extensive plateau in Argentinian Patagonia. *Phymaturus yachanana*, *P. calcogaster*, and *P. camilae* form a subclade , which is nested within the *somuncurensis* clade not forming an independent lineage (indicated as *calcogaster* group in Morando *et al*., 2013) . On the other hand, the *spurcus* clade is composed by *P. spurcus*, *P. spectabilis*, *P. excelsus,* and *P. manuelae* (Lobo *et al*., 2018). Lobo *et al*. (2012b) considered *P. agilis* Scolaro *et al*. (2008) as a junior synonym of *P. spectabilis* based on the lack of morphometric differences between these species for the same meristic characters studied by their authors. They also described and provided photographs of the dorsal color pattern of two neonates —one with the uniform dorsal pattern of *P. agilis*, the other with the bold pattern of *P. spectabilis*— born from a single female assignable to *P. spectabilis*. Corbalán *et al*. (2016) used the mitochondrial locus cytochrome c oxidase I to test if this molecular marker would reliably distinguish lizard species of the *patagonicus* group of *Phymaturus* (18 described species and two populations of unidentified species included in their study). They calculated intra- and inter-population genetic distances for all species and performed phylogenetic reconstructions. Based on the low genetic distances found, the authors concluded that *P. agilis, P. excelsus*, *P. spectabilis*, and *P. spurcus* are a single species with high polymorphism. Becker *et al*. (2018) arrived to a similar conclusion based on analyses of COI, fragments of Cytb, ND1, ND2 and eight transfer RNAs of *P. agilis*, *P. excelsus*, *P. manuelae*, *P. spectabilis,* and *P. spurcus*. Both Corbalán *et al*. (2016) and Becker *et al*. (2018) restricted their morphological observations to only one character: the presence/ absence of brown morphs. More recently, in a contribution that studied the evolution of body size and shape of the *patagonicus* group, González Marín *et al*. (2018) analyzed multiple loci simultaneously (mitochondrial and nuclear data). They found that their molecular analysis provided support for divergence among the species *P. spectabilis*, *P. spurcus* and *P. excelsus* and considered them valid species. Morando *et al*. (2020) considered that those species formed a single polymorphic species, following Corbalán *et al*. (2016) and Becker *et al*. (2018).

Assignment to a clade of the *patagonicus* group is uncertain in two cases: *P. curivilcun* Scolaro *et al*. (2016) and *P. katenke* Scolaro *et al*. (2021); both species were described for Chubut province. *Phymaturus curivilcun*, for which no DNA information is available, has never been included in a phylogenetic analysis, whereas *Phymaturus katenke* was included in an analysis using only COI information (sp. 1 in Corbalán *et al*., 2016).

Taking into account the contradictory conclusions drawn from the last molecular analyses (especially in the case of the *spurcus* clade), we conducted a study to provide new morphological information, expand observations made in previous works and clarify certain interpretations. We carried out an exhaustive analysis of these species, studied the type series, performed statistical analyses, and showed the diagnostic characters that differentiate the species involved. We completed morphological data for *Phymaturus curivilcun*, *P camilae*, *P. sinervoi*, and *P. katenke*, and two unnamed populations, and added new DNA sequences. With this new evidence, we analyzed the phylogenetic relationships of all species mentioned and describe two new species for the genus *Phymaturus*, one belonging to the *somuncurensis* clade and the other to the *spurcus* clade. One of these two species was considered as candidate species (sp13) by Morando *et al.* (2013).

Materials and methods

We examined 356 specimens belonging to 19 species of *Phymaturus* of the *patagonicus* group, including all members of the *somuncurensis* and *spurcus* clades, and two unnamed populations: 1) on RP N° 23, approx. 10 km N from Maquinchao town (41° 11.300' S, 68° 38.386' W, 876 m), Veinticinco de Mayo department, Río Negro province, Argentina, and 2) on RN N° 1s40 (former RN° 40), approx. 7 km S of Las Bayas village (41° 29.238' S, 70° 41.557' W, 1139 m), climbing the plateau Meseta Chenqueniyén from Las Bayas, Ñorquinco department, Río Negro province, Argentina (see Appendix 1). We updated the morphological data matrix of *Phymaturus*, which includes 271 characters (see character lists in Lobo and Quinteros 2005a; Lobo, *et al.* 2012a, 2016, 2018, 2019, 2021). Part of this variation, 42% of those characters, involves informative features within the *patagonicus* group (114); these characters were revisited across the species included in the present study. Continuous characters were coded and scored following the method of Goloboff *et al.* (2006), as in previous studies. Fifty-three characters are continuous, and 218 characters are discrete: 192 of external morphology (characters are of color pattern, scale counts, scale morphology and ornamentation, scale organs, integumentary glands, skin folding), 73 are anatomical (skeleton, muscles, hemipenis, viscera) and 6 are miscellaneous (chromosomes, fecundity, salt excretion). With respect to our contribution of Lobo *et al.* (2018), for this study, we improved our samples for the whole morphology of *P. camilae*, *P. curivilcun*, *P. calcogaster*, *P. yachanana*, *P. somuncurensis*, *P. ceii*, *P. sinervoi,* and *P. katenke*, taking data from MLP (Museo de La Plata, Argentina), MCN-UNSa (Museo de Ciencias Naturales, Universidad Nacional de Salta, Argentina) and IBIGEO (Reptile collection deposited at Instituto de Bio y Geociencias del NOA, Salta, Argentina).The molecular dataset includes sequences of Cytb, COI, 12S, ND4, NTF3, PNN, PRLR, C-mos, and seven anonymous nuclear loci: Phy38, Phy41, Phy60, Phy64, Phy84, Phy87, Phy89, which were recorded by Morando *et al.* (2013), Corbalán *et al.* (2016), Lobo *et al.* (2018; 2021), and this study (see Appendix 2). To improve the molecular dataset, new DNA sequences of five markers (12S, COI, Cytb, ND4, C-mos) were obtained for *P. curivilcun*, *P. katenke*, and for the unnamed population from Maquinchao area. For the unnamed population from Chenqueniyén plateau, only the ND4 fragment was amplified and combined with available markers labeled as *Phymaturus* sp. 13 uploaded by Morando *et al.* (2013). The genomic DNA was extracted from 96% ethanol-preserved muscle tissue samples using the phenol/chloroform method (Sambrook and Russell, 2001). Molecular markers were amplified following standard polymerase chain reaction (PCR) procedures, using the following primers: G73 (5´-GCGGT AAAGC AGGTG AAGAAA-3´) and G78 (5´- AGRGT GATRW CAAAN GARTA RATGTC-3´) for the nuclear fragment of C-mos (Saint et al. 1998); ND4 (5´- CACCT ATGAC TACCA AAAGC TCATG TAGAAGC-3´) and Leu (5´-CATTA CTTTT ACTTG GATTT GCACCA-3´) for the ND4 fragment (Arévalo et al. 1994); 12e (5´-GTRCG CTTAC CWTG TTACG ACT-3´) and tPhe (5´- AAAGC ACRGC ACTGA AGATGC-3´) for the 12S fragment (Wiens et al. 1999); and GLUDGL (5´-TGACT TGAAR AACCA YCGTTG-3´) and CB3–3´ (5´-GGCAA ATAGG AARTA TCATTC-3´) for Cytb (Palumbi, 1996), and T3-AnF1 (5´-AATAA CCCTC ACTAA AGACH AAYCA YAAAG AYATY GG-3´) and T7-AnR1 (5´-AATAC GACTC ACTAT AGCCR AARAA TCARA ADARR TGTTG-3´) for COI (Lyra *et al.*, 2017). Sequencing reactions were run using Big Dye Terminators 3.1 in an ABI 3130 Genetic Analyzer (Applied Biosystems). All samples were sequenced in both directions and the contigs were made using DNA BASER 3 (HeracleBioSoft, Pitesti, Romania). Sequences were edited with BioEdit (Hall, 1999) and each gene was aligned with Clustal W (Thompson *et al.*, 1994), and later concatenated using SequenceMatrix 1.7 (Vaidya *et al.*, 2011). In total, 10,799 bp were obtained (Appendix 2).

To find the best-fitting model for each marker, we used Partition Finder v2 (Lanfear *et al.*, 2017) according to the Akaike Information Criterion (AIC) and using a greedy searching scheme (Lanfear *et al.*, 2012). In each protein-coding gene, the codon positions were treated as a separate partition. We performed two independent analyses using default prior values in MrBayes v3.2 (Ronquist *et al.*, 2012), each one with four independent Monte Carlo Markov chains of 20 000 000 generations, sampled every 2000 generations with a burn-in of 25% of trees. We examined the stationarity of parameters using TRACER 1.5 (Rambaut *et al.*, 2018). Both Partition Finder and MrBayes were run on CIPRES Science Gateway website (Miller *et al.*, 2010). Uncorrected *p-distance* was estimated using the software MEGA 7 (Kumar *et al.*, 2016).

We performed a total evidence analysis including all the available morphological and DNA evidence (a data matrix used in a previous study for the *patagonicus* group, Lobo *et al.*, 2018, adding new information and terminal taxa). Our population sample from Meseta de Chenqueniyén has no morphological differences from samples of Museum of Vertebrate Zoology, California University (MVZ) collected by R. Sage at Alto del Escorial, located at 3 km in straight line on the same plateau; therefore, we consider that they are the same species. We also consider that the sample mentioned as candidate species by Morando *et al.* (2013) (as *P.* sp13, 41°330' S, 70°400' W), about 6 km south of our finding is the same species. We analyzed our data matrix with TNT v. 1.5 applying strict parsimony (Goloboff *et al.*, 2008). The search for the most parsimonious trees was performed applying equal weights (EW) to all characters. We made a "traditional search" applying tree bisection and reconnection (TBR) with 10.000 replications (saving 20 trees per replication). Including five species of *Liolaemus* (*L. archeforus*, *L. lineomaculatus*, *L. buergeri, L. kingii, L. petrophilus*), and three spp of the *palluma* group (*P. palluma*, *P. vociferator*, *P. mallimaccii* see Appendix 2) as outgroup taxa. Support for individual nodes was assessed with symmetric resampling (Goloboff *et al.*, 2003) using 1000 replicates and a deletion value of 25%.

To explore the morphological diversity within each clade, we used continuous characters: 18 morphometric measurements and 32 scale counts. The following measurements were taken: abdominal width (AW); fourth toe´s claw length (CL); eye length (measured between anterior and posterior commissural angles formed by ciliary scales) (EL); eye-auditory meatus distance (EM); foot length (FL); height head (HH); head length (HL); humerus length (HU); humerus width (Hu); head width (HW); internasal distance (measured between both medial borders of nasal openings (IN); interorbital distance (IO); auditory meatus height (MH); neck length (NL); snout-vent length (SVL); tibia length (Tb); trunk length (TL) and tail length (Tl). All measurements were taken using digital calipers at 0.02 mm of precision. Pictures of live specimens were taken in the field using a digital camera, and most character details were examined under a stereomicroscope. The scale counts included were: scales along lateral neck fold till the antehumeral fold (AF); scales contacting interparietal (CI); scales contacting nasal (CN); scales contacting mental (CM); dorsal scales along the dorsal midline of trunk (counted along a head length distance) (DT); enlarged scales on the anterior margin of the auditory meatus (EM); subdigital plates of fourth finger (FF); scales between frontals and superciliaries (FS); subdigital plates of fourth toe (FT); gular scales taken in ventral view between both auditory openings (GS); number of scales counted along midline over dorsum of head (Hellmich´s index) (He); infralabial scales (IS); internasal scales (Is); lorilabial scales in contact with subocular (LO); lorilabial scales (LS); number of scales counted around midbody (MS); neck scales counted along lateral neck fold (NS); postmental scales (PM); postocular scales (PO); number of precloacal pores in males (PP); postrostral scales (PR); scales between frontals and rostral scale (RF); superciliaries scales (SC); the superciliary scale juxtaposed on both ends can be the fourth, fifth or sixth scale (Sc); average of scale organs counted on postrostral scales (SO); scales separating preocular from lorilabial row (SP); subocular row (SR); supralabial scales (SS); temporal scales (TS); temporal scales counted along a vertical line between labial commissure and the level of superciliaries (TV); upper ciliary scales (UC) and ventral scales (VS). Raw data of all the studied species of *somuncurensis* and *spurcus* clades are provided in S1 and S2 (Supplementaries files). With the only addition of the two measurements of humerus, all these continuous characters (scale counts and measurements) were included in the block of continuous characters in the phylogenetic matrix in previous works and also used for taxonomic comparisons (Lobo *et al.*, 2018, Lobo *et al.*, 2021, among the most recent publications). Principal component analyses (PCAs) were performed separately for scale counts and measurements, and for both the *somuncurensis* and the *spurcus* clades. The variables that most contributed to explain the variability were selected to perform the statistical test. The criterion used to choose the variables was that they contribute 80% or more to each principal component, considering the maximum contribution (100%) that of the character with the highest value. On the other hand, some variables were statistically tested but did not appear in the PCA; this is because some species did not have any data for this variable and to avoid eliminating the entire species from the PCA the variable was better removed. For more details see Supplementary file 3 (S3). The missing variable values were completed with the average obtained from the specimens of the species that did have that data. In the case of the measurements, the same procedure was followed but the sex of each species was considered for the average. We performed Kruskal Wallis tests. All analyses were performed using the statistical package INFOSTAT (Di Rienzo *et al.*, 2016). The procedure used to judge the significance of multiple comparisons and postulated contrasts in Kruskal-Wallis analysis is that described in Conover (1999). Since differences were found in SVL between the analyzed species, the residuals of the regression of each measurement character vs SVL were obtained to remove the possible effects of size across analyses.

Results

Relationships within the *somuncurensis* and *spurcus* clades and phylogenetic position of *Phymaturus katenke* and *P. curivilcun*.

Phylogenetic analyses included the addition of DNA sequences (five markers) for the two new species, as well as for *Phymaturus curivilcun* and *P. katenke*, and new morphological information collected for the two clades. Figure 1A shows the tree obtained in a total evidence analysis that included morphology and molecules (parsimony) and Figure 1B depicts the Bayesian tree built based on all the molecular information available. The phylogenetic analyses found that each of the unnamed populations studied (Maquinchao and Chenqueniyén) belongs to two different clades. *Phymaturus* sp. nov. from Maquinchao is found nested within the *somuncurensis* clade in the two analyses performed (total evidence and Bayesian). In the analysis of total evidence (Fig. 1A), *Phymaturus tenebrosus* is the sister taxon of all remaining species of the *somuncurensis* clade: then, towards the terminal branches, there are two sister subclades, the *calcogaster* subclade (formed by *P. calcogaster*, *P. yachanana* and *P. camilae*) and the *ceii* subclade (including the remaining species of the clade *somuncurensis*). Within the *calcogaster* subclade, *P. camilae* is the sister taxon of *P. yachanana*, within the *ceii* subclade, *P. ceii* is the sister taxon of *P. somuncurensis* and *P.* sp. nov. from Maquinchao is more closely related to *P. etheridgei* and *P. sinervoi*.

In the Bayesian analysis (Fig. 1B), *Phymaturus tenebrosus* is the sister taxon of the remaining species of the clade *somuncurensis*; then, towards the terminal branches, *P. calcogaster* is more basal (the *calcogaster* subclade is not recovered), *P. yachanana* is the sister taxon of *P. camilae*; within the *ceii* subclade, *P. ceii* is a sister taxon to *P. somuncurensis*, *P.* sp. nov from Maquinchao is more closely related to *P. etheridgei*, and *P. sinervoi* is the sister taxon of *P.* sp22a.

Phymaturus sp. nov. from Chenqueniyén is recovered into the *spurcus* clade. In the total evidence analysis, *P. curivilcun* is the sister taxon of all remaining species; then *P.* sp. nov. is sister taxon of all remaining species (96% Symmetric Resampling), then towards the terminal branches, *P. spurcus* is related to the pair of species formed by *P. excelsus* and *P. spectabilis* (76% SR). *Phymaturus manuelae* is not included in this clade. There is no strong statistical

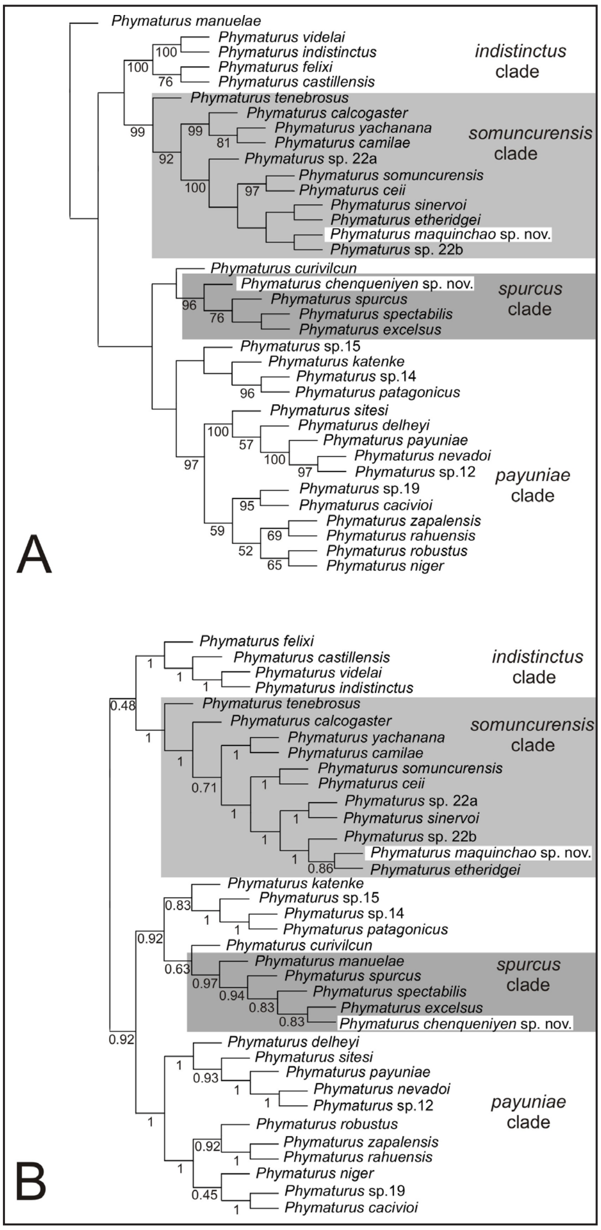

Figure 1. Phylogenetic tree showing phylogenetic relationships within the *patagonicus* after the update of Lobo *et al.* (2018) study including more DNA sequences, morphological data and taxa (*P. curivilcun*, *P. katenke*, *P. chenqueniyen* sp. nov. and *P. maquinchao* sp. nov.). A- Total evidence analysis of parsimony including all DNA data plus morphological information (running TNT). B- Bayesian tree obtained only for molecules (running MrBayes). The new species described in the present contribution and the *somuncurensis* and *spurcus* clades to which they belong are highlighted. Numbers under branch are support values (A: symmetric resampling, B: posterior probabilities).

support for the position of *P. curivilcun*.

In the Bayesian tree (Fig. 1B), *P. curivilcun* is weakly-supported as sister taxon of all other members of the *spurcus* clade (0.63 pp); then *P. manuelae* is sister taxon of the remaining species (0.97 pp), *P. spurcus* is sister taxon of the other three species (0.94 pp), and *P. spectabilis* is sister taxon (0.83 pp) of the pair of species formed by *P.* sp. nov. (from Chenqueniyén) and *P. excelsus* (0.83 pp).

Because we extended the information available for *P. katenke* (only known for COI data in Corbalán *et al*., 2016) by sequencing Cytb, 12S, ND4, C-mos and revisiting all morphological characters (based on new samples), we checked its phylogenetic position. In the two analyses, *P. katenke* was recovered related to *P*. sp15, *P*. sp14 and *P. patagonicus*. The Bayesian tree showed *P. katenke* as the sister taxon of the other three species (0.83 pp); in the total evidence analysis *P. katenke* was recovered as closely related to *P*. sp14-*P. patagonicus* with low support. In the case of *P. curivilcun*, we included DNA information of the species that was unknown until the present study, and we revisited its morphology based on the type series and a new sample. In the two analyses, *P. curivilcun* was recovered as sister of the well-supported *spurcus* clade.

The *somuncurensis* clade: morphological comparisons among its members

Within the *somuncurensis* clade, several characters related to pattern and colors are quite informative for taxonomic purposes and carry phylogenetic information, as proved in previous articles (Lobo *et al*., 2012a; 2016; 2018). In those phylogenetic articles, 77 characters referred to pattern and colors were described for the entire genus. Here, we highlight only the main and more significant ones for this clade. Species of this clade can exhibit dorsal ocelli but less conspicuous than in the *spurcus* clade (see Lobo *et al*., 2018: Figure 5A and C), sometimes more often in females than in males. The number of ocelli in this group counted between shoulders and thighs is larger than in the *spurcus* clade (character 130). *Phymaturus somuncurensis*, *P. tenebrosus*, *P. ceii* and *P. sinervoi* share a black coloration along the flank (dark lateral band -character 120-, Lobo and Quinteros, 2005a), which is inconspicuous or absent in the remaining species. This character state also occurs in the species members of the *payuniae* clade (*P. payuniae*, *P. nevadoi*, *P. sitesi*, *P. delheyi*, *P. robustus*, *P. rahuensis*, *P. zapalensis*, *P. cacivioi*, *P. niger*). *Phymaturus camilae*, *P*. sp. (from Maquinchao) and *P. calcogaster* show a pattern on dorsum of occipital region of head formed by black transversal bars (“head star pattern”) (character 296), which is shared with species of the *indistinctus* clade and with *P. katenke*. Dark pigmentation of infradigital lamellae concentrated between central keels (remarked as a dark central line) (described in Lobo *et al*., 2010) is observed only within the clade in *P. ceii*, *P*. sp. (from Maquinchao) and *P. tenebrosus*. This character state is highly homoplastic, occurring in species of all the other clades within the *patagonicus* group. A mixed dorsal pattern consisting of small and medium-sized white spots (character 270) is present in *P*. sp. (from Maquinchao), *P. etheridgei*, the *calcogaster* subclade (*P. calcogaster*, *P. yachanana* and *P. camilae*), and within other clades in *P. patagonicus*, *P. katenke* and the northern subclade of the *payuniae* clade. A dorsal tail pattern of males (character 118) is also very informative within the *somuncurensis* clade; the *calcogaster* subclade exhibits ocellated/variegated tails, whereas the tail pattern of the remaining species is markedly ringed or with almost inconspicuous ringing. *Phymaturus tenebrosus*, the sister species of all other members of the clade, lacks a dorsal tail pattern. Variation in the throat pattern was recorded. This pattern can consist of lines densely disposed but disrupted in *P. sinervoi*, *P. etheridgei*, *P. yachanana* and *P.* sp. (from Maquinchao). This throat pattern may be scarce but formed by thick lines interrupted, as in P. camilae and P. calcogaster., as in *P. camilae* and *P. calcogaster.* In the latter species, the throat pattern can be absent in some individuals, as in *P. tenebrosus*, *P. somuncurensis* and *P. ceii* (see Figures in Lobo *et al*., 2020). Belly coloration of males is quite informative (character 127). It is yellow in *P. calcogaster* and *P. ceii*, orange in *P. sinervoi*, *P. etheridgei*, *P. yachanana*, and *P. camilae*, and mustard or red in *P. tenebrosus*. In *P*. sp. (from Maquinchao) most males have orange bellies, but an individual with yellow coloration was found. Other characters not referred to color pattern are also useful, i.e. opening of nares with a wide superficial platform inside (character 247); this character state is present in almost all species, except for *P. tenebrosus*, *P. somuncurensis* and *P*. sp. (from Maquinchao). Those miscellaneous characters described above plus all anatomical characters were already included in character lists in Lobo *et al.* (2012a; 2016; 2018).

Several continuous characters exhibit significant variation among species. Table 1 shows characters that exhibited significant variation among species of the two clades (in S1 we provide the raw results given by the analyses). Characters that exhibit significant variation among species were 17 of the 32 studied scale counts and 9 of 18 measurements taken. Table 2 shows characters that exhibited significant differences between all pairs of species within the *somuncurensis* clade after our statistical comparisons (scale counts and morphometric characters are indicated below and above the diagonal, respectively). We did not find differences in scale counts between *P. sinervoi* and *P. ceii*. *Phymaturus somuncurensis*, *P. ceii* and *P. sinervoi* differed in only one measurement character (interorbital distance –IO-). Body measurements did not show significant differences among *P. somuncurensis*, *P. ceii*, *P. sinervoi*, *P*. sp. (from Maquinchao), and *P. camilae*, but the latter two differ in scale count characters. *Phymaturus ceii* and *P. somuncurensis* are closely related (Fig. 1), which may explain the lack of greater morphological differentiation. Their morphological discrimination is based on a few characters, mostly of coloration patterns. Of the total pairwise comparisons (scale counts), characters that are discriminant in most comparisons were: scale organs counted on postrostral scales (SO= 15 times), scales along lateral neck fold till the antehumeral fold (AF= 15), neck scales counted along lateral neck fold (NS= 14), and ventral scales (VS= 13). Of the total pairwise comparisons (measurements), characters that were found as more discriminant in most comparisons were: abdominal width (AW= 13), internasal distance (IN= 10), interorbital distance (IO= 11), and humerus length (HU= 9). The PCA allows us to explain the morphological variation in the *somuncurensis* clade based on four components. The S3 file shows the variables with the highest value (of the list of variables used in the analysis, the limit value selected to consider the characters that most contribute was the one calculated up to at least 80% of the maximum value found among the variables). All characters that exhibited significant differences among species are the same as those that exhibit the highest values in the PCA procedure.

Based on the significant amount of evidence provided by our morphological revision (color pattern, scale counts, and morphometry) and the genetic information available (Table 3), we are able to describe the population called *P*. sp. (from Maquinchao) so far in this article as a new species.

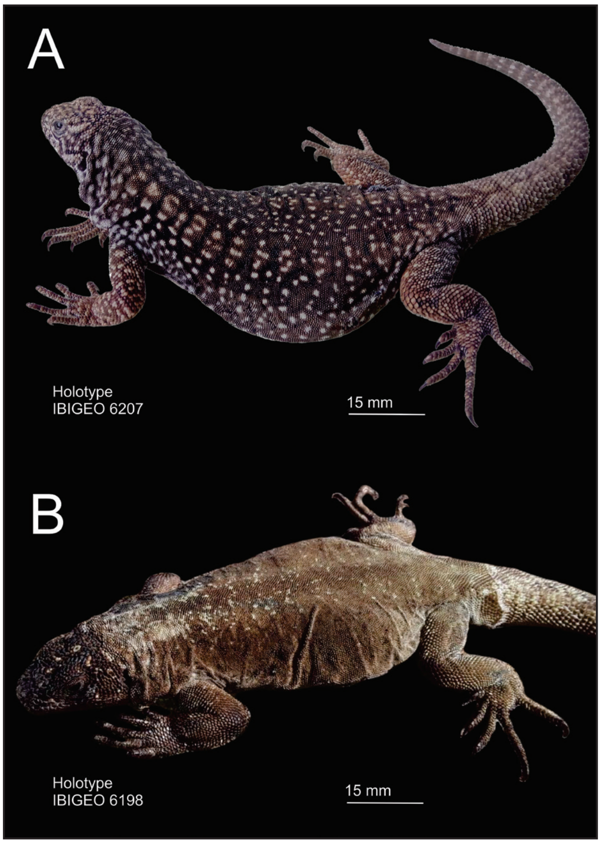

Figure 2. A- Holotype of *Phymaturus maquinchao* sp. nov. showing its typical color pattern. IBIGEO 6207 (male). Snout-vent length: 89.09 mm. B- Holotype of *Phymaturus chenqueniyen* sp. nov. IBIGEO 6198 (male). Snout-vent length: 90.00 mm.

Species description

Phymaturus maquinchao sp. nov.
Holotype.— IBIGEO 6207. Male (Fig. 2A). Deposited at the Reptile collection of the Instituto de Bio y Geociencias del Noa (IBIGEO), Salta, Argentina. Provincial Route (PR) N° 23, approx. 10 km N from Maquinchao (41° 11.300' S, 68° 38.386' W; altitude: 876 m.) Veinticinco de Mayo department, Río Negro province, Argentina.

Paratypes.— IBIGEO 6203, 6205-06, 6208, 6210, 6213, 6215 (4 adult males, 3 juvenile males) and IBIGEO 6209, 6211-12, 6214, 6216 (4 adult females, 1 juvenile female). Deposited at the Reptile collection of the Instituto de Bio y Geociencias del Noa (IBIGEO), Salta, Argentina. Provincial Route (PR) N° 23, approx. 10 km N from Maquinchao (41° 11.300' S, 68° 38.386' W; altitude: 876 m.) Veinticinco de Mayo department, Río Negro province, Argentina. DNA samples: IBIGEO 6211, 6214.

Diagnosis (Figs. 2, 3 & 4. Table 2, S1).— *Phymaturus maquinchao* sp. nov. belongs to the *patagonicus* group of *Phymaturus* because it exhibits flat and imbricated superciliary scales, smooth tail scales, and a set of enlarged scales projected onto the auditory meatus (Etheridge, 1995; Lobo and Quinteros, 1995). Within the *patagonicus* group, *Phymaturus maquinchao* sp. nov. belongs to the *somuncurensis* clade, supported by four morphological and 62 molecular sinapomorphies. The *somuncurensis* clade comprises nine species: *P. calcogaster*, *P. camilae*, *P. ceii*, *P. etheridgei*, *P. maquinchao* sp. nov., *P. sinervoi*, *P. somuncurensis*, *P. tenebrosus* and *P. yachanana*, and was found as a monophyletic group (Fig. 1; see also Lobo *et al.*, 2018 Figure 1).

Phymaturus maquinchao sp. nov. is discriminated from the most closely related members of the *somuncurensis* clade (Fig. 1), *P. ceii*, *P. somuncurensis*, *P. sinervoi* and *P. etheridgei*, as follows: *Phymaturus ceii* shows more conspicuous black coloration along its flank; males are ventrally yellow (most males of *P. maquinchao* sp. nov. are orange); there are completely melanic individuals (no melanism in *P. maquinchao* sp. nov.). The throat pattern in *P. ceii* is absent or inconspicuous (thin conspicuous pattern in males and females of *P. maquinchao* sp. nov.). *Phymaturus maquinchao* sp. nov. also shows statistically significant differences (with overlapping ranges) from *P. ceii* in the following characters: it has fewer scales along lateral neck fold counted up to the antehumeral fold (AF), more enlarged scales in the anterior margin of the auditory meatus (EM) and shorter forelimbs (HU). *Phymaturus somuncurensis* exhibits a quite homogenous dorsal pattern; no ocelli are evident, as in *P. maquinchao* sp. nov. *Phymaturus somuncurensis* shows black coloration along its flank more conspicuous than in *P. maquinchao* sp. nov. (inconspicuous or absent). *Phymaturus somuncurensis* lacks throat reticulation, which is conspicuous in *P. maquinchao* sp. nov. Males and females of *P. somuncurensis* show pink ventral coloration, but in males of *P. maquinchao* sp. nov. it is orange or yellow (Fig. 3). *Phymaturus maquinchao* sp nov. exhibits significant differences from *P. somuncurensis* in two squamation characters: more scales contacting mental scale (CM) and enlarged scales on the anterior margin of the auditory meatus (EM), and in two morphometric characters: smaller eyes (EL) and shorter head (HL). No differences in scale counts were found between these species (Table 2). *Phymaturus sinervoi* lacks sexual dimorphism in its color pattern; it also lacks dorsal ocelli, which are present in all females of *P. maquinchao* sp. nov., and even in males, but are no so evident. *Phymaturus sinervoi* shows more conspicuous black coloration along its flank than *P. maquinchao* sp. nov., in which it is inconspicuous or absent. The anterior gular fold is almost conspicuous in *P. sinervoi* (absent in *P. maquinchao* sp. nov.). Enlarged scales on the anterior margin of auditory meatus are directed backwardly in *P. sinervoi*, but they are perpendicular to temporals in *P. maquinchao* sp. nov. There are two differences in scale counts: *P. maquinchao* sp. nov. exhibits more scales contacting mental scale (CM) and more enlarged scales on the anterior margin of the auditory meatus (EM); there are no differences in morphometry between these species (Table 2). Males of *P. sinervoi* and *P. maquinchao* sp. nov. can exhibit orange or yellow ventral color; in *P. etheridgei*, ventral coloration is orange, and it can exhibit yellow mustard only in ventral surfaces of thighs. Contrary to *P. maquinchao* sp. nov., *P. etheridgei* has a dorsal black to dark brown coloration with very small white spots, contrasting the light brown coloration of tails; no dorsal ocelli are present in this species. Ventral coloration of *P. etheridgei* males is orange, turning to bright mustard/yellowish on ventral surfaces of

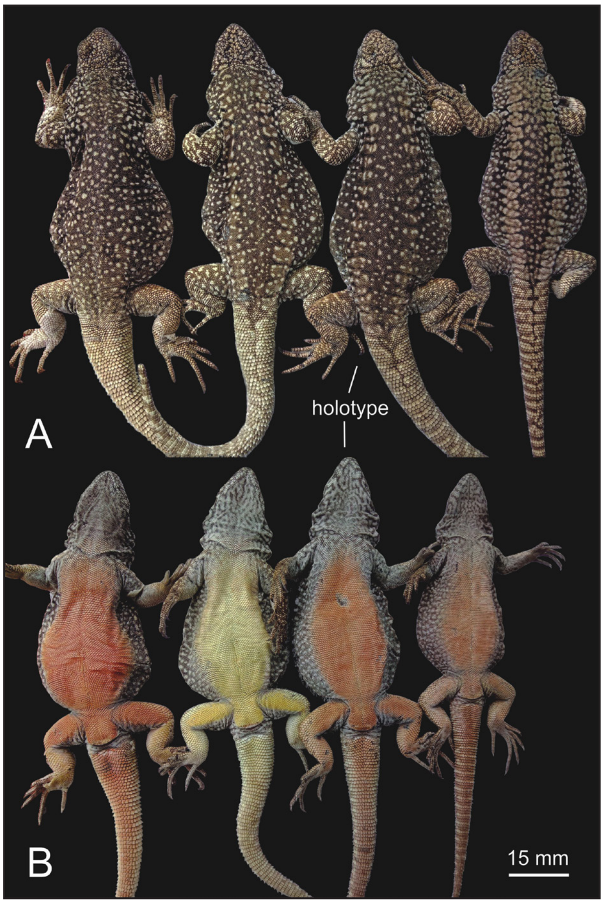

Figure 3. A- Male individuals of the type series of *Phymaturus maquinchao* sp. nov. Dorsal and ventral views.

thighs and cloaca; in females, ventral coloration is pink, whereas in *P. maquinchao* sp. nov., both males and females exhibit orange ventral coloration (brighter in males), and some males have yellow coloration (Fig. 3). *Phymaturus etheridgei* presents the white dorsal spotting in two sizes: small to very small spots irregularly scattered and densely covering their backs; this condition of fore and hind limbs found in *P. etheridgei* is shared with *P. yachanana*. *P. maquinchao* sp. nov. shows a similar condition of white spotting but scarcely distributed and not extended onto the limbs. *Phymaturus maquinchao* sp. nov. also differs from *P. etheridgei* in six scale count characters: it has more postrostral scales (PR), scales contacting nasal (CN), scales contacting mental scale (CM), scales in the subocular row (SR) and fewer scales along lateral neck fold up to the antehumeral fold (AF), and ventral scales (VS). They also differ in four morphometric characters: *P. maquinchao* sp. nov. has smaller eyes (EL), shorter head (HL), lower internasal distance (IN), and greater abdominal width (AW).

Phymaturus maquinchao sp. nov. is discriminated from all other members of the *somuncurensis* clade as follows: *Phymaturus maquinchao* sp. nov. exhibits a quite different color pattern from that of *P. tenebrosus*; the latter species exhibits brown or red morphs, or even black individuals (Cerro Alto) with thin and homogeneously distributed white spotting. Brown morphs of *P. tenebrosus* can exhibit black coloration in flanks, as *P. ceii*, *P. sinervoi* and *P. somuncurensis*. *Phymaturus tenebrosus* never exhibits dorsal ocelli. *Phymaturus maquinchao* sp. nov. also differs from *P. tenebrosus* in having a higher number of: scales contacting nasal (CN), scales contacting mental (CM), dorsal scales along the dorsal midline of trunk (counted along a head length distance) (DT), enlarged scales on the anterior margin of the auditory meatus (EM), more scales counted along midline over dorsum of head (Hellmich's index) (He), internasal scales (Is), lorilabial scales (LS), neck scales (NS), postrostral scales (PR), the superciliary scale juxtaposed on both ends tends to be the sixth rather than the fifth (Sc), and ventral scales (VS), and fewer scale organs on postrostral scales (SO). *P. maquinchao* shows a longer neck (NL), greater forelimb width (Hu) and abdominal width (AW). Dorsal white spots of *P. calcogaster* are large (formed by 9 to 16 scales, see fig. 2 in Lobo *et al.*, 2018), contrary to *P. maquinchao* sp. nov. (no more than six or seven scales). The throat pattern consists of a dark thick reticulation (especially in males) in *P. calcogaster* (thin reticulation in *P. maquinchao* sp. nov.), dorsal pattern of head reticulated in *P. calcogaster*, inconspicuous in *P. maquinchao* sp. nov. Males have yellow chests and abdomen in *P. calcogaster* (orange in 3 of 4 individuals in *P. maquinchao* sp. nov.). Dorsal ocelli are absent in *P. calcogaster*. In *P. calcogaster*, transversal rows of white spots are evident (absent in *P. maquinchao* sp. nov.). *Phymaturus maquinchao* sp. nov. also differs from *P. calcogaster* in four scale count characters: more postrostral scales (PR) and scales contacting nasal (CN), fewer scales organs in postrostrals (SO), and fewer scales along lateral neck fold up to the antehumeral fold (AF). No morphometric characters exhibiting significant differences between these species were found. Dark brown to black dorsal pattern of trunk and limbs in *P. camilae*, differs from brown to light brown in *P. maquinchao* sp. nov. Dorsal pattern of head marked in *P. camilae* (light and almost inconspicuous in *P. maquinchao* sp. nov.), white scales on dorsum of tail contrasting from the brown background coloration in *P. camilae*

Figure 4. Female paratypes of *Phymaturus maquinchao* sp. nov. Dorsal and ventral views.

Table 1. Characters of squamation (scale counts) and measurements that exhibit significant differences among members of the somuncurensis and spurcus clades after performing Kruskal Wallis tests. We provide the raw data for all species studied in Supplementary files1 and 2. Abbreviations of scale counts are as follow: Scales along lateral neck fold up to the antehumeral fold (AF); contacting nasal (CN); contacting mental (CM); dorsal scales along the dorsal midline of trunk (counted along a head length distance) (DT); enlarged scales at the anterior margin of the auditory meatus (EM); subdigital plates of fourth finger (FF); gular scales, between both auditory openings (GS); number of scales counted along midline over dorsum of head (Hellmich´s index) (He); infralabial scales (IS); internasal scales (Is); lorilabial scales in contact with subocular (LO); lorilabial scales (LS); number of scales counted around midbody (MS); neck scales counted along lateral neck fold (NS); postmental scales (PM); postocular scales (PO); postrostral scales (PR); scales between frontals and rostral scale (RF); superciliary scale juxtaposed to the others in both endings (Sc); average of scale organs counted on postrostral scales (SO); subocular row (SR); supralabial scales (SS); temporal scales counted in a vertical line between labial commissure and the superciliaries level (TV); upper ciliary scales (UC); ventral scales (VS). Measurements: abdominal width(AW); fourth toe´s claw length(CL); eye length (EL measured between anterior and posterior commissural angles formed by ciliary scales); height head (HH); head length (HL); humerus length (HU); humerus diameter (Hu); head width (HW); internasal distance (IN measured between both medial borders of nasal openings); interorbital distance (IO); auditory meatus height (MH); neck length (NL); trunk length (TL) and tail length (Tl).

	***somuncurensis* clade**		***spurcus* clade**	
Character	H	P	F	P
AF	H=51.89	<0.0001	H=40.31	<0.0001
CM	H=34.86	<0.0001		
CN	H=21.21	0.0027	H=13.07	0.0052
DT	H=30.35	0.0002		
EM	H=22.98	0.0023		
FF	H=17.20	0.0208	H=16.81	0.0017
GS			H=15.55	0.0036
He	H=38.80	<0.0001	H=27.98	<0.0001
IS	H=23.58	0.0006		
Is	H=23.74	<0.0001	H=10.75	0.0043
LO			H=20.63	0.0001
LS	H=34.21	<0.0001	H=13.22	0.0075
MS			H=16.20	0.0027
NS	H=46.86	<0.0001	H=29.01	<0.0001
PM	H=17.32	0.0160	H=14.77	0.0015
PO			H=15.46	0.0002
PR	H=48.88	<0.0001	H=19.24	0.0001
RF			H=21.43	0.0001
Sc	H=11.52	0.0370		
SO	H=68.92	<0.0001	H=26.45	<0.0001
SR	H=27.25	<0.0001		
SS			H=16.12	0.0006
TV			H=14.51	0.0040
UC			H=16.38	0.0018
VS	H=49.03	<0.0001		
AW	H=44.74	<0.0001	H=15.87	0.0032
CL			H=13.50	0.0091
EL	H=22.41	0.0042	H=24.58	0.0001
HH	H=18.50	0.0178		
HL	H=26.79	0.0008		
HU	H=31.98	0.0001		
Hu			H=24.41	0.0001

HW			H=9.76	0.0448
IN	H=39.92	<0.0001		
IO	H=25.21	0.0014		
MH			H=14.32	0.0063
NL	H=23.68	0.0006	H=11.41	0.0223
TL	H=15.81	0.0451		
Tl			H=32.84	<0.0001

(inconspicuous in *P. maquinchao* sp. nov.). *Phymaturus maquinchao* sp. nov. also differs from *P. camilae* in seven scale count characters: it has more scales contacting mental scale (CM), scales contacting nasal (CN), enlarged scales on the anterior margin of the auditory meatus (EM), postmental scales (PM) and postrostral scales (PR), fewer scales along lateral neck fold up to the antehumeral fold (AF) and scale organs on postrostral scales (SO). No morphometric characters exhibiting significant differences between these species were found. *Phymaturus maquinchao* sp. nov. has no reddish/clay coloration on dorsum, which is commonly found in *P. calcogaster* and *P. yachanana*. In *P. yachanana* dorsum of females has black transversal bars forming a longitudinal paired series, no ocelli are evident (they can be present in some females of *P. maquinchao* sp. nov. but as margins of light ocelli). Chest and abdomen of most individuals with light gray spotting in *P. yachanana* (immaculate in *P. maquinchao* sp. nov.). *Phymaturus maquinchao* sp. nov. also differs from *P. yachanana* in one scale count character: it has more neck scales (NS), ventral scales (VS) and the superciliary scale juxtaposed on both ends tends to be the sixth rather than the fifth (Sc). They differ in two morphometric characters: *P. maquinchao* sp. nov. smaller eye (EL) and greater head height (HH).

Description of holotype (Fig. 2A).— Male. SVL 89.09 mm. Head length: 15.42 mm. Head width: 14.98 mm. Head height (at parietal): 8.15 mm. Axilla-groin length: 47.93 mm (53.80 % of SVL). Tail length (complete, not regenerated): 97.88 mm.. Body moderately wide, trunk width: 37.74 mm (42.4 % of SVL). Twenty-two smooth dorsal head scales. Three scale organs in each of the four postrostrals. Nasal bordered by nine scales, not in contact with rostral. Canthal separated from nasal by two scales. Loreal region flat. Eight enlarged supralabial scales, none contacting subocular. Eight enlarged infralabials. Auditory meatus oval (height: 4.2 mm; width: 2.4 mm) with six enlarged, flat and smooth perpendicular scales projecting on the anterior margin. Auricular scale absent. Ten convex, juxtaposed temporals. Auditory meatus - ciliary scales distance: 5.2 mm. Rostral undivided. Mental scale sub-pentagonal, in contact with six scales. Interparietal scale bordered by eight scales, larger than postparietals. Frontal region without an azygous scale. Supraorbital semicircles inconspicuous. No distinctly enlarged supraoculars. Nine juxtaposed superciliaries, 15 upper ciliaries and 11 lower ciliaries. Subocular unique (not fragmented). Eleven lorilabials, the 11th contacting subocular. Preocular smaller than canthal, these two scales separated by another one. Preocular separated from lorilabial row by two scales. Scales of throat round, small, and juxtaposed. Seventy-nine gulars between auditory meata. Lateral nuchal folds well developed, with granular scales on longitudinal fold. Antehumeral pocket well developed. Seventy-seven scales between auditory meatus and shoulder. Sixty scales between antehumeral fold and shoulder. In ventral view, anterior gular fold absent, posterior gular fold present with its anterior margins with two enlarged scales on their borders. Dorsal scales round, smooth and juxtaposed. Thirty-eight dorsal scales along midline of the trunk in a length equivalent to head length. Scales around midbody: 209. Ventral scales larger than dorsal scales. Ventral scales between mental and precloacal pores: 185. Seven precloacal pores in an undivided row without supernumerary pores. Four moderately enlarged postcloacal scales. Brachial and antebrachial scales smooth, with round posterior margins. Supracarpals laminar, round and smooth. Subdigital lamellae of fingers have three keels. Subdigital lamellae of finger (left manus) IV: 22. Supradigital lamellae convex, imbricate. Infracarpals and infratarsals have round margins and 2–3 keels. Supracarpals and supratarsals smooth, with rounded posterior margins. Subdigital lamellae of toe (left pes) IV: 27. Claws moderately long (fourth toe's claw: 1.9 mm).

Coloration (in life).—The holotype exhibits a

Table 2. Continuous characters that exhibit significant variation between all pairs of species within the *somuncurensis* clade (17 of 32 scale counts studied and 9 of 18 measurements studied). Below the diagonal are scale counts characters, and above measurement characters. Same abbreviations of Table 1.

	P. maquinchao	***P. calcogaster***	***P. camilae***	***P. ceii***	***P. etheridgei***	***P. sinervoi***	***P. somuncurensis***	***P. tenebrosus***	***P. yachanana***
P. maquinchao		_	_	HU	AW EL HL IN	_	EL HL	AW NL	EL HH
P. calcogaster	AF CN PR SO		IO NL	_	AW EL HL IN IO	IO	HL HU	AW HU	HH
P. camilae	AF CM CN EM PM PR SO	EM PM		IO HU	AW EL IN	_	_	AW IO NL	AW HH IO
P. ceii	AF EM	EM IS PR SO	Is PM PR SO		AW EL IO	IO	HU	AW HU TL	HU IN TL
P. etheridgei	AF CM CN PR SR VS	He IS SR	PM VS	PR SR VS		AW EL HL IN	IN TL	EL HU Is IO NL TL	AW HH HL IN IO TL
P. sinervoi	CM EM	EM SO	SO	_	SO SR		_	AW HU NL IO	HH IN IO
P. somuncurensis	CM EM	EM	VS	FF	FF He SR	_		_	HH HL IN
P. tenebrosus	CM CN DT EM He Is LS NS PR Sc SO VS	AF CM DT EM He IS Is LS NS PR SO VS	AF DT He NS PM	AF CM DT FF He Is LS NS PR SO VS	AF DT FF He Is LS NS PR SO SR VS	AF DT He Is LS NS PR SO VS	DT He Is IS LS NS PR SO VS		AW HH IN NL
P. yachanana	NS Sc VS	AF He NS VS	AF CN CM DT NS PM	AF FF NS	AF CM FF NS SR VS	AF CM NS PM SO VS	AF CM He NS PM VS	CM CN He Is LS PR SO	

brown color as dorsal background, with a pair of longitudinal rows of ocelli (14 between shoulders and thighs). On the anterior ocelli (anterior half of trunk) transversal rows of white scales are conspicuous. Upper half of flanks darker, almost black, with irregularly scattered white spots. The dorsal body pattern consists of white spots of two sizes is more evident on the vertebral band between the rows of ocelli (also commonly found in other species of the clade). The "star pattern" is conspicuous on the back of neck and nuchal region. Tail and limbs exhibit a light brown coloration. There is no evident pattern on the tail. In ventral view, throat, anterior half of chest and forearms are light gray. The throat exhibits thin reticulation. Posterior half of chest, abdomen, cloacal region, hind limbs and tail orange.

Variation.— Squamation based on 13 specimens (8 males and 5 females), including four juvenile individuals (3 males and 1 female); measurements only based on adult individuals. SVL 78.9–95.3 mm (mean = 88.09; SD = 5.8). Head length 16.3–18.2% (mean = 17.4%; SD = 0.1) of SVL. Tail length 1.04–1.26 (mean = 1.15; SD = 0.08) times SVL. Scales around midbody 204–231 (mean = 216.6; SD = 9.11). Dorsal head scales (Hellmich's index) 19–26 (mean = 22.6; SD = 1.7). Ventral scales 161–188 (mean = 176.2; SD = 8.6). Scales surrounding interparietal 6–8 (mean = 7.2; SD = 0.7). Scales surrounding nasal 7–10 (mean = 8.9; SD = 0.8). Number of scale organs on postrostrals 2–5 (mean = 3.3; SD = 0.8). Superciliaries 7–11 (mean = 9.6; SD = 1.2). Subocular never fragmented (a single scale in all individuals). Mental scale in contact with six scales in all the samples. Number of chinshields 6–8 (mean = 6.7; SD = 0.7). Enlarged scales on the border of the posterior gular fold: 2–5 (mean = 3.3; SD = 1.0). Lorilabials 11–14 (mean = 11.8; SD = 1.1). Enlarged scales on the anterior border of the audi-

Table 3. Estimates of Evolutionary Divergence between Sequences. The number of base differences per site from between sequences are shown. The analysis involved 9 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 838 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. Above: Cytb pairwise comparisons; below: 12S.

somuncurensis clade									
	P. calcogaster	*P. camilae*	*P. ceii*	*P. etheridgei*	***P. maquinchao***	*P. sinervoi*	*P. somuncurensis*	*P. tenebrosus*	*P. yachanana*
P. calcogaster		1.45	2.17	2.90	3.26	2.65	2.90	3.38	2.29
P. camilae	0.36		1.93	2.65	2.53	2.05	2.65	2.90	2.17
P. ceii	1.43	1.55		2.90	3.02	2.17	1.21	2.90	2.29
P. etheridgei	1.07	1.07	1.07		1.57	2.41	3.14	3.38	3.02
P. maquinchao	1.07	1.07	1.07	0.24		2.29	3.26	3.74	3.14
P. sinervoi	1.31	1.31	1.07	0.48	0.48		2.65	3.14	2.53
P. somuncurensis	1.31	1.31	0.36	0.72	0.72	0.72		3.14	2.53
P. tenebrosus	1.19	1.19	1.91	1.31	1.07	1.55	1.55		2.77
P. yachanana	0.95	1.07	1.91	1.55	1.55	1.79	1.79	1.67	

spurcus clade						
	P. chenqueniyen	*P. curivilcun*	*P. excelsus*	*P. manuelae*	*P. spectabilis*	*P. spurcus*
P. chenqueniyen	2.32	1.22	2.32	1.46	1.46	
P. curivilcun	1.56		1.83	2.20	1.83	1.83
P. excelsus	1.08	1.20		1.59	0.24	0.24
P. manuelae	0.96	1.32	0.84		1.83	1.83
P. spectabilis	0.96	1.08	0.36	0.72		0.24
P. spurcus	0.96	1.08	0.36	0.72	0.00	

tory meatus 4–9 (mean = 6.8; SD = 1.7). Scales of neck along longitudinal fold from posterior border of auditory meatus to shoulder 74–88 (mean = 80.2; SD = 4.9). Gulars 68–89 (mean = 77.3; SD = 6.0). Scales between rostral and frontal 9–11 (mean = 9.7; SD = 0.8). Subdigital lamellae on fourth finger 21–26 (mean = 23.2; SD = 1.6). Subdigital lamellae on fourth toe 26–31 (mean = 28.1; SD = 1.6). Males with 7–9 precloacal pores (mean = 8.0; SD = 1.0). One female shows precloacal pores (3). Measurements: Eye length 3.4–3.7 (3.5; SD = 0.1). Head length 14,3–15,8 (mean =15.3; SD =0.5). Neck length 10.9–14.8 (mean = 13.4; SD = 1.3). Head width 12.8–15.5 (mean = 14.6; SD = 0.9). Head height 7.6–9.3 (mean = 8.5; SD = 0.6). Internares distance 2.4–2.8 (mean = 2.6; SD = 0.1). Interorbita distance 6.2–7.9 (mean = 7.1; SD = 0.5). Trunk length 37.5–50.6 (mean = 44.9; SD = 4.2). Humerus length 12.5–15.5 (mean = 13.9; SD = 1.0). Humerus width 5.2–7.5 (mean = 6.5; SD = 0.7). Tibia length 14.9–17.5 (mean = 16.3; SD = 1.1). Foot length 22.8–24.8 (mean = 24.1; SD = 0.7). Tail length 93.9–114.0 (mean = 99.9; SD = 6.6). Eye -auditory meatus distance 4.4–5.3 (mean = 5.0; SD = 0.3). Auditory meatus height 3.3–4.2 (mean = 3.8; SD =0.3). Fourth toe´s claw length 1.7–2.1 (mean = 1.9; SD = 1.1). Abdominal width 31.0–43.6 (mean = 37.2; SD = 3.9).

Males exhibit a brown background coloration (Fig. 3); small to medium-sized white spots are widespread all over their backs, dorsum of neck, fore and hind limbs, but fading towards the tail. All males except one show a pair of longitudinal rows of dorsal ocelli that are different from the background coloration because of their lighter coloration. Poorly conspicuous black transversal lines separate ocelli, and sometimes white small spots form a transversal line inside ocelli. Half of the males exhibit a conspicuous “star head pattern”, forming a reticulum in the posparietal area. There is no evident pattern on limbs, except for the white regular spotting. No pattern is evident on the tail, except for one specimen (ringed pattern). The throat is light gray, with slender black lines forming a reticulum; one specimen has no pattern. Three males have homogeneously orange chest and bellies; one male has yellow ventral coloration. All males exhibit small orange precloacal pores. Poscloacal enlarged scales are more or less conspicuous. Orange or yellow coloration of their ventral surfaces is extended on the cloacal region, tail and thighs. Dorsal pattern of females is similar to that of males (Fig. 4), but ocelli rows are more conspicuous; the upper half of flanks is darker (lateral black band usually present in *P. somuncurensis*, *P. ceii*, *P. sinervoi* and *P. tenebrosus* and in the *payuniae* clade), the white spotting is continuous on tails, and half of the females exhibit ringed tails. Ventral surfaces are of similar pattern and coloration to those of males.

Etymology.— Maquinchao refers to the locality where this species was found; it is an ancient native language word that means “site of wintering”.

Distribution (Fig. 5).—This species inhabits the northwestern margin of Meseta de Somuncurá and is only known for the type locality. Morando *et al.* (2013) sampled individuals from two sites 35 km south to this place (candidate species *P.* sp22a and *P.* sp22b); further studies are needed to evaluate the identity of these populations. The Somuncurá plateau covers a vast territory of about 25,000 km^2 in Argentine Patagonia. This geological structure is located more than 1000 meters above sea level; it is a formation with several canyons generated by different water streams that drain in the lowlands. It is a basaltic plateau, with reliefs of volcanic cones, mountain ranges, hills that are almost 1900 meters above sea level, interspersed with temporary and clay lagoons. Figure 5 shows how this diversified clade of lizards (*somuncurensis* clade outlined in orange) inhabits mainly inside this plateau and all around its margins.

The *spurcus* clade: morphological comparisons among its members

Analysis of continuous characters taken from squamation and measurements. — Continuous characters exhibit significant variation among species. Table 1 shows characters that exhibited significant variation among species of both clades; scale counts characters are displayed below the diagonal and the morphometric ones, above this diagonal (we provide the raw results of the analyses in S2). The characters that exhibit significant variation among species were 18 of the 32 scale counts studied and 8 of the 18 measurements taken. Table 4 shows characters that exhibited significant differences between all pairs of species within the *spurcus* clade. We did not find differences in measurements between *P*. sp (Chenqueniyén) and *P. spectabilis* (but they differ in 12 scale counts). Of all the pairwise comparisons (scale counts), the characters that are most discriminant and present in most comparisons were: scales between frontals and rostral scale (RF= 6 times), number of scales counted around midbody (MS= 6); scales along lateral neck fold up to the antehumeral fold (AF= 6); lorilabial scales in contact with subocular (LO= 4), and number of scale organs in postrostral scales (SO= 6). Of all the pairwise comparisons (measurements), the characters that were found as most discriminant and present in most comparisons were: tail length (Tl= 7), fourth toe´s claw length (CL= 6), abdominal width (AW= 5), auditory meatus height (MH= 5) and humerus width (Hu= 4). The PCA allows us to explain the morphological variation in the *spurcus* clade based on three components that account for 100%. The S3 file shows the variables with the highest value. All characters that showed significant differences among species are the same as those that exhibit higher values in the PCA. Figure 6 shows one of the PCA graphics obtained (squamation); in this case, PC1xPC2, PC2 discriminates *P. spurcus*, *P. spectabilis* and *P. excelsus* from each other, whereas PC1 discriminates *P. manuelae* and *P. chenqueniyen* from the other three. Tables with individual values of characters and for each component and accumulated percentages are included in S3.

Identification of brown morphs in the *spurcus* clade (Fig. 7).— Since coloration pattern was an essential topic in a recent publication about this group of *Phymaturus*, we considered it important to revisit it and make the necessary remarks and observations to avoid confusion. In Lobo and Quinteros (2005a) we described *P. excelsus* and *P. spectabilis* from a restricted area of Río Negro province in Argentina. At that time, we considered certain brown morphs as a variation within *P. spectabilis* species. In the

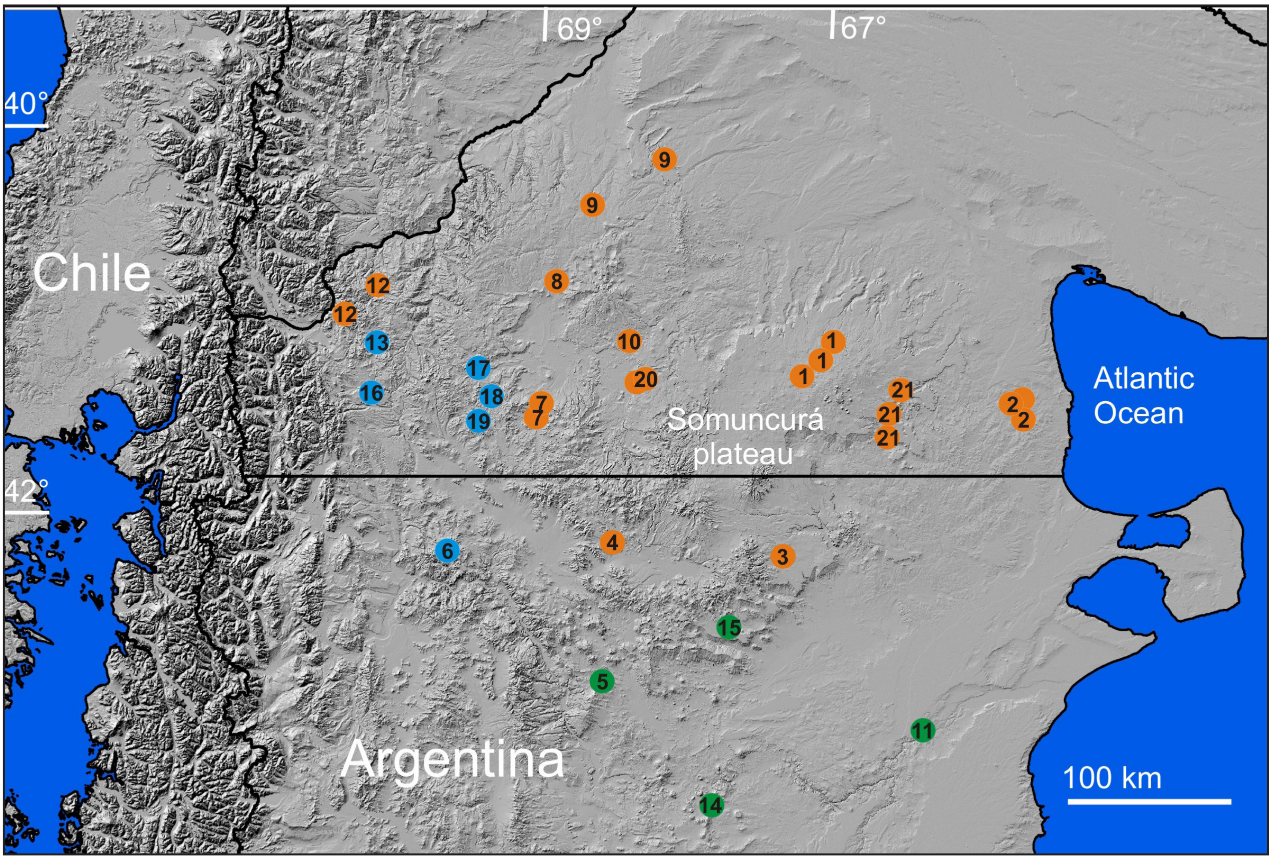

Figure 5. Map of distribution of the *somuncurensis* (orange), *spurcus* (light blue), and *P. katenke* and related species (green) in Rio Negro and Chubut provinces of Argentina (Somuncura plateau is indicated, one of the most massive geomorphological formations in Patagonia). Numbers indicate: 1-*P. somuncurensis*, 2- *P. yachanana*, 3- *P. calcogaster*, 4- *P. camilae*, 5- *P. katenke*, 6- *P. curivilcun*, 7- *P. etheridgei*, 8- *P. sinervoi*, 9- *P. ceii*, 10- *P. maquinchao* sp. nov., 11- *P. patagonicus*, 12- *P. tenebrosus*, 13- *P. manuelae*, 14- *P.* sp14, 15- *P.* sp15, 16-*P. chenqueniyen* sp. nov., 17- *P. spurcus*, 18- *P. spectabilis*, 19- *P. excelsus*, 20- *P.* sp22a and *P.* sp22b. "*P.* sp." are Morando *et al.* (2013) candidate species.

case of *P. excelsus*, we found only three individuals in Ojo de Agua (its type locality) exhibiting brown morphs; since these brown morphs in particular resemble *P. spurcus* (Huanualuan), a species we resurrected in another article (Lobo and Quinteros, 2005b), we assumed that *P. spurcus* could be syntopic to *P. excelsus*. Later, after the description of *P. agilis* provided by Scolaro *et al.* (2008), we analyzed (Lobo *et al.*, 2012b) this new species statistically for different continuous characters and because we found a female giving birth both patterns "*agilis*" (brown morph) and "*spectabilis*" (ocellated morph), we concluded that these two species are synonymous. At that time, we also considered the brown morphs of *P. excelsus* as being part of that species, rejecting the idea of *P. spurcus* being syntopic with *P. excelsus*. We discriminated brown morphs of *P. spectabilis* from *P. excelsus* (shown in Lobo *et al.*, 2012b Fig. 2). At that time, we considered the brown morphs of the species to be different from each other and very constant for each species. Figure 7A shows an individual of *P. excelsus* from the MCN collection, and an individual of "*agilis*" deposited at the MLP collection (Fig. 7B). As can be seen, the brown pattern of *P. spectabilis* (= *agilis*) shows constancy in dorsal pattern, which is difficult to confuse with the *P. spurcus* one; this "*agilis* pattern" is a brown dorsal pattern with darker coloration on both sides of the back, named in Lobo *et al.* (2018) as a kind of "*elongatus* pattern" because it resembles the pattern found in members of the *Liolaemus elongatus* group. This color pattern is not present in any other *Phymaturus* species (*P. spurcus*, *P. excelsus* or *P.* sp. nov. from Chenqueniyén described in the present article). All specimens of the type series of *P. agilis* deposited at MLP, several other individuals deposited at the same museum, and all *P. spectabilis* specimens with brown pattern deposited at FML collection exhibit the same pattern. Pictures published in the description of *P. agilis* by Scolaro *et al.* (2008) are quite illustrative of

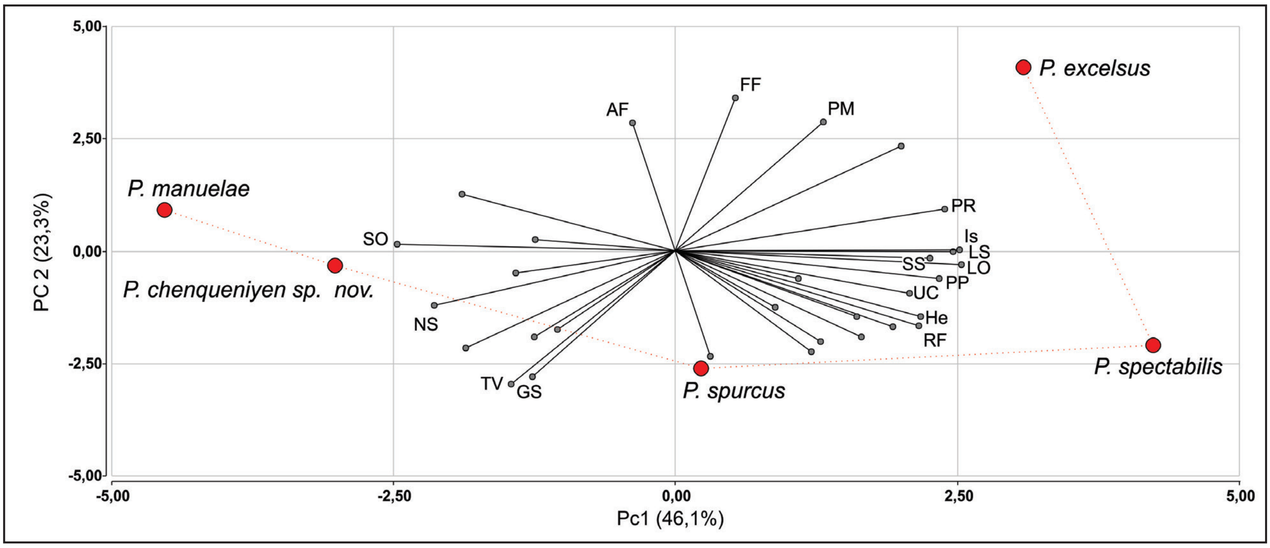

Figure 6. PC1 versus PC2 of squamation characters. *Phymaturus excelsus* discriminated from *P. spectabilis* (PC2) and *P. chenqueniyen* sp. nov. from the three *P. spurcus*, *P. spectabilis*, and *P. excelsus* (PC1). Tables with individual values of characters and for each component and accumulated percentages are shown in S3.

this pattern. Therefore, because this design of brown patterns is different among *P. spurcus*, *P. excelsus* and *P. spectabilis*, it is a mistake to call all of them simply "*spurcus*" morphotype based only on the color without considering the design, as in Becker *et al.* (2018). The most similar brown morphs are the ones of *P. excelsus* with *P. spurcus*. For this reason, the brown *excelsus* found at that time was assigned to *P. spurcus* (Lobo and Quinteros, 2005a). But later, another case of intraspecific polymorphism was considered (Lobo et al., 2012b). All the examined individuals (MCN-UNSa and FML collections) of brown morphs of *P. excelsus* exhibit a fading ocellated pattern in a lighter brown shade than the darker background color similar to that of individuals of *P*. sp. nov. from Chenqueniyén; in *P. spurcus* this fading ocellated pattern is absent or inconspicuous, but present in most newborns and small juveniles. This pattern of fading ocelli in brown morphs is completely absent in *P. spectabilis* brown morphs. Interparietal scale is white in both morphs of *P. excelsus*, in ocellated individuals of *P. spectabilis* and in a few individuals of *P*. sp. nov. from Chenqueniyén, but never in *P. spurcus* or in brown morphs of *P. spectabilis*. The throats do not exhibit a pattern in most individuals of *P. excelsus* or *P. spurcus*; when there is a pattern, in *P. excelsus* it is a vanished reticulation (light brown almost inconspicuous) but in *P. spurcus* it consists of small light brown dots. In addition, the abdominal region is yellow in *P. spurcus* males versus orange in those of *P. excelsus* and *P. spectabilis*.

How to distinguish the ocellated patterns of *P. spectabilis* and *P. excelsus* (Figs. 7C-D).—The occurrence of specific brown morphs is not the only feature of color patterns that discriminates *P. spectabilis* from *P. excelsus*. Ocelli on dorsum of *P. spectabilis* and *P. excelsus* are constant in number. Lobo *et al.* (2012a, Fig. 6A) used this information for phylogenetic analysis (character 130) and described the variation found within the *patagonicus* group. Species belonging to the *somuncurensis* clade, *payuniae* clade and *P. manuelae* (Lobo *et al.*, 2018) showed more ocelli along their backs (9-11) counted between hips and shoulders, whereas *P. spectabilis* and *P. excelsus* had fewer ocelli, between 6-8 (Lobo *et al.*, 2012a). Fading pattern of ocelli (almost inconspicuous) on brown morphs of *P. excelsus*, *P. spurcus* and in *P*. sp. nov. (from Chenqueniyén) are 6-8, the same numbers of ocellated morphs of *P. spectabilis* and *P. excelsus*. The presence of this character in all species, except for *P. manuelae* (higher number of ocelli) or *P. curivilcun* (melanic), is an apomorphy of the subclade of the *spurcus* clade (with the addition of the occurrence of brown morphs, fixed in all population in *P. spurcus* and *P.* sp. (Chenqueniyén), and in part of the population in *P. spectabilis* and *P. excelsus*). Even though the number of ocelli on the backs of these two species is similar, they are different in size and coloration. In most *P. spectabilis* individuals, ocelli are larger, formed by more cream to white scales (Fig. 7D) than those of *P. excelsus*.There are more white scales irregularly

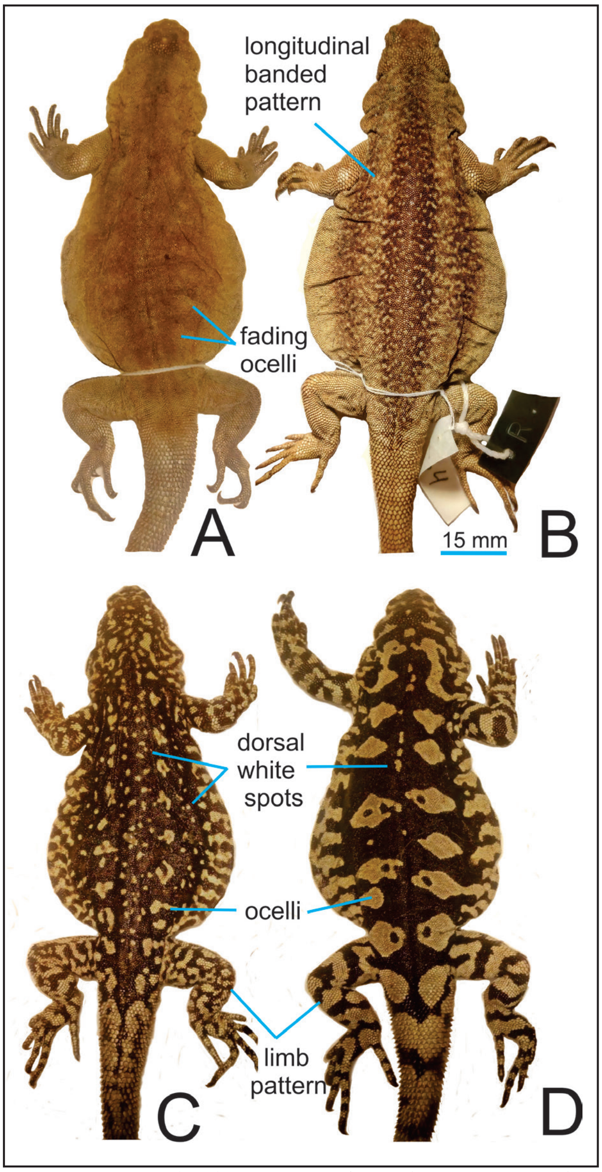

Figure 7. Typical color pattern of A- *Phymaturus excelsus* (MCN-UNSa 1385). B- Brown morph of *Phymaturus spectabilis* (MLP 5346). C and D ocellated morphs of C- *Phymaturus excelsus* (MCN-UNSa 1336) and D- *Phymaturus spectabilis* (MCN-UNSa 1205). Typical banded pattern is present in all samples of brown morphs of *P. spectabilis*, absent in *P. spurcus* and *P. excelsus*. Ocelli are usually larger in *P. spectabilis*, limb pattern consisting of thicker lines, and small white spots more concentrated on the vertebral area.

scattered on the background black coloration of the back in *P. excelsus* (Fig. 7C) while in *P. spectabilis* these white scales are much fewer and concentrated along the vertebral midline. The rostral scale is cream or almost white in *P. excelsus*, but dark brown to black in *P. spectabilis*. Chinshields (posmental row) are white and differentiated in color from the rest of the throat in *P. excelsus*, but not differentiated in *P. spectabilis*. Fore and hind limbs with a reticulated pattern of black slender bands is typical of *P. excelsus*, being formed by thick bands and on a wider/extended cream background in *P. spectabilis*. Based on the study of a population sampled from Meseta de Chenqueniyén and samples deposited at MVZ collection (considered in previous articles as *P. spurcus*), as well as on morphological (see Table 4) and genetic differences (Table 3) found in our comparisons, we conclude that these lizards represent an independent lineage (see phylogenetic tree in Figure 1) that deserves a formal description.

Species description

Phymaturus chenqueniyen sp. nov.
(Figs. 2B and 8).
Phymaturus spurcus: Lobo and Quinteros (2005a), in part; Lobo *et al.* (2012a) in part; Lobo *et al.* (2018), in part.

Phymaturus sp. 13: Morando *et al.* (2013).

Holotype.— IBIGEO 6198. Male (Fig. 2B). Between Las Bayas village and Las Bayas hill (also named as Alto del Escorial), at the edge of the Chenqueniyén plateau (41° 29.238' S, 70° 41.557' W; 1139 m) Ñorquinco department, Río Negro province, Argentina. Date 16 January 2020. Deposited at the Reptile collection of the Instituto de Bio y Geociencias del Noa (IBIGEO), Salta, Argentina.

Paratypes.— IBIGEO 6184, 6186, 6200; MVZ 188904-05, 247102-03, 247105 (6 adult males, 2 juvenile males); IBIGEO 6185, 6196-97, 6199; MVZ 247101, 247104, 247106, 188906-07(6 adult females, 3 juvenile females). Deposited at the Reptile collection of the Instituto de Bio y Geociencias del Noa (IBIGEO), Salta, Argentina, and Museum of Vertebrate Zoology (Berkeley, California, USA). IBIGEO samples collected from Meseta Chenqueniyén, same site of Holotype between Las Bayas and Alto el Escorial or Cerro Las Bayas (41° 29.238' S, 70° 41.557' W; 1139 m). Ñorquinco department, Río Negro province, Argentina. Date 16 January 2020. MVZ samples: MVZ 188904-07. Ñorquinco department, along rimrock, 4 km S and 1 km E Alto del Escorial, elevation: 1100 m. Río Negro province, Argentina. 25 February 1982. MVZ 247101-07. Ñorquinco department, along rimrock, 4 km S and 1 km E Alto del Escorial, elevation: 1100 m. Río Negro province, Argentina. 23 November 1986. DNA samples: IBIGEO 6197, 6199.

Diagnosis (Fig. 2B and 8; Table 4; S2).— *Phymaturus chenqueniyen* sp. nov. belongs to the *patagonicus* group of *Phymaturus* because it exhibits flat and imbricated superciliary scales, smooth tail scales, and a set of enlarged scales projected onto the auditory meatus (Etheridge, 1995; Lobo and Quinteros, 1995). Within the *patagonicus* group, *Phymaturus chenqueniyen* sp. nov. belongs to the *spurcus* clade because it shares the following morphological apomorphies (according to the total evidence analysis, excluding *P. manuelae*): females exhibit ocellated (not ringed) tails, presence of two longitudinal rows of ocelli in both sexes, and scale organs in rostral scale (six changes in DNA positions also support this clade). Since *P. manuelae* is included in the group (with high support value) in the Bayesian analysis, we also include this species in our comparisons *Phymaturus chenqueniyen* sp. nov. has dark brown coloration all over its head, body and limbs; half of the males studied exhibit irregularly distributed light brown markings (mostly on shoulders or neck); belly and chests of *P. chenqueniyen* sp. nov. are dark brown (light gray to white immaculate in all the other members of the *spurcus* group). It is the only species within the *spurcus* clade with melanic individuals, but at a low frequency (3/17, 17%). This melanism was reported in *P. cacivioi*, *P. ceii*, *P. curivilcun, P. niger* and *P. tenebrosus* (Lobo *et al.*, 2021). *Phymaturus curivilcun* is sister to the other taxa of the group but without support. *Phymaturus chenqueniyen* sp. nov. differs from *P. manuelae* because it lacks any kind of dorsal pattern, its coloration is dark brown with almost inconspicuous light brown ocelli (*P. manuelae* exhibits white small scattered spots, irregularly distributed or forming transversal rows, as well as conspicuous white dorsal ocelli along the paravertebral region between thighs and shoulder). The interparietal scale is white in *P. chenqueniyen* sp. nov. but brown and inconspicuous in *P. manuelae*. *Phymaturus chenqueniyen* sp. nov. has spinier tail scales than *P. manuelae* (in this species, tail scales are not different from those of the *somuncurensis* or *payuniae* clades). Most of specimens of *P. chenqueniyen* sp. nov. have enlarged (3-5) scales on the posterior gular fold margin (absent in *P. manuelae*), *Phymaturus chenqueniyen* sp. nov. has conspicuous anterior gular fold, whereas it is absent in *P. manuelae*; *P. chenqueniyen* sp. nov. has more scales contacting nasal (CN), postocular scales (PO), scales between frontals and rostral scale (RF), and fewer scales counted around midbody (MS). *Phymaturus chenqueniyen* sp. nov. differs from *P. manuelae* in five body measurements: slender trunk (AW), lower auditory meatus height (MH), shorter fourth´s toe claw (CL), neck (NL), and tail (Tl). *Phymaturus chenqueniyen* sp. nov. differs from *P. spurcus* in that it exhibits a darker brown dorsal coloration; belly and chests are dark brown in *P. chenqueniyen* sp. nov. and light gray /white immaculate in *P. spurcus*. The collected males or females of *P. chenqueniyen* sp. nov. did not exhibit any abdominal coloration typical of the *patagonicus* group (males and some females of *P. spurcus* have yellow abdomen). The interparietal scale is white in *P. chenqueniyen* sp. nov. but brown and inconspicuous in *P. spurcus*. Enlarged scales at the anterior border of the auditory meatus are perpendicular but in *P. spurcus* they extend backward, covering part of the auditory opening;

Table 4. Continuous characters that exhibit significant variation between all pairs of species within the *spurcus* clade (18 of 32 scale counts studied and 8 of 18 measurements studied). Below the diagonal are scale counts significant characters, and above measurement significant characters. Same abbreviations of Table 1.

	P. chenqueniyen	*P. excelsus*	*P. manuelae*	*P. spectabilis*	*P. spurcus*
P. chenqueniyen		CL EL Hu HW	AW CL MH NL Tl	_	AW Tl
P. excelsus	GS NS PM PR SO SS TV		AW EL Hu HW MH NL Tl	CL EL Hu HW MH	AW CL EL Tl
P. manuelae	CN MS PO RF	AF FF GS LO MS PM PR SO TV		CL MH NL Tl	CL Hu MH Tl
P. spectabilis	AF He Is LO LS NS PO PR RF SO SS UC	AF FF GS He PM RF TV UC	AF CN He Is Ls LO MS NS PR RF SO UC		AW Tl
P. spurcus	FF MS PO PR SO SS	AF FF GS MS NS PM TV	RF SO	AF He LO LS MS NS RF UC	

Figure 8. Colors in life of *Phymaturus chenqueniyen* sp. nov. (individuals photographed on rocks of their typical environment). A- Dorsal view of a male (IBIGEO 6200); B- ventral view of the same individual; C- Dorsal view of a female (IBIGEO 6185); D- ventral view of the same individual; E- Dorsal view of a melanic female (IBIGEO 6196); F- ventral view of the same individual.

most of *P. chenqueniyen* sp. nov. individuals have a dorsal fading tail pattern (absent in *P. spurcus*); 8/17 individuals exhibit a fading ocellated pattern in a lighter brown tone with respect to the darker color of background (absent or inconspicuous in adult *P. spurcus,* but present in most newborns and small juveniles); dorsal scales of tail more spiny and ending in dark brown spine (less spiny and with less developed light brown spine in *P. spurcus*); all ventral surface of fingers and toes dark brown to black (in *P. spurcus* a central longitudinal row is darker than the rest of finger or toes, see Lobo *et al.* (2010, Fig. 8D). *Phymaturus chenqueniyen* sp. nov. shows significant differences from *P. spurcus* in the following continuous characters (eight characters): fewer scales counted around midbody (MS), supralabial scales (SS), postrostral scales (PR) and more scale organs over postrostral scales (SO), postocular scales

(PO), and subdigital plates of fourth finger (FF) and morphometric: slender trunk (AW) and longer tail (Tl). *Phymaturus chenqueniyen* sp. nov. differs from *P. spectabilis* in that all *P. chenqueniyen* sp. nov. are dark brown; they may exhibit some lighter coloration on the shoulders or neck and a fading (almost inconspicuous) pattern of ocelli, whereas the brown morphs of *P. spectabilis* show a brown dorsal pattern, speckled with lighter brown or some almost white scales scattered irregularly, sometimes concentrated on sides of dorsum of the trunk (Fig. 6B). Belly and chests are dark brown in *P. chenqueniyen* sp. nov., and light gray/white immaculate in *P. spectabilis*. Ocellated morphs of *P. spectabilis* have black background coloration of the back, with large cream almost white ocelli. The interparietal scale is white in *P. chenqueniyen* sp. nov. but brown in brown morphs of *P. spectabilis. Phymaturus chenqueniyen* sp. nov. did not exhibit any abdominal coloration that is typical in the *patagonicus* group (males and females of *P. spectabilis* show abdominal orange color). *Phymaturus chenqueniyen* sp. nov. shows significant differences from *P. spectabilis* in the following continuous characters (12 characters): fewer scales on dorsum of head (He), postrostral scales (PR), supralabials (SS), upper ciliary scales (UC), scales between rostral and frontal (RF), lorilabial scales in contact with subocular (LO), lorilabial scales (LS) internasal scales (Is) more scales along lateral neck fold till the antehumeral fold (AF), neck scales (NS), postocular scales (PO) and scale organs counted on postrostral scales (SO). No morphometric differences between these two species were found. *Phymaturus chenqueniyen* sp. nov. differs from *P. excelsus* in the homogeneous dark brown coloration on the head, body and limbs, sometimes with lighter coloration on shoulders and neck, whereas *P. excelsus* brown morphs have lighter background coloration; the interparietal scale is white in *P. chenqueniyen* sp. nov. but brown and inconspicuous in brown morphs of *P. excelsus*. Belly and chests are dark brown in *P. chenqueniyen* sp. nov. and light gray/white immaculate in *P. excelsus*; the collected males and females of *P. chenqueniyen* sp. nov. did not exhibit any abdominal coloration (males and females of *P. excelsus* show abdominal orange color). *Phymaturus chenqueniyen* sp. nov. shows significant differences from *P. excelsus* in the following continuous characters (11 characters): fewer neck scales (NS), postrostral scales (PR), supralabial scales (SS), postmental scales (PM), more scale organs in postrostrals (SO), gulars (GS), and temporal scales counted in a vertical line between labial commissure and the superciliaries level (TV). Morphometric: shorter fourth´s toe claw (CL), smaller eyes (EL), wider head (HW), and wider forelimbs (Hu).

Description of holotype (Fig. 2B).— Male. SVL 89.09 mm. Head length: 16.88 mm. Head width: 16.07 mm. Head height at parietal level 8.46 mm. Axilla-groin length 45.01 mm (44.4 % of SVL). Tail length (complete, not regenerated) 100.22 mm. Body moderately wide, trunk width 34.17 mm (38.3 % of SVL). Nineteen smooth dorsal head scales. Five, two and three scale organs in three postrostrals. Nasal bordered by seven scales, not in contact with rostral. Canthal separated from nasal by two scales. Loreal region flat. Nine enlarged supralabial scales, none contacting subocular. Eight enlarged infralabials. Auditory meatus oval (height 3.8 mm; width 2.7 mm) with six enlarged, flat and smooth perpendicular projecting scales on the anterior margin. Auricular scale absent. Nine convex, juxtaposed temporals. Auditory meatus - ciliary scale distance: 5.5 mm. Rostral scale undivided. Mental scale subpentagonal, in contact with four scales. Interparietal scale bordered by eight scales, larger than postparietals. Frontal region without an azygous scale. Supraorbital semicircles inconspicuous. No distinctly enlarged supraoculars. Six juxtaposed superciliaries, 14 upper ciliaries, and 12 lower ciliaries. Subocular fragmented in two scales. Ten lorilabials, ninth to tenth contacting subocular. Preocular of the same size as that of canthal, in contact. Preocular contacting lorilabial row. Scales of throat round, small and juxtaposed. Eighty-three gulars between auditory meata. Lateral nuchal folds well developed, with granular scales on longitudinal fold. Antehumeral pocket well developed. Ninety-three scales between auditory meatus and shoulder. Seventy-four scales between antehumeral fold and shoulder. In ventral view, anterior gular fold absent, posterior gular fold present with its anterior margins with three enlarged scales on their borders. Dorsal scales round, smooth and juxtaposed. Forty dorsal scales along midline of the trunk in a length equivalent to head length. Scales around midbody: 218. Ventral scales larger than dorsal scales. Ventral scales between mental and precloacal pores: 174. Nine precloacal pores in an undivided row without supernumerary pores. Two enlarged postcloacal scales. Brachial and antebrachial scales smooth, with round posterior margins. Supracarpals laminar, round and smooth. Subdigital lamellae of fingers have three keels.

Subdigital lamellae of finger (left manus) IV: 22. Supradigital lamellae convex, imbricate. Infracarpals and infratarsals have round margins and 2–3 keels. Supracarpals and supratarsals smooth, with rounded posterior margins. Subdigital lamellae of toe (left pes) IV: 28. Claws moderately long (fourth toe's claw: 2.4 mm).

Coloration in life (Fig. 2B).— The holotype exhibits a homogeneous brown dorsal background, with light brown cream colored scales on dorsum of neck and shoulders. Toward the trunk, these cream scales become more scattered and irregularly distributed, and are almost absent towards the posterior half of trunk. Dorsum of tail of the same creamy coloration (no obvious ringed or variegated pattern). Head uniformly dark brown, with this coloration extended on the lateral neck folds. Interparietal scale white. Two large melanic spots are conspicuous on the right side, one anterior to the forearm and the other on the shoulder. Flank,fore and hind limbs brown, as the rest of body. Throat immaculate light gray with no variegation. Chest, abdomen, ventral surface of limbs, and tail light gray. Ventral surface of tail does not have any pattern.

Variation.— Squamation based on thirteen adult specimens (7 males and 6 females) and five juvenile individuals (2 males and 3 females); measurements only based on adult individuals. SVL 75.1–99.4 mm (mean = 91.4; SD = 6.8). Head length 16.4–19.9% (mean = 17.7%; SD = 0.9) of SVL. Tail length 0.95–1.12 (mean = 1.05; SD = 0.05) times SVL. Scales around midbody 185–234 (mean = 207.4; SD = 11.9). Dorsal head scales (Hellmich's index) 17–21 (mean = 19.1; SD = 1.2). Ventral scales 155–182 (mean = 167.2; SD = 6.5). Scales contacting interparietal 7–9 (mean = 7.7; SD = 0.6). Scales surrounding nasal 7–11 (mean = 8.6; SD = 1.1). Number of scale organs on postrostrals 3–8 (mean = 4.8; SD = 1.2). Superciliaries 6–9 (mean = 7.6; SD = 0.8). Subocular fragmented in 1–4 scales (mean = 2.6; SD = 0.9). Mental scale in contact with 4–5 (mean = 4.1; SD = 0.3). Number of chinshields 4–7 (mean = 5.9; SD = 0.9). Twelve of 18 specimens exhibit enlarged scales on the border of the posterior gular fold: 0–5 (mean = 2.4; SD = 1.7). Lorilabials 8–13 (mean = 10.1; SD = 1.4). Enlarged scales on the anterior border of the auditory meatus 4–8 (mean = 6.8; SD = 1.3). Scales of neck along longitudinal fold from posterior border of auditory meatus to shoulder 77–97 (mean = 87.4; SD = 5.8). Gulars 71–96 (mean = 78.7; SD = 6.3). Scales between rostral and frontal 7–10 (mean = 8.5; SD = 0.9). Subdigital lamellae on fourth finger 19–27 (mean = 22.9; SD = 2.2). Subdigital lamellae on fourth toe 23–31 (mean = 27.4; SD = 1.9). Males with 8–11 precloacal pores (mean = 9.0; SD = 1.1). No females show precloacal pores. Eye length 3–4.1 (mean =3.4; SD = 0.4). Head length 13,4–17,3 (mean =15.9; SD =1.0). Neck length 10.7–16.4 (x = 13.3; SD = 1.2). Head width 13.2–16.9 (mean = 15.5; SD = 1.1). Head height 7.0–9.9 (mean = 8.5; SD = 0.8). Internares distance 2.3–3.3 (mean = 2.9; SD = 0.3). Interorbital distance 5.9–7.6 (mean = 7.1; SD = 0.5). Trunk length 35.6–54.5 (mean = 46.9; SD = 4.9). Humerus length 12.2–16.4 (mean = 13.8; SD = 1.0). Humerus width 4.7–6.7 (mean = 5.9; SD = 0.7). Tibia length 13.5–18.8 (mean = 16.9; SD = 1.4). Foot length 19.9–25.6 (mean = 24.2; SD = 1.5). Tail length 85.2–104.7 (mean = 96.8; SD = 5.8). Eye -auditory meatus distance 4.3–5.9 (mean = 5.3; SD = 0.4). Auditory meatus height 3.1–4.2 (mean = 3.8; SD =0.3). Fourth toe´s claw length 2.3–0.3 (mean = 2.3; SD = 0.3). Abdominal width 28.9–44.3 (mean = 35.7; SD = 4.3). Individuals of *Phymaturus chenqueniyen* sp. nov. (Fig. 8) exhibit a brown coloration all around their trunks. This color is extended on the tail and fore and hind limbs. Four males (of a total of nine) also exhibit an irregular spotting of a lighter coloration (cream) on neck and shoulders. Eight of 18 individuals (independent of sexes) show a vanishing pattern of lighter brown ocelli than the dark brown background coloration. In addition, three individuals are completely melanic (Fig. 8E and F). Only six specimens have a white interparietal. Five individuals show few scattered black scales on throats. Tails of half of the sample of individuals exhibit reticulated pattern (not very conspicuous), with irregular light brown cream spots scattered on the darker brown coloration. Females with dorsal background coloration brown all over head, trunk, tail and limbs. Interparietal brown, inconspicuous. Light brown coloration speckled on flank and on the mid-dorsal line. Most of the ventral surfaces immaculate, brown to light brown. Darker coloration on ventral surfaces of jaws than in the middle of throat. Tail light-brown creamy, no pattern is evident.

Etymology.—Its name refers to the Patagonian plateau called Chenqueniyén.

Distribution (Fig. 5).— The species occurs in Meseta de Chenqueniyén, a flat elevated plateau area of about 260 square kilometers. Known from three close sites along National Road 1s40 (ex 40), with less than 6-km distance between the two extreme sites.

Discussion

Relationships within the *somuncurensis* and *spurcus* clades

Relationships among clades of the *patagonicus* group remain uncertain, with none of the hypothesis obtained in the last years having support to be accepted with confidence. Our inclusion of two new taxa, more sequences (five DNA markers of four species) and additional morphological data for several species has not modified this situation.

Our results about the *somuncurensis* clade agree with those obtained by Lobo *et al.* (2018). *Phymaturus tenebrosus* is the sister taxon of the other members of the *somuncurensis* clade and the subclade *calcogaster* (*P. calcogaster*, *P. camilae*, and *P. yachanana*) is sister to the remaining species. The relationships found among most terminal taxa differ from those reported by Lobo *et al.* (2018). *Phymaturus etheridgei* is sister taxon of *P*. sp. 22b and *P. sinervoi* of *P*. sp. 22a in Lobo *et al.* (2018) but it is sister taxon of *P. sinervoi* in the present total evidence analysis and of *P. maquinchao* sp. nov. in the bayesian tree (Fig 1B). The inclusion of *P. maquinchao* sp. nov. with additional sequences and morphology changes this position (Fig. 1). Our Bayesian analysis breaks the monophyly of the *calcogaster* subclade recovered in Lobo *et al.* (2018); the remaining relationships are the same, i.e. *P. maquinchao* sp. nov. is sister taxon of *P*. sp. 22b. The inclusion of new data (taxa, morphology, and DNA sequences) can affect the results of the analyses and, consequently, the topologies; however, we consider that such effect is not very drastic. The completion of our knowledge of morphologies and DNA markers in candidate species, and the more careful exploration of this clade will probably provide a more complete picture of the *somuncurensis* clade, its composition and evolutionary relationships.

The inclusion of *Phymaturus curivilcun* and *P. chenqueniyen* sp. nov. did not affect the main topology; in the total evidence analysis, *P. manuelae* is not related to *somuncurensis* clade, and *P. curivilcun* –a species that was not previously evaluated– is recovered as sister of the remaining species of the *spurcus* clade but with weak statistical support. Then, *P. chenqueniyen* sp. nov. (*P*. sp. 13 in Morando *et al.*, 2013) is the sister taxon of the remaining species (in Lobo *et al.*, 2018, sister taxon of *P. excelsus*), and *P. spurcus* is the sister taxon of the clade formed by *P. excelsus* and *P. spectabilis*. In comparison to Lobo *et al.* (2018), with the inclusion of *P. curivilcun* and *P. chenqueniyen* sp. nov, the Bayesian topology only changed in the position of *P. spurcus*, now placed as sister taxon of *P. spectabilis* plus the clade formed by *P. excelsus* and *P. chenqueniyen* sp. nov. In contrast to findings reported by González Marín *et al.* (2018), we do not consider *P. calcogaster* as an independent clade because 1) *P. yachanana*, *P. camilae* and *P. calcogaster* (forming or not a monophyletic grouping) are nested within the *somuncurensis* clade, and 2) the authors include *P. patagonicus* within their *calcogaster* clade, and our previous phylogenetic analysis does not support this relationship (*P. patagonicus* is outside the *somuncurensis* clade and more related to *P. katenke*, and *P.* sp14 and *P.* sp15.) (Lobo *et al.*, 2018; Morando *et al.*, 2020). In addition, we considered *P. tenebrosus* as member of the *somuncurensis* clade (recovered in both the total evidence analysis and the Bayesian tree), González Marín *et al.* (2018) included this species in the *spurcus* clade.

More information on morphology is available for the study of *Phymaturus*

Morphological data can be used for many purposes, sometimes for studying the evolution and the effect of selection in certain systems of characters (e.g. Tulli *et al.*, 2011; Reaney *et al.*, 2018; Valdecantos *et al.*, 2019; among others), for resolving taxonomic problems or the description of the diversity of a group (e.g. Avila *et al.*, 2011; Scolaro *et al.*, 2012; Avila *et al.*, 2014; Scolaro *et al.*, 2016; González Marín *et al.*, 2016a; 2016b; among others), or for providing phylogenetic information (Lobo and Quinteros, 2005a; Lobo *et al.*, 2012a; 2016; 2018). Morphology is not restricted to the revision of a limited set of characters, as is done in certain publications, but involves much more diverse data. Therefore, authors' conclusions in those examples often suggest the power of morphology in this group as highly conservative and not very informative. Their conclusions sometimes are based on the study of a subset of characters or systems of characters, which should not be taken as a general morphological rule, but which were obviously useful just for meeting the objectives that they set. In fact, in a recent study, González Marín *et al.* (2018)

analyzed the body shape morphology (based on 11 linear measurements and the geometric analysis of heads in species of the *patagonicus* group). "*In this study we quantify levels of morphological divergence (size and shape) among the multiple species relative to interspecific molecular divergence, and show that most species have not diverged significantly in size and/or shape to permit unambiguous species diagnosis with morphological data alone*". It is a type of generalization that we do not agree with the authors' conclusion indicates that morphological data are insufficient to make species diagnosis, since diagnoses are usually built based on squamation and color pattern characters. They studied a reduced dataset with respect to the 271 characters described in the literature (see character lists in Lobo and Quinteros 2005; Lobo *et al.*, 2012a; 2016; 2018; 2019; 2021) for the genus *Phymaturus*; of those characters, 114 were found informative for the *patagonicus* group. The need for such claims is unclear, since, to our knowledge, there is no description of *Phymaturus* based solely on body size or body shape characters. Similarly, if we study only a couple of DNA markers and state that genomes of these species are invariant or constant, probably we may be arbitrary in our conclusions. After 15 years of morphological, taxonomic and phylogenetic studies, we have incorporated new characters that we can be used to address new questions. Over this period, we have gathered a body of more than 300 characters that can be used (see character lists in Lobo *et al.*, 2012a; 2019; 2021). Anyway, our results show that most morphometric (linear) characters have ranges of values that overlap among species and are not useful to elaborate a diagnosis of a species, but they are very informative to determine the taxonomic status of different populations (when added to other sources of evidence) see Tables 1-2 and 4. In the present contribution, we have added this information to qualitative characters derived from scalation, bones, hemipenis, precloacal glands, colors, and color patterns (see Lobo *et al.*, 2021). Curiously, González Marín *et al.* (2018, Table 2) found significant differences in measurements among most of the species of the *patagonicus* group, even though these characters alone cannot be used to make a taxonomic diagnosis. How does evolution occur through the different character systems? Do integumentary traits, like scale numbers or ornamentation features, exhibit the same evolutionary degree of change as that of color and patterns, or morphometric traits?. In Lobo *et al.* (2021) we measured those sets of characters separately for the entire *patagonicus* group. In the phylogenetic trees inferred for the *patagonicus* group, we estimated the evolutionary lability index (ELI) for the whole morphological data set *sensu* Poe and Wake (2004), who proposed this method for ontogenetic changes in evolution. We calculated the index for each character as follows: (number of changes – number of stases) / (number of changes + number of stases). A stasis is a branch of the tree that does not exhibit a change in the character. For example: character 120 (presence/absence of dark lateral band) changed three times on the tree, whereas the number of branches in stasis is 67, so the lability index is (3 – 67) / (3 + 67) = −0.914. Values range between -1 and 1. Continuous characters were divided into two subsets: morphometric (20 characters) and scale counts (21). We found the first subset to be more conservative, given that the average ELI of all morphometric characters was −0.885 versus −0.764 for the scale counts. The discrete characters were divided into scalation (33 characters) versus pattern and color (43 characters). The scalation characters were slightly less conservative than pattern and color characters (scalation = −0.801; pattern and colors = −0.852).

According to these results, when reviewing the literature of species descriptions of *Phymaturus* and phylogenetic studies, we found that scale characters (scale counts and discrete characters), the most widely used were those that exhibited most of the changes during the evolution of the group (after calculating ELI), followed by the characters referring to pattern designs and colors. However, our analyses in Lobo *et al.* (2021) are preliminary. Have we addressed only a part of the general morphology of these animals, and what would happen if we also analyzed the anatomical features, like skeletal, muscular, or genital characters? Regarding the taxonomy objectives of this article, we demonstrate that overall morphology is more informative than can be expected, even in clades and subclades of closely related species like in *Phymaturus* (see Tables1-2 and 4). This amount of information, along with other qualitative characters, like scale ornamentation and color patterns, is useful for analyzing the taxonomic status of the described species, even when mtDNA distances can be small in some complexes.

The truly integrative taxonomy

Corbalán *et al.* (2016) remarked the low genetic distance among *P. ceii*, *P. somuncurensis*, and *P. sinervoi*, as for species of the *spurcus* clade (*P. spurcus*, *P. excelsus,* and *P. spectabilis*), but they made an asymmetric decision, since they proposed only the synonymy for all species of the latter clade. Contrary to DNA information, our morphological analyses of continuous characters including scale counts and morphometry (see Tables 1, 2 and 4) show more morphological divergence among species of the *spurcus* clade than that detected among the three species of the *somuncurensis* clade. In fact, within the *somuncurensis* clade we found: *P. camilae-P. calcogaster* (scalation: 0 character; morphometry: 1); *P. camilae-P. sinervoi* (1/0); *P. sinervoi-P. ceii* (0/1), *P. camilae-P. somuncurensis* (0/0)*; P. somuncurensis-P. maquinchao* sp. nov. (0/2); *P. somuncurensis-P. calcogaster* (0/4); *P. sinervoi-P. somuncurensis* (1/0), *P. ceii-P. somuncurensis* (2/1).

Within the *spurcus* clade we found: *P. excelsus-P. spectabilis* (8/5), *P. excelsus-P. spurcus* (6/3) and *P. spectabilis-P. spurcus* (8/2). We consider here all taxa (of the two clades) as valid, taking into account that, beyond the comparisons we made on continuous characters, there are also characters of color and patterns that discriminate all these species.

Brown morphs of *Phymaturus spectabilis*, always show the striped type, unlike those of *P. spurcus* and *P. excelsus*, which exhibit the homogeneous brown, not striped type (Fig. 7). That was probably one of reasons why Scolaro *et al.* (2008) described *Phymaturus agilis*. In Becker's (2018) contribution, the authors advocated, even in the title, for the integrative taxonomy as the preferred tool to discriminate and/or delimit species, but in this pursue they just made some remarks on what they call the "*spurcus*" morph. They restricted their analysis of integrative taxonomy to a single morphological character (presence or absence of a "*spurcus* morphotype"). Here we analyzed 49 continuous characters of squamation and measurements plus four color pattern characters, and revised the brown morphs of all species, the only morphological character studied by Becker *et al.* (2018). Confusion arose and led authors to wrong conclusions because they assigned the same character state to all members of the group, *P. spurcus*, *P. excelsus* and *P. spectabilis*, since all of them exhibited the same "*spurcus* morphotype". Their observations on the offspring recorded from *P. agilis* and *P. spectabilis* confirmed our observations (Lobo *et al.* 2012b). Their observations on brown morph giving birth to an *excelsus* in Ojo de Agua is also not surprising, since we pointed out that it is the polymorphism of that species. Unfortunately, Becker *et al.* (2018) did not show pictures of patterns of the female and the newborn. Becker *et al.* (2018) were not able to discriminate that brown morph (*spurcus*) from the *spectabilis* one. What we never found until today is a single *P. spurcus* individual from its type locality (Estancia Huanuluan) showing the ocellated pattern. Noticeably, some brown morphs of other species of the *patagonicus* group are more similar to *spurcus* than to *spectabilis* or *excelsus* ones (see Fig. 5 in Lobo *et al.*, 2018). Here, based on different collection samples, we demonstrate that each of the above mentioned species have its particular brown morph (see Fig. 7). In addition, we provide morphometric and squamation characters that show statistically significant differences among species (Tables 1, 2 and 4), and five characters of color pattern: abdominal life color (yellow in *P. spurcus* but orange in *P. spectabilis* and *P. excelsus*), brown morphs, limb pattern, size and shape of dorsal ocelli, and dorsal white spots distribution (Fig. 7). Morphology can provide more information than that used so far; regarding this aspect, Becker *et al.* (2018) indicate in the discussion section: "*The P. spurcus populations studied here might be useful in identifying similar processes of incipient speciation, given that their dorsal pattern polymorphisms are also associated with ventral color polymorphisms (Fernández JB, Boretto JM, Ibargüengoytía NR, and Sinervo B, unpublished observations).*"

No shared haplotypes among species supported by morphology

In both analyses, Corbalán *et al.* (2016) and Becker *et al.* (2018), built their tree using COI, and recovered the monophyly of *P. spurcus* and *P. excelsus*, but not of *P. spectabilis* and/or *P. agilis*. Because this complex of species does not match their BIN definition (barcoding index), they concluded that *P. spurcus*, *P. spectabilis*, *P. agilis* and *P. excelsus* are the same species. Corbalán *et al.* (2016) after their comparisons considered *P. cei-P. somuncurensis-P. sinervoi*; *P. indistinctus- P. videlai*; and *P. payuniae-P. nevadoi* as valid species despite having a very short genetic distance (such as the genetic distance found between *P. spurcus-P. excelsus-P. spectabilis*). Their interpretation was different for both clades only based on the fact that the species of the *somuncurensis* clade are geographically separated by greater distances. We consider that geographic/spatial isolation can occur between populations and cause speciation at a much smaller geographic scale than the one exhibited by for example between *P. ceii* and *P. somuncurensis*. In their final considerations, Corbalán *et al.* (2016) indicate that "*species delimitation ideally requires data from many different sources such as morphology, behavior, and multiple molecular markers (Funk and Omland, 2003; Hajibabaei et al., 2007)*" and: "*Therefore, DNA barcoding can fail to identify species when introgression, incomplete lineage sorting, or complex species are involved (Vences et al., 2005a, b; Smith et al., 2008). In such cases, nuclear loci are necessary to reliably identify species (Hebert et al., 2003a; Murphy et al., 2013).*" In their Discussion section, Becker *et al.* (2018) remark: "*Despite their relatively narrow distribution, gene flow seems to be restricted among P. spurcus populations as revealed by genetic structure, except between the North and South Yuquiche Hill, whose haplotypes were found in common. Thus, P. spurcus appears to be a single highly structured species whose populations seem to be experiencing a process of divergence in morphometric and meristic characteristics and dorsal color patterns from a common, ancestral population. The star-shaped haplotype network agrees with this hypothesis, and the small genetic pairwise distances among haplotypes are in accordance with a recent diversification.* "

Becker *et al.* (2018) found no shared haplotypes among *spurcus* type locality, *excelsus* and *spectabilis* / *agilis*, but they only found shared haplotypes between south and north of Yuquiche Hill (individuals identified as *spectabilis* and *agilis*), in agreement with Lobo *et al.* (2012b), who showed that *P. spectabilis* and *P. agilis* are synonyms, based on the lack of statistical differences in measurements or squamation, and on the evidence of a mother giving birth individuals with both patterns. Divergence in morphometric and meristic characteristics as well as color patterns suggests that these lizards may be distinguishable species; in such a case, we agree that it must be a recent process of isolation. Anyway introgression and the existence of shared haplotypes have been detected among different closely related vertebrate species (i.e. Méndez Rodríguez *et al.*, 2021 in bats, Chen *et al.*, 2009 in frogs, etc.); therefore, this fact cannot be a sufficient argument to synonymize the described species. We agree that the genetic distance is quite low for mitochondrial DNA among *P. spurcus*, *P. excelsus* and *P. spectabilis* (Table 3).

González Marín *et al.* (2018) performed a combined analysis of mitochondrial and nuclear markers, and indicated that the limitation of these two studies (Corbalán *et al.*, 2016; and Becker *et al.*, 2018) to detect the independence of lineages in the case of the *spurcus* clade is that they are almost exclusively based on mtDNA. In fact, our morphological results agree with findings of González Marín *et al.* (2018, Fig.2), who provided molecular support for divergence for the species *P. spectabilis*, *P. spurcus*, and *P. excelsus*. In a recent contribution to the taxonomy of a *Liolaemus* complex of species (*leopardinus* group) made by Esquerré *et al.* (2019b), they found strong conflicting signals between phylogenetic analyses of the nuclear and mtDNA data, but they discovered a consistent match between nuclear and morphological data. The authors stressed the importance of using multiple lines of evidence to resolve evolutionary histories, and the potential misleading results from relying solely on mtDNA. Our results are similar to previous findings in other groups of vertebrates, such as those reported by Pedraza Marrón *et al.* (2019). The authors combined different molecular sources of evidence and discriminated two species of fishes that were previously lumped after only mtDNA analyses. The conclusions of Pedraza Marrón *et al.* (2019) are in agreement with the old morphology-based taxonomy of the group that discriminated these two species just based on two morphological traits.

Conclusion

Phymaturus katenke is related to *P. patagonicus* and other two candidate species (*P.* sp. 14 and *P.* sp. 15), one of them close to the locality of El Sombrero. Lobo and Quinteros (2005b) collected a sample of specimens from that locality and at that time assigned it to *P. patagonicus*, indicating some differences with the type locality population (Dolavon, Chubut province), such as the presence of two rows of dorsal ocelli. Further studies are needed to revise those two candidate species. This natural group might be recognized as the *katenke* clade (Fig. 1 and Fig. 5 green circles), but its relationships with other clades is uncertain and not well supported. *Phymaturus curivilcun* is recovered in both analyses as sister to all members of the *spurcus* clade, but without support; indeed, because of the extreme melanism of this species, it was not possible to record color characters and patterns that are so informative in the systematics of this group. Its phylogenetic position continues to be a subject pending investigation. The *somuncurensis* clade still needs more studies. *P.*

sinervoi, *P. ceii*, and *P. somuncurensis* exhibit quite low morphological differentiation among them; DNA markers studied to date also show restricted to null distance among them (Corbalán *et al*., 2016). A similar situation is observed within the *spurcus* clade, but in the latter case more morphological differentiation is evident (see Tables 2 and 4). *Phymaturus maquinchao* sp. nov. is closely related to *P. etheridgei* and *P*. sp. 22b; the morphology of *P*. sp. 22a and *P*. sp. 22b is lacking and should be analyzed. It would also be very useful to add ND4 sequences because they were proved to be very informative for recovering phylogenetic relationships (see Lobo *et al*., 2018: Table 2). Although the title of Becker *et al.* (2018) claims "An integrative approach to elucidate the taxonomic status...", the work analyzed a single morphological character (presence/absence of "*spurcus* morphotype. This "*spurcus* morphotype" was wrongly interpreted as being the same for all taxa, and, what is more controversial, the authors arrived to that conclusions without studying the type series of the involved species. Furthermore, they did not show vouchers of individuals that allow the scrutiny of other researchers about the identity of specimens, and the correspondence between phenotypes, haplotypes and localities. They indicate: "*Species assignation was based on external morphology. Most lizards (N = 130) were released at their exact site of capture within 48 h after tissue sampling from the tip of the tail, and only seven individuals were euthanized and tissue samples taken from liver*." Their Appendix 2 shows the number of DNA extracts deposited at the MACN barcoding laboratory, but no vouchers of specimens are mentioned. The lack of vouchered samples does not allow other authors to corroborate their interpretations and the proper taxonomic identification. Because results shown in the present study (statistical analysis of continuous characters and the revision of color pattern variation) and results obtained by Becker *et al.* (2018) that did not find shared haplotypes among species (only within *spectabilis-agilis* samples), we consider that the taxonomic status of *P. spurcus* Barbour 1921, *P. excelsus* Lobo and Quinteros 2005, *P. spectabilis* Lobo and Quinteros 2005 should be maintained. Our results indicate several morphological traits showing significant differences among species/ populations within the *spurcus* clade. If these entities are not species, is it possible to have such levels of morphological differentiation? If these cannot be considered full species, then can we say that we are able to discriminate populations within *Phymaturus* based on morphological characters? In terms of conservation, recent diversification processes should be considered, reported and classified. From the point of view of conservation, in the case of *P. spurcus*, *P. excelsus* and *P. spectabilis*, if we consider them only a single species distributed in a vast extension, this would require less concern and conservation priorities than if we valued them as they really are: independent entities that carry their own particular phenotypic diversity. There are no sufficient arguments to change the taxonomy of the *spurcus* clade as it is known up to now (Lobo and Quinteros, 2005a; Lobo *et al*., 2012a; Morando *et al*., 2013; González Marín *et al*., 2018; Lobo *et al*., 2018).

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SUPLEMENTARY FILES

Supplementary materials cited in this article are available upon request from FL

Supplementary 1. All character information (average, standard deviation, and sample size) taken for comparisons among species of the *somuncurensis* clade and outgroup species.

Supplementary 2. All character information (average, standard deviation, and sample size) taken for comparisons among species of the *spurcus* clade and outgroup species.

Supplementary 3. PCA tables resuming information of analyses.

APPENDIX 1

Specimens included in the present study (type series data of *Phymaturus chenqueniyen* sp. nov. and *Phymaturus maquinchao* sp. nov. are provided in their respective descriptions).

Phymaturus calcogaster (n = 16)

MACN 39990–91 (paratypes), JAS-DC 799, 803,1096–97: Laguna de las Vacas, Telsen Dept, Chubut Province, Argentina; JAS-DC 1154–55, Bajo Amarillo, Telsen Dept, Chubut Province, Argentina. MCN-UNSa 4295–98, 4301–04, Laguna de las Vacas, southwestern end of the lake (42°29054.60''S, 67°21007.03''O, 651 m), Telsen Dept, Chubut Province, Argentina.

Phymaturus camilae (n = 4)

MLP5786 (holotype), MLP 5787-89 (paratypes). In volcanic rocky outcrops (1100 m) of Sacanana stream bridge, adjacent to Provincial Road 4, (42º27'55.4"S, 68º43'33.3"W), Chubut Province, Argentina.

Phymaturus ceii (n = 21)

MCN-UNSa 910–18, RP No. 8, 17 km S of San Antonio del Cuy, 25 de Mayo Dept, Río Negro Province, Argentina. MACN 44738 (ex MCN-UNSa 3914), MACN 44739 (ex MCN-UNSa 3918), MACN 44740 (ex MCN-UNSa 3921), MACN 44741 (ex MCN-UNSa 3923), MACN 44742 (ex MCN-UNSa 3928), MACN 44743 (ex MCN-UNSa 3941), MCN-UNSa 3913, 3916, 3920, 3939–40, 3942, on RP No. 6 (40°20047.1''S, 68°58050.3''W, 1,194 m), El Cuy Dept, Río Negro Province, Argentina.

Phymaturus curivilcun (n = 8)

MLP 6339 (holotype). 6340-41, 6342 (three individuals), 6343. Paraje El Mirador (42º 27' S; 70º 03' W; 1100 m, datum = WGS84), Provincial road Nº 4, approximately 80 km NW of Gastre, Cushamen Department, Chubut Province, Argentina. IBIGEO 6180. Paraje El Mirador 42° 26.980' S 70° 02.154' W. 1178m. Provincial road Nº 4, approximately 80 km NW of Gastre, Cushamen Department, Chubut Province, Argentina.

Phymaturus etheridgei (n = 17)

FML 23495 (holotype) FML 23496–501 (paratypes), MCN-UNSa 4305, 07–08, 10, on RP No. 76, between Ingeniero Jacobacci and Moligüe (41°34047.2''S, 69°23033.0''W, 818 m), 25 de Mayo Dept, Río Negro Province, Argentina. FML 8435, MCN-UNSa 3109–13, 43 km N of Moligüe (41°35.8800S, 69°22.6280W), 25 de Mayo Dept, Río Negro Province, Argentina.

Phymaturus excelsus (n = 13)

MCN-UNSa 1582 (holotype), MCN-UNSa 1582-89, RP No. 6, 1 km NW from Ojo de Agua (41°32030''S, 69°51033''W, 1,141 m), Ñorquinco Dept, Rio Negro Province, Argentina.

MCN-UNSa 1590. RP No. 6, 1 km NW from Ojo de Agua (41°32030''S, 69°51033''W, 1,141 m), Ñorquinco Dept, Rio Negro Province, Argentina. MCN-UNSa 1386,88. from Ojo de Agua, Ñorquinco Dept, Rio Negro Province, Argentina. MCN-UNSa 1385,87. from Ojo de Agua, Ñorquinco Dept, Rio Negro Province, Argentina.

Phymaturus indistinctus (n = 24)

IBA 666-1, (Holotype), IBA 666-2–3, 2 km W Lago Munsters, Las Pulgas (700–800 m), Sarmiento Dept., Chubut Province, Argentina. MCN-UNSa 1274–77, Las Pulgas, Sarmiento Dept, Chubut Province, Argentina. MCN-UNSa 3943–55. RP No. 20, 19 km W to Los Manantiales (45°270S, 69°420W, 669 m).

Phymaturus katenke (n = 8) IBIGEO 6165-72. Los Adobes, Paso de Indios, Chubut Province. Argentina. (43°15'37.59"S; 68°53'16.83"W 839m).

Phymaturus manuelae (n = 7)

UNCo-PH 201–02 (paratypes), JAS-DC 1251, 26 km W Comallo, adjacent to RN No. 23, Pilcaniyeu Dept, Río Negro Province, Argentina. MCN-UNSa 3929–30, 3932–33, between Pilcaniyeu and Las Bayas on RN1S40 (ex- RN No. 40; 41°12011.1''S, 70°41030.9''W, 1,014 m), Pilcaniyeu Dept, Río Negro Province, Argentina.

Phymaturus patagonicus (n = 37)

MLP 778 (lectotype), MLP 777 (paralectotype), Chubut Province, Patagonia, Argentina. FML 10079–85, 1 km W from juction of RP 53 and RP 90, 2.2 km SW Meseta El Sombrero, Paso de Los Indios Dept, Chubut Province, Argentina. IADIZA 80, 40 km W Dolavon, 350 m, Gaiman Dept, Chubut Province, Argentina. IBA 783(4), IBA 785, 20 km W from Sombrero, Paso de Los Indios Dept, Chubut Province, Argentina. IBA 787, IBA 789 (7), MCN-UNSa 1284– 86, 40 km W Dolavon, Gaiman Dept, Chubut Province, Argentina. MCN-UNSa 1250–58, 1261, hills in front of El Sombrero, Paso de Los Indios Dept, Chubut Province, Argentina. SDSU 1980, 40 km WSW Dolavon, Gaiman Dept, Chubut Province, Argentina.

Phymaturus payuniae (n = 45)

IBA 769-2, 769-4–8, 769–10, 76912, 769–17, 769–20, 769–24, 769–26 (type series), Payún Plateau (2,000 m), 5 km from Volcán Payún Malargüe Dept, Mendoza Province, Argentina. IADIZA 87-8–9, 20 km SE Volcán Payún (1,800 m) Malargüe Dept, Mendoza Province,

Argentina. MCZ 152079–81, basaltic rocks of the Payún Plateau, Malargüe Dept, Mendoza Province, Argentina. REE-SDSU 2330–32, 2339, SDSU 1981–84, 10 km SW base of Volcán Payún, Mendoza Province, Malargüe Dept, Argentina. MCN-UNSa 3648–51, 3665–79, on RP No. 183, 16 km S to Payún vulcano (36°40020.8''S, 69°16010.9''W, 1,737 m).

Phymaturus sinervoi (n=9)

MLP 5660 (holotype); MLP 5664; 5920-22; 5929 (paratypes). In rocky outcrops (1000 m) of Cari Laufquen basaltic Tableland in Abi-Saad farm (41°02'12"S, 70°24'30.6"W), adjacent to Provincial Road 6, 61 km north of Ingeniero Jacobacci town, Rio Negro Province, Argentina.

Phymaturus spectabilis (n = 38)

MCN-UNSa 1203 (holotype), MCN-UNSa 1204–11, 1214 (paratypes), on RP No. 6, 28 km S Ingeniero Jacobacci, 25 de Mayo Dept, Rio Negro Province, Argentina. MCN-UNSa (agilis) 1212-13, 1215. on RP No. 6, 28 km S Ingeniero Jacobacci, 25 de Mayo Dept, Rio Negro Province, Argentina. MLP (agilis) 5343 (Holotype), 5344-46, collected in rocky tableland (41° 25' 40" S; 69° 45' 07" W; 1030 m), close to Provincial road 6 south of Ingeniero Jacobacci, Rio Negro Province, Argentina. 10 March 2006. MLP (agilis) 5880-83. 41,4342 S; 69,7534 W. 25 de Mayo Dept, Rio Negro Province, Argentina. 5/2/2011. MLP 5877-79. 41,4342 S; 69,7534 W. 25 de Mayo Dept, Rio Negro Province, Argentina 5/2/2011. FML 23502–15. On provincial road 6, approximately 27 km S of intersection with provincial road 23, Río Negro, Argentina (41°25'43.25"S, 69°45'24"W; 924 m). FML (agilis) 23503–23505, 23508– 09. On Ruta Prov. 6, approximately 27 km S of intersection with Ruta Prov. 23, Río Negro, Argentina (41°25'43.25"S, 69°45'24"W; 924 m).

Phymaturus spurcus (n = 17)

MCZ 14791 (Holotype), MCZ 14915 (paratype) Huanuluan, Pilcaniyeu Dept, Rio Negro Province, Argentina. MCN-UNSa 1237–44, 1246– 49, hills opposite of Estancia Huanuluan, RN No. 23, 22 km W from Ingeniero Jacobacci, 25 de Mayo Dept, Rio Negro Province, Argentina. MVZ 6177. Huanuluan, Pilcaniyeu Dept, Rio Negro Province, Argentina.

Phymaturus somuncurensis (n = 29)

IBA 470, IBA 472 (type series), MACN 37436–40, MCZ 156909, 170443–44, Laguna Raimunda, Meseta de Somuncurá, 9 de Julio Dept, Rio Negro Province, Argentina. FML 1038, Laguna Raimunda, Meseta de Somuncurá (1400 m) 9 de Julio Dept., Rio Negro Province Argentina. IADIZA 212, Meseta de Somuncurá, Cerro Corona, 9 de Julio Dept., Rio Negro Province, Argentina. IBA 507, 4, Laguna Raimunda, Meseta de Somuncurá, Rio Negro Province, 9 de Julio Dept., Argentina. MACN 37431–35, 2 km N Casco Cecchi, Meseta de Somuncurá, 9 de Julio Dept, Rio Negro Province Argentina. REE-SDSU 2433–35, N from Laguna Raimunda, Meseta de Somuncurá. 9 de Julio Dept, Rio Negro Province, Argentina. SDSU 1780–83, 2 km N Laguna Raimunda, Meseta Somuncurá, 9 de Julio Dept, Rio Negro Province, Argentina. MCN-UNSa 4550 (SJ 25) (41°12013.95''S, 66°53031.94''W, 1060 m), Meseta Somuncurá. 9 de Julio Dept, Rio Negro Province, Argentina.

Phymaturus tenebrosus (n = 18)

MCN-UNSa 1271 (Holotype), MCN-UNSa 1264–70, 1272–73 (paratypes), RN No. 40, 20 km S Cerro Alto; Pilcaniyeu Dept, Río Negro Province, Argentina. MCN-UNSa 1591–95, 1597–99, RN No. 23 between San Carlos de Bariloche and Pilcaniyeu, Pilcaniyeu Dept, Río Negro Province, Argentina.

Phymaturus yachanana (n = 14)

MLP 2636 (holotype). 1.74 km South of the Sierra Grande town, east of National Road 3 (41°37'S, 65°20'W, 270 m, datum = WGS 84), San Antonio department, Río Negro province, Argentina. MCN-UNSa 1334-35. Eight kilometer north from junction of RP No. 8 and RP No. 4, Sierra Colorada, Telsen Dept, Chubut Province, Argentina. MCN-UNSa 3281, MCN-UNSa 4314, 4319–20, 8 km north of junction between RP No. 8 and RP No. 4 (on RP No. 8–42°41040.9''S, 65°49017.7''W), Telsen Dept, Chubut Province, Argentina. MCN-UNSa 3272,3274, 3276-78, 3280. Provincial road 8, 80 km NW from intersection of provincial road 4. 42° 11' 20.8" S; 66° 23' 6.7"W. IBIGEO 6228. A 64 km de Pto Madryn cerca del cruce rutas 4 y 8, sobre ruta 8.

Outgroups (DNA sequences)

L. archeforus LJAMM–CNP 9240; *L. buergeri* LJAMM–CNP 2744; *L. kingii* LJAMM–CNP 326/LJAMM3040; *L. lineomaculatus* LJAMM–CNP 7471/SDSU4268; *L. petrophilus* LJAMM–CNP 11121/BYU47098. *P. palluma* MCN 3627. *P. vociferator* LJAMM–CNP 3432. *P. mallimaccii* LJAMM–CNP 2035/MCN1741.

Morphology data:

Liolaemus kingii: SDSU 1670-71, 3378. MCZ 150291. Golfo de San José, Pen. Valdéz, Chubut, Argentina. MCZ 11837, 39-40. Patagonia. MCZ 18948-49. Ultra Cautín, Prov. Cautín Chile. MCN 1545-50. Rio Seco, Ruta Nac. 3 entre San Julián y Tres Cerros, S 48°31.817'; O 67°44.081'. MCN 1551-52. Tres Cerros, S48°07.160'; O67°38.384'. MCN 1324. Las

Pulgas (Cerro frente a Gruta de la virgen) Dpto. Sarmiento, Prov. Chubut, Argentina.

Phymaturus mallimaccii (N = 17): FML 21114. Camino entre Famatina y Mina la Mejicana, 3460 m a.s.l. 28°58'45,9"; S67°42'58,1"W. FML 21117. Camino entre Alto Carrizal y Cueva de Pérez, pasando Famatina 8 kms al sur. 3460 m a.s.l. 28°58'45,9"S; 67°42'58,1"W. FML 1721. (6 ejemplares) Nevado de Famatina, Cueva de Pérez. 3800-4000 m a.s.l. MCN-UNSa 920 (LA 2779) on the road to La Mejicana (28°54'43'S, 67°42'47'W, 3430 m a.s.l.), Sierra de Famatina, Famatina Department, La Rioja Province, Argentina. MCN-UNSa 1483-84 (LA 2035 2002). Camino a la Mejicana, 3430 m a.s.l. 28°54'43"S; 67°42'47"W. Dpto. Famatina. Prov. de La Rioja (CS). MCN-UNSa 1741, Cueva de Pérez, Famatina Department, La Rioja Province, Argentina. MCN-UNSa 3567 (LA 2196). Camino a Mina La Mejicana, 3420 m a.s.l. 28º54´43´´S; 67º42´47´´W. Famatina Department, Prov. La Rioja. MLP 5360. Quebrada de Ampallados, Cerro de la Cueva de Pérez. S 28 99798; W 67 73719. REE–CSUN 183, 489–491, Sierra de Famatina, Cueva de Pérez, Famatina Department, La Rioja Province, Argentina.

Phymaturus palluma (= *Phymaturus gynechlomus*; N = 32). MCN 3130–3131, Portillo Argentino (Cordón del Portillo, 33°36'53.8''S, 69°29'16.7''W), Mendoza Province, Argentina. MCN 3612–13, 3619–22, Portillo Argentino, Arroyo Guardia Vieja (33°36'53.8''S, 69°29'16.7''W), Mendoza Province, Argentina. MVZ 126991, Valle Hermoso (35°20'S, 70°15'W), Malargüe Departament, Mendoza Province, Argentina. MVZ 126992–126894, Lago de la Niña Encantada (33°18'S, 69°83'W, 2000 m a.s.l.), 6 km east of Molles, Mendoza Province, Argentina. MVZ 126995, at the north end of Valle Hermoso (35°11'S, 70°10'W), Malargüe Departament, Mendoza Province, Argentina. MVZ 126996–126999, 4 km NW from Cerro Chupasangral (33°21'S, 69°51'W, 2800 m a.s.l.), Quebrada de Chupasangral, Tupungato Department, Mendoza Province, Argentina. MVZ 127025–127027, 2 km east from Agua Botada (35°62'S, 69°95'W), Malargüe Departament, Mendoza Province, Argentina. MVZ 180771–180774, Quebrada Cruz de Piedra (34°26'S, 68°90'W), San Carlos Departament, Mendoza Province, Argentina. MCN 3627–30, 3635–43, 3645, Road to Laguna Diamante (34°14'33.6''S, 69°24'00.0''W), San Carlos Departament, Mendoza Province, Argentina.

Phymaturus vociferator (N= 7) (*P.* cf. *palluma* CH, *P* sp2., *P.* sp chi in Lobo and Quinteros, 2005; Lobo et al 2012; Lobo et al 2016). MVZ 199435-38 & 230992. Hotel Termas de Chillán. Región VIII (= Región del Bío Bío), Chile. MCZ 165456. Cordillera de Chillán. Chile. MCZ 169935. Chile.

APPENDIX 2. List of all the species, voucher numbers and Genbank accession numbers of the sequences employed in this study, new sequences obtained in this study are marked in bold. Observations: *P.* sp. 11 of Morando *et al.* (2013) here is named as *P. camilae* (Scolaro *et al.*, 2013); *P.* sp. 16 of Morando *et al.* (2013) is named here as *P. rahuensis* (González Marín *et al.*, 2016); *P.* sp. 17 of Morando *et al.* (2013) here is named as *P. robustus* (Lobo *et al.*, 2021); *P.* sp. 18 of Morando *et al.* (2013) is named here as *P. cacivioi* (Lobo & Nenda, 2015); *P.* sp. 20 of Morando *et al.* (2013) is named here as *P. sinervoi* (Scolaro *et al.*, 2012); *P.* sp. 21 of Morando *et al.* (2013) is named here as *P. yachanana* (Avila *et al.*, 2014); *P. felixi*_b of Morando *et al.* (2013) is named here as *P. felixi*. *Asterisks mark species with fragments belonging to different vouchers specimens. The accession numbers AY173912, KF967760, KF967803, KF967809, KF967837, JF272897, JF272908, KF967611 and KF967641 could be aligned only when reverse complement transformation was used.

Species	Vouchers	COI	12S	Cytb	ND4	Cmos	NTF3	PLRL	PNN	Phy38	Phy41	Phy60	Phy64	Phy84	Phy87	Phy89
L. archeforus	LJAMM–CNP 9240		KF969004	KF968826		KF968633		JF272897	KF967760							
L. buergeri	LJAMM–CNP 2744		AY173912	AY173843	AY367868	AY367896										
*L. kingii**	LJAMM–CNP 326/ LJAMM3040		KF969053	JN614929	DQ237465	KF968680		JF272908	KF967803							
*L. lineomaculatus**	LJAMM–CNP 7471/ SDSU4268		JX522193	JX522338	AY367875	KF968687		KF967611	KF967809							
*L. petrophilus**	LJAMM–CNP 11121/ BYU47098		KF969091	JN847092	AY367849	KF968714		KF967641	KF967837							
P. palluma	MCN 3627		KT203839	KT203834	KT203854	KT203821										
P. vociferator	LJAMM–CNP 3432		JX969067	JX969016		JX969524	JX969602	JX969504	JX969425	JX969130	JX969322	JX969177	JX969117	JX969223		JX969274
*P. mallimaccii**	LJAMM–CNP 2035/ MCN1741		JX969062	JX969011	KT203847	JX969519	JX969597	JX969499	JX969422		JX969317	JX969173	JX969112	JX969218		JX969269
*P. cacivioi**	LJAMM–CNP 5549/ MCN3938		JX969106	JX969055	MG888420	JX969559	JX969590	JX969492	JX969461	JX969166	JX969360	JX969211	JX969385	JX969262	JX969415	JX969310
*P. calcogaster**	CHIVC472/ LJAMM–CNP 6856/ MCN4301	KU565043	JX969081	JX969030	MG888406	JX969535	JX969565	JX969467	JX969438	JX969144	JX969336	JX969187	JX969366	JX969237	JX969391	JX969287
P. camilae	LJAMM–CNP 3408		JX969110	JX969059		JX969563	JX969594	JX969496	JX969465	JX969170	JX969364	JX969215	JX969389		JX969419	JX969314
*P. castillensis**	CHIVC345/ MCN3976	KU565051	MG888427	MG888428	MG888407	MG888430										
*P. ceii**	CHIVC444/ LJAMM–CNP 1584/ MCN3940	KU565055	JX969082	JX969031	MG888408	JX969536	JX969566	JX969468	JX969439	JX969145	JX969337		JX969367	JX969238	JX969392	JX969288
*P. chenqueniyen**	LJAMM–CNP 3507/ IBIGEO6197	OP422205	JX969101	JX969050	OP433649	JX969554	JX969585	JX969487	JX969457	JX969161	JX969355	JX969206	JX969380	JX969257	JX969411	JX969306
P. curivilcun	IBIGEO6180	OP422206	OP453356	OP425723	OP425726	OP425729										

*P. delheyi**	LJAMM–CNP 5221/ MCN4970		JX969098	JX969047	MG888409	JX969551	JX969582	JX969484	JX969455	JX969158	JX969352	JX969203	JX969379	JX969254	JX969408	JX969303
*P. etheridgei**	CHIVC461/ LJAMM–CNP 5897/ MCN4308	KU565058	JX969080	JX969029	MG888410	JX969534	JX969564	JX969466	JX969437	JX969143	JX969335	JX969186	JX969365	JX969236	JX969390	JX969286
*P. excelsus**	CHIVC476/ LJAMM–CNP 2265/ MCN1388	KU565061	JX969083	JX969032	MG888411	JX969537	JX969567	JX969469	JX969440	JX969146	JX969338	JX969188	JX969368	JX969239	JX969393	JX969289
*P. felixi**	CHIVC350/ LJAMM–CNP 3825/ MCN3988	KU565064	JX969095	JX969044	MG888412	JX969549	JX969579	JX969481	JX969452	JX969156	JX969349	JX969200	JX969377	JX969251	JX969405	JX969300
*P. indistinctus**	CHIVC356/ LJAMM–CNP 2124/ MCN3954	KU565066	JX969084	JX969033	MG888413	JX969538	JX969568	JX969470	JX969441	JX969147	JX969339	JX969189	JX969369	JX969240	JX969394	JX969290
P. katenke	IBIGEO6165	OP422207	OP453357	OP425724	OP425727	OP425730										
P. maquinchao	IBIGEO6211	OP422208	OP453358	OP425725	OP425728	OP425731										
*P. manuelae**	LJAMM–CNP 5448/ MCN3932		JX969085	JX969034	MG888414	JX969539	JX969569	JX969471	JX969442			JX969190	JX969370	JX969241	JX969395	JX969291
*P. nevadoi**	CHIVC110/ LJAMM–CNP 4431/ MCN3656	KU565080	JX969086	JX969035	MG888415	JX969540	JX969570	JX969472	JX969443	JX969148	JX969340	JX969191		JX969242	JX969396	JX969292
P. niger	MCN 5565		MW310691	MW310692	MW310694											
*P. patagonicus**	CHIVC362/ LJAMM–CNP 3205/ MCN1285	KU565083	JX969087	JX969036	MG888416	JX969541	JX969571	JX969473	JX969444	JX969149	JX969341	JX969192	JX969371	JX969243	JX969397	JX969293
*P. payuniae**	CHIVC78/ LJAMM–CNP 4437/ MCN3649	KU565110	JX969088	JX969037	MG888417	JX969542	JX969572	JX969474	JX969445	JX969150	JX969342	JX969193	JX969372	JX969244	JX969398	JX969294
P. rahuensis	LJAMM–CNP 5379		JX969104	JX969053		JX969557	JX969588	JX969490	JX969459	JX969164	JX969358	JX969209	JX969383	JX969260	JX969413	JX969308
*P. robustus**	LJAMM–CNP 8916/ MCN 5548		JX969105	JX969054	MW310693	JX969558	JX969589	JX969491	JX969460	JX969165	JX969359	JX969210	JX969384	JX969261	JX969414	JX969309
*P. sitesi**	LJAMM–CNP 10367/ MCN4758		JX969100	JX969049	MG888418	JX969553	JX969584	JX969486	JX969456	JX969160	JX969354	JX969205		JX969256	JX969410	JX969305

P. sinervoi	LJAMM– CNP 6543		JX969108	JX969057		JX969561	JX969592	JX969494	JX969463	JX969168	JX969362	JX969213	JX969387	JX969264	JX969417	JX969312
*P. somuncurensis**	CHIVC494/ LJAMM– CNP 4453/ MCN4550	KU565117	JX969089	JX969038	MG888419	JX969543	JX969573	JX969475	JX969446	JX969151	JX969343	JX969194	JX969373	JX969245	JX969399	JX969295
P. sp. 12	LJAMM– CNP 7975		JX969099	JX969048		JX969552	JX969583	JX969485		JX969159	JX969353	JX969204		JX969255	JX969409	JX969304
P. sp. 14	LJAMM– CNP 3459		JX969102	JX969051		JX969555	JX969586	JX969488		JX969162	JX969356	JX969207	JX969381	JX969258	JX969412	JX969307
P. sp. 15	LJAMM– CNP 8190		JX969103	JX969052		JX969556	JX969587	JX969489	JX969458	JX969163	JX969357	JX969208	JX969382	JX969259		
P. sp. 19	LJAMM– CNP 5541		JX969107	JX969056		JX969560	JX969591	JX969493	JX969462	JX969167	JX969361	JX969212	JX969386	JX969263	JX969416	JX969311
P. sp. 22a*	LJAMM– CNP 6538–6256		JX969096	JX969045			JX969580	JX969482	JX969453	JX969157	JX969350	JX969201	JX969378	JX969252	JX969406	JX969301
P. sp. 22b	LJAMM– CNP 6257		JX969097	JX969046		JX969550	JX969581	JX969483	JX969454		JX969351	JX969202		JX969253	JX969407	JX969302
*P. spectabilis**	CHIVC449/ LJAMM– CNP 3600/ MCN1215	KU565120	JX969090	JX969039	MG888421	JX969544	JX969574	JX969476	JX969447		JX969344	JX969195		JX969246	JX969400	JX969296
*P. spurcus**	CHIVC450/ LJAMM– CNP 3586/ MCN1248	KU565124	JX969091	JX969040	MG888422	JX969545	JX969575	JX969477	JX969448	JX969152	JX969345	JX969196		JX969247	JX969401	JX969297
*P. tenebrosus**	CHIVC440/ LJAMM– CNP 5426/ MCN1272	KU565128	JX969092	JX969041	MG888423	JX969546	JX969576	JX969478	JX969449	JX969153	JX969346	JX969197	JX969374	JX969248	JX969402	JX969298
*P. videlai**	CHIVC359/ MCN4203	KU565131	MG888426	MG888429	MG888424	MG888431										
P. yachanana	LJAMM– CNP 3234		JX969109	JX969058		JX969562	JX969593	JX969495	JX969464	JX969169	JX969363	JX969214	JX969388		JX969418	JX969313
*P. zapalensis**	LJAMM– CNP 8067/ MCN3850		JX969093	JX969042	MG888425	JX969547	JX969577	JX969479	JX969450	JX969154	JX969347	JX969198	JX969375	JX969249	JX969403	

Nota

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First record of myiasis in *Physalaemus cuvieri* Fitzinger, 1826 (Anura: Leptodactylidae) by Diptera

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ABSTRACT
Myiasis is a parasitic disease caused by some species of Diptera, whose larvae feed on their host's tissues. Records of myiasis in Brazilian anurans are scarce in the literature with single records in 12 species divided into 5 families. We report herein the observation of the first case of myiasis in the frog *Physalaemus cuvieri* from an individual found dead.

Key Words: Ecology; Interaction; Natural History; Parasitism.

Myiasis is a parasitic disease caused by larvae of some species of Diptera. In anurans, the most representative families of these parasites are Calliphoridae, Chloropidae, Muscidae, and Sarcophagidae (Eizemberg *et al*., 2008). The larvae feed on tissues of their host, frequently causing death, but in unusual cases individuals can survive (Eaton *et al.* 2008; Souza-Pinto *et al.* 2015).

Records of dipteran infestations are well documented in humans and domestic animals for the reason of their impacts in public health and economics (Hall and Wall, 1995). Despite several records of myiasis in wild animals have been made, studies on Neotropical amphibians are not so common (Travers and Townsend, 2010; Pinto *et al*., 2017). Among them, there are some records in anurans of the Brachycephalidae (*Brachycephalus ephippium*), Bufonidae (*Rhinella margaritifera, Rhinella pombali*), Leptodactylidae (*Leptodactylus latrans, Physalaemus albonotaus*), Hylidae (*Aplastodiscus arildae, Boana beckeri, Boana atlantica, Scinax fuscovarius, Scinax ruber, Dryaderces inframaculata*) and Ranidae (*Lithobates catesbeianus*) families (Schwartz and Sebben, 1992; Eizemberg *et al.* 2008; Souza Jr. *et al.* 1990; Carvalho-Filho *et al.* 2010; Mello-Patiu and Luna-Dias 2010; Oliveira *et al.* 2012, Müller *et al.* 2015; Souza-Pinto *et al.,* 2015; Pinto *et al.* 2017, Mulieri *et al.,* 2018). In this work we report the first case of myiasis in *Physalaemus cuvieri.*

Physalaemus cuvieri is widely distributed throughout Brazil, parts of Argentina, Bolivia, Paraguay, and Venezuela (Frost, 2021). On April 3, 2021, at 11:30 h, during fieldwork in the municipality of Pinheiros (-18,4111233, -40,3013780, WGS 84; 131 m), Espírito Santo state, southeastern Brazil, we found an individual of *P. cuvieri* (Fig. 1A) parasitized by larvae Diptera apparently of the Sarcophagidae family (Fig. 1B). The larvae were not collected, and for this reason specific identification of the specimens was not possible; only adult individuals of this dipteran family can be confidently identified (Mello-Patiu and Luna-Dias, 2010). The individual of *P. cuvieri* was found dead in the leaf litter. When handled, two larvae left the host (one through the mouth and another one through a hole on the left side of its abdomen) (Fig. 1C). During the observation of the event, many other juvenile individuals of *P. cuvieri* were jumping on the litter,

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Figure 1. A) Individual of *Physalaemus cuvieri* (juvenile) killed by the larvae of Diptera; B) Close view of parasitic larva; C) Larvae abandoning the anuran carcass from a hole on the left side of its abdomen; D) Live juvenile of *P. cuvieri* from the same locality.

which confidently allowed the identification of the host (Fig. 1D). The study locality is part of the Atlantic Forest biome domains (Garbin *et al.*, 2017), and corresponds to an understory area of dense ombrophylous forest with some invasive species such as *Acacia*. The climate is tropical humid with a monthly average of 95 mm (Governo-ES, 2021; Climatempo, 2021). There is also a small water body that flows into a permanent lake.

The frog *P. cuvieri* is the second species of the genus registered as parasitized by Diptera. Previously, *Physalaemus albonotatus*, was observed to be attacked by *Lepidodexia* (*Notochaeta*) *adelina* (Mulieri *et al.*, 2018). In South America, Sarcophagidae is the main cause of anuran myiasis (Kraus, 2007; Mello-Patiu and Luna-Dias, 2010). However, only three groups, one genus and two subgenera, were registered parasitizing amphibians: *Sarcophaga*, *Peckia* (*Sarcodexia*) and *Lepidodexia* (*Notochaeta*) (Souza *et al.*, 1989; Hagman *et al.*, 2005).

Records generally point to the death of the anuran by the end of the parasitic stage, or after the larvae abandonment (Lopes 1942; Crump and Pounds 1985; Eizemberg *et al.*, 2008). This fact is probably due to the voracity of the last larval instar, characteristic of dipterans, especially of parasitic and parasitoid species (Coupland and Barker, 2004). Mello-Patiu and Luna-Dias (2010) pointed to the rapid death of anurans, followed by the host abandonment of the larvae hours after capture. This occurrence, also observed in other works, suggests the possibility that parasites respond to the stress caused on the host (Muratori *et al.* 2010).

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Nota

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New additions to the anuran fauna of the Cancão Municipal Natural Park, Serra do Navio, state of Amapá, Brazil

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ABSTRACT

Twelve species of anurans (*Amazophrynella teko*, *Rhinella castaneotica*, *Hyalinobatrachium mondolfii*, *H. tricolor*, *Pristimantis gutturalis*, *P. inguinalis*, *Ranitomeya variabilis*, *Osteocephalus leprieurii*, *Scinax proboscideus*, *Leptodactylus petersii*, *Chiasmocleis hudsoni*, *Synapturanus mirandaribeiroi*), into the seven families (Bufonidae, Centrolenidae, Craugastoridae, Dendrobatidae, Hylidae, Leptodactylidae, Microhylidae) are reported for the first time from Cancão Municipal Natural Park, state of Amapá, North Brazil. The total number of species of anurans known from the park now stands at 61 species. Our results contribute to an increase in the knowledge of the anuran fauna of Eastern Amazonia and Guiana Shield.

Key Words: Amphibians; Natural History; Protected Areas; Amazonia.

The state of Amapá is located in the extreme northeast of the Brazilian Amazon and belonging to the Guiana Shield region. Amapá plays an important role in Brazil's conservation with more than 95% of its original vegetation well-preserved and close to 70% of its extent lying within protected areas (Hilário *et al.,* 2017). The state has 14 protected areas (except private reserves) and five indigenous reserves (Drummond *et al.,* 2008). Despite this good conservation status, little is known about the diversity of anurans of this part of eastern Amazonia (Azevedo-Ramos and Galatti, 2002; Benício and Lima, 2017; Costa-Campos and Freire, 2019). Thus, inventories regarding the biodiversity are a conservation priority, especially because several studies based on deforestation have detected changes in the habitats potentially contributing to declines of some species (Becker *et al.,* 2016).

Cancão Municipal Natural Park is a Municipal Protection Conservation Unit that composes the Protected Areas Mosaic of the eastern Brazilian Amazonia, located in the municipality of Serra do Navio, in the northwest center portion of Amapá state, North Brazil. The Protected Areas that make up the Mosaic of the Eastern Amazon are: Tumucumaque Mountains National Park, Amapá National Forest, Iratapuru River Sustainable Development Reserve, Amapá State Forest, Cancão Municipal Natural Park, Extractive Reserve Beija-Flor Brilho de Fogo and Indigenous Land Wajãpi, Tumucumaque Mountains National Park e Rio Paru D'Este (Drummond *et al.,* 2008).

Of the Protected Areas that make up the Mosaic of the Eastern Amazon only four have amphibians' inventories: Tumucumaque Mountains National Park (Lima, 2008); Amapá National Forest (Benício and Lima, 2017), Cancão Municipal Natural Park (Silva-e-Silva and Costa-Campos, 2018), and Extractive Reserve Beija-Flor Brilho de Fogo (Pedroso *et al.,* 2019). For Iratapuru River

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Sustainable Development Reserve only records and new amphibian's species distributions have been registered (Costa-Campos *et al.,* 2020; Figueiredo *et al.,* 2020; Figueiredo *et al.,* 2021; Tavares-Pinheiro *et al.,* 2021).

Until recently the only literature report on anuran richness from municipality of Serra do Navio was a survey conducted by Silva-e-Silva and Costa-Campos (2018), and although the sampling effort was limited, they recorded significant richness (n = 49 species) for four areas in the Cancão Municipal Natural Park. Here, we provide new additions to the anurans of the municipality of Serra do Navio, in Amapá state, Brazil, and with some comments on the natural history and taxonomic notes.

The sampled area was conducted in the Cancão Municipal Natural Park (0°54'8.82"N; 52°0'19.62"W). The park has an area of 370,26 ha of *terra firme* forest, belonging to the Amazonian forest domain. The climate is classified as Am (Equatorial, Köpper-Geiger classification), with average temperature of 26.1 ºC and rainfall annual of 2,450 mm (NHMET database, 2022).

The first fieldwork was conducted in the park during January to December 2013 (see Silva-e-Silva and Costa-Campos, 2018), whereas the second fieldwork occurs during March 2018 to February 2019 and July 2022. We using "visual encounter surveys" during the day (6:00 – 9:00 hs) and about the first three hours after dark on three nights. Surveys were made in two trails (Fig. 1): i) trail at Cancão forest (0°54'9.72"N, 52°0'17.64"W); and ii) right margin of the River Amapari trail (0°54'2.88"N, 52°0'48.44"W).

Specimens were anesthetized with 5% lidocaine, fixed with 10% formalin and preserved in 70% ethanol (Heyer *et al.,* 1994). Voucher specimens were deposited in the Herpetological Collection of Universidade Federal do Amapá (CECC). The taxonomic nomenclature applied herein follows Segalla et al. (2021) for amphibians with modifications made by Dubois (2017). The conservation status of species was obtained from the Red List of endangered species (IUCN, 2022).

We recorded 12 species of anurans (Fig. 2) belong to families Bufonidae (*Amazophrynella teko*, *Rhinella castaneotica*), Centrolenidae (*Hyalinobatrachium mondolfii*, *H. tricolor*), Craugastoridae (*Pristimantis gutturalis*, *P. inguinalis*), Dendrobatidae (*Ranitomeya variabilis*), Hylidae (*Osteocephalus leprieurii*, *Scinax proboscideus*), Leptodactylidae (*Leptodactylus petersii*), and Microhylidae (*Chiasmocleis hudsoni*, *Synapturanus mirandaribeiroi*), undetected in previous inventories at the Cancão Municipal Natural Park (*see* Silva-e-Silva and Costa-Campos, 2018).

Amazophrynella teko Rojas, Fouquet, Ron, Hernandez-Ruz, Melo-Sampaio, Chaparro, Vogt, Carvalho, Pinheiro, Avila, Farias, Gordo & Hrbek, 2018. Is a small toad of the family Bufonidae (SVL 12.9–15.8 mm in males and 17.9–21.5 mm in females; Rojas *et al.,* 2018). This species occurs in French Guiana, southern region of Suriname and Brazil (Amapá state), at elevations ranging from 70–350 m. The species is diurnal and crepuscular (Rojas *et al.,* 2018). The conservation status of this species is Not Evaluated under IUCN Red List of Threatened Species. Voucher number CECC 3168.

Rhinella castaneotica (Caldwell, 1991). A medium-sized species of the *Rhinella margaritifera* species group (SVL 18.4–23.6 mm in males and 18.9–26.3 mm in females; Caldwell, 1991). It is known from the Amazon Basin in Bolivia, Colombia, eastern Peru, Brazil (Amazonas, Amapá, Pará, and Rondônia), but likely occurs wider in the upper Amazon Basin. *Rhinella castaneotica* are nocturnal and terrestrial toads and natural habitats are tropical moist old-growth lowland forests. It is a forest floor species that breeds in Brazil nut capsules and temporary pools (Caldwell, 1993). There are no known significant threats to this species. *Rhinella castaneotica* is listed as Least Concern under IUCN Red List of Threatened Species. Voucher number CECC 2145.

Hyalinobatrachium mondolfii Castroviejo-Fisher, Vilà, Ayarzagüena, Blanc & Ernst, 2011. Is a small glassfrog (SVL 20.7–23.0 mm in adult males; unknown in females; Castroviejo-Fisher *et al.,* 2011). The species inhabits primary tropical floodplain forest of the eastern Guiana Shield (15–200 m) and the western Amazon. It has exclusively been found in vegetation associated with streams. This species has a broad distribution through the lowland Amazon rainforests, occurring in Bolivia, Brazil (Amapá state), Colombia, French Guiana, Guyana, Suriname and Venezuela (Castroviejo-Fisher *et al.,* 2011; Figueiredo *et al.,* 2020). Calling males were found perched on the underside of leaves at night, at a height of 3 m above ground. *Hyalinobatrachium mondolfii* is listed as Least Concern under IUCN

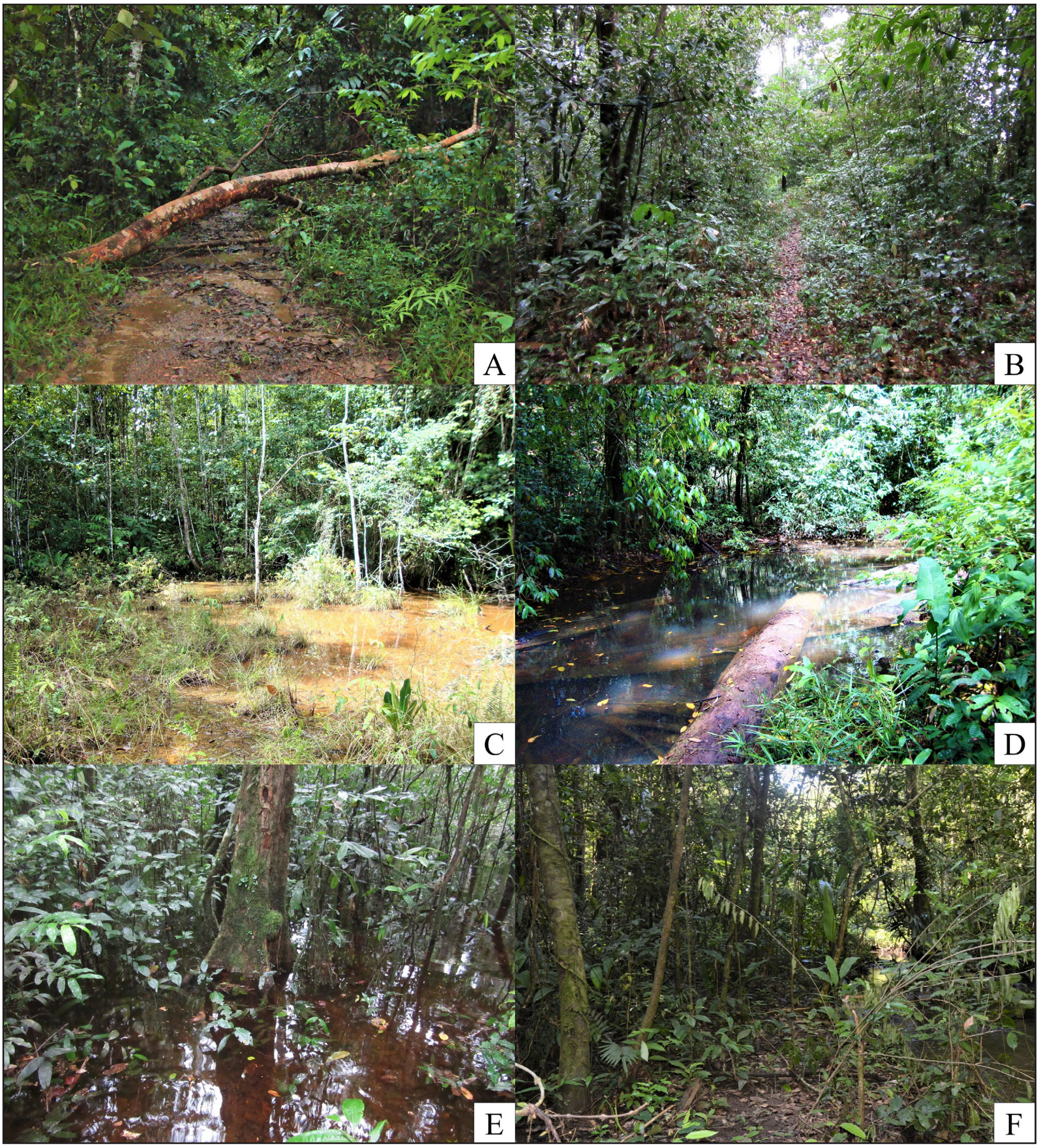

Figure 1. Trails and habitats sampled at the Cancão Municipal Natural Park, municipality of Serra do Navio, Amapá state: **A)** trail at Cancão forest; **B)** right margin of the River Amapari trail; **C)** Treefall gap at Cancão forest; **D)** temporary pond at river Amapari trail; **E)** igapó forest; **F)** *terra firme* forest.

Red List of Threatened Species. Voucher number CECC 1813.

Hyalinobatrachium tricolor Castroviejo-Fisher, Vilà, Ayarzagüena, Blanc & Ernst, 2011. Is a small glassfrog (SVL 20.3–21.0 mm in adult males; unknown in females; Castroviejo-Fisher *et al.,* 2011). Males call on vegetation 4–5 m above streams of 0.5–1.5 m deep. This species occurs in a riparian zone on the right bank of the Amapari River, which has some anthropic disturbance (Costa-Campos *et al.*, 2021) in the Amapá state, and in Crique Wapou, Kaw, French Guiana (type locality) at low elevations to 100 m (Castroviejo-Fisher *et al.*, 2011; Vacher *et al.,* 2020). Males call from the underside of leaves

Figure 2. Anurans recorded at the Cancão Municipal Natural Park, municipality of Serra do Navio, Amapá state: **A)** *Amazophrynella teko*; **B)** *Rhinella castaneotica*; **C)** *Hyalinobatrachium mondolfii*; **D)** *H. tricolor*; **E)** *Pristimantis gutturalis*; **F)** *P. inguinalis*); **G)** *Ranitomeya variabilis*); **H)** *Osteocephalus leprieurii*; **I)** *Scinax proboscideus*; **J)** *Leptodactylus petersii*; **K)** *Chiasmocleis hudsoni*; **L)** *Synapturanus mirandaribeiroi*.

during night and close to egg clutches. *Hyalinobatrachium tricolor* is listed as Least Concern under IUCN Red List of Threatened Species. Voucher number CECC 2783.

Pristimantis gutturalis (Hoogmoed, Lynch & Lescure, 1977). A medium-sized species belongs to Craugastoridae family (SVL 19.0–20.3 mm in males and 17.9–40.9 mm in females; Hoogmoed *et al.,* 1977). Is a diurnal and terrestrial frog found in leaf litter in tropical moist lowland forests and is known from Northern Brazil (Amapá), southern French Guiana, and eastern Surinam (Ouboter and Jairam, 2012; Frost, 2022). *Pristimantis gutturalis* is listed as Least Concern under IUCN Red List of Threatened Species. Voucher number CECC 3486.

Pristimantis inguinalis (Parker, 1940). Adult males measure on average 20.2 mm (Fouquet *et al.,* 2013). Is a arboreal frog of the family Craugastoridae. It is found in Guyana, Suriname, French Guiana, and northern Brazil (Amapá state). Occurs in primary forests at elevations of 50–700 m. Males call from trees 4–6 m above the ground (Cole *et al.,* 2013; Fouquet *et al.,* 2013). It is a common species, and no significant threats to it are known. Its range overlaps with several protected areas (IUCN SSC Amphibian Specialist Group, 2018). *Pristimantis inguinalis* is listed as Least Concern under IUCN Red List of Threatened Species. Voucher number CECC 3498.

Ranitomeya variabilis (Zimmermann & Zimmermann, 1988). Is a small (adult males has a mean SVL 17.4 mm and 18.0 in females; Brown *et al.,* 2008), diurnal and semi-arboreal dendrobatid, with inhabits in primary and secondary rainforests in the understory, canopy, and sometimes in leaf litter at elevations of up to 900-1200 m above sea level (Brown *et al.,* 2008). Its habitat is in the Amazon Rainforest. It most frequently uses bromeliads for breeding, approximately 2.5 m above the forest floor (Brown *et al.,* 2011; Simões *et al.,* 2019). Known distribution consists of widely separated populations, one in northeastern Amazonian Peru and extreme southeastern Colombia, and expected in the adjacent Brazil, Venezuela; extreme southern Guyana; eastern French Guiana; the mouth of the Amazon in Brazil (Frost, 2022; Muell *et al.,* 2022). *Ranitomeya variabilis* is listed as Data Deficient under IUCN Red List of Threatened Species. Voucher number CECC 2609.

Osteocephalus leprieurii (Duméril & Bibron, 1841). The species is a moderate-sized treefrog (SVL max size 63 mm, unknown sex; Lescure and Marty, 2000; Ouboter and Jairam, 2012) distributed throughout the Guiana Shield in French Guiana, Surinam, Guyana, Venezuela, Ecuador, Peru, Bolivia, and northern Brazil in Amazonas and Amapá (Santana *et al.,* 2008; Barrios-Amorós *et al.,* 2019; Figueiredo *et al.,* 2021). This species is an explosive breeder associated to lowland rainforest, where it occurs both in *terra firme* and in seasonally flooded forest (Jungfer and Hödl, 2002). *Osteocephalus leprieurii* is listed as Least Concern under IUCN Red List of Threatened Species. Voucher number CECC 3843.

Scinax proboscideus (Brongersma, 1933). The species is a moderate-sized (SVL max size 46 mm, unknown sex; Lescure and Marty, 2000; Ouboter and Jairam, 2012). Is a species of frog in the family Hylidae, currently known from French Guiana, Guyana, Suriname, and expected to be found in Brazil (Frost, 2022). Its natural habitats are subtropical or tropical moist lowland forests and intermittent freshwater marshes (Lescure and Marty, 2000). This species is categorized as Least Concern according to the IUCN Red List of Threatened Species. Voucher number CECC 2740.

Leptodactylus petersii (Steindachner, 1864). Is a small to moderate size (SVL 26.6–41.1 mm in males and 31.2–51.3 mm in female; De Sá *et al.,* 2014). Is a species of frog in the family Leptodactylidae found widely in the Guianas and the Amazon Basin. It has found in tropical rainforest, forest edge, open areas, savanna enclaves in the tropical rainforest, and open cerrado formations below 600 m (De Sá *et al.,* 2014). This nocturnal frog is usually found on the ground near water. Eggs are laid in a foam nest near water, to which the tadpoles will later move (Lima *et al.,* 2012). *Leptodactylus petersii* is listed as Least Concern under IUCN Red List of Threatened Species. Voucher number CECC 2398.

Chiasmocleis hudsoni Parker, 1940. Is a small-sized (SVL 14.9–16.4 mm in males and 19.9 mm in the single female; Costa-Campos *et al.,* 2019) common species living in tropical rainforests at elevations below 300 m, nocturnal, terrestrial and fossorial that inhabits streamside ponds (Rodrigues *et al.,* 2008). Is found in French Guiana, Suriname, Guyana, Guianan Venezuela, Colombia (Amazonas), and in Brazil at southern Amazonas, as well as at several localities in central and center-east Pará and from Serra do Navio municipality, Amapá state (Lima *et al.,* 2012; Peloso *et al.,* 2014; Costa-Campos *et al.,* 2019). *Chiasmocleis hudsoni* is listed as Least Concern under IUCN Red List of Threatened Species. Voucher number CECC 2262.

Synapturanus mirandaribeiroi (Nelson & Lescure, 1975). Is a medium-sized (SVL 26.2–30.8 in males and 28.6–34.4 in females; Fouquet *et al.,* 2021). The species is found in *terra firme* forests between 100–400 m above sea from the eastern Guiana Shield in Guyana, Suriname, south to Parque Estadual Rio Negro, Amazonas, Brazil; probably occurs in the northern part of the states of Amapá, Pará, and Roraima, in Brazil (Fouquet *et al.,* 2021).

Synapturanus mirandaribeiroi is listed as Least Concern under IUCN Red List of Threatened Species. Voucher number CECC 3847.

Previous research in the Cancão Municipal Natural Park represents the only officially published list of anurans present in the area (Silva-e-Silva and Costa-Campos, 2018). The authors recorded 49 anuran species of anurans belonging to 11 families: Allophrynidae (1); Aromobatidae (2), Bufonidae (5), Centrolenidae (1), Craugastoridae (5), Dendrobatidae (2), Eleutherodactylidae (1), Hylidae (18), Leptodactylidae (10), Phyllomedusidae (3) and Pipidae (1). In our study, we identify 12 species not registered in the previous study (i.e., Silva-e-Silva and Costa-Campos, 2018) increasing a known richness of 61 anurans species for the area.

Although the Cancão Municipal Natural Park is located on the edge of the urban expansion area of the municipality of Serra do Navio (Drummond *et al.,* 2008), the current number of 61 anuran species uncovered a representative sample of the anurofauna, when compared to other conservation units that are part of the Mosaic of the Eastern Amazon: 25 species listed for Extractive Reserve Beija-Flor Brilho de Fogo (Pedroso-Santos *et al.,* 2019); 53 species listed for Amapá National Forest (Benício and Lima, 2017), and 70 species listed for Tumucumaque Mountains National Park (Lima, 2008). The presence of new records in the study area (e.g., *Hyalinobatrachium mondolfii*, *H. tricolor* and *Chiasmocleis hudsoni*) according to previously studies (Costa-Campos *et al.,* 2019; Figueiredo *et al.,* 2020; Costa-Campos *et al.,* 2021) evidence the incipience of knowledge in the context of anuran fauna regional and revealing the importance of the anurans inventories in areas not samples.

Our results contribute to an increase in the knowledge of the anuran fauna of Eastern Amazonia and Guiana Shield, in which a lowland tropical forest in the municipality of Serra do Navio is inserted. With the increase in deforestation and logging in Brazilian Amazonia (Fearnside, 2005), the protection of these areas is importance for the conservation of the local anurofauna, considering the descriptions from new species (Taucce et al., 2020; Carvalho *et al.,* 2022) and the extension restricted and endemism of anurans in the region (Costa-Campos *et al.,* 2016; Pezzuti *et al.,* 2022). We recommended that future sampling designs include these areas to better characterize the amphibian diversity of Amazonian fauna.

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Nota

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Amelanism in *Amphisbaena darwinii* Duméril & Bibron, 1839 (Squamata: Amphisbaenidae)

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ABSTRACT
Color anomalies are rarely reported in Amphisbaenia. We present the first record of amelanism in this group based on a specimen of *Amphisbaena darwinii* from Argentina. The photos were uploaded to a citizen science platform, reinforcing the positive impact of citizen science to filling gaps in our knowledge about biodiversity.

Key Words: Amphisbaenia; Citizen Science; Color Anomaly; Hypopigmentation; iNaturalist

Conspicuous chromatic anomalies occur due to pigmentation production disturbances causing aberrant coloration of the skin (Rook *et al.*, 1998). Such anomalies are not common in wild squamates, but have been frequently reported for snakes, especially cases of hypopigmentation (Borteiro *et al.*, 2021). Traditionally, hypopigmentation anomalies were classified as albinism, leucism, and piebaldism. In albinism, there is a complete absence of pigmentation of the skin and eyes caused by hereditary disposition compromising melanocytes, responsible for melanin production (Griffiths *et al.*, 2016). On the other hand, in leucism and piebaldism, which are also known as 'partial albinism', the eyes are pigmented, but there is an almost complete absence of pigmentation in skin (leucism) or there is a pattern of unpigmented patches along the body (piebaldism) (Prüst, 1984; Bechtel, 1991; Lamoreux *et al.*, 2010; Abreu *et al.*, 2013). Recently, Borteiro *et al.* (2021) reviewed color anomalies in Neotropical snakes and proposed a standardized terminology to be used in reptiles, particularly in cases of hypopigmentation: amelanism, albinism, hypomelanism, leucism, and piebaldism.

Hypopigmentation is rarely reported in worm lizards (Amphisbaenia), although it is suggested that such color anomalies would be of little adaptative harm to fossorial species (Sazima & Di-Bernardo, 1991; Kornilios, 2014; Perez & Alvares, 2020). There are records of hypopigmentation in *Amphisbaena munoai* (Perez & Alvares, 2020), *A. darwinii* (cited as *A. d. trachura*) (Chalkidis & Di-Bernardo, 2004), *Blanus strauchi* (Avcý *et al.*, 2018; Kazilas *et al.*, 2018), and *B. vandellii* (cited as *B. cinereus*) (Malkmus, 1997; Cabana & Vázquez, 2008). With the exception of an albino specimen of *B. strauchi* (Avcý *et al.*, 2018), those reports refer to cases of piebaldism, sometimes cited as partial albinism (Malkmus, 1997; Chalkidis & Di-Bernardo, 2004; Cabana & Vázquez, 2008) or even complete albinism (Fig. 1 in Cabana & Vázquez 2008) (Table 1).

On 24 October 2021, at 6:16 p.m., in La Capilla, Buenos Aires Province, Argentina (34.8925° S, 58.2827° W), the father of MC was shoveling in the backyard, when he unearthed a specimen of *Amphisbaena darwinii* which was buried about 50 cm deep (Fig. 1). The specimen was photographed by MC and released. The photos were uploaded to the citizen science website iNaturalist (https://www.inaturalist.org/observations/99297581), where they caught the attention of the remaining authors.

Amphisbaena darwinii is known to occur from

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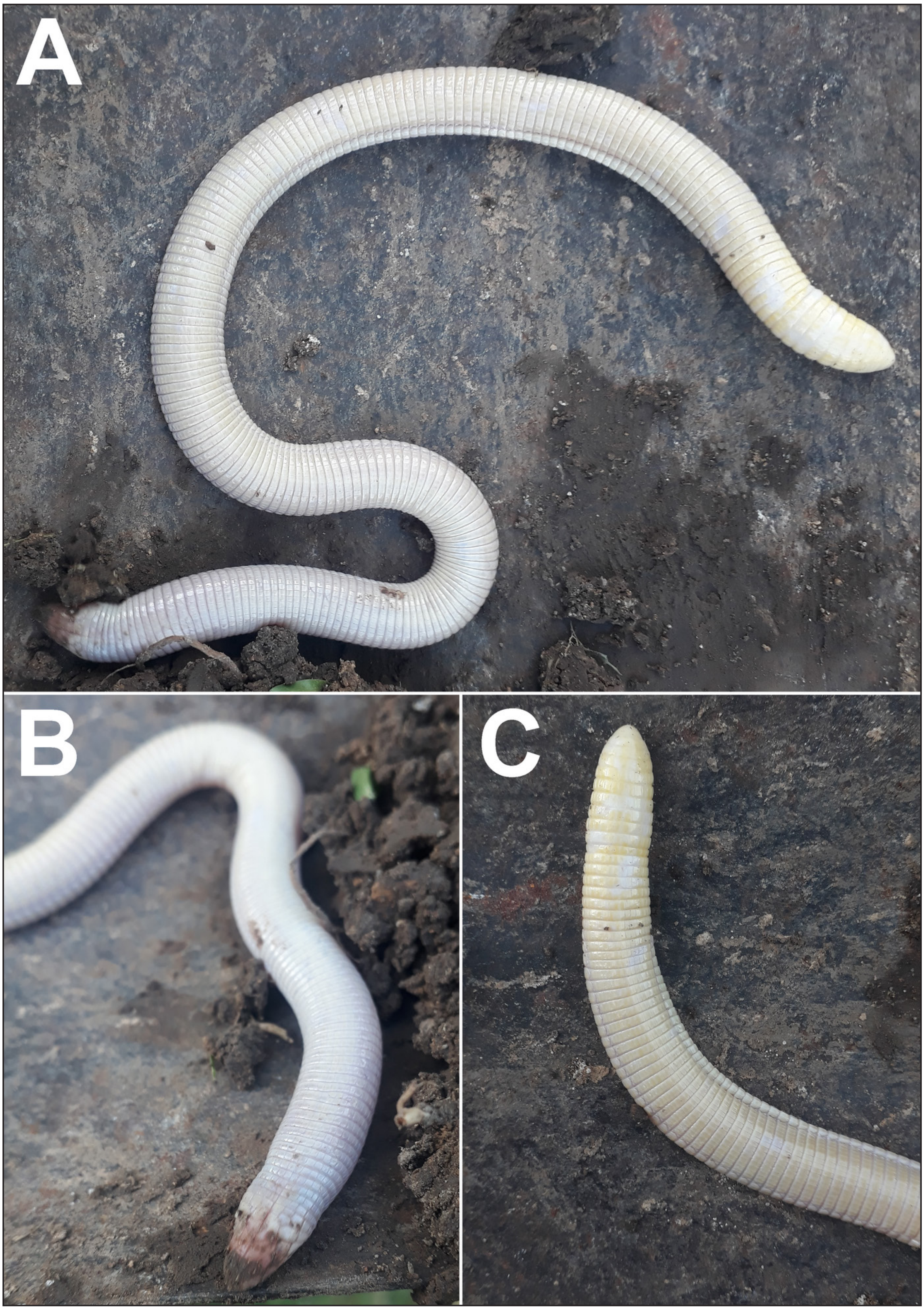

Figure 1. Amelanistic specimen of *Amphisbaena darwinii* unearthed at La Capilla, Buenos Aires Province, Argentina, while a backyard was being shoveled. A) dorsal view of the specimen; B) detail of the head and anterior portion of the body; C) detail of the posterior portion of the body and the tail (note that the tail is not tuberculate and exhibits a pale-yellow color).

Table 1. Published reports of color anomalies in Amphisbaenia.

Taxon	Color anomaly	Source
Amphisbaena darwinii “heterozonata”	Amelanism	This study
Amphisbaena darwinii “trachura”	Piebaldism	(Chalkidis and Di-Bernardo, 2004)
Amphisbaena munoai	Piebaldism	(Perez and Alvares, 2020)
Blanus strauchii	Albinism	(Avcý et al., 2018)
Blanus strauchii	Piebaldism	(Kazilas et al., 2018)
Blanus vandellii	Piebaldism	(Malkmus, 1997; Cabana and Vázquez, 2008)

eastern Bolivia to central Argentina (Montero, 2016). Three subspecies were traditionally recognized: *A. d. darwinii* Duméril & Bibron, 1839, *A. d. heterozonata* Burmeister, 1861 and *A. d. trachura* Cope 1885 – although *darwini* is used by many authors (Gans, 1966; Vanzolini, 2002; Montero, 2016), the original spelling is *darwinii* (Duméril & Bibron, 1839). Some authors have considered these subspecies as valid species (Vanzolini, 2002; Gans, 2005; Perez *et al.*, 2012), but Montero (2016) argued that differences observed are due to clinal variation in morphology, synonymizing *A. heterozonata* and *A. trachura* with *A. darwinii*, without recognition of subspecies. The taxonomy of these taxa was recently reviewed, but still not formally published (Perez, 2016).

Although the photographed specimen was not collected, we can confidently assign its identity to *A. darwinii* due to the following characters that fall within the known variation of the species (Montero, 1996; Vanzolini, 2002): snout rounded, annuli (body+lateral+(autotomy)caudal), 186+2+(8)17; dorsal segments, 13. The number of body annuli (186) falls slightly below the known range of 188–209 in populations from central-eastern Argentina (traditionally assigned to *A. d. heterozonata*; Montero, 1996), but is consistent with the low count observed in the southernmost populations; nevertheless, the counts may be inaccurate as they are based on a photo in dorsal view (Fig. 1A), and the position of the cloaca was estimated based on the posterior end of the lateral sulci. The specimen exhibits a smooth surface at the end of the tail, in congruence with central-eastern Argentina populations (tuberculated in Brazilian populations, traditionally assigned to *A. d. trachura*). We can eliminate the other amphisbaenian species that may occur in the area (*A. kingii* and *A. angustifrons angustifrons*) (Montero, 1996), based on the rounded head shape (keeled in *A. kingii*) and the presence of a well-marked autotomy annulus (absent in *A. a. angustifrons*) (Gans & Diefenbach, 1972).

Typical specimens of *A. darwinii* are brown dorsally (with pigmentation more concentrated in the center of each dorsal segment/scale), darker in the head and tail (Gans, 1966). The photographed specimen reported here clearly lacks normal pigmentation, except for some pale-yellow segments on the posteriormost portion of the body, including the tail (Fig. 1C). Based on the terminology proposed by Borteiro *et al.* (2021), the present case cannot be considered albinism, as there is not a ‘total absence of pigments’, evidenced by the presence of yellowish stains on the body. The absence of melanin rules out that it may be a case of hypomelanism, leucism or piebaldism (Borteiro *et al.*, 2021). Therefore, we can assume the individual here reported was amelanistic in the sense of Borteiro *et al.* (2021). To the best of our knowledge, this is the first published report of amelanism in Amphisbaenia (Table 1). In the past decades, RM examined hundreds of *A. darwinii* in collections (Montero, 2016) and never recorded naturally unpigmented specimens, suggesting hypopigmentation is a rare condition in this taxon.

Citizen science is gaining space in the last years as an important tool to increase our understanding of biodiversity (Suprayitno *et al.*, 2017; Rowley 2020; Maritz & Maritz, 2020; Yves *et al.*, 2021), and iNaturalist stands out as one of the main platforms for citizen scientists (Hochmair *et al.*, 2020; Maritz & Maritz, 2020; Marshall *et al.*, 2020). Our report reinforces the relevance and positive impact of citizen science in contributing to filling gaps in our knowledge about the natural world, including the secretive worm lizards.

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Cuad. herpetol. 36 (2): 251-257 (*2022)*

Hidden among bromeliads in the Brazilian semiarid: first records of *Phyllopezus lutzae* for the Caatinga domain and its predation by *Tropidurus hispidus*

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ABSTRACT

During wildlife rescue and monitoring activities, we recorded 142 individuals of *Phyllopezus lutzae* in the municipalities of Tucano and Nova Soure, state of Bahia, Northeastern Brazil. These records are the first of this species in the Caatinga domain. Moreover, an adult individual of *Tropidurus hispidus* was recorded attempting to subdue an adult *P. lutzae*. Beyond to expand the known distribution range of the species, our records show that *P. lutzae* inhabits an ecological and climate domain different from Atlantic Forest where it was previously known, and that it is a potential prey of *T. hispidus*.

Key Words: Caatinga; Distribution; Predator-prey interaction; Squamata.

The Caatinga domain (Queiroz *et al.*, 2017) was recognized as little diverse in Squamata reptiles, being represented by a set of species shared with other domains of the diagonal of open formations in South America (Vanzolini, 1974, 1988). In recent years, this comprehension has changed, with an increase in the number of known species and endemisms for Caatinga (Guedes *et al.*, 2014; Mesquita *et al.*, 2017). Currently, it is known that the heterogeneity of vegetation types and morphoclimatic conditions within the Brazilian semiarid region contribute to a representative diversity, one of the most important among semiarid areas around the world (Silva *et al.*, 2017a). However, there are still deep scientific gaps about the composition and geographic distribution of species, the Wallacean Shortfall (Lomolino, 2004), including regarding reptiles.

Phyllodactylidae comprises 160 species belonging to 10 genera (Dubeux *et al.*, 2022; Uetz *et al.*, 2022), of which 14 species are recorded in Brazil (Costa *et al.*, 2022 “2021”; Dubeux *et al.*, 2022). *Phyllopezus* comprises eight species distributed throughout South America (Dubeux *et al.*, 2022; Gamble *et al.*, 2012; Uetz *et al.*, 2022), six of which occur in the Brazilian territory (Costa *et al.*, 2022 “2021”; Dubeux *et al.*, 2022). *Phyllopezus lutzae* (Loveridge, 1941) (Fig. 1A) is endemic to Brazil and has its distribution restricted to the Northeastern

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Figure 1. A. Adult individual of *Phyllopezus lutzae* registered in Tucano, Bahia, Nortehastern Brazil; B. Clump of *Aechmea* bromeliads in the studied area; C. Adult male of *Tropidurus hispidus* approaching of an adult *P. lutzae*; D. A *T. hispidus* biting *P. lutzae* on the head in an attempted predation.

Atlantic Forest, occurring from the state of Paraíba to Southern Bahia (Albuquerque *et al.*, 2019) (Table 1, Fig. 2). This species inhabits rainforests and restinga habitats (vegetation types with marine influence and associated with coastal sand deposits; Costa *et al.*, 2018), intimately associated with bromeliads (Albuquerque *et al.*, 2019; Loveridge, 1941). Here, we report the first records of this species in the Brazilian semiarid and its attempted predation by the lizard *Tropidurus hispidus* (Spix, 1825).

During wildlife rescue and monitoring activities for the installation of wind power plants (Complexo Eólico Tucano) between February and November 2021, 141 individuals of *P. lutzae* were registered in the municipality of Tucano (11°11’ 58.80” S, 38°46’57.02’ ‘ W, 405 m elevation) and one in the municipality of Nova Soure (11°17’04.67” S, 38°28’07.14” W, 239 m elevation), in the Raso da Catarina ecoregion (Veloso *et al.*, 2002; Silva *et al.*, 2017b), Bahia, Brazil. The region is located in the part of Brazil most affected by drought, known as the “Polygon of Droughts” (Ab’Sáber, 2003). The climate of both municipalities is classified as DdA´a´ according Thornthwaite (1948), with average annual rainfall of 561.2 mm in Tucano and 891 mm in Nova Soure (SEI, 1999). During the wildlife rescue, the individuals were captured manually during vegetation suppression activities, and later they were released in areas close to the activity sites. For wildlife monitoring activity, individuals were recorded during nocturnal searches. The specimens were always found associated to ground bromeliads, on the leaves, inside rosettes or in the vicinities of them when felled during vegetation removal.

The taxonomic determination of individuals was based on the following diagnostic characters: presence of undivided interdigital lamellae, absence of dorsal tubercles, rudimentary or absent pollex, and the dorsal typical color pattern of the species (gray to orange dorsal background with darker small marks, almost regular in size and spacing; Loveridge, 1941; Dubeux *et al.*, 2022). We collected five individuals between 20 and 22 November 2021 in the municipality of Tucano as voucher specimens. They

Table 1. Details of the geographic records of *Phyllopezus lutzae*. The present record is highlighted in bold. * Type Locality.

Municipality	State	Latitude (S)	Longitude (W)	Reference
Flexeiras	Alagoas	09°22'00.0"	035°45'00.0"	Avila et al., 2010
Ibateguara	Alagoas	09°00'02.0"	035°51'12.0"	Silva, 2008
Quebrangulo/Chã Preta/Lagoa do Ouro	Alagoas/Pernambuco	9°13'54.37"	36°25'38.6"	Roberto et al., 2015
Cairú	Bahia	13°36'47.1"	038°56'11.6"	Dias and Rocha, 2014
Camaçari	Bahia	12°38'03.0"	038°04'32.0"	Dias and Rocha, 2014
Cruz das Almas	Bahia	12°40'25"	39°06'05"	Protázio et al., 2021
Cumuruxatiba	Bahia	17°06'00.0"	039°11'00.0"	Rodrigues, 1987
Jandaíra	Bahia	11°40'28.0"	037°29'03.0"	Dias and Rocha, 2014
Lauro de Freitas	Bahia	12°53'9.6"	38°18'30.0"	Freitas, 2014
Maraú	Bahia	14°06'22.6"	038°59'23.0"	Dias and Rocha, 2014
Mata de São João	Bahia	12°31'40.9"	038°18'03.1"	Couto-Ferreira et al., 2011; Freitas, 2014; Gamble et al., 2012
Prado	Bahia	17°19'56.6"	039°13'31.1"	Vrcibradic et al., 2000
Salvador*	Bahia	12°38'03.0"	038°04'32.0"	Loveridge, 1941; Dias and Rocha, 2014; Freitas, 2014
Santa Cruz Cabrália/Porto Seguro	Bahia	16°23'13.0"	039°10'11.4"	Franco et al., 1998; Reis, 2017
Saubara	Bahia	12°50'00.0"	038°49'00.0"	Soeiro, 2013
Simões Filho	Bahia	12°50'00.0"	038°25'00.0"	Vrcibradic et al., 2000
Trancoso	Bahia	16°39'00.0"	039°06'00.0"	Vrcibradic et al., 2000
Nova Soure	**Bahia**	**11°17'4.67"**	**38°28'7.14"**	**Present study**
Tucano	**Bahia**	**11°11'58.80"**	**38°46'57.03"**	**Present study**
Caaporã	Paraíba	07°25'40.2"	34°57'51.6"	Albuquerque et al., 2019
Pedras de Fogo	Paraíba	07°24'53.2"	34°57'56.3"	Albuquerque et al., 2019
Iguarassu	Pernambuco	07°50'00.0"	34°54'00.0"	Vanzolini, 1972
Recife	Pernambuco	08°05'45.6"	34°57'04.9"	Oliveira et al., 2016; Santos et al., 2017
São Lourenço da Mata	Pernambuco	08°02'09.6"	35°11'56.4"	Albertim et al., 2010; Teixeira et al., 2013
Areia Branca	Sergipe	10°45'54.6"	037°20'19.4"	Carvalho et al., 2005

were euthanized with lidocaine injection, had muscle tissue samples preserved in 100% alcohol, were fixed in 10% formalin and are preserved in 70% alcohol at the Coleção Herpetológica do Semiárido, Universidade Federal Rural do Semi-Árido, Mossoró, Rio Grande do Norte, Brazil, under following identification codes: CRSAR 1870 (38.7 mm snout-vent length [SVL]), 1871 (44.3 mm SVL), 1872 (60.6 mm SVL), 1874 (39.0 mm SVL) and 1876 (50.5 mm SVL).

These present records constitute the first occurrence of *P. lutzae* outside the Atlantic Forest and the first record for the Caatinga domain. Our records in Tucano and Nova Soure municipalities extend the species distribution range 151 and 116 km, respectively, Northwest of Jandaíra, Bahia, the closest record reported in the literature (Dias and Rocha, 2014). The area where *P. lutzae* was found can be defined as a mosaic of Caatinga and Cerrado vegetation and has a high density of bromeliads of the genus *Aechmea* Ruiz & Pav. (Fig. 1B). The majority of records were made in Tucano because most of the wildlife rescue and monitoring activities are focused there, but the species can also be abundant in Nova Soure and other sites in the region with high density of bromeliads.

Bromeliads are recognized as a suitable environment for shelter and foraging for many species, as the arrangement of their leaves allows the accumulation of water and form a microenvironment that supports the development of invertebrates and

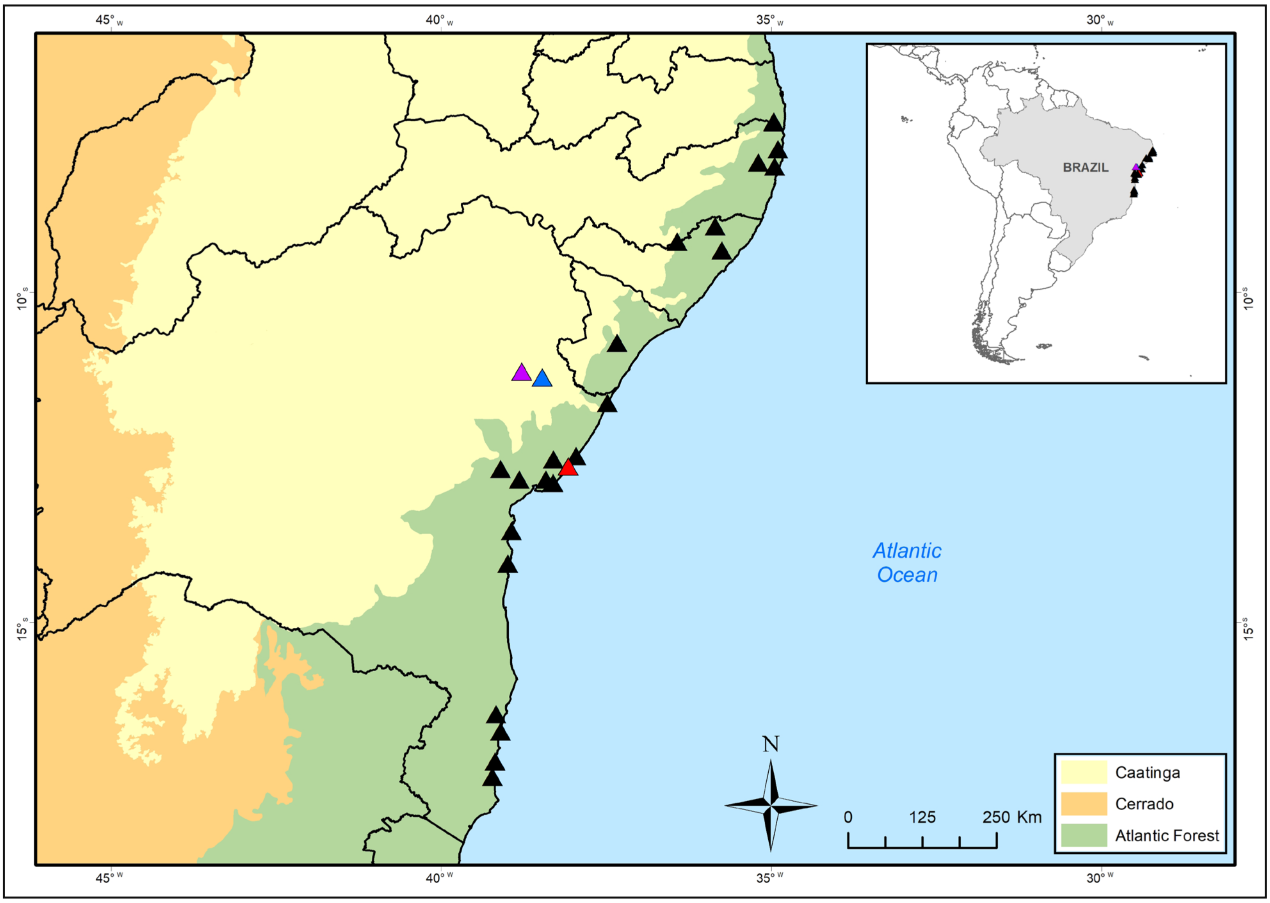

Figure 2. Updated distribution map of *Phyllopezus lutzae*. Red triangle = Type locality, black triangles = previously known records, purple triangle = new record in Tucano municipality, blue triangle = new record in Nova Soure municipality.

vertebrates (Rocha *et al.*, 2000; Jorge *et al.*, 2020; Jorge *et al.*, 2021a; Jorge *et al.*, 2021b). As *P. lutzae* was always recorded in association with bromeliads, we suggest that the presence and high population density of bromeliads in the sampled area is the main factor that allows the species inhabiting this semiarid region. These findings highlight the importance of bromeliads irrespective the considered domain (Schineider and Teixeira, 2001; Sabagh *et al.*, 2017; Jorge *et al.*, 2021a), and show how the suitable management of fauna during licensing activities can be important sources of knowledge about biodiversity.

On 24 February, 2021, at around 10 a.m., during a wildlife rescue in the municipality of Tucano, an adult male of *Tropidurus hispidus* was found approaching an adult of *P. lutzae* disturbed after vegetation removal (Fig. 1C). The *T. hispidus* began to subdue *P. lutzae* with bites on the head (Fig. 1D), but released the prey and ran away after perceiving the approach of observers.

Tropidurus hispidus is one of the most common Squamata species in the Brazilian semiarid region (Passos *et al.*, 2016a). It is a diurnal sit-and-wait predator with a primarily insectivorous but generalist diet (Kolodiuk *et al.*, 2010; Ribeiro and Freire, 2011), including many vertebrate prey. For instance, anurans (Beltrão-Mendes, 2017), birds (Guedes *et al.*, 2017), mammals (Virgínio *et al.*, 2017), snakes (Santos *et al.*, 2017), and lizards (Zanchi *et al.*, 2012; Passos *et al.*, 2016b; Pergentino *et al.*, 2017), including conspecifics (Sales *et al.*, 2011; Sousa *et al.*, 2021) are among the documented prey of *T. hispidus*. Despite the behavioral interaction observed was an unsuccessful predation attempt, this finding provides evidence that *P. lutzae* also composes the list of potential prey of *T. hispidus*. In this regard, other phyllodactylid lizards as *Gymnodactylus geckoides* and *Phyllopezus pollicaris* were already consumed by *T. hispidus* (Pergentino *et al.*, 2017; Dubeux *et al.*, 2020).

Our new records of *P. lutzae* not only expand its known distribution range, but demonstrate that it may inhabit an ecological and climate dominion different than was reported in the scientific literature so far. The prey-predator interaction reported also highlights the predator potential of *T. hispidus*, reinforcing this species is able to prey upon any smaller vertebrate. The semiarid of North of the state of Bahia still has areas with little known biodiversity and our findings constitute one of the first works on reptiles from this region.

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Novedad zoogeográfica

Cuad. herpetol. 36 (2): 259-264 (*2022*)

Novos registros dos lagartos *Ameivula cipoensis* Arias *et al.*, 2014, *Enyalius capetinga* Breitman *et al.*, 2018, *Psilops paeminosus* (Rodrigues, 1991) e *Tupinambis quadrilineatus* Manzani & Abe, 1997 (Squamata) para o estado de Minas Gerais, Brasil, através da ciência cidadã

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Localidade.— *Ameivula cipoensis*: Brasil, Minas Gerais, Santana de Pirapama, Latitude -18.9448°, Longitude -43.8214°. Registrado em 24/11/2009 por Leandro Moraes e publicado na plataforma de ciência cidadã iNaturalist (https://web.archive.org/web/20220330210904/https://www.inaturalist.org/observations/68863211). *Enyalius capetinga*: Brasil, Minas Gerais, Rio Paranaíba, Latitude -19.2097°, Longitude -46.1325°. Registrado em 27/09/2019 por Marcelo Ribeiro e publicado na plataforma de ciência cidadã iNaturalist (https://web.archive.org/web/20220401132551/https://www.inaturalist.org/observations/33681732). *Psilops paeminosus*: Brasil, Minas Gerais, Botumirim, Latitude -16.9021°, Longitude -43.1019°. Registrado em 02/2018 por João Menezes e publicado na plataforma de ciência cidadã iNaturalist (https://web.archive.org/web/20220401133338/https://www.inaturalist.org/observations/12928102). *Tupinambis quadrilineatus*: Brasil, Minas Gerais, Chapada do Norte, Latitude -17.1717°, Longitude -42.4374°. Registrado em 23/01/2015 por Adelton Nunes Nascimento e publicado na plataforma de ciência cidadã iNaturalist (https://web.archive.org/web/20220401133842/https://www.inaturalist.org/observations/40750076). São Gonçalo do Abaeté, Latitude -18.016°, Longitude -45.4144°. Registrado em 17/03/2021 por Adelton Nunes Nascimento e publicado na plataforma de ciência cidadã iNaturalist (https://web.archive.org/web/20220401134320/https://www.inaturalist.org/observations/72153824). São Sebastião do Paraíso, Latitude -20.9172°, Longitude -46.9841°. Registrado em 28/03/2017 por Aline Horikawa e publicado na plataforma de ciência cidadã iNaturalist (https://web.archive.org/web/20220401134828/https://www.inaturalist.org/observations/75405331). Couto de Magalhães, Latitude -17.9489°, Longitude -43.449°. Registrado em 02/02/2018 por Adelton Nunes Nascimento e publicado na plataforma de ciência cidadã iNaturalist (https://web.archive.org/web/20220401135158/https://www.inaturalist.org/observations/106175732).

Comentários.— Trabalhos contendo atualizações de distribuição geográfica são ferramentas úteis para preencher lacunas do conhecimento da biodiversidade, como o déficit Wallaceano – conhecimento inadequado sobre a distribuição geográfica das espécies (Hortal *et al.*, 2015). Atualmente, iniciativas e plataformas de ciência cidadã têm se mostrado grandes aliadas na busca por dados de distribuição e história natural ainda não publicadas na literatura científica (e.g., Messas *et al.*, 2021; Richter *et al.*, 2021; Rowley *et al.*, 2019; Wangyal *et al.*, 2020). O iNaturalist é uma plataforma de ciência cidadã criada para registrar qualquer observação de animais, plantas, fungos e até micro-organismos. O banco de dados desta plataforma inclui imagens e sons que são adicionadas pelos usuários e confirmados por outros usuários, muitos deles especialistas no táxon em questão (Jones *et al.*, 2019; Laufer *et al.*, 2021). O iNaturalist tem se mostrado uma ferramenta importante para trabalhos que envolvam dados de biogeografia e história natural (Laufer *et al.*, 2021; D´Angiolella *et al.*, 2021; Messas *et al.*, 2021; Mesaglio *et al.*, 2021; Jones *et al.*, 2019). Através desta plataforma nós detectamos novas ocorrências para quatro espécies de lagartos no estado de Minas Gerais, Brasil: *Ameivula cipoensis*, *Enyalius capetinga*, *Psilops paeminosus* e *Tupinambis quadrilineatus*. Além disso, atualizamos o mapa de distribuição de todas as espécies com base em dados da literatura (Fig. 1, Apêndice 1).

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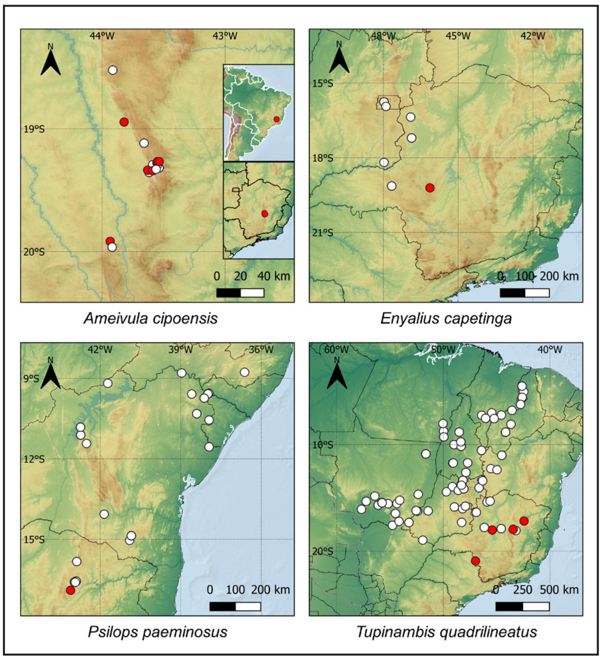

Ameivula cipoensis

Enyalius capetinga

Psilops paeminosus

Tupinambis quadrilineatus

Figura 1. Mapas de distribuição atualizados para *Ameivula cipoensis*, *Enyalius capetinga*, *Psilops paeminosus* e *Tupinambis quadrilineatus*. Novos registros em vermelho. Para detalhes sobre os registros de literatura, vide Apêndice 1.

Ameivula cipoensis (Teiidae) é endêmico da porção meridional da Serra do Espinhaço em Minas Gerais, tendo sua distribuição conhecida apenas para o Parque Nacional da Serra do Cipó e nos municípios de Belo Horizonte e Gouveia, Minas Gerais (Arias *et al.*, 2014; Moura & Cruz, 2017; Mol *et al.*, 2021), onde ocorre em baixas densidades nos campos rupestres, em moitas de vegetação ou áreas de solo arenoso (Filogonio *et al.*, 2010; Arias *et al.*, 2014). O novo registro que identificamos em Santana de Pirapama, MG, preenche uma lacuna de 70 Km entre Gouveia e o Parque Nacional da Serra do Cipó. A foto em questão (Fig. 2) mostra um Teiinae em vista dorsal, cujo padrão de listras não deixa dúvidas de que se trata de um "*Cnemidophorus*" *sensu lato*. *Ameivula cipoensis* é a única espécie do grupo esperada para a região (Arias *et al.*, 2018) e o padrão de coloração observado na fotografia é condizente com a diagnose da espécie: linha vertebral ausente, campos e linhas paravertebrais e dorsolaterais presentes, e linha lateral superior presente (Arias *et al.*, 2014). Outros registros presentes no iNaturalist foram realizados em locais onde a presença de *A. cipoensis* já era conhecida: a Serra do Cipó (localidade tipo) (https://web.archive.org/web/20220402151153/https://www.inaturalist.org/observations/70615653; https://web.archive.org/web/20220402152237/https://www.inaturalist.org/observations/77276466; https://web.archive.org/web/20220402153239/https://www.inaturalist.org/observations/90435849; https://web.archive.org/web/20220402153515/https://www.inaturalist.org/observations/92768583; https://web.archive.org/web/20220404141123/https://www.inaturalist.org/observations/72227938) e Belo Horizonte (https://web.archive.org/web/20220402145330/https://www.inaturalist.org/observations/41883396).

Enyalius capetinga (Leiosauridae) é endêmico do cerrado brasileiro, habita principalmente matas de galeria, podendo ocorrer também em áreas de cerrado *sensu stricto* e cerradão (Breitman *et al.*, 2018; Oliveira-Filho & Ratter 2002). Possui ocorrência em Goiás (Catalão), Distrito Federal (Brasília) e municípios próximos em Minas Gerais (Paracatu, Unaí e Nova Ponte) (Breitman *et al.*, 2018). O novo registro que localizamos em Rio Paranaíba, MG, representa o novo limite oriental da distribuição conhecida da espécie, cerca de 160 Km a leste do registro mais próximo, em Nova Ponte, MG. O padrão de coloração observado pelo indivíduo fotografado (Fig. 3) está de acordo com o conhecido para *E. capetinga* e, embora outros caracteres diagnósticos não estejam visíveis na imagem, inferimos que se trate dessa

Figura 2. Indivíduo de *Ameivula cipoensis* fotografado em Santana de Pirapama, Minas Gerais. Foto: Leandro Moraes / iNaturalist (reproduzida com autorização).

Figura 3. Indivíduo de *Enyalius capetinga* fotografado em Rio Paranaíba, Minas Gerais. Foto: Marcelo Ribeiro / iNaturalist (CC BY-NC 4.0).

espécie por uma questão biogeográfica, sendo *E. capetinga* o único táxon de *Enyalius* com ocorrência esperada na região (Breitman *et al.*, 2018).

Psilops paeminosus (Gymnophthalmidae) ocorre na Caatinga e em áreas de transição com o Cerrado e a Mata Atlântica, do leste de Pernambuco ao norte de Minas Gerais (Rodrigues *et al.*, 2017; Thomassen *et al.*, 2017). Habita principalmente áreas de solo arenoso, mas pode ser encontrado também sob o folhiço (Rodrigues, 1991a; Delfim *et al.*, 2006). Localizamos um novo registro em Botumirim, MG, novo limite meridional da distribuição conhecida da espécie, cerca de 35 Km a sudoeste do registro mais próximo, em Grão Mogol, MG. O dorso castanho claro com flancos mais escuros e a cauda em tom castanho-avermelhado (Fig. 4) são típicos de *P. paeminosus* (Rodrigues, 1991a; Rodrigues *et al.*, 2017). Entre os Gymnophthalmini com ocorrência possível na região, *Micrablepharus maximiliani* (Reinhardt & Lütken, 1861) apresenta a cauda azulada (Moura *et al.*, 2010) e *Vanzosaura savanicola* Recoder *et al.*, 2014 possui a cauda mais avermelhada e listras dorsais (Recorder *et al.*, 2014). *Procellosaurinus* spp., embora não esperados para a região (Rodrigues, 1991b; Delfim *et al.*, 2011), têm um padrão de cor próximo ao de *Psilops*; contudo, apresentam escamas frontoparietais, as quais estão ausentes em *Psilops* (Rodrigues, 1991b; Rodrigues *et al.*, 2017), estado de caráter observável em uma das fotografias disponíveis do indivíduo aqui reportado. *Psilops mucugensis* Rodrigues *et al.*, 2017 possui a cauda mais avermelhada e duas linhas dorsolaterais conspícuas percorrendo o corpo, ausentes em *P. paeminosus* (Rodrigues *et al.*, 2017) e no indivíduo fotografado. Uma cauda vermelha também estaria presente em indivíduos adultos de *P. seductus* Rodrigues *et al.*, 2017, mas a própria descrição da espécie apresenta fotografias de indivíduos com a cauda marrom (Rodrigues *et al.*, 2017). Outros caracteres que diferenciam essa espécie de *P. paeminosus* não estão visíveis nas imagens (contagens de escamas, lamelas subdigitais e número de poros femorais) (Rodrigues *et al.*, 2017). Contudo, a localização geográfica do registro aqui citado nos dá segurança de se tratar de *P. paeminosus*.

Tupinambis quadrilineatus (Teiidae) ocorre do leste do Pará ao leste de Minas Gerais, ao longo do Cerrado – com registros escassos na Caatinga –, habitando principalmente matas de galeria (Morato *et al.*, 2015; Silva *et al.*, 2018). Os quatro novos registros de *T. quadrilineatus* em Minas Gerais representam: Chapada do Norte (Fig. 5), novo limite oriental da distribuição conhecida da espécie, cerca de 125 Km nordeste do registro mais próximo em São Gonçalo do Rio Preto; São Gonçalo do Abaeté a 85 Km oeste do registro mais próximo em João Pinheiro; São Sebastião do Paraíso (Fig. 6), novo limite meridional da distribuição conhecida da espécie, cerca de 360 Km sudoeste do registro mais próximo em Lassance; e Couto de Magalhães a 30 Km noroeste do registro mais próximo em São Gonçalo do Rio Preto. *Salvator merianae* Duméril & Bibron, 1839 é a outra única espécie de Tupinambinae possivelmente simpátrica nessas localidades, sendo as duas facilmente diferenciadas pelo padrão geral de coloração (Manzani & Abe, 1997).

Todos estes registros reforçam a importância de plataformas de ciência cidadã para auxiliar a reduzir a lacuna do conhecimento biogeográfico da nossa biodiversidade. Portanto, reiteramos que o uso destas ferramentas pela população deva ser incentivado pela comunidade acadêmica.

Figura 4. Indivíduo de *Psilops paeminosus* fotografado em Botumirim, Minas Gerais. Foto: João Menezes / iNaturalist (CC BY-NC-SA 4.0).

Figura 5. Indivíduo de *Tupinambis quadrilineatus* fotografado em Chapada do Norte, Minas Gerais. Foto: Adelton Nunes Nascimento / iNaturalist (reproduzida com autorização).

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Figura 6. Indivíduo de *Tupinambis quadrilineatus* fotografado em São Sebastião do Paraíso, Minas Gerais. Foto: Aline Horikawa / iNaturalist (reproduzida com autorização).

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Apêndice 1

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