# New Species of Leaf-litter Toad of the Rhinella margaritifera Species Group (Anura: Bufonidae) from Amazonia

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# *Copeia*

# New Species of Leaf-litter Toad of the *Rhinella margaritifera* Species Group (Anura: Bufonidae) from Amazonia

Miquéias Ferrão[1,2], Albertina Pimentel Lima[2], Santiago Ron[3], Sueny Paloma dos Santos[3], and James Hanken[1]

We describe through integrative taxonomy a new Amazonian species of leaf-litter toad of the *Rhinella margaritifera* species group. The new species inhabits open lowland forest in southwest Amazonia in Brazil, Peru, and Bolivia. It is closely related to a Bolivian species tentatively identified as *Rhinella* cf. *paraguayensis*. Both the new species and *R. paraguayensis* share an uncommon breeding strategy among their Amazonian congeners: each breeds in moderate to large rivers instead of small streams or ponds formed by rainwater. The new species is easily differentiated from other members of the *R. margaritifera* species group by having a strongly developed bony protrusion at the angle of the jaw, a snout–vent length of 63.4–84.7 mm in females and 56.3–72.3 mm in males, well-developed supratympanic crests with the proximal portion shorter than the parotoid gland in lateral view, a divided distal subarticular tubercle on finger III, and multinoted calls composed of groups of 7–9 pulsed notes and a dominant frequency of 1,012–1,163 Hz. Recent studies have shown that the upper Madeira Basin harbors a megadiverse fauna of anurans, including several candidate species. This is the first member of the *R. margaritifera* species group to be described from this region in recent years, and at least two additional unnamed species await formal description.

THE *Rhinella margaritifera* species group is characterized by a pronounced expansion of the posterior ramus of the pterygoid bone in the skull (Pramuk, 2006). Currently, this species group is composed of 20 species distributed in Panama, Ecuador, Peru, Venezuela, French Guiana, Guyana, Surinam, Bolivia, Paraguay, and Brazil (Frost, 2020). Brazil harbors the most species, 16, which occur mostly in Amazonia but also in Atlantic Forest, Cerrado, and Pantanal (Frost, 2020). Although the presence of cephalic crests is not a synapomorphy of the *R. margaritifera* species group, this character allows the designation of two nonmonophyletic morphological subgroups: (1) species with moderate to well-developed supratympanic crests—*R. gildae, R. margaritifera, R. martyi, R. paraguayensis, R. roqueana, R. sclerocephala, R. sebbeni,* and *R. stanlaii;* and (2) those with poorly developed supratympanic crests—*R. acutirostris, R. alata, R. castaneotica, R. dapsilis, R. hoogmoedi, R. lescurei, R. magnussoni, R. parecis, R. proboscidea, R. ocellata, R. scitula,* and *R. yunga.*

Most taxonomic problems regarding the *R. margaritifera* group concern the identification of the species *R. margaritifera* (Lavilla et al., 2013; Moravec et al., 2014; Santos et al., 2015). Until recently, its holotype was presumed lost for more than two centuries, and its type locality was inaccurately interpreted as Brazil. This situation led to a number of synonymizations and revalidations. It has also complicated the description of additional species, especially those with well-developed cephalic crests. Finally, anuran checklists conducted in Amazonia have commonly but inconsistently applied the name *R. margaritifera* both to populations with prominent cephalic crests as well as to those with poorly developed ones.

After an exhaustive but ultimately unsuccessful attempt to locate the holotype of *R. margaritifera*, and wishing to resolve this taxonomic and systematic problem, Lavilla et al. (2013) designated an adult female housed in the Museu Nacional, Rio de Janeiro (MNRJ 71538) from the municipality of Humaitá (Amazonas, Brazil) as the species' neotype. This action followed an intense literature review to determine the most probable type locality. However, shortly before the publication of Lavilla et al. (2013), ZISP 257.1, a specimen housed in the Academy of Sciences in St. Petersburg, Russia, was identified as the holotype of *R. margaritifera* (Milto and Barbanov, 2011). Lavilla et al. (2017) subsequently recognized this specimen as the one depicted by Seba (1734) and used by Laurenti (1768) to erect *R. margaritifera*, thus invalidating the neotype designated by Lavilla et al. (2013) and attributing to *R. margaritifera* the status of *species inquirenda*. While discovery of the holotype will facilitate descriptions of some related species, continued uncertainty regarding the type locality will likely hamper the description of those that are morphologically similar to *R. margaritifera*.

Despite the chaotic taxonomy that surrounds *Rhinella margaritifera*, its species group has attracted increased attention from Neotropical taxonomists and systematists. Fouquet et al. (2007a) revealed through molecular data that as many as 11 cryptic species were concealed under the names *R. margaritifera* and *R. castaneotica* in northern South America. Jansen et al. (2011) showed in an integrative inventory that a Bolivian population of *R. margaritifera* probably represents *R. paraguayensis* or another closely related species. As part of a revision of *R. margaritifera* (*sensu lato*) from Panama and Ecuador, Santos et al. (2015) redescribed *R. alata* using integrative taxonomy and argued that the identities of some clades in their phylogeny remain

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unresolved and that some probably represent undescribed species. Ávila et al. (2018) described the morphological variation, advertisement call, and phylogenetic position of *R. gildae* and showed that this species is more widely distributed than previously thought. In total, ten new species of the *R. margaritifera* species group have been described since 2006 (Caramaschi and Pombal, 2006; Fouquet et al., 2007b; Lima et al., 2007; Ávila et al., 2010, 2020; Moravec et al., 2014; Vaz-Silva et al., 2015), and additional candidate species await formal description.

Over the last 15 years, two of us (A.P.L. and M.F.) have collected several specimens of a species belonging to the *R. margaritifera* species group from the east bank of the upper Madeira River, Brazil. This species was previously identified mainly as *Bufo* sp. II (*margaritifer* complex) by Moravec and Aparicio (2005) based on one specimen from Bolivia (CBF5800), as "*Bufo typhonius*" by Duellman (2005), *R.* cf. *margaritifera* "5" by Pramuk (2006), and *R. margaritifera* by Mendelson et al. (2011) based on Peruvian specimens from Puerto Maldonado (KU 215145–46), and recently as *R.* cf. *margaritifera* by Moravec et al. (2014) based on a Peruvian specimen from Masisea (NMP6V 74915). Our specimens strongly differ morphologically from both the holotype (ZISP 257.1) and the former neotype (MNRJ 71538) of *R. margaritifera*, as well as from other close relatives. Herein, we describe this taxon as a new species by integrating morphological, bioacoustic, phylogenetic, and ecological traits.

## MATERIALS AND METHODS

*Sampling.*—Eighteen individuals of the new species were collected between 2009 and 2014 through visual encounters in three RAPELD sampling modules along the east bank of the upper Madeira River, Porto Velho municipality, Rondônia, Brazil (Magnusson et al., 2013; Fig. 1). Two of the modules were located along the east bank of the Jací-Paraná River, a tributary of the upper Madeira River: Jací Novo Sampling Module (09°24′45″S, 64°26′33″W; 133 m a.s.l.) and Jací Direito Sampling Module (09°27′44″S, 64°23′32″W; 122 m a.s.l.). The third site, Morrinhos Sampling Module, was located on the east bank of the Madeira River (09°04′34″S, 64°14′46″W; 100 m a.s.l.). Three additional specimens were collected in January 2017 at Três Praias Camp (09°27′11″S, 64°25′04″W; 77 m a.s.l.), east bank of the Jací-Paraná River. Specimens were euthanized with 2% benzocaine topical solution, fixed in 10% neutral-buffered formalin, and preserved in 70% ethanol. Tissue samples were collected before fixation, stored in 100% ethanol, and housed at Albertina Lima's laboratory at Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil. Specimens are deposited in the Herpetology Collection of INPA (INPAH).

*Measurements and morphological analysis.*—Sex of specimens was determined by the presence or absence of vocal slits. Maturity was assessed by examination of gonads or when specimens were actively calling. The following 35 morphometric measurements were taken to the nearest to 0.1 mm by using digital calipers. Eight measurements followed Duellman (1970): SVL, snout–vent length; HL, head length; HW, head width; EL, horizontal eye diameter; TYMH, horizontal tympanum diameter; HAND3, hand length on finger III;

FOOT4, foot length on toe IV; TL, tibia length. Four measurements followed Heyer et al. (1990): FAL, forearm length; UAL, upper arm length; THL, thigh length; TAL, tarsus length. Seventeen measurements followed Caldwell and Lima (2003) and Caramaschi and Niemeyer (2003): EN, eye–nostril distance; IN, inter-nostril distance; IOD, interorbital distance; UEW, upper eyelid width; TYMV, vertical tympanum diameter; PGW, parotoid gland width; PGL, parotoid gland length; TED, tympanum–eye distance; FOOT1, foot length on toe I; FOOT2, foot length on toe II; FOOT3, foot length on toe III; FOOT5, foot length on toe V; HAND1, hand length on finger I; HAND2, hand length on finger II; HAND4, hand length on finger IV; PTL, palmar tubercle length; PTW, palmar tubercle width. Additionally, six other measurements were measured and defined as: BPD, distance between bony protrusions of the jaw, measured ventrally between the lateral tips of the protrusions; POCL, supratympanic crest length, measured between anterior and posterior crest margins; POCD, distance between supratympanic crests, measured at the posterior extremities; SOCL, supra-orbital crest length, measured between anterior and posterior crest margins; SH, snout height, measured from the tip of the snout to the border of the upper lip; APO, number of emerging dorsal vertebral apophyses. Toe webbing was scored according to the formula of Savage and Heyer (1967) as modified by Myers and Duellman (1982). External morphological terminology follows Heyer et al. (1990) and Kok and Kalamandeen (2008). Coloration in life was taken from photographs and field notes.

*Call recordings and acoustic analysis.*—Advertisement calls of two males (INPAH 41331 and INPAH 41332) were recorded on the east bank of the Jací-Paraná River at Três Praias Camp on 31 January 2017. Recordings were made at 1800 h with a Sennheiser K6/ME66 unidirectional microphone (Sennheiser, Germany) and a Marantz PMD660 digital recorder (Marantz, Japan). The microphone was positioned approximately 1.5 m from each male. Recordings were made at a sampling rate of 44.1 kHz and a sample size of 16 bits and stored in WAV format. Temperature during recording was 25°C.

Calls were analyzed using Raven Pro © v.1.5 software (The Cornell Lab of Ornithology, available from https://ravensoundsoftware.com) with the following configuration: window = Blackman, 3 dB Filter Bandwidth = 80 Hz, overlap = 80%, hop size = 4.1 ms, and DFT size = 2,048 samples. The following temporal and spectral traits were quantified from eight calls of each male: call duration, inter-call interval, call repetition rate (calculated as 60 seconds/[call duration + inter-call interval]), number of notes, note duration (quantified for the first, middle, and last notes), inter-note interval (quantified between the first and second notes and between the middle and consecutive notes), note repetition rate (calculated as 1 seconds/[note duration + inter-note interval]), pulses per note (quantified for the first, middle, and last note), call dominant frequency (measured along all the call), and call bandwidth (using 20 dB as threshold). Sound graphs were produced in R v.3.5 (R Core Team, 2016) using the packages *seewave* v.2.1 (Sueur et al., 2008) and *tuneR* v.1.3.2 (Ligges et al., 2018). Seewave was set as follows: window = Hanning, FFT size = 256 samples, and FFT overlap = 85%.
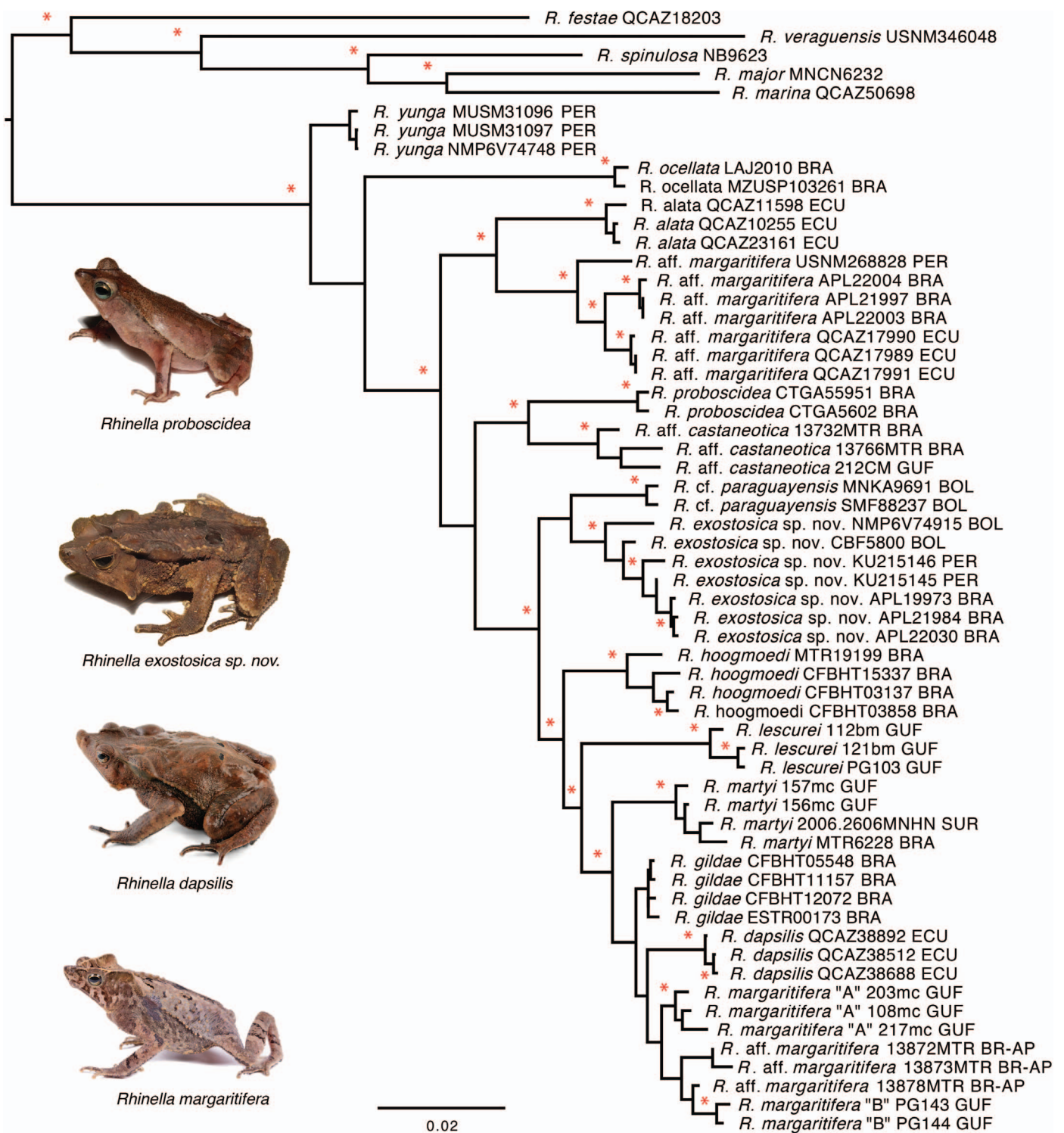
**Fig. 1.** Phylogeny of species of the *Rhinella margaritifera* species group based on one nuclear and three mitochondrial genes (2,997 bp) and reconstructed through Bayesian inference. Posterior probabilities above 0.80 are shown close to nodes and those greater than or equal to 0.95 are represented with an asterisk. Scale bar represents substitutions/site. Photographs by Santiago Ron, bioweb.bio (*R. dapsilis*, *R. margaritifera*), Anthony S. Ferreira (*R. proboscidea*) and Albertina P. Lima (*Rhinella exostosica*, new species). See Data Accessibility for tree file.

***DNA sequencing and phylogenetic analyses.***—Total DNA of samples of three specimens of the new species from the east bank of the upper Madeira River and three specimens of *R.* aff. *margaritifera* from the west bank was extracted from liver or muscle preserved in 100% ethanol following the protocol of Santos et al. (2015). The mitochondrial genes 16S rRNA

(primers *16Sc* and *16Sd*; Pauly et al., 2004) and cytochrome c oxidase I (COI; primers *LEP-F1* and *LEP-R1*; Hebert et al., 2004) and the nuclear gene tyrosinase (Tyr; primers *Tyr1C* and *Tyr1G*; Bossuyt and Milinkovitch, 2000) were amplified through polymerase chain reaction for all specimens. Amplifications were sequenced by Macrogen Inc. (Seoul,

Korea). Forward and reverse sequences were assembled and edited manually using GeneiousPro 5.4.6 (Biomatters Ltd.).

BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare sequences of the new species with other species of the *R. margaritifera* group deposited in GenBank. Sequences of two specimens from Puerto Maldonado and one from Masisea, Peru (KU 215145–46, NMP6V 74915, respectively) and one specimen from Bolivia (CBF5800) were retrieved with high similarity (>99%) and referred to the new species based on morphological and phylogenetic similarity. In order to infer phylogenetic relationships among the new species and its close relatives, sequences from 2–5 specimens were selected from each nominal and candidate new species of the *R. margaritifera* species group previously published and available in GenBank. Although sequences of the mitochondrial gene 12S rRNA were not generated in the present study, this gene was included in the dataset to better estimate phylogenetic relationships within the focal species group. Sequences from species belonging to the *R. festae*, *R. granulosa*, *R. marina*, *R. spinulosa*, and *R. veraguensis* species groups were used as outgroups. Specimens, sampling localities, and GenBank accession numbers are listed in Appendix 1.

Gene datasets were individually aligned using Clustal W (Thompson et al., 1994) as implemented in Bioedit (Hall, 1999) and using default settings. Alignments were posteriorly concatenated in Mesquite 3.2 (Maddison and Maddison, 2019), resulting in a final matrix comprising 60 terminals and 2,996 bp (16S, 870 bp; 12S, 899 bp; COI, 678 bp; Tyr, 550 bp). PartitionFinder v. 1.1.1 (Lanfear et al., 2017) was used to estimate the best partitioning scheme and best-fit molecular evolution model for each partition (see Appendix 2). Codon partitioning was applied to protein-coding genes. Best scheme and models were identified by using the PhyML algorithm and Bayesian information criterion (BIC). Phylogenetic relationships were inferred through Bayesian Inference (BI) in MrBayes (Ronquist et al., 2012) by using four independent runs of 20 million generations with four Metropolis-coupled Markov-chain Monte Carlo algorithms (MCMCMC). Probabilities were sampled every 1,000 generations, and stationary of posterior distributions (Effective Sample Sizes $\geq$ 200) were accessed in Tracer v.1.6 (Rambaut et al., 2018). The 50% majority rule consensus tree was calculated after discarding the first 25% of trees as burn-in. Interspecific pairwise p-distances and Kimura-2-Parameters distances (Kimura, 1980) were calculated using the 16s rRNA gene in Mega 6 (Tamura et al., 2013).

## RESULTS

The phylogeny reconstructed through Bayesian inference recovers with strong support the *Rhinella margaritifera* species group as monophyletic (Fig. 1). The most basal species are *R. yunga* and *R. ocellata*, respectively. In addition, three major clades of species within the *R. margaritifera* species group are moderately to well supported (posterior probabilities [PP] > 0.93). The first major clade (PP = 1) groups the trans-Andean *R. alata* and three other cis-Andean species from Brazilian, Peruvian, and Ecuadorian Amazonia with moderately to well-developed supratympanic crests. The second clade (PP = 0.93) is composed of small-sized Amazonian species with poorly developed supratympanic crests: *R.* aff. *castaneotica* and *R. proboscidea*. Finally, the third major clade includes all remaining cis-Andean species with moderately to well-developed supratympanic crests distributed in Amazonia, Pantanal, Atlantic Forest, and Savanna. However, phylogenetic relationships among the major clades are poorly resolved.

Samples of the new species from the east bank of the upper Madeira River (Brazil) cluster with two Peruvian samples from Puerto Maldonado previously identified as *Rhinella* aff. *margaritifera* (PP = 0.97; Fig. 1). Samples previously identified as *R.* aff. *margaritifera* from Masisea, Peru (NMP6V 74915) and another from Bolpebra, Bolivia (CBF5800) group with the clade Brazil + Puerto Maldonado (PP = 0.98; Fig. 1). Pairwise genetic distances between Brazilian samples and those from Puerto Maldonado and CBF5800 are very low (K2P and p-distances = 0.2% in both pairs). The CBF5800 also shows low genetic distance from samples from Puerto Maldonado (K2P and p-distance = 0.2%). Conversely, NMP6V 74915 presents higher p-distances to samples from Brazil (0.9%), Puerto Maldonado (0.9%), and Bolpebra (0.7%). Based on morphological similarity, genetic distance, and phylogenetic position, we refer the Bolivian and Peruvian specimens to the new species.

The new species is placed in sister position to a clade composed of samples from Bolivian lowland tentatively attributed to *Rhinella* cf. *paraguayensis* (Fig. 1). Nevertheless, this relationship is poorly supported (PP = 0.82). Pairwise genetic distance between the new species and *R.* cf. *paraguayensis* is low (K2P and p-distances = 1.9%). In contrast to the low genetic distance, these taxa show strongly divergent morphology. The species inhabiting the west bank of the upper Madeira River is not closely related to the new species according to our phylogeny, and instead is grouped as sister to *R.* aff. *margaritifera* from Ecuador. Additionally, genetic distances between samples of *R.* aff. *margaritifera* from the west bank of the upper Madeira River and those from the new species are high (K2P and p-distances = 5.1 and 4.9%, respectively).

Unlike several other Neotropical genera of anurans and similarly to treefrogs of the genus *Osteocephalus* (see Jungfer et al., 2013), pairwise genetic distances among species of *Rhinella* included in this study are moderately low (Appendix 3). For example, a low genetic distance is observed between the new species and *R. hoogmoedi* (Table 1), a medium-sized species from the Atlantic coast of Brazil (p-distance = 2.4%). The highest genetic distances are between *R. ocellata* and other species of the *R. margaritifera* species group; they range from 4.8 to 7.7% (p-distance). See Table 1 for K2P genetic distances.

### *Rhinella exostosica*, new species
urn:lsid:zoobank.org:act:FDE2D615-4BA5-4A19-969D-EF1B-BA5BFE09
Figures 1–3, 4A–D, 5A–B, 6–9, 10A–C, 11; Tables 1–2

*Bufo typhonius*: Duellman and Salas (1991); Duellman (2005).
*Bufo* sp. (*margaritifer* complex): Moravec and Aparicio (2000).
*Bufo* sp. II (*margaritifer* complex): Moravec and Aparicio (2005).
*Rhinella* cf. *margaritifera* 5: Pramuk (2006); Moravec et al. (2014).
*Rhinella margaritifera* (=*typhonius*): Mendelson et al. (2011).
*Rhinella* cf. *margaritifera*: Moravec et al. (2014); Santos et al. (2015); Cusi et al. (2017).

**Table 1.** Uncorrected (p-distance) and Kimura-2-Parameters (K2P) pairwise genetic distances between *Rhinella exostosica*, new species, and other species of the *R. margaritifera* species group included in our phylogenetic analyses. Distances are based on the 16S rRNA mitochondrial gene and expressed as percent difference.

| Species | p-distance | K2P |
|---|---|---|
| *Rhinella ocellata* | 6.0 | 6.3 |
| *Rhinella* aff. *margaritifera* PER | 5.1 | 5.3 |
| *Rhinella* aff. *margaritifera* ECU | 4.4 | 4.6 |
| *Rhinella* aff. *margaritifera* BR-AP | 3.1 | 3.2 |
| *Rhinella* aff. *margaritifera* BRA | 4.9 | 5.1 |
| *Rhinella lescurei* | 4.0 | 4.1 |
| *Rhinella alata* | 4.4 | 4.6 |
| *Rhinella margaritifera* "A" | 3.0 | 3.1 |
| *Rhinella dapsilis* | 2.4 | 2.5 |
| *Rhinella martyi* | 3.1 | 3.2 |
| *Rhinella margaritifera* "B" | 3.3 | 3.4 |
| *Rhinella hoogmoedi* | 2.7 | 2.7 |
| *Rhinella gildae* | 2.7 | 2.8 |
| *Rhinella yunga* | 4.0 | 4.2 |
| *Rhinella* aff. *castaneotica* | 4.9 | 5.1 |
| *Rhinella proboscidea* | 4.4 | 4.6 |
| *Rhinella* aff. *paraguayensis* BOL | 1.9 | 1.9 |

*Rhinella* sp. (*Rhinella margaritifera* complex): Moravec et al. (2016).

*Rhinella margaritifera*: Ávila et al. (2018).

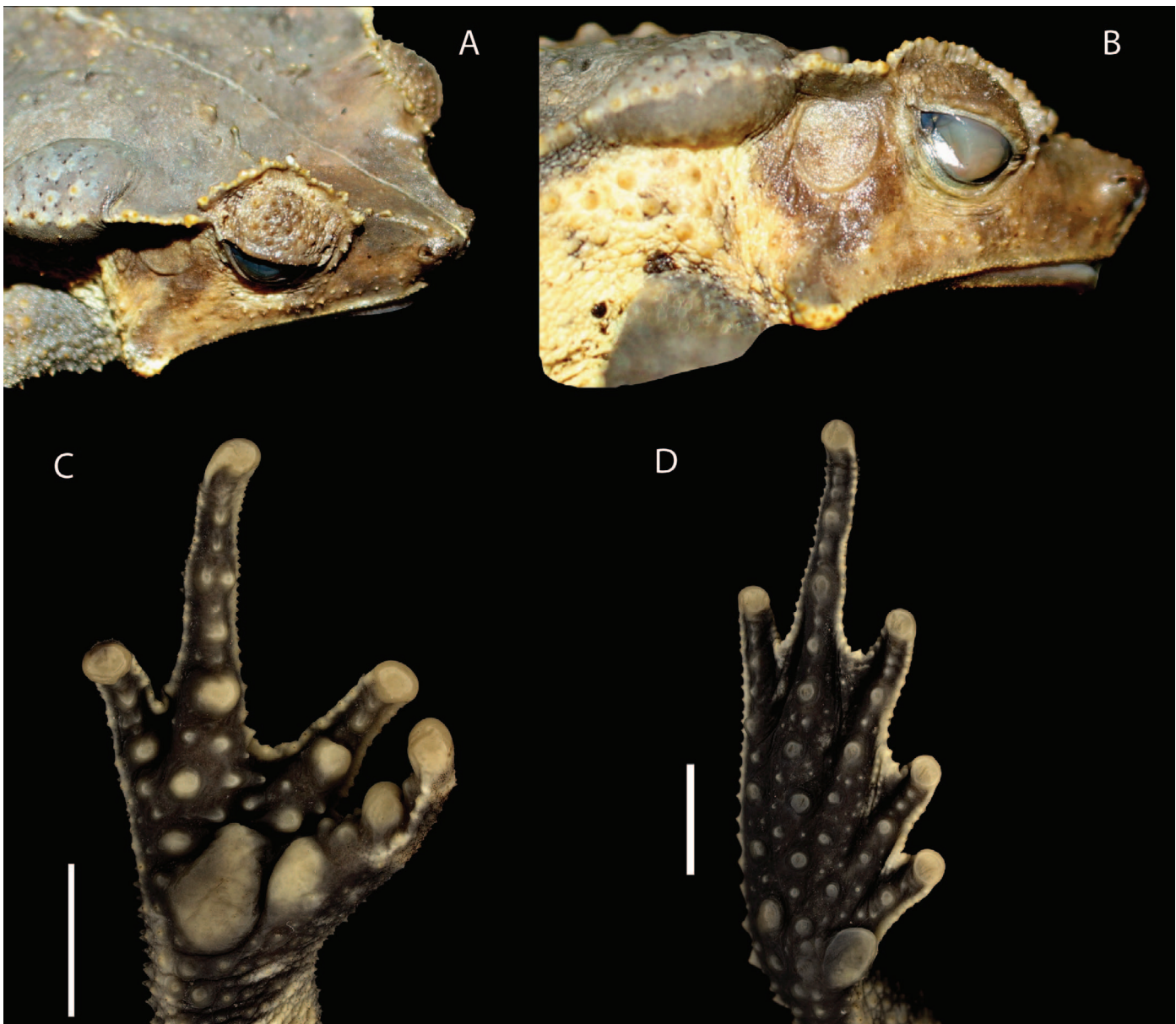**Holotype.**—INPAH 41323 (field number APL 19973), adult male from the Jací Direito Sampling Module, 09°27′44″S, 64°23′32″W, east bank of the Jací-Paraná River (a tributary of the east bank of the upper Madeira River), municipality of Porto Velho, Rondônia, Brazil, A. P. Lima, 7 November 2013 (Figs. 1, 2, 3A, B).

**Paratopotypes.**—Five adult specimens, all collected by A. P. Lima, same locality as the holotype: INPAH 41317 (field number APL 17632), female, 25 May 2011; INPAH 41321 (field number APL 19688), male, 25 May 2013; INPAH 41322 (field number APL 19697), female, 25 March 2013; INPAH 41326–27 (field numbers APL 21152–53), male and female (respectively), 11 November 2014.

**Paratypes.**—15 adult specimens. 7 specimens, Jací Novo Sampling Module, 09°24′45″S, 64°26′33″W, all collected by A. P. Lima: INPAH 41318–19 (field numbers APL 19409–10), females, 13 February 2013; INPAH 41320 (field numbers APL 19650), female, 22 March 2013; INPAH 41324 (field number APL 20029), male, 13 November 2013; INPAH 41325 (field number APL 21133), male, 8 November 2014; INPAH 41328–29 (field numbers APL 21154–55), females, 12 November 2014. 5 specimens, Morrinhos Sampling Module, 09°04′34″S, 64°14′46″W: INPAH 41312 (field number APL 15907), female, A. P. Lima, 9 November 2010; INPAH 41313 (field number APL 16422), male, R. Fraga, 13 January 2011; INPAH 41314 (field number APL 16468), female, R. Fraga, 14 January 2011; INPAH 41315–16 (field numbers APL 16473–74), male and female (respectively), A. P. Lima, 14 January 2011. 3 specimens, Três Praias Camp, 09°27′11″S, 64°25′04″W, all collected by A. P. Lima and M. Prestes: INPAH 41330 (field
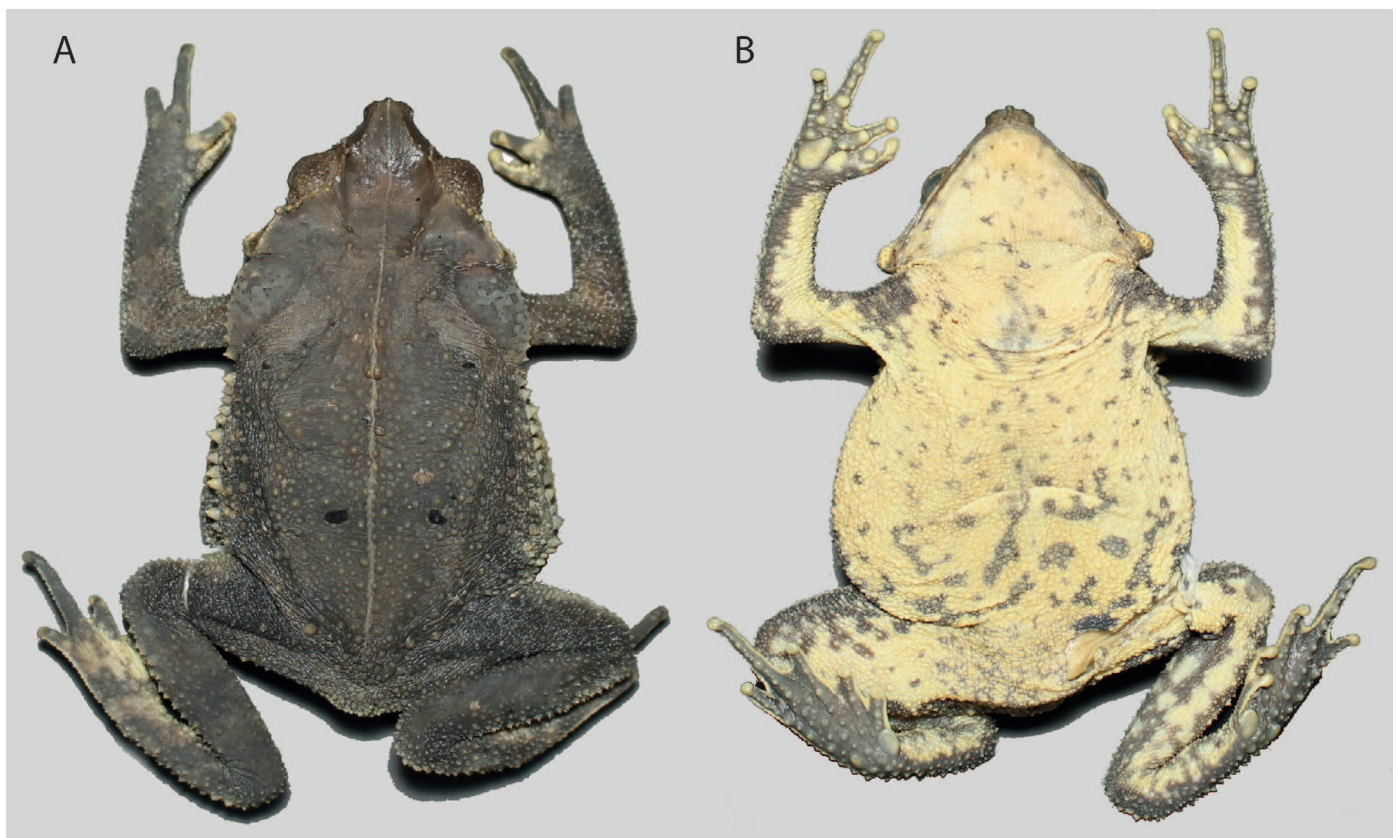


**Fig. 2.** Dorsal (A) and ventral (B) views of the male holotype of *Rhinella exostosica*, new species, INPAH 41323, SVL 68.7 mm.

**Fig. 3.** Dorsolateral (A) and lateral (B) views of the head, and ventral views of the hand (C) and foot (D) of the male holotype of *Rhinella exostosica*, new species, INPAH 41323. Scale bars, 5 mm.

number APL 21984), 11 January 2017; INPAH 41331–32 (field numbers APL 22030–31), males, 31 January 2017.

***Referred material.***—Six adult specimens. Bolivia: NMP6V 70687–8, 2 males, 5–6 km NE of Riberalta, Beni, 11°00′S, 66°05′W, J. Moravec and J. Aparicio, 26–27 November 1999; CBF 5800, male, Bolpebra, 10°57′S, 69°34′W, Nicolas Suaréz, Pando, J. Moravec, and J. Aparicio, between 30 January and 3 February 2005. Peru: NMP6V 74915, male, Regional Conservation Imiría, 17.4 km S of Masisea, 08°36′18″S, 74°18′23″W, Ucayali, J. Moravec and I. A. Tuanama, 27 September 2011; KU 215145–46, 2 unsexed specimens, 15 km E Puerto Maldonado, 12°32′38″S, 69°03′23″W, Cusco Amazónico, Madre de Dios, W. E. Duellman, 1989.

***Etymology.***—The specific epithet *exostosica* is derived from the Latin "exostosis" and a reference to the strongly

developed bony protrusion at the angle of the jaw of the new species.

***Diagnosis.***—*Rhinella exostosica* is a large-sized species of the *R. margaritifera* species group (Fig. 1; Pramuk, 2006). The species is diagnosed by the following combination of characters: 1) SVL 63.4–84.7 mm in females, 56.3–72.3 mm in males; 2) snout subacuminate in dorsal view; 3) snout lacks pronounced fleshy proboscis; 4) upper jaw curved upward in lateral view; 5) strongly developed bony protrusion at angle of jaw; 6) tympanic membrane and tympanic annulus present and evident; 7) supratympanic crests well developed; 8) proximal portion of supratympanic crest same height or shorter than parotoid gland in lateral view; 9) canthal crests poorly developed; 10) large parotoid glands; 11) 3–6 dorsal vertebral apophyses; 12) divided distal subarticular tubercle on finger III; 13) relative length of fingers III > IV > II > I; 14) skin on dorsum granulated with conical tubercles; 15)
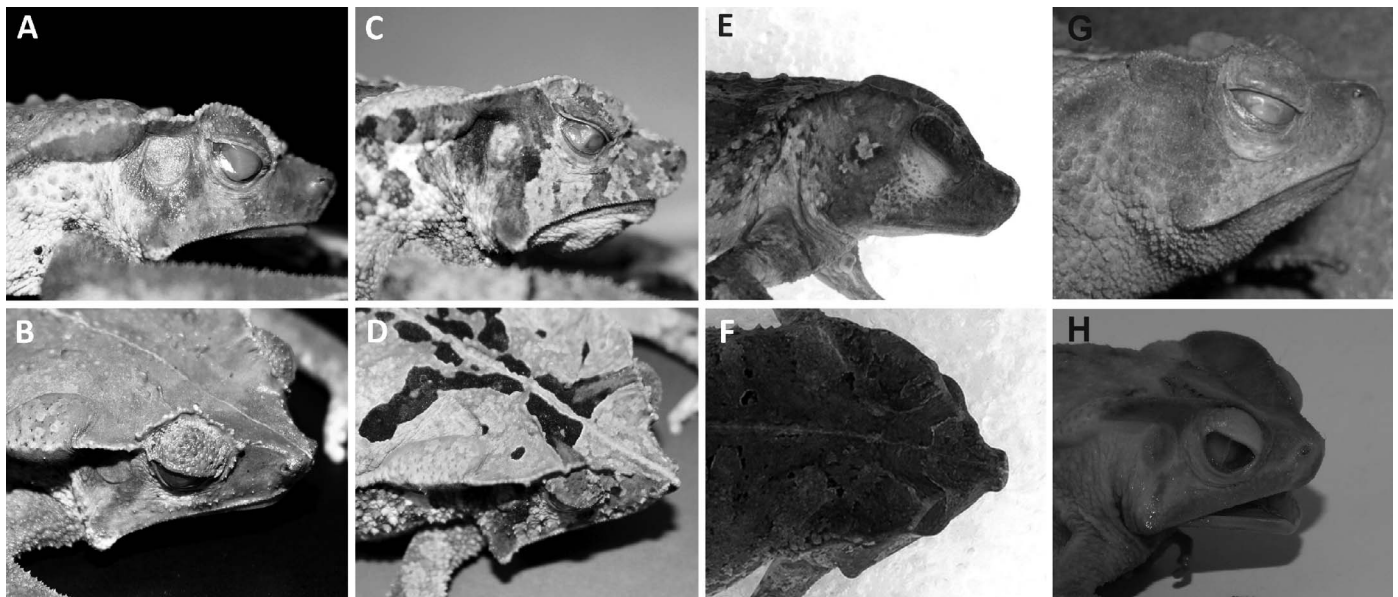
**Fig. 4.** Lateral and dorsolateral views of the head of *Rhinella exostosica*, new species (A–D), *R. martyi* (E, F), *R. paraguayensis* (G), and *R. margaritifera* (H). (A, B) Male holotype, INPAH 41323, SVL 68.7 mm. (C, D) Female, INPAH 41320, SVL 77.1 mm. (E, F) Female holotype, MNHN 2006.2601, SVL 66.5 mm. (G) Male holotype, UFMT 7430, SVL 50.4 mm. (H) Holotype, ZISP 257.1. Photographs by Miquéias Ferrão (A–D), Fréderic Braux, MNHN (E, F), Robson Ávila (G), and Konstantin D. Milto (H).

advertisement call duration 295–394 ms (339±26 ms), composed of groups of 7–9 (7.8±0.6) pulsed notes, with the last note consisting of 2–4 (2.9±0.8) pulses, and a dominant frequency of 1,012–1,163 Hz (1,081±63 Hz).

***Comparisons.***—We compare the new species with all nominal species of the *Rhinella margaritifera* species group, with particular attention to *R. martyi* and *R. paraguayensis* due to their morphological similarity and tentative phylogenetic placement, respectively. *Rhinella exostosica* can be distinguished from all members of the *R. margaritifera* species group by its combination of a strongly developed bony protrusion at the jaw angle, supratympanic crest shorter than or the same height as the parotoid gland in lateral view, and bifid distal subarticular tubercle on finger III. Diagnostic characters of compared species are enclosed in parentheses or brackets unless stated otherwise.

Maximum snout–vent length (SVL) of male *Rhinella exostosica* is 72.3 mm, which is much larger than *R. acutirostris* (holotype, 47 mm), *R. alata* (43.2 mm; Santos et al., 2015), *R. castaneotica* (41.9 mm; Caldwell, 1991), *R. gildae* (64.5 mm; Ávila et al., 2018), *R. hoogmoedi* (52 mm;



**Fig. 5.** Dorsolateral (above) and dorsal (below) views of *Rhinella exostosica*, new species (A, B) and *R.* aff. *margaritifera* (C, D) from the upper Madeira River, municipality of Porto Velho, Rondônia, Brazil. (A) Adult female, INPAH 41322. (B) Adult male, INPAH 41321. (C) Adult female, INPAH 41333. (D) Adult male, INPAH 41334. Localities: A, B, Jací Direito Sampling Module; C, D, west bank of the upper Madeira River. Photographs by Albertina P. Lima.
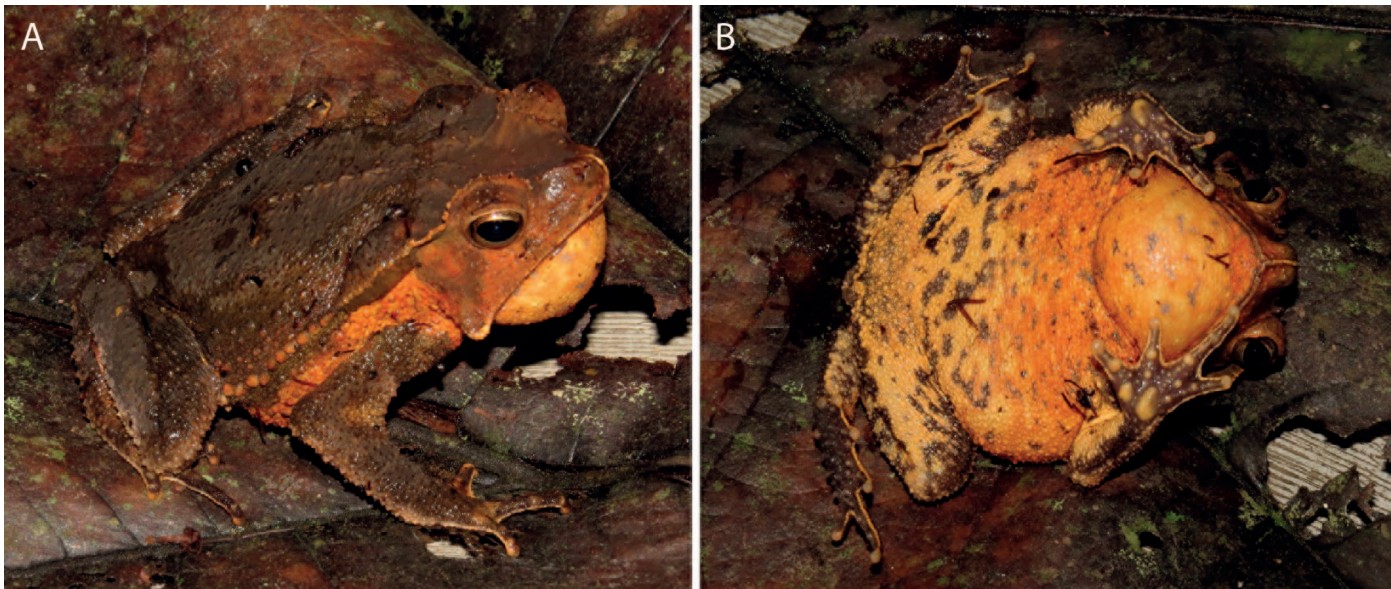
**Fig. 6.** Coloration in life of the adult male holotype of *Rhinella exostosica*, new species, INPAH 41323 (field number APL 19973). Dorsolateral (A) and ventral (B) views. Photographs by Albertina P. Lima.

Caramaschi and Pombal, 2006), *R. lescurei* (34.6±4.3 mm; Fouquet et al., 2007b), *R. magnussoni* (45.3 mm; Lima et al., 2007), *R. parecis* (53.5 mm; Ávila et al., 2020), *R. proboscidea* (54 mm; Lima et al., 2006), *R. scitula* (46.1 mm; Caramaschi and Niemeyer, 2003), *R. sebbeni* (59.7 mm; Vaz-Silva et al., 2015), and *R. stanlaii* (54 mm; Lötters and Köhler, 2000).

*Rhinella exostosica* differs from *R. roqueana* by having the upper jaw curved upward in lateral view and a divided distal subarticular tubercle on finger III (jaw straight and single distal subarticular tubercle; Melin, 1941); from *R. yunga* by having a tympanic membrane and annulus (absent), a divided distal subarticular tubercle on finger III (single distal subarticular tubercle), and dorsal vertebral apophyses (absent; Moravec et al., 2014); from *R. dapsilis* by lacking a pronounced fleshy proboscis on the snout (present), dorsal skin granulated with conical tubercles (dorsum smooth), supratympanic crests well developed (poorly developed), and maximum SVL 84.7 mm in females (77 mm; Myers and Carvalho, 1945; Hoogmoed, 1986); from *R. sclerocephala* by having a divided distal subarticular tubercle on finger III (single), the upper jaw curved upward in lateral view (straight), and maximum SVL 72.3 mm in males and 84.7 mm females (67.3 mm and 77.4 mm in males and females, respectively; Mijares-Urrutia and Arends, 2001); from the holotype of *R. margaritifera* by its subacuminate snout in dorsal view (truncate), proximal portion of the supratympanic crest the same height or shorter than the parotoid gland in lateral view (supratympanic crest higher than the parotoid gland; Fig. 4A, C, H), and a strongly developed bony protrusion at the angle of the jaw (protrusion moderately developed).

*Rhinella exostosica* differs from *R. martyi* in relative finger length III > IV > II> I (III > I > II > IV), the proximal portion of the supratympanic crest the same height or shorter than the parotoid gland in lateral view (higher than parotoid gland; Fig. 4A, C, E), parotoid gland large (small) and thenar tubercle ovoid (round). Males of *R. exostosica* present wider IOD than males of *R. martyi* (IOD/SVL = 0.18±0.01 in *R. exostosica*; IOD/SVL = 0.14±0.01 in *R.*

*martyi*). The advertisement call of *R. exostosica* is emitted in groups of 7.8±0.6 pulsed notes with a call duration of 339±26 ms, and the last note is composed of 2–4 pulses (maximum 6 notes per call, call duration 295±13 ms, and the last note has up to 6 pulses; Fouquet et al., 2007b).

*Rhinella exostosica* differs from *R. paraguayensis sensu stricto* by having the proximal portion of the supratympanic crest the same height or shorter than the upper limit of the parotoid gland in lateral view (higher; Fig. 4A, C, G), maximum SVL 72.3 mm in males and 84.7 mm in females (52.6 mm and 53.3 mm in males and females, respectively), snout subacuminate in dorsal view (rounded), parotoid glands large (small), vertebral apophyses present (absent), strongly developed bony protrusion at the angle of the jaw (poorly developed and straight), and an advertisement call with mean dominant frequency of 1,081±63 Hz (1,439±71 Hz; Ávila et al., 2010). *Rhinella exostosica* is readily distinguished from *R.* cf. *paraguayensis* from Bolivian lowland (*sensu* Jansen et al., 2011) by having a strongly developed bony protrusion at the angle of the jaw (poorly developed), dorsal vertebral apophyses (absent), and snout subacuminate in dorsal view (rounded; specimens reported in Jansen et al., 2011).

The new species is easily distinguished from *Rhinella* aff. *margaritifera* from the west bank of the upper Madeira River (Fig. 5) by having the proximal portion of the supratympanic crest the same height or shorter than the parotoid gland in lateral view (higher), a divided distal subarticular tubercle on finger III (single), a strongly developed bony protrusion at the angle of the jaw (poorly developed), canthal crests poorly developed (well developed), and parotoid glands large (small).

***Description of holotype.***—INPAH 41323 (field number APL 19973), adult male, SVL 68.7 mm (Figs. 2, 3, 4A–B, 6). Head wider than long (HW/HL = 1.1); HL 35% of SVL. Snout protruding in lateral view and subacuminate in dorsal view; dorsal surface slightly concave; nasal opening directed dorsolaterally; internarial distance 38% of interorbital dis-
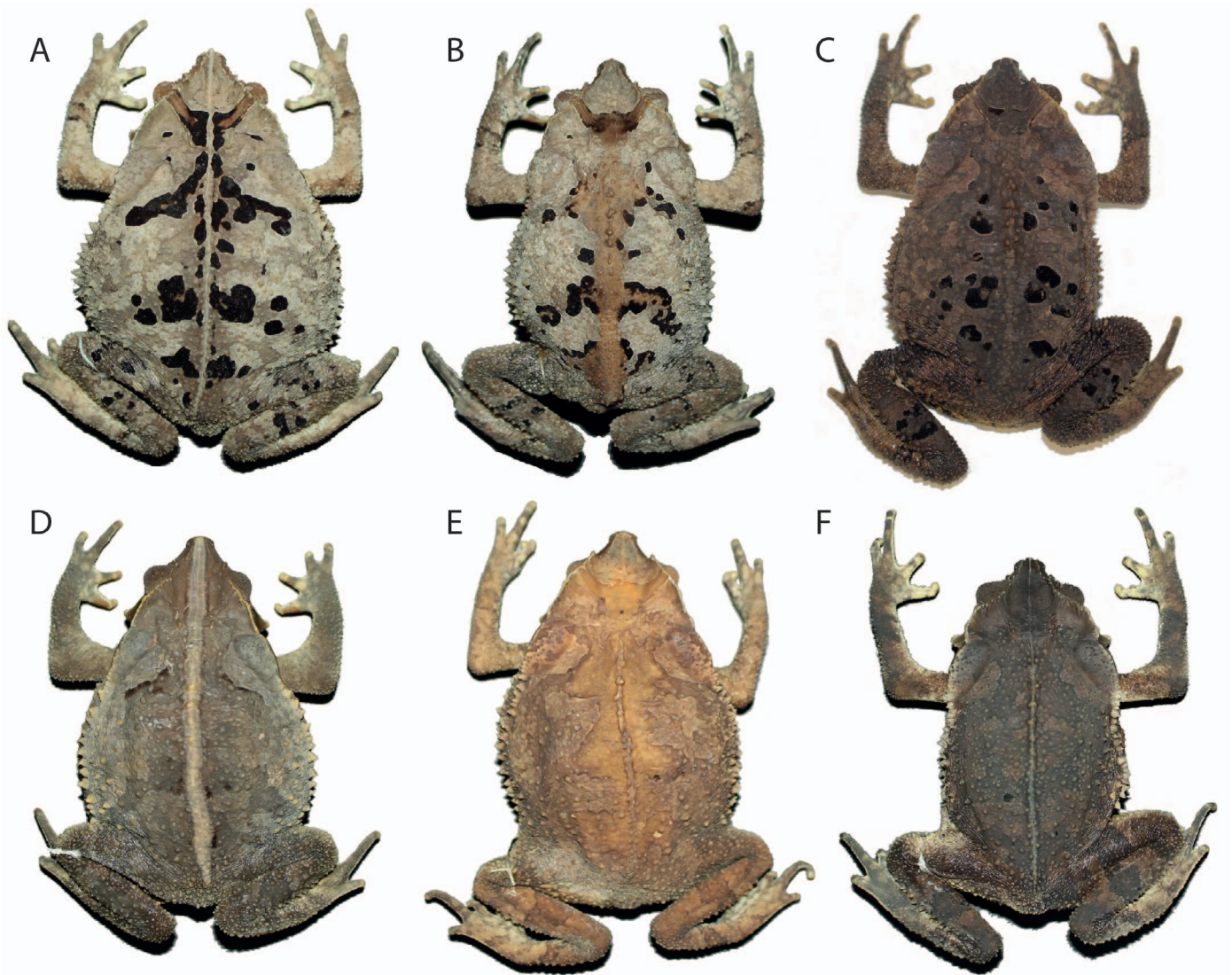
**Fig. 7.** Variation in dorsal coloration of preserved *Rhinella exostosica*, new species, from Brazil. (A) Adult female, INPAH 41320, SVL 77.1 mm. (B) Adult male, INPAH 41321, SVL 56.7 mm. (C) Adult male, INPAH 41327, SVL 84.4 mm. (D) Adult male, INPAH 41313, SVL 72.3 mm. (E) Adult female, INPAH 41312, SVL 84.2 mm. (F) Adult female, INPAH 41329, SVL 72.1 mm.

tance. Canthus rostralis delimited by a poorly developed canthal crest; loreal region concave. Eye–nostril distance 114% of eye diameter, 150% of horizontal tympanum diameter, and 142% of upper eyelid width. Eyes protuberant, wider than tympanum (EL/TYMH = 1.45; EL/TYMV = 1.30); eye diameter 137% of UEW. Absence of projections on upper eyelid; UEW 44% of IOD. A strongly developed and curved

bony protrusion at the angle of the jaw is visible in dorsal, ventral, and lateral views; distance between bony protrusions equals 116% of HW. Preorbital and canthal crests poorly developed; supraorbital, supratympanic, and parietal crests well developed; proximal portion of supratympanic crest shorter than the parotoid gland in lateral view; distance between supratympanic crests slightly larger than head
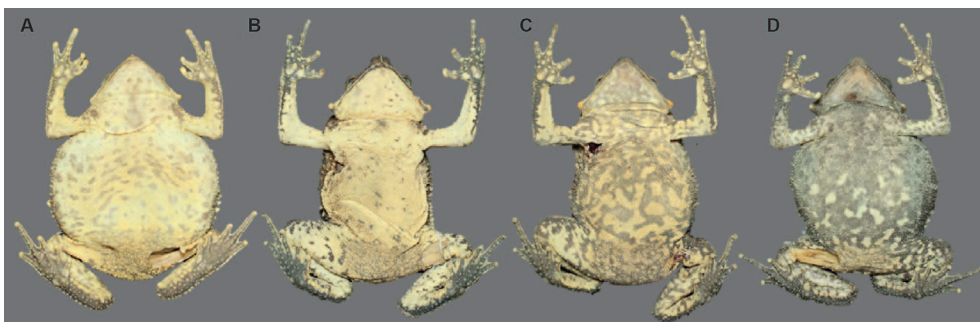


**Fig. 8.** Variation in ventral coloration of preserved *Rhinella exostosica*, new species. (A) Adult female, INPAH 41314, SVL 83.4 mm. (B) Adult female, INPAH 41329, SVL 72.1 mm. (C) Adult female, INPAH 41327, SVL 84.4 mm. (D) Adult female, INPAH 41322, SVL 68.7 mm.
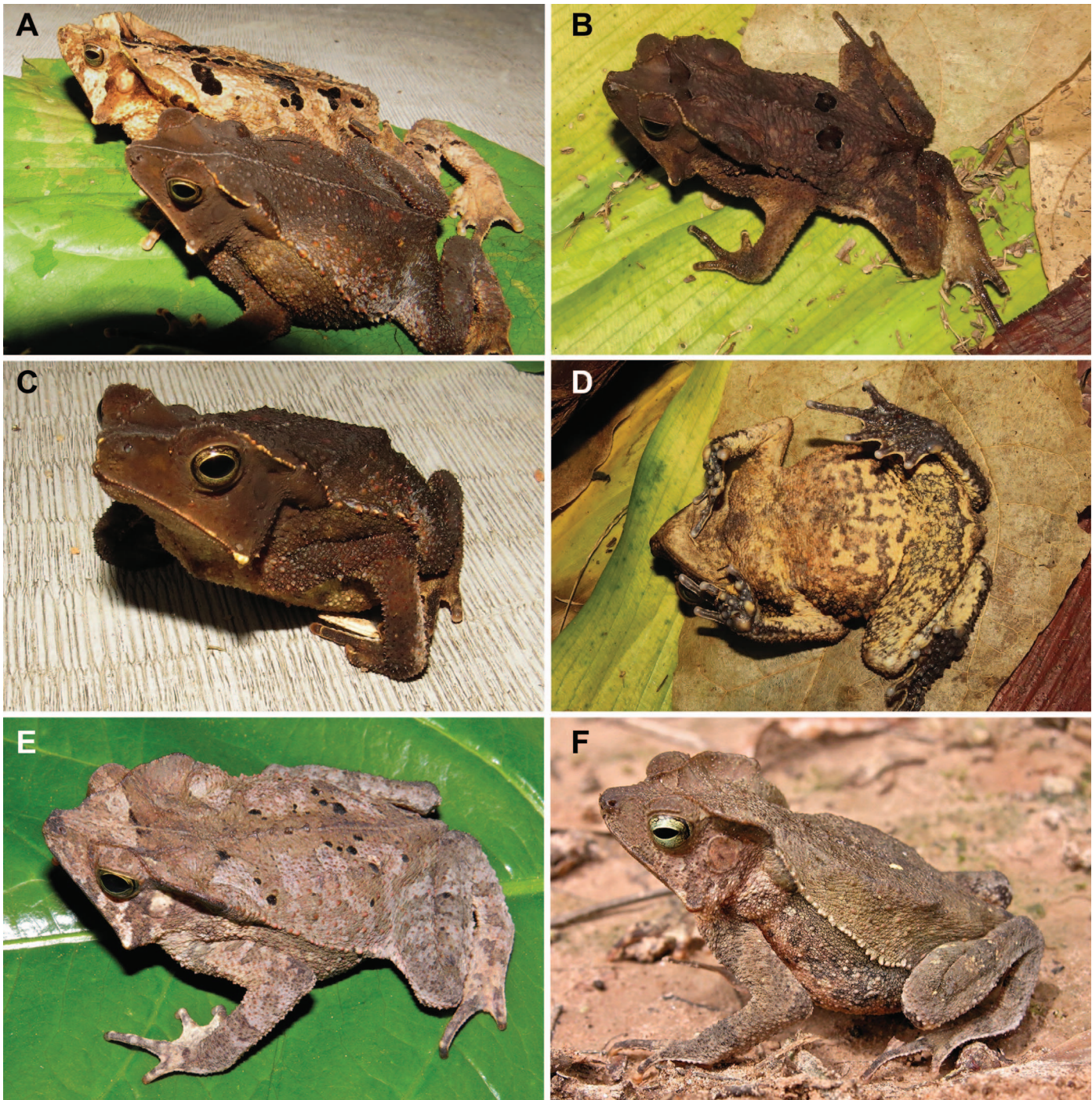
**Fig. 9.** Color variation in life of *Rhinella exostosica*, new species, from Brazil (A–D), Peru (E), and Bolivia (F). (A) Adult females: INPAH 41320 (above), SVL 77.1 mm; INPAH 41322 (below), SVL 68.7 mm; (B) Adult female, INPAH 41318, SVL 79.1 mm. (C) Adult female, INPAH 41322. (D) Adult female, INPAH 41318. (E) Subadult, NMP6V 74915. (F) Adult male, CBF 5800. Photographs by Albertina P. Lima (A–D) and Jiří Moravec (E–F).

width (POCD/HW = 1.01) but smaller than distance between bony protrusions (POCD/BPD = 0.87). Tympanum large, vertically oval (TYMV/TYMPH = 1.12), with a distinct annulus. Parotoid gland well developed, subtriangular in dorsal view and elliptic in lateral view; in dorsal view, twice as long as wide (PGL/PGW = 2.04); parotoid gland length 240% of POCL. Parotoid gland bordered by a line of small conical tubercles; a lateral line of large conical tubercles extends from the proximal corner of the parotoid gland to the groin. Two vertebral apophyses expanded dorsally. External choanae

small, oval, and laterally positioned; separated by approximately four times their width. Tongue oval, four times longer than wide. Vocal slits present; vocal sac single and subgular.

Anterior limbs robust; forearm as robust as upper arm; a line of small conical tubercles borders the forearm. Hand long; HAND3 90% of UAL; relative lengths of fingers III > IV > II > I (Fig. 3C); lateral fringes developed, especially on fingers I, II, and IV, with small conical tubercles extending from the outer lateral of finger I to the external lateral of finger IV. Fingertips poorly expanded; palmar tubercle large,
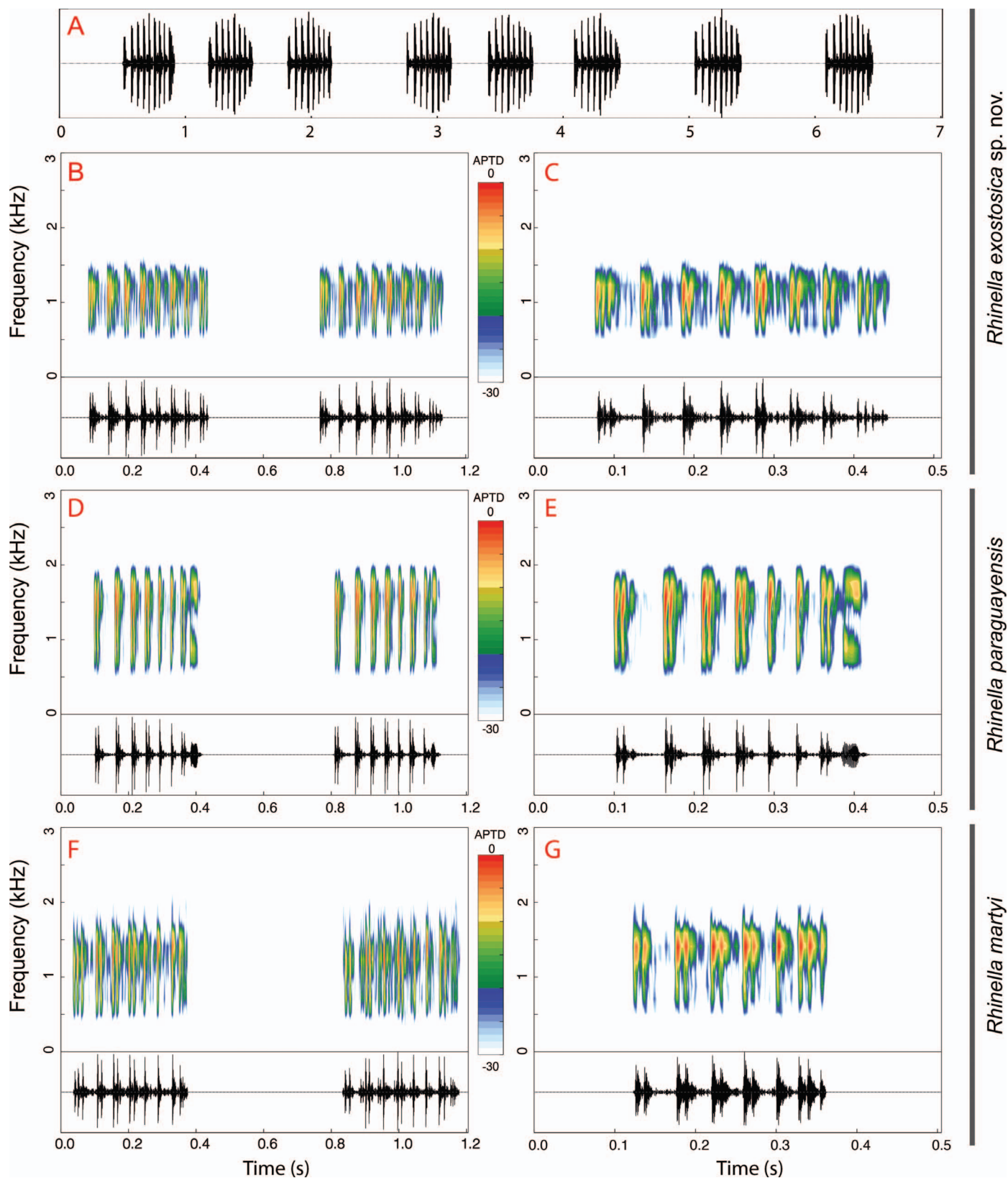
**Fig. 10.** Advertisement calls of *Rhinella exostosica*, new species (A–C), *R. paraguayensis* (D–E), and *R. martyi* (F–G). (A) Oscillogram of a series of eight-note calls of *R. exostosica*, new species. Detailed views of two (B) and one (C) calls of *R. exostosica*, new species. Detailed views of two (D) and one (E) calls of *R. paraguayensis*. Detailed views of two (F) and one (G) calls of *R. martyi*. Recordings: (A–C) INPAH 41331, Três Praias Camp, east bank of the Jací-Paraná River (affluent of the east bank of the Madeira River), Porto Velho, Rondônia, Brazil; (D–E) UFMT 2112, east bank of the Sepotuba River, Caceres, Mato Grosso, Brazil; (F–G) MNHN 2001.2006, Brownsberg Nature Park, Brokopondo district, Suriname.

**Table 2.** Morphometric measurements (in mm) of *Rhinella exostosica*, new species. Values are presented as mean ± standard deviation (minimum–maximum). Measurement abbreviations are defined in Materials and Methods. *n*, number of specimens.

| | Holotype | Females (*n* = 11) | Males (*n* = 10) |
| --- | --- | --- | --- |
| SVL | 68.7 | 77±7.1 (63.4–84.7) | 64.2±6.3 (56.3–72.3) |
| HL | 23.9 | 25.9±1.9 (21.9–28.4) | 21.9±1.9 (18.7–23.9) |
| HW | 26.6 | 27.8±2.4 (23.2–32.1) | 23.9±1.9 (20.7–26.6) |
| BPD | 30.9 | 32.5±2.2 (27.5–35.8) | 27.8±2.2 (24.6–30.9) |
| EL | 7.3 | 7.5±1.1 (5.7–9.5) | 6.5±0.5 (5.9–7.3) |
| IN | 4.4 | 4.9±0.4 (4.2–5.5) | 4.2±0.2 (3.9–4.4) |
| ED | 7 | 6.8±0.6 (6.1–7.8) | 6.3±0.6 (5.5–7.0) |
| IOD | 11.6 | 13.2±2.2 (10.6–18.1) | 11.5±0.9 (10.6–12.7) |
| UEW | 5.1 | 5.1±0.4 (4.6–5.6) | 4.8±0.4 (4.3–5.4) |
| TYMH | 4.8 | 4.3±0.4 (3.5–4.8) | 4.1±0.4 (3.6–4.8) |
| TYMV | 5.4 | 5.4±0.5 (4.2–6.2) | 4.8±0.6 (4.1–6.6) |
| TED | 2.1 | 3.3±0.5 (2.5–4.4) | 2.5±0.3 (2.1–2.8) |
| SH | 5.2 | 5.8±0.5 (5.0–6.6) | 4.8±0.3 (4.3–5.2) |
| POCL | 5.7 | 7.1±1.1 (4.9–8.8) | 6.2±0.5 (5.7–7.0) |
| POCD | 27 | 29.9±2.9 (24.8–33.3) | 24.1±2 (21.2–27.0) |
| SOCL | 11 | 11.8±0.9 (9.7–12.9) | 10.5±1 (9.3–12.0) |
| PGL | 13.7 | 14.7±2.0 (11.4–18.9) | 12.3±1.2 (10.4–13.7) |
| PGW | 6.7 | 8±2.1 (5.9–13.1) | 5.8±1.2 (3.4–6.9) |
| FAL | 20.2 | 21.6±1.9 (17.7–24.7) | 18.4±1.6 (16.4–20.2) |
| UAL | 15.5 | 15.9±2.0 (12.9–19.6) | 14.1±1.4 (12.9–16.5) |
| HAND1 | 11.4 | 13±1.1 (10.8–14.6) | 10.1±0.9 (8.9–11.4) |
| HAND2 | 12.1 | 13.3±1.1 (10.7–14.4) | 10.7±1 (9.5–12.1) |
| HAND3 | 18.2 | 19.9±1.3 (17.2–21.9) | 16.7±1.4 (15.0–18.8) |
| HAND4 | 12.9 | 13.9±1.2 (11.6–15.8) | 11.6±1.3 (10.2–13.9) |
| PTL | 4.6 | 5±0.7 (3.9–6.0) | 4.1±0.5 (3.3–4.6) |
| PTW | 3.1 | 3.5±0.4 (2.7–4.0) | 2.9±0.3 (2.4–3.4) |
| THL | 32.1 | 33.6±3.3 (27.6–37.8) | 29.1±2.6 (25.3–32.1) |
| TL | 29.2 | 30.5±2.8 (25.5–34.4) | 26.3±2.3 (23–29.2) |
| TAL | 18.4 | 19.3±1.7 (16.0–21.6) | 16.4±1.6 (14.0–18.4) |
| FOOT1 | 9.5 | 10.7±1.2 (8.5–12.5) | 9.0±0.8 (7.9–10.1) |
| FOOT2 | 12.1 | 13.5±1.3 (10.9–15.3) | 11.5±1.1 (9.8–12.9) |
| FOOT3 | 16.3 | 18.3±1.9 (10.9–15.3) | 15.5±1.2 (13.7–17) |
| FOOT4 | 23.9 | 26.7±2.1 (23–29.6) | 22.6±1.6 (19.8–24.7) |
| FOOT5 | 16.1 | 17.7±1.6 (14.6–19.5) | 14.9±1.2 (12.8–16.4) |
| APO | 3 | 5.0±0.9 (3–6) | 3.9±0.7 (3–5) |

tear-shaped, flat and smooth; thenar tubercle ovoid, pronounced, approximately 65% of palmar tubercle length. Subarticular tubercles developed on all fingers, single on fingers I, II, and IV, divided on distal articulation of finger III, single on proximal. Supernumerary tubercles conical, varied in size, and irregularly distributed.

Hind limbs robust. Thigh longer than tibia (THL/TL = 0.91); thigh length 47% of SVL, tibia length 42% of SVL. Tarsus length 77% of FOOT4 and 27% of SVL. Foot relatively short; FOOT4 35% of SVL. Relative lengths of toes IV > III > V > II > I; lateral fringes present and developed on toes, with small conical tubercles extending from the distal portion of the inner metatarsal tubercle on toe I to external toe V; toes with moderate webbing, webbing formula I 1–2$^+$ II 1–2$^{1/2}$ III 1–3$^{1/2}$ IV 3$^{1/2}$–1$^{1/2}$ V. Subarticular tubercles conical, single on all toes; outer metatarsal tubercle small and subconical; inner metatarsal tubercle large and ovoid, approximately twice the size of the outer metatarsal tubercle. Supernumerary tubercles present, varied in size, and irregularly arranged.

Skin granulated with conical and flat tubercles of varied size irregularly distributed on dorsum and flanks, granulated with small conical tubercles on limbs. Tubercles absent on interorbital region. Upper eyelids with small conical tuber-

cles. Tiny conical tubercles on subocular region, lips, and on bony protrusions at jaw angle. Ventral surface granulated.

In life, dorsal surface of body and limbs brown; dorsal surface of head orangish brown; lateral surfaces of head brownish orange; crests orange; lateral line of conical tubercles orange; lateral fringes on toes and fingers orangish cream (Fig. 6). Iris tan without black reticulations. Flanks orange tan. Chin, throat, and chest orange with inconspicuous light gray and cream blotches; ventral surface of arms cream; belly and ventral surface of thigh with conspicuous dark gray blotches irregularly distributed; ventral surface of tarsus dark gray. Palmar and plantar surfaces dark gray; thenar and palmar tubercles cream; subarticular and large supernumerary tubercles cream on hand; inner and outer metatarsal tubercles cream; subarticular and supernumerary tubercles dark brown on foot.

In preservative (Fig. 3, 4A, B), the brown dorsal coloration becomes gray; dorsal surface of head and snout brown; lateral surface of head brownish cream (Fig. 3A, B). Flanks cream; lateral line of tubercles light gray. Lateral fringes on toes and fingers cream. Chin, throat, chest, and ventral surfaces of thigh cream with gray and light gray blotches; ventral surface of arms cream. Palmar and plantar surfaces dark gray;
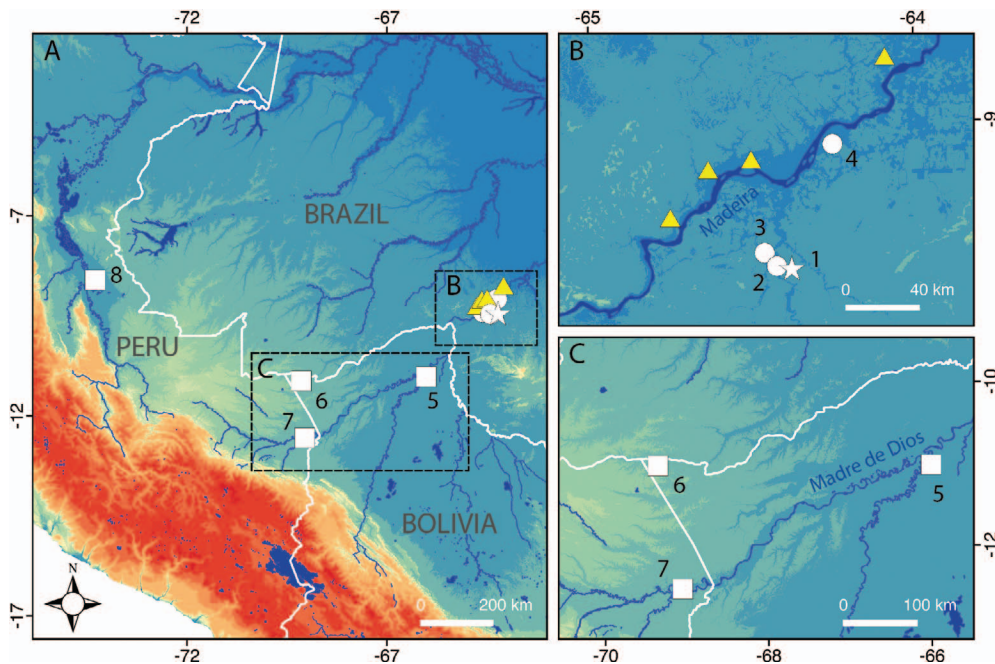
**Fig. 11.** Geographic distribution of *Rhinella exostosica*, new species (stars, circles, and squares) and *Rhinella* aff. *margaritifera* BRA (triangles). Localities: 1, type locality of *R. exostosica*, new species, Jací Direito Sampling Module; 2, Três Praias Camp; 3, Jací Novo Sampling Module; 4, Morrinhos Sampling Module; 5, Riberalta; 6, Bolpebra; 7, Puerto Maldonado; 8, Masisea.

tubercles on hand cream to light gray; tubercles on foot light gray.

***Color and morphological variation.***—In preservative, dorsal color varies from grayish cream (44% of specimens), dark brown (25%), orangish cream (19%), and dark gray (6%) to light gray (6%). Dorsolateral line of tubercles is grayish cream (44%), light brown (37%), dark brown (13%), or gray (6%). A dead-leaf pattern with dark blotches is present in 69% of specimens (Fig. 7A–C) but faded or inconspicuous in the rest (Fig. 7D–F). A cream-colored vertebral line extending from the snout to the urostyle is present in 81% of specimens. Dorsal surface of hind limbs with dark blotches or bars is seen in 81% of specimens. Ventral surfaces of chin, throat, chest, and thighs are colored by different shades of cream with light to dark gray blotches (which range from scarce to densely concentrated) in 94% of specimens (Fig. 8), but these surfaces are light gray with cream and gray blotches in the rest (Fig. 8). Although coloration in life is more vivid, the basic pattern is generally retained in preservative (Fig. 9).

Morphologically, all of the type series of *Rhinella exostosica* resembles the holotype, although the species exhibits sexual dimorphism in several characters (Table 2). Females are larger than males (SVL, t = –3.9643, df = 11.224, $P = 0.002$) and have more vertebral apophyses (APO, t = –2.6784, df = 13.705, $P = 0.018$) and a longer finger I (HAND1/SVL, t = –2.6222, df = 8.7776, $P = 0.028$). Conversely, males have longer supraocular crests (SOCL/SVL, t = 2.5907, df = 13.766, $P = 0.021$), wider tympanums (TYMH/SVL, t = 2.3596, df = 7.9322, $P = 0.046$), larger eyes (EL/SVL, t = 2.5296, df = 9.0854, $P = 0.032$), and wider upper eyelids (UEW/SVL, t = 3.2986, df = 13.029, $P = 0.005$) than females.

***Advertisement call.***—The advertisement call of *Rhinella exostosica* is emitted in a series of 9±5 calls (5–12, $n = 7$) with a call duration of 339±26 ms (295–394 ms; $n = 16$) and an inter-call interval of 483±209 ms (254–980 ms; $n = 16$; Fig. 10A–C). Calls are composed of 7.8±0.6 pulsed notes (7–9, $n =$ 16) with a note duration of 18±8 ms (7–47 ms; $n = 48$) and an inter-note interval of 36±4 ms (28–45 ms; $n = 32$). Overall, notes are formed by 2.3±0.8 pulses (1–4 pulses, $n = 48$) with a pulse duration of 8±1 ms (7–12 ms; $n = 48$). The number of pulses per note varies during the call; the first note (2.3±0.4 pulses [2–3 pulses, $n = 16$]) and the last note (2.9±0.8 pulses [2–4 pulses, $n = 16$]) usually contain more pulses than notes in the middle of the call (1.6±0.5 pulses [1–2 pulses, $n = 16$]). Calls have a dominant frequency of 1,081±63 Hz (1,012–1,163 Hz, $n = 16$) and a bandwidth of 423±17 Hz (409–452 Hz, $n = 16$).

***Tadpoles.***—Tadpoles of *Rhinella exostosica* were described by Duellman (2005).

***Distribution and natural history.***—*Rhinella exostosica* inhabits forests of the eastern portion of the upper Madeira Basin in Brazil, Bolivia, and Peru (Fig. 11). In Brazil, males and females of *R. exostosica* are active during the day on leaf litter within open lowland forest. At night, specimens are usually found on green leaves of shrubs or at the base of small trunks up to ~1 m high. Calling males were unsuccessfully sought close to small streams and temporary puddles within disturbed and intact forests at the sampling sites in Brazil. After seven years of field surveys, we finally came upon an explosive breeding event on 31 January 2017 in a bay of the Jací-Paraná River during heavy rain at the Três Praias Camp. Males began calling at ~1600 h sitting alongside the bank river or while floating within shallow water. We also found males calling on dense stands of macrophytes floating above deeper waters.

In Bolivia, *Rhinella exostosica* inhabits the forest of the Madre de Dios Basin and Acre Basin. In Riberalta (Beni Department), the species was recorded in a *terra firme* forest on the east banks of the Beni River close to the junction with the Madre de Dios River (Moravec and Aparicio, 2000). In Bolpebra (Pando Department), a calling male of *R. exostosica* was collected by Moravec and Aparicio (2005) in a temporary

pond surrounded by secondary forests along the east bank of the Acre River.

In Peru, Duellman (2005) recorded 450 individuals of *Rhinella exostosica* along a trail paralleling the Madama Stream, close to the junction with the Madre de Dios River (Cusco Amazónico, Madre de Dios Department). All individuals were found in a *terra firme* forest. Explosive breeding events also occur after heavy rains between November and February. Most calling males were found sitting on shallow backwaters or adjacent banks of the Madama Stream.

## DISCUSSION

*Rhinella exostosica* is the twenty-first described species of the *R. margaritifera* species group. In Brazilian Amazonia, the species is known only from the east bank of the Madeira River. In the last decade, our research group has repeatedly sampled 18 RAPELD sampling modules along both banks of the upper Madeira River in Rondônia and Amazonas, Brazil, especially those along the west bank (166 sampling sites distributed in 15 modules). No specimen of *R. exostosica* has been recorded in the west bank. Conversely, no specimen of *R.* aff. *margaritifera* BRA has been collected in the east bank. Therefore, we do not expect the new species to occur in the west bank of the upper Madeira River in Brazil.

*Rhinella exostosica* also occurs in Peru and Bolivia. Intraspecific pairwise genetic distances between Brazilian and Peruvian samples of *R. exostosica* from Puerto Maldonado are very low, as are those between these samples and the one from Bolpebra, Bolivia. In other hand, the Peruvian sample from Masisea shows higher intraspecific genetic distances to all other samples. Despite such genetic differentiation, morphology of the specimen from Masisea falls into the variation observed in the type series, as well as those from Puerto Maldonado and Bolivia.

*Rhinella* cf. *paraguayensis* (*sensu* Jansen et al., 2011) was recovered with low support as sister to *R. exostosica*. Genetic divergence between *R. exostosica* and *R.* cf. *paraguayensis* (p-distance = 1.9%) is low compared to the usual threshold of 3% used as evidence for heterospecificity in Neotropical frogs (Fouquet et al., 2007c; Vacher et al., 2020). Nevertheless, morphological characters show that these taxa unambiguously represent distinct species. Such disparity between morphological and molecular data is not uncommon in Neotropical frogs (e.g., Jungfer et al., 2013; Silva et al., 2020), and is also observed between *R. martyi* and *R. gildae*. It demonstrates that low genetic divergence should not be used without further data (e.g., morphology, behavior) to decide whether populations within the *R. margaritifera* species group are conspecific.

The strongly developed bony protrusion at the angle of the jaw of *Rhinella exostosica* easily distinguishes the species from most nominal congeners. The only exceptions are *Rhinella martyi* (northwestern Amazonia) and females of *R. dapisilis* (western Amazonia), from which *R. exostosica* differs mainly by having the proximal portion of the supratympanic crest shorter than the upper limit of the parotoid gland in lateral view, by its advertisement call, and by its breeding behavior. Also, *R. exostosica* is not closely related to *R. martyi* or *R. dapsilis* according to our multilocus phylogeny.

Males of *Rhinella exostosica* were found calling while perched on vegetation alongside the Jací-Paraná River, and also on dense stands of macrophytes floating above deeper water. To the best of our knowledge, these calling sites have not been reported for any other nominal species of the *R. margaritifera* species group in Amazonia. Large species within this species group usually breed in small to large ponds connected or unconnected to small streams (Fouquet et al., 2007b; Ávila et al., 2018). However, the use of dense stands of macrophytes has been described for *R. paraguayensis* in the Brazilian Pantanal (Ávila et al., 2010). Thus, this behavior may be a synapomorphy for *R. paraguayensis* and *R. exostosica*. Validation of this hypothesis awaits the inclusion of confirmed specimens of *R. paraguayensis* in a future phylogenetic reconstruction.

## DATA ACCESSIBILITY

Supplemental material is available at https://www.copeiajournal.org/ch2020043.

## MATERIAL EXAMINED

*Rhinella acutirostris:* Brazil: "flumen Amazonum" (=Amazon River), ZSM 1147/0 (holotype, photo).

*Rhinella alata:* Panama: Obispo, MNHN 84285 (holotype, photo).

*Rhinella castaneotica:* Brazil: Pará, Altamira, 7 km S of the Xingu River Ferry (APL 14104–05, 14453–55), Altamira Airport (APL 14477), CEPB 10043–51, 10053–58, 10061–62, 10064–65, 10068, MZUSP 67156–61, 67163–65 (paratopotypes, photo), 67162 (holotype, photo); Trairão, APL 21730, 21745; Treviso, APL 12307.

*Rhinella gildae:* Brazil: Maranhão, São Pedro da Água Branca, MNRJ 23838 (holotype, photo).

*Rhinella lescurei:* French Guiana: Haute Wanapi, Saut wanapi, MNHN 2006.2608 (holotype, photo).

*Rhinella magnussoni:* Brazil: Pará, Belterra, Highway BR-163, km 89, INPAH 19534, 19537–40 (paratypes).

*Rhinella margaritifera:* Brazil: ZISP 257.1 (holotype, photo), 257.2.

*Rhinella* aff. *margaritifera* BRA: Brazil: Rondônia, Porto Velho, INPAH 41333–37.

*Rhinella martyi:* Suriname: Brokopondo district, Brownsberg Nature Park, MNHN 2006.2601 (holotype, photo).

*Rhinella paraguayensis:* Brazil: Mato Grosso, Pantanal National al Park, UFMT-A 7430 (holotype, photo).

*Rhinella proboscidea:* Brazil: "flumen Solimoens" (=Solimoes River), ZSM 1145/0 (holotype, photo).

*Rhinella sebbeni:* Brazil: Goiás, Goiânia, MNRJ 53073 (holotype, photo).

*Rhinela yunga:* Peru: Pasco, Oxapampa, MUSM 31096 (paratype, photo), 31097 (holotype, photo).

## ACKNOWLEDGMENTS

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**Appendix 1.** Species, vouchers, localities, and GenBank accession numbers of samples used in molecular analysis.

| Species | Localities | Vouchers | TYR | 16S | 12S | COI | References |
|---|---|---|---|---|---|---|---|
| R. aff. castaneotica | Brazil: Amapá, Serra do Navio | 13766MTR | JN692098 | JN691352 | JN690745 | — | Fouquet et al. (2012a) |
| R. aff. castaneotica | Brazil: Amapá, Serra do Navio | 13732MTR | JN692095 | JN691359 | JN690752 | — | Fouquet et al. (2012a) |
| R. aff. castaneotica | French Guiana: Montagne des singes | 212CM | EF364351 | EF364290 | EF364264 | — | Fouquet et al. (2007a) |
| R. aff. margaritifera BRA | Brazil: Rondônia, Porto Velho, Pedras | INPAH 41335 | MT799550 | MT796078 | — | MT796631 | This study |
| R. aff. margaritifera BRA | Brazil: Rondônia, Porto Velho, Pedras | INPAH 41337 | MT799551 | MT796079 | — | MT796632 | This study |
| R. aff. margaritifera BRA | Brazil: Rondônia, Porto Velho, Pedras | INPAH 41336 | MT799552 | MT796080 | — | MT796633 | This study |
| R. aff. margaritifera "B" | French Guiana: Patawa | PG143 | EF364311 | EF364302 | EF364276 | — | Fouquet et al. (2007a) |
| R. aff. margaritifera "B" | French Guiana: Patawa | PG144 | EF364312 | EF364302 | EF364276 | — | Fouquet et al. (2007a) |
| R. aff. margaritifera BR-AP | Brazil: Amapá, Lourenco | 13873MTR | JN692017 | JN691391 | — | — | Fouquet et al. (2012a) |
| R. aff. margaritifera BR-AP | Brazil: Amapá, Lourenco | 13872MTR | JN692016 | JN691390 | — | — | Fouquet et al. (2012a) |
| R. aff. margaritifera BR-AP | Brazil: Amapá, Lourenco | 13878MTR | JN692019 | JN691392 | — | — | Fouquet et al. (2012a) |
| R. aff. margaritifera ECU | Ecuador: Napo, E. Biologica Jatun Sacha | QCAZ17989 | KR012530 | KR012622 | — | KR012565 | Santos et al. (2015) |
| R. aff. margaritifera ECU | Ecuador: Napo, E. Biologica Jatun Sacha | QCAZ17990 | KR012531 | KR012623 | KR012593 | KR012557 | Santos et al. (2015) |
| R. aff. margaritifera ECU | Ecuador: Napo, E. Biologica Jatun Sacha | QCAZ17991 | KR012532 | KR012614 | — | KR012543 | Santos et al. (2015) |
| R. aff. margaritifera PER | Peru: Madre de Dios | USNM268828 | — | DQ158490 | DQ158490 | — | Pramuk (2006) |
| R. aff. paraguayensis | Bolivia: Santa Cruz, San Sebastián | SMF88237 | — | JF790186 | — | — | Jansen et al. (2011) |
| R. aff. paraguayensis | Bolivia: Santa Cruz, San Sebastián | MNKA9691 | — | JF790185 | — | — | Jansen et al. (2011) |
| R. alata | Ecuador: Esmeraldas, Protectora La Chiquita | QCAZ11598 | KR012526 | KR012618 | KR012603 | KR012550 | Santos et al. (2015) |
| R. alata | Ecuador: Esmeraldas, Protectora La Chiquita | QCAZ10255 | KR012525 | KR012617 | KR012602 | KR012570 | Santos et al. (2015) |
| R. alata | Ecuador: Esmeraldas, San Lorenzo | QCAZ23161 | KR012534 | KR012626 | KR012577 | KR012562 | Santos et al. (2015) |
| R. dapsilis | Ecuador: Pastaza, Comunidad Kurintza | QCAZ38892 | KR012519 | KR012640 | KR012608 | KR012566 | Santos et al. (2015) |
| R. dapsilis | Ecuador: Pastaza, Villano K4 | QCAZ38688 | KR012517 | KR012638 | KR012607 | KR012575 | Santos et al. (2015) |
| R. dapsilis | Ecuador: Pastaza, Villano BII | QCAZ38512 | KR012514 | KR012635 | KR012587 | KR012558 | Santos et al. (2015) |
| R. exostosica, new species BOL | Bolivia: Bolpebra | CBF5800 | — | KY912609 | — | — | Cusi et al. (2017) |
| R. exostosica, new species BRA | Brazil: Rondônia, Porto Velho, Três Praias Camp | INPAH 41330 | MT799547 | MT796075 | — | MT796628 | This study |
| R. exostosica, new species BRA | Brazil: Rondônia, Porto Velho, Três Praias Camp | INPAH 41331 | MT799548 | MT796076 | — | MT796629 | This study |
| R. exostosica, new species BRA | Brazil: Rondônia, Porto Velho, Jací Direito | INPAH 41323 | MT7995489 | MT796077 | — | MT796630 | This study |
| R. exostosica, new species PER | Peru: Madre de Dios, 15 km E Puerto Maldonado | KU215145 | — | DQ158491 | DQ158491 | — | Pramuk (2006) |
| R. exostosica, new species PER | Peru: Madre de Dios, 15 km E Puerto Maldonado | KU215146 | — | HM563858 | HM563816 | JN867978 | Mendelson et al. (2011) |
| R. festae | Ecuador: Napo, E. Biologica Jatun Sacha | QCAZ18203 | KR012510 | KR012624 | KR012609 | KR012574 | Santos et al. (2015) |
| R. gildae | Brazil: Maranhão, Carolina | ESTR00173 | — | JN867574 | JN867548 | — | Fouquet et al. (2012b) |
| R. gildae | Brazil: Pará, Maraba | CFBHT12072 | — | KU495515 | — | KU494722 | Lyra et al. (2017) |
| R. gildae | Brazil: Maranhão, Estreito | CFBHT05548 | — | KU495519 | — | KU494726 | Lyra et al. (2017) |
| R. gildae | Brazil: Ceará, Guaramiranga | CFBHT11157 | — | KU495518 | — | KU494725 | Lyra et al. (2017) |
| R. hoogmoedi | Brazil: Sao Paulo, Sao Sebastao | CFBHT03137 | — | KU495508 | — | KU494715 | Lyra et al. (2017) |
| R. hoogmoedi | Brazil: Sao Paulo, Sao Sebastao | CFBHT03858 | — | KU495507 | — | KU494714 | Lyra et al. (2017) |

**Appendix 1.** Continued.

| Species | Localities | Vouchers | TYR | 16S | 12S | COI | References |
|---|---|---|---|---|---|---|---|
| *R. hoogmoedi* | Brazil: Bahia, Serra Bonita, Camacan | MTR16199 | – | JN867571 | JN867545 | – | Fouquet et al. (2012b) |
| *R. hoogmoedi* | Brazil: Espírito Santo, Linhares | CFBHT15337 | – | KU495504 | – | KU494711 | Lyra et al. (2017) |
| *R. lescurei* | French Guiana: Litany | 112BM | EF364343 | EF364305 | EF364279 | – | Fouquet et al. (2007a) |
| *R. lescurei* | French Guiana: Saül | 121BM | EF364341 | EF364304 | EF364278 | – | Fouquet et al. (2007a) |
| *R. lescurei* | French Guiana: Saül | PG103 | EF364339 | EF364304 | EF364278 | – | Fouquet et al. (2007a) |
| *R. major* | Bolivia: Cochabamba, Chapare | MNCN6232 | – | KP685219 | KP685219 | – | Pereyra et al. (2016) |
| *R. margaritifera* "A" | French Guiana: Kaw | 108MC | EF364333 | EF364292 | EF364266 | – | Fouquet et al. (2007a) |
| *R. margaritifera* "A" | French Guiana: Saül | 217MC | EF364329 | EF364299 | EF364273 | – | Fouquet et al. (2007a) |
| *R. margaritifera* "A" | French Guiana: Grand Santi | 203MC | EF364327 | EF364295 | EF364269 | – | Fouquet et al. (2007a) |
| *R. marina* | Ecuador: Puerto Cayo | QCAZ50698 | KR012508 | KR012643 | KR012579 | KR012545 | Santos et al. (2015) |
| *R. martyi* | French Guiana: Trijonction | 156MC | EF364337 | EF364303 | EF364277 | – | Fouquet et al. (2007a) |
| *R. martyi* | French Guiana: Trijonction | 157MC | EF364338 | EF364303 | EF364277 | – | Fouquet et al. (2007a) |
| *R. martyi* | Suriname: Road to Apura | 2006.2606 MNHN | JN692043 | JN691373 | JN690766 | – | Fouquet et al. (2012a) |
| *R. martyi* | Brazil: Amapá, Igarape Camaipi | MTR6228 | JN692055 | JN691377 | JN690770 | – | Fouquet et al. (2012a) |
| *R. ocellata* | Brazil: Tocantins, UHE Lajeado | LAJ2010 | – | JN867572 | JN867546 | – | Fouquet et al. (2012b) |
| *R. ocellata* | Brazil: Tocantins, Peixe | MZUSP103261 | – | DQ158479 | DQ158479 | – | Pramuk (2006) |
| *R. proboscidea* | Brazil: Amazonas, Manaus | CTGA5602 | – | MG806046 | – | – | Motta et al. (2018) |
| *R. proboscidea* | Brazil: Amazonas, Manaus | CTGA5595 | – | MG806045 | – | – | Motta et al. (2018) |
| *R. spinulosa* | Argentina: Neuquén | NB96-23 | – | AY680263 | AY680263 | – | Pauly et al. (2004) |
| *R. veraguensis* | Peru: Cusco | USNM346048 | – | DQ158497 | DQ158497 | – | Pramuk (2006) |
| *R. yunga* | Peru: Cordillera Yanachaga | MUSM31096 | – | KF992150 | – | – | Moravec et al. (2014) |
| *R. yunga* | Peru: Cordillera Yanachaga | MUSM31097 | – | KF992151 | – | – | Moravec et al. (2014) |
| *R. yunga* | Peru: Cordillera Yanachaga | NMP6V74748 | – | KF992152 | – | – | Moravec et al. (2014) |

**Appendix 2.** Best-fit partition schemes and nucleotide evolution models determined by PartitionFinder. Numbers after backslashes represent protein coding marker codons.

| Schemes | Best model | Subset partitions | Subset alignment |
|---|---|---|---|
| 1 | GTR+I+G | 16S, 12S | 1–870, 871–1768 |
| 2 | F81 | COI\1 | 1769–2446 |
| 3 | GTR+G | COI\2 | 1770–2446 |
| 4 | SYM+I | COI\3 | 1771–2446 |
| 5 | JC | Tyr\1, Tyr\2 | 2447–2996, 2448–2996 |
| 6 | HKY+G | Tyr\3 | 2449–2996 |

**Appendix 3.** Uncorrected p-distances and Kimura-2-Parameters (K2P) pairwise genetic distances among *Rhinella exostosica*, new species, and other species of the *R. margaritifera* species group. Distances, expressed as a percentage, are based on 16S rRNA mitochondrial gene sequence data.

| | Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *R. ocellata* | | 6.5 | 6.3 | 6.4 | 6.5 | 6.6 | 6.5 | 6.3 | 7.7 | 6.1 | 6.5 | 4.8 | 6.6 | 6.8 | 6.8 | 6.4 | 6.3 | 6.0 |
| 2 | *R. aff. margaritifera* PER | 6.8 | | 1.7 | 1.8 | 6.5 | 6.4 | 6.2 | 4.5 | 4.3 | 5.1 | 6.4 | 4.1 | 7.0 | 5.7 | 6.6 | 5.2 | 4.1 | 5.1 |
| 3 | *R. aff. margaritifera* ECU | 6.7 | 1.7 | | 0.9 | 6.3 | 6.2 | 6.0 | 5.0 | 4.2 | 4.6 | 5.9 | 4.3 | 6.8 | 5.4 | 6.2 | 4.9 | 3.3 | 4.4 |
| 4 | *R. aff. margaritifera* BRA | 6.7 | 1.8 | 0.8 | | 6.5 | 6.4 | 6.2 | 5.2 | 4.4 | 5.1 | 6.2 | 4.3 | 7.0 | 5.7 | 6.4 | 5.3 | 3.7 | 4.9 |
| 5 | *R. aff. margaritifera* BR-AP | 6.9 | 6.9 | 6.6 | 6.9 | | 0.6 | 0.5 | 5.8 | 4.9 | 6.1 | 3.8 | 4.7 | 2.6 | 2.7 | 0.6 | 1.0 | 4.0 | 3.1 |
| 6 | *R. margaritifera* "A" | 7.0 | 6.8 | 6.5 | 6.8 | 0.6 | | 1.1 | 5.7 | 4.9 | 5.4 | 3.7 | 4.8 | 2.7 | 2.8 | 0.9 | 0.9 | 3.9 | 3.0 |
| 7 | *R. margaritifera* "B" | 6.9 | 6.6 | 6.3 | 6.6 | 0.5 | 1.1 | | 5.8 | 5.5 | 6.1 | 4.3 | 4.7 | 3.1 | 3.2 | 1.1 | 1.5 | 4.2 | 3.3 |
| 8 | *R. aff. castaneotica* | 6.6 | 4.7 | 4.8 | 5.0 | 5.5 | 5.4 | 5.5 | | 5.2 | 3.5 | 4.9 | 4.5 | 5.6 | 4.9 | 5.9 | 4.5 | 4.4 | 4.9 |
| 9 | *R. alata* | 8.2 | 4.4 | 4.1 | 4.6 | 4.7 | 4.7 | 5.2 | 5.5 | | 4.2 | 5.9 | 3.9 | 4.7 | 4.5 | 4.7 | 4.4 | 3.5 | 4.4 |
| 10 | *R. proboscidea* | 6.4 | 5.3 | 4.7 | 5.3 | 5.8 | 5.2 | 5.8 | 3.6 | 4.3 | | 5.9 | 4.2 | 5.8 | 4.9 | 5.2 | 4.4 | 3.8 | 4.4 |
| 11 | *R. lescurei* | 6.8 | 6.8 | 6.2 | 6.5 | 3.7 | 3.6 | 4.2 | 5.1 | 6.2 | 6.2 | | 6.2 | 4.5 | 3.2 | 3.6 | 3.2 | 4.1 | 4.0 |
| 12 | *R. yunga* | 5.0 | 4.2 | 4.4 | 4.4 | 4.5 | 4.6 | 4.5 | 4.7 | 4.0 | 4.3 | 6.5 | | 5.0 | 4.8 | 4.6 | 3.4 | 3.7 | 4.0 |
| 13 | *R. martyi* | 7.0 | 7.5 | 7.2 | 7.5 | 2.6 | 2.6 | 3.1 | 5.8 | 4.9 | 6.1 | 4.6 | 5.3 | | 3.6 | 2.4 | 3.1 | 4.0 | 3.1 |
| 14 | *R. hoogmoedi* | 7.3 | 6.0 | 5.6 | 6.0 | 2.8 | 2.9 | 3.3 | 5.1 | 4.7 | 5.1 | 3.2 | 5.1 | 3.7 | | 2.5 | 2.1 | 2.7 | 2.7 |
| 15 | *R. gildae* | 7.2 | 7.0 | 6.5 | 6.8 | 0.6 | 0.9 | 1.2 | 6.2 | 4.9 | 5.5 | 3.7 | 4.8 | 2.4 | 2.6 | | 1.2 | 3.4 | 2.7 |
| 16 | *R. dapsilis* | 6.8 | 5.4 | 5.2 | 5.6 | 1.0 | 0.9 | 1.5 | 4.7 | 4.5 | 4.6 | 3.3 | 3.5 | 3.1 | 2.1 | 1.2 | | 2.6 | 2.4 |
| 17 | *R. cf. paraguayensis* | 6.6 | 4.2 | 3.4 | 3.8 | 4.1 | 4.0 | 4.4 | 4.6 | 3.6 | 3.9 | 4.3 | 3.8 | 4.1 | 2.8 | 3.5 | 2.6 | | 1.9 |
| 18 | *R. exostosica*, new species | 6.3 | 5.3 | 4.6 | 5.1 | 3.2 | 3.1 | 3.4 | 5.1 | 4.6 | 4.6 | 4.1 | 4.2 | 3.2 | 2.7 | 2.8 | 2.5 | 1.9 | |