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Two new species of the *Rhinella margaritifera* species group (Anura: Bufonidae) from the Eastern Guiana Shield

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Abstract. We herein describe two new species of the *Rhinella margaritifera* group from the eastern Guiana Shield. These two species have low cephalic crests, no vertebral apophyses or bony protrusion at jaw angles and were consistently recovered as candidate species in recent integrative studies combining morphological, acoustic and molecular data. The first species, *Rhinella guianensis* sp. nov., is related to *R. proboscidea*, *R. castaneotica* and *R. teotoniensis* and is characterized by a rounded snout in dorsal view, acute in lateral view and the absence of cranial crests, except for a low supratympanic crest. The second species, *Rhinella latebrosa* sp. nov., is related to *R. unapensis* and is characterized by a truncate snout in dorsal view, acute in lateral view, and the presence of small cranial crests. Both species are known to occur in French Guiana and the state of Amapá in Brazil, where they co-occur with *Rhinella lescurei* and the *Rhinella margaritifera/dapsilis/martyi* complex. The description of these two new species brings the number of currently recognized nominal species in the *R. margaritifera* group to 25. However, many species remain to be described in this clade and some boundaries need to be clarified using genomics, as well as the taxonomic status of modern populations using museomics.

Keywords. Amphibia, Neotropics, rainforest, systematics, taxonomy.

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Introduction

The taxonomy of toads of the *Rhinella margaritifera* species group is notoriously difficult and has been a challenge for a long time (Hoogmoed 1989, 1990; Fouquet *et al*. 2007a, 2007b; Ávila *et al*. 2018). Recently, several attempts to unlock some taxonomic problems inherent to this clade have been made combining bioacoustics, morphology and molecular data (dos Santos *et al*. 2015; Avila *et al*. 2018; Pereyra *et al*. 2021; Fouquet *et al*. 2024).

Currently, 23 species distributed in Central and South America are recognized (Frost 2025): *Rhinella acutirostris* (Spix, 1824), *R. alata* (Thominot, 1884), *R. angeli* Rojas, Peña, Avila, Carvalho, Perez, Farias, Gordo & Hrbek, 2022, *R. castaneotica* (Caldwell, 1991), *R. cristinae* (Vélez-Rodríguez & Ruiz-Carranza, 2002), *R. dapsilis* (Myers & Carvalho, 1945), *R. exostosica* Ferrão, Lima, Ron, Santos & Hanken, 2020, *R. hoogmoedi* Caramaschi & Pombal, 2006, *R. iserni* (Jiménez de la Espada, 1875), *R. lescurei* Fouquet, Gaucher, Blanc & Vélez-Rodriguez, 2007, *R. magnussoni* Lima, Menin & Araújo, 2007, *R. margaritifera* (Laurenti, 1768), *R. martyi* Fouquet, Gaucher, Blanc & Vélez-Rodriguez, 2007, *R. ocellata* (Günther, 1858), *R. proboscidea* (Spix, 1824), *R. roqueana* (Melin, 1941), *R. parecis* Ávila, Morais, Perez, Pansonato, Carvalho, Rojas, Gordo & Farias, 2020, *R. sclerocephala* (Mijares-Urrutia & Arends, 2001), *R. sebbeni* Vaz-Silva, Maciel, Bastos & Pombal, 2015, *R. sternosignata* (Günther, 1858), *R. stanlaii* (Lötters & Köhler, 2000), *R. teotoniensis* Ferrão, Sousa, Hanken & Lima, 2022 and *R. unapensis* Rojas, Peña, Avila, Carvalho, Perez, Farias, Gordo & Hrbek, 2022.

However, several studies highlighted the underestimation of the actual number of species in the group (Fouquet *et al*. 2007a, 2024; dos Santos *et al*. 2015; Avila *et al*. 2020; Ferrão *et al*. 2020; Pereyra *et al*. 2021). Recently, Pereyra *et al*. (2021) hypothesized the existence of 11 additional undescribed species, while Fouquet *et al*. (2024) indicated six undescribed confirmed candidate species including two from the eastern Guiana Shield within a total of 54 MOTUs. In the present paper, we formally describe these two new species.

Material and methods

Morphological analyses

We used the large dataset of Fouquet *et al.* (2024), complemented by recently collected material. Thus, the external morphology of 2028 specimens housed at museum collections (see list of abbreviations) was examined, including types of most nominal species (Suppl. file 1).

ABAM = Acervo Biológico da Amazônia Meridional da Universidade Federal de Mato Grosso, Sinop, Brazil
CHUFC-A = Coleção Herpetológica da Universidade Federal do Ceará, Fortaleza Brazil
CHUFMG = Coleção Herpetológica da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
CTGA = Coleção de Tecidos de Genética Animal da Universidade Federal do Amazonas, Manaus, Brazil
CZDP = Coleção Zoológica Delta do Parnaíba da Universidade Federal Delta do Parnaíba, Parnaíba, Brazil
INPA-H = Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil
MNHN-RA = Reptiles and Amphibians collection of the Muséum national d'Histoire naturelle, Paris, France
MNRJ = Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil
MZUSP = Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil
UFMT-A = Coleção Zoológica da Universidade Federal de Mato Grosso, Cuiabá, Brazil
UFOPA = Coleção Herpetológica da Universidade Federal do Oeste do Pará, Santarém, Brazil

UFRGS = Coleção de répteis e anfíbios do Departamento de Zoologia da Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
UFRPE = Coleção Herpetológica e Paleoherpetológica da Universidade Federal Rural de Pernambuco, Recife, Brazil
URCA-H = Coleção de Herpetologia da Universidade Regional do Cariri, Fortaleza, Brazil
USNM = United States National Museum, Smithsonian Institution, Washington, USA
ZISP = Herpetological collection of the Zoological Institute, Russian Academy of Sciences, St Petersburg, Russia
ZUEC = Coleção de Anfíbios do Museu de Zoologia da UNICAMP, Campinas, Brazil

Additionally, we examined specimens from field series: Antoine Fouquet (prefixes: AF, CAAM), Afonso Pedroso dos Santos (prefix: APSJ), Christine Strüssmann (prefix: LH), Marcelo Gordo (prefixes: IR, NS, MN, Taracuá, MG, IR, J, JTA, Ipixuna) and Miguel Trefaut Rodrigues (prefixes: MTR, ESTR) (Suppl. file 1). Comparisons were also supplemented with original descriptions.

Sex was determined by examination of dark gular and chest color in males, nuptial excrescences (Leão & Cochran 1952; Hoogmoed 1990; Caramaschi & Niemeyer 2003; Lima *et al*. 2007; Avila *et al*. 2020), and the presence of vocal slits in males. Terminology for head shape follows Heyer *et al*. (1990), and webbing formulae follow Savage & Heyer (1967) as modified by Myers & Duellman (1982).

After exclusion of juveniles and damaged specimens, all 18 morphometric measurements (see abbreviations) from 1195 specimens (777 adult males and 418 adult females) were taken with digital calipers to the nearest 0.1 mm following Avila *et al*. (2020) by the first author (RWA).

Abbreviations for measurements

ED = eye diameter
END = eye to nostril distance
FAL = forearm length
FL = foot length
HAL = hand length
HL = head length
HTD = horizontal tympanum diameter
HW = head width
IND = internarial distance
PGL = parotoid gland length
POCH = postorbital crest height
POCL = postorbital crest length
SVL = snout–vent length
UEW = upper eyelid width
TAL = tarsal length
THL = thigh length
TL = tibia length
VTD = vertical tympanum diameter

To assess morphological differences, a descriptive analysis was performed to summarize the variation in characters between species. Morphometric data were transformed using log-shape ratios (Claude 2013) for the entire set, obtained through the *lsr* function in the MorphoInd package (Baylac 2008). We performed two independent Principal Component Analyses (PCA), each including morphometric data from the currently recognized species belonging to the clades to which the new species are associated (see below), in order to assess the variables that contributed most to morphological differences. The

analyses were conducted using the *prcomp* function in R (stats package). Because of sexual dimorphism we treated males and females separately in the PCA analyses. All statistical analyses were conducted in the software R ver. 4.3.3 (R Development Core Team 2024).

In addition, we used 12 qualitative morphological characters following Fouquet *et al*. 2024: (1) development of the apical ridge of the snout (low, large and very large with a proboscis); presence and development of (2) canthal, (3) preocular, (4) supraocular, (5) supratympanic, (6) postocular, and (7) parietal cranial crests (absent, inconspicuous with markings or tubercles only, low, high, or hypertrophied); (8) parotoid gland morphology (triangular or elongated), (9) presence of tubercles on the parotoid gland edge (present or absent); (10) presence and development of neural spines (absent, inconspicuous with markings or tubercles only, low, or high); (11) presence and development of the protrusion at the mouth angles (absent, developed, or hypertrophied); and (12) morphology of the dorsolateral line (tuberculated fold with scattered tubercles only, tuberculated fold with distinctive round tubercles, or spinose with developed pointed tubercles).

Molecular phylogenetic analyses

We included representatives of the MOTU's delimited Fouquet et al. (2024), complemented by newly collected material (n = 21) that filled previous sampling gaps, especially on the right margin of the Amazon River (450 sequences in total; Suppl. file 2). For phylogenetic analyses, we preferentially included sequences from the holotype, paratype or topotype, and/or specimens from recent description or taxonomic revision studies, besides individuals of the new taxa. We obtained partial sequences of 16S rDNA gene (578 base pairs) by polymerase chain reaction (PCR) for the new taxa following the protocol described in Ávila *et al*. (2018). Products of successful amplifications were purified and sequenced by ACTGene, Porto Alegre, Brazil.

The sequences were aligned with Geneious R9 (Biomatters) using the MAFFT algorithm default setting (Katoh 2013). An analysis of Bayesian Inference was performed to obtain a phylogenetic tree using the program BEAST 2 (Bouckaert *et al*. 2019) with the GTR+I+G model of substitution selected via the Akaike information criterion as implemented in JModelTest ver. 2.1.7 (Darriba *et al*. 2012). We used two independent chains of 100 million iterations, recording every 1000 iterations and a lognormal relaxed clock, without specific rates or time calibrations as priors. We combined the log and tree files discarding the first 25% iterations as burn-in and resampling 10% of the trees, using LogCombiner ver. 2.5 (Bouckaert *et al*. 2014). Convergence of the Markov chains and effective sample sizes were checked with Tracer ver. 1.7 (Rambaut *et al.* 2018). Uncorrected pairwise genetic distances between species within the *R. margaritifera* group were retrieved from Fouquet *et al*. (2024).

Bioacoustic analysis

We primarily used the dataset of advertisement call recordings from specimens associated with the *Rhinella proboscidea* clade (19 males) from Fouquet *et al*. (2024). Additional calls (n = 2) were recorded with Tascam DR-40X digital recorders equipped with external directional microphones. Digital recordings were sampled at a 44.1 kHz sampling rate and a 16 bit resolution, saved as uncompressed. wav files, and deposited in the Coleção Bioacústica of the Núcleo Regional de Ofiologia da Universidade Federal do Ceará (CBUFC, Fortaleza, Ceará, Brazil). We visualized the spectrograms using Raven Pro 1.3 (www.birds.cornell.edu/raven), with the following settings: DFT size of 256-point samples, time grid overlaps of 50%, and Hanning window.

We followed Fouquet *et al*. (2024) and measured 10 temporal parameters (in ms) from the waveform and two spectral parameters (in Hz) from a spectral trace extracted from entire calls (terminology follows the call-centered definition of Köhler *et al.* 2017; see abbreviations).

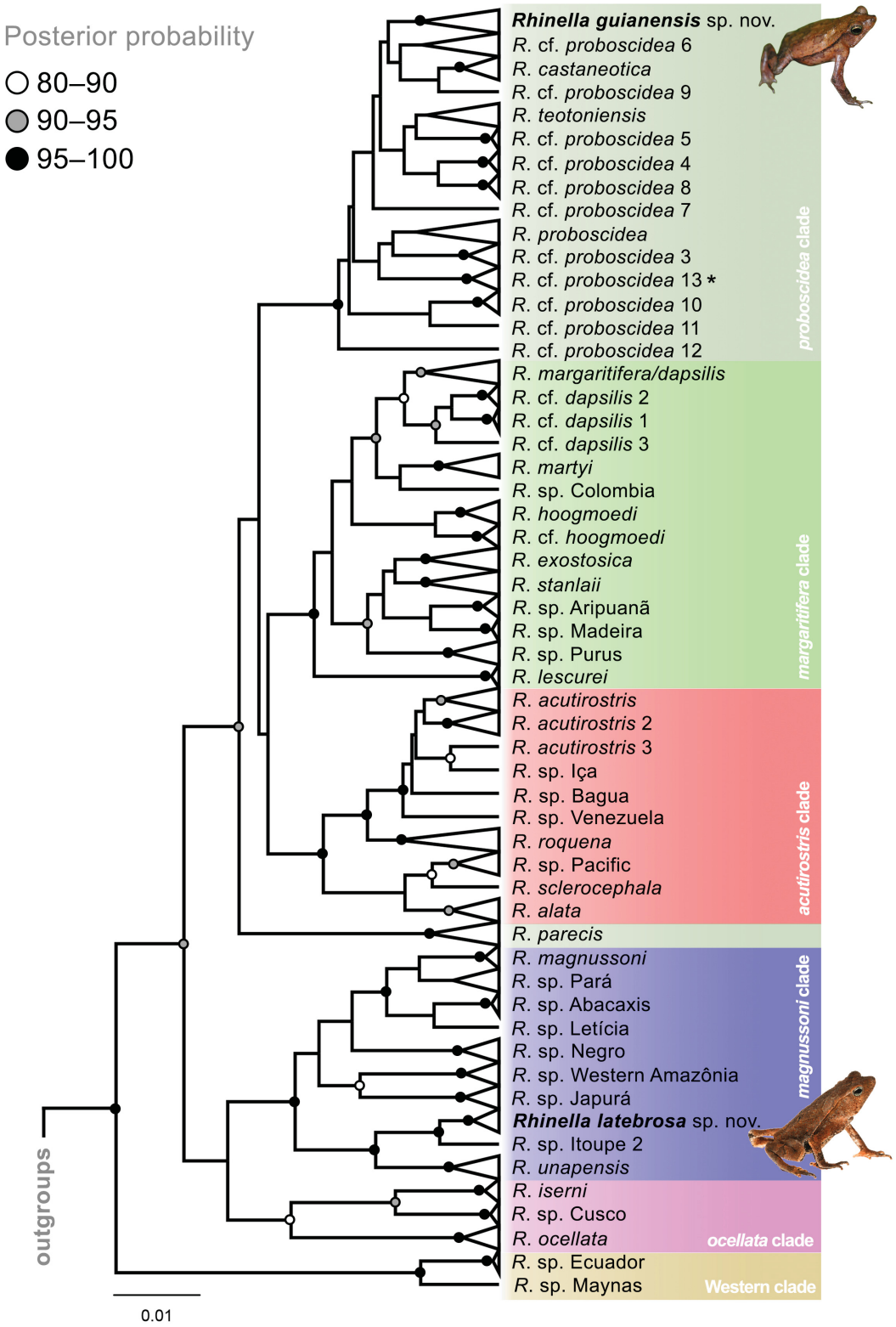


Fig. 1. Bayesian phylogenetic tree of the *Rhinella margaritifera* species group based on a partial 16S rRNA sequence (578 bp), showing the phylogenetic position of *Rhinella guianensis* sp. nov. and *Rhinella latebrosa* sp. nov. (posterior probabilities are indicated by the presence and shading of circles circles at each node). Branches are collapsed according to the molecular operational taxonomic units delimited by Fouquet *et al*. 2024. * Denotes a new lineage found in this study.

B90 = bandwidth 90%
CD = call duration
DF = dominant frequency
IC = inter-call interval
LI = maximum inter-note interval
LND = longest note duration per call
MaxCS = maximum number of calls per series
NN = number of notes per call
NP1 = number of pulses in the first note
NP2 = number of pulses in the last note
SI = minimum inter-note interval
SND = shortest note duration per call

We performed a PCA using acoustic data from the new species and currently recognized species of the *proboscidea* clade, including *Rhinella proboscidea* sensu stricto, *R. castaneotica*, *R. teotoniensis*, and *R. parecis*. We used the mean values of the variables for each male to identify which variables contributed most to the observed bioacoustic variation. Data were standardized using the 'scale. = TRUE' option using the *prcomp* function in R (stats package).

Results

The reconstructed tree from the Bayesian Inference analysis is similar to those from previous studies and shows many candidate species yet to be resolved, including two medium-sized species from the eastern Guiana Shield: one nested within the *Rhinella proboscidea* clade and the other one within the *R. magnussoni* clade (Fig. 1).

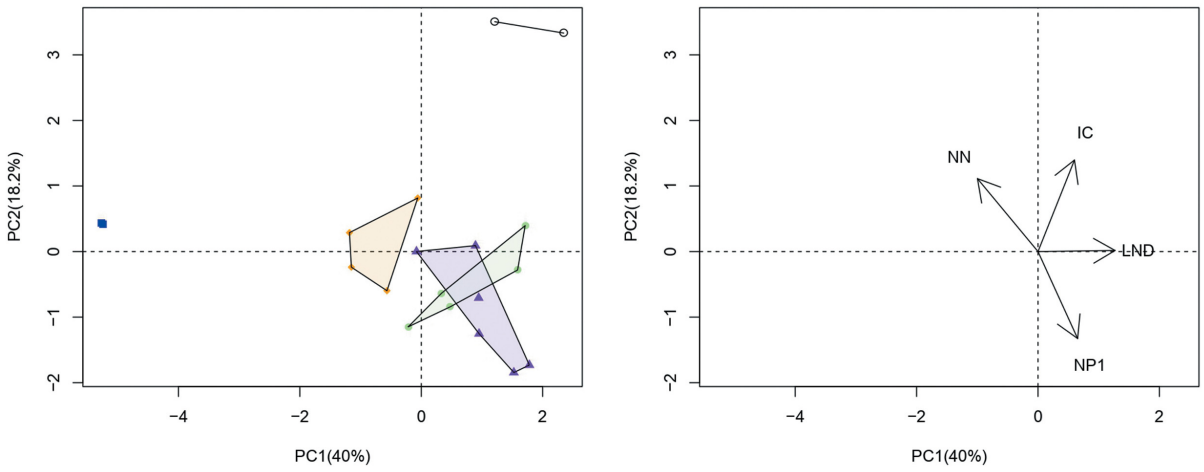


Fig. 2. Principal Components Analysis (PCA) scores plot (left) of the bioacoustic data of *Rhinella guianensis* sp. nov. (green circles) and the currently recognized species of the *R. proboscidea* clade, including: *R. proboscidea* stricto sensu (purple triangles), *R. castaneotica* (blue squares), *R. teotoniensis* (orange diamonds), and *R. parecis* (white circles). Polygons represent convex hulls around every species. In the right, loading plot with variable loadings (arrows) showing best informative variables: number of notes (NN), intercall intervals (IC), longest note duration per call (LND) and number of pulses in the first note (NP1).

Within the *Rhinella proboscidea* clade, the genetic distance between the first new species and its relatives range from 1.7 to 4.5%, being the lowest with *R. castaneotica* and the highest with *R. parecis*. Within the *Rhinella proboscidea* clade, the genetic distance between the first new species and its relatives range from 1.7 to 4.5%, being the lowest with *R. castaneotica* and the highest with *R. parecis* and is 2.5% with *R. proboscidea*. Within the *R. magnussoni* clade, the genetic distance between the second new species and *R. unapensis* is 3.8% and 3.9% with *R. magnussoni*.

The new species nested in the *Rhinella proboscidea* clade overlap with relatives in bioacoustics parameters and quantitative morphology (Figs 2–3), mostly with *R. proboscidea*, although the dominant

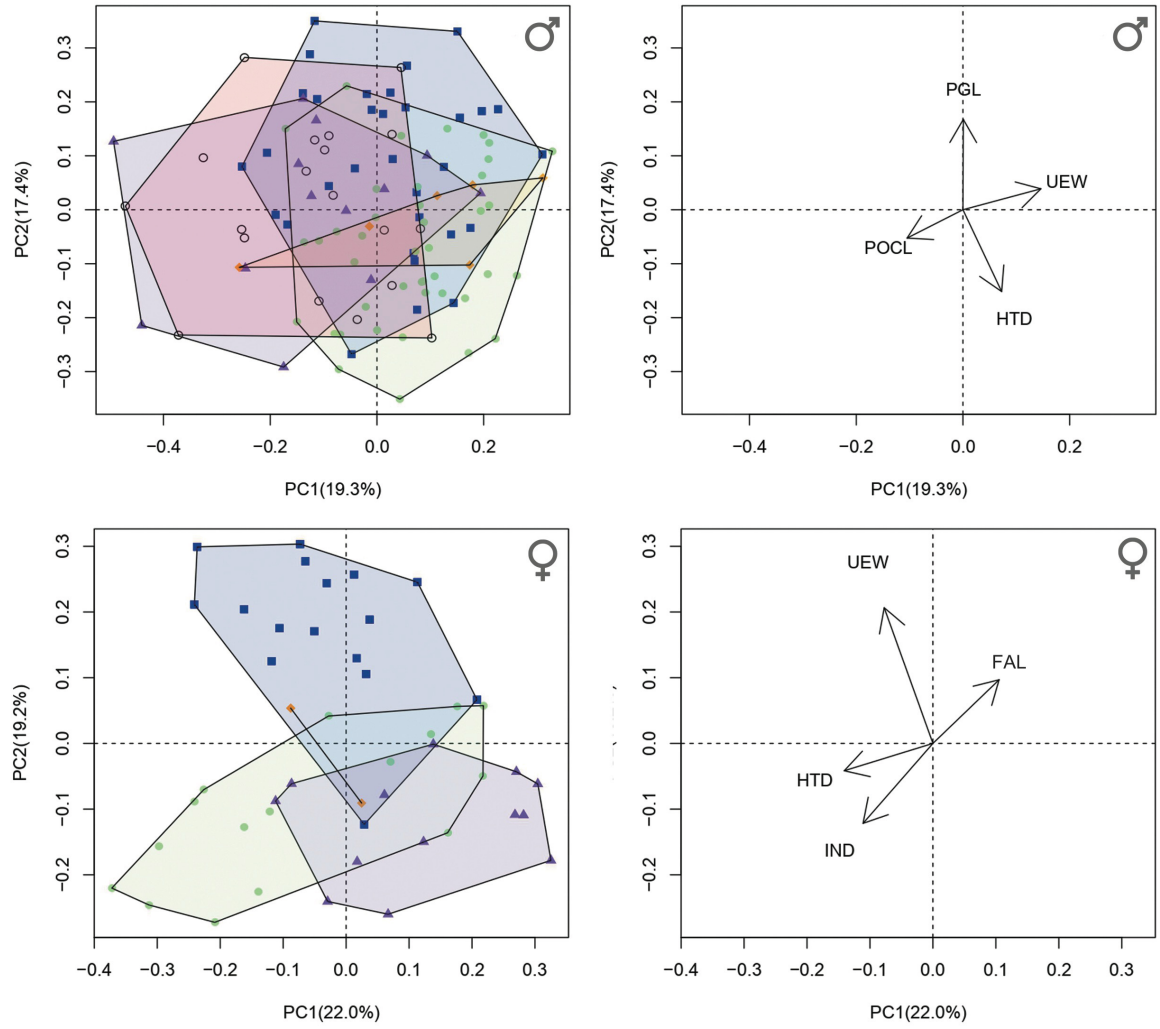


Fig. 3. Principal Components Analysis (PCA) scores plot (left) of the morphometric data from males and females of *Rhinella guianensis* sp. nov. (green circles) and the currently recognized species of the *R. proboscidea* clade, including: *R. proboscidea* (purple triangles), *R. castaneotica* (blue squares), *R. teotoniensis* (orange diamonds), and *R. parecis* (white circles). Polygons represent convex hulls around every species. In the right, loading plot with variable loadings (arrows) showing best informative variables: parotoid gland length (PGL), upper eyelid width (UEW), postorbital crest length (POCL) and horizontal tympanum diameter (HTD) for males and UEW, HTD, internarial distance (IND) and forearm length (FAL) for females.

frequency and qualitative morphology are different (see the diagnosis below). The second new species nested in the *R. magnussoni* clade does not overlap with relatives in bioacoustics parameters neither in quantitative (Fig. 4) or qualitative morphology. We thus consider that we have enough evidence that the two species are distinct from any nominal species in the *R. margaritifera* species group and describe them below as new species.

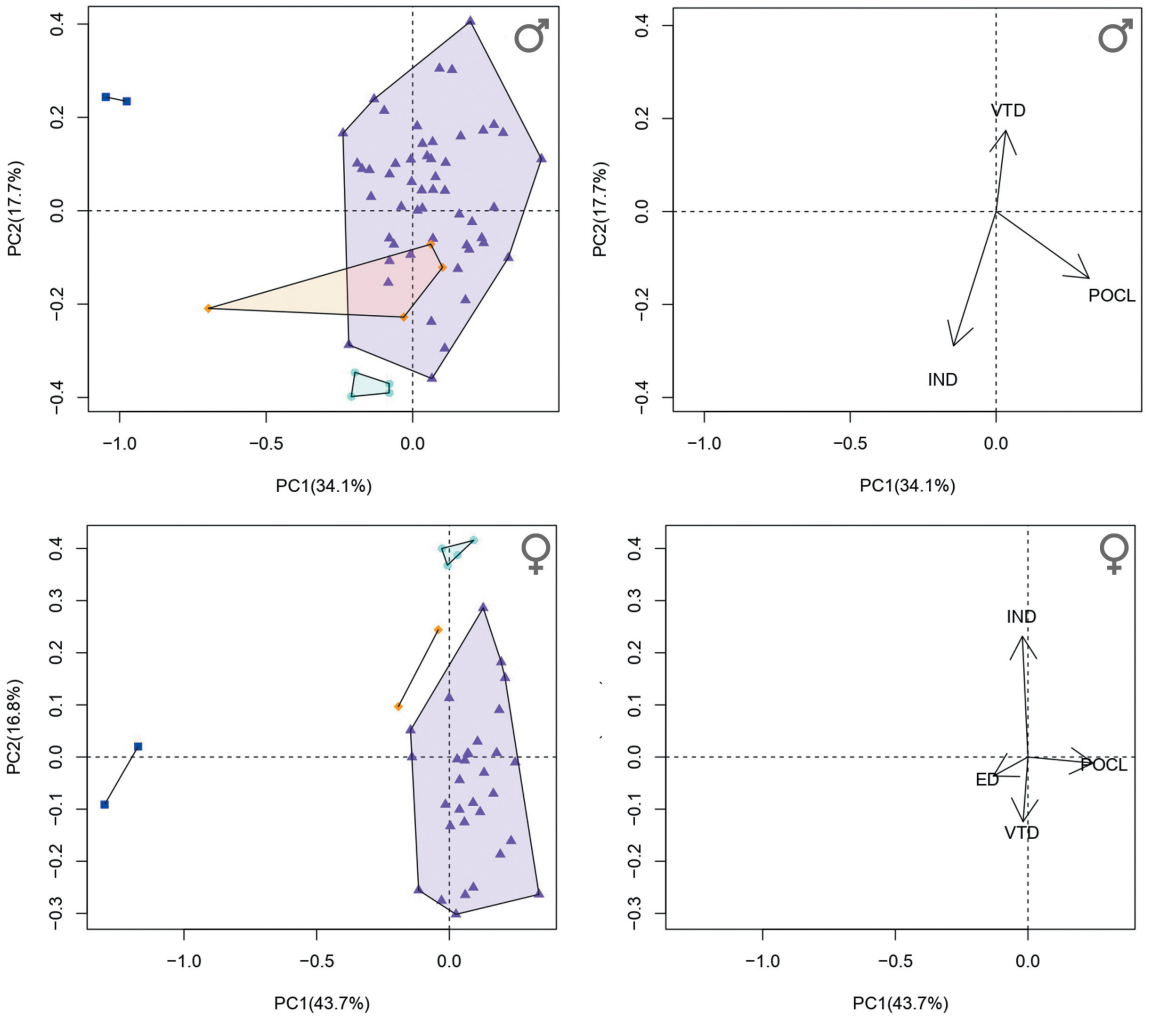


Fig. 4. Principal Components Analysis (PCA) scores plot (left) of the morphometric data from males and females of *Rhinella latebrosa* sp. nov. (light blue circles) and the currently recognized species of the *R. magnussoni* clade, including: *R. magnussoni* (purple triangles), *R. angeli* (blue squares) and *R. unapensis* (orange diamonds). Polygons represent convex hulls around every species. In the right, loading plot with variable loadings (arrows) showing best informative variables: postorbital crest length (POCL), internarial distance (IND) and vertical tympanum diameter (VTD) for males and IND, POCL, VTD and eye diameter (ED) for females.

Taxonomy

Class Amphibia Linnaeus, 1758
Order Anura Fischer von Waldheim, 1813
Family Bufonidae Gray, 1825
Genus *Rhinella* Fitzinger, 1826

Rhinella guianensis sp. nov.
urn:lsid:zoobank.org:act:E7B1443F-DCC1-41DE-998A-C433A98A234C
(Figs 5A–E, 6)

Bufo typhonius – Lescure & Marty 2000: 66.
Rhinella sp. E – Fouquet *et al.* 2007a: 5, fig. 3.
Rhinella margaritifera E – Fouquet *et al.* 2007b: 5, fig. 1.
Rhinella castaneotica – Lima 2008: 45. — Fouquet *et al.* 2015: 365, fig. 2. — Benício & Lima 2017: 629. — Vacher *et al.* 2020: Sup. 16. — Fouquet *et al.* 2020: 6. — Ferrão *et al.* 2020: 969, fig. 1. — de Figueiredo *et al.* 2022: 35–39.
Rhinella cf. *castaneotica* – Dewynter *et al.* 2008: 1. — Fouquet *et al.* 2012: 7, fig. 2.
Rhinella aff. *castaneotica* – Queiroz *et al.* 2011: 7, fig. 6. — Taucce *et al.* 2022: 108.
Rhinella gr. *margaritifera* – Valentim *et al.* 2013: 3, fig. 2.
"Rhinella castaneotica" – Pereyra *et al.* 2021: 68, fig. 14
Rhinella castaneotica "GS" – Ferrão *et al.* 2022: 7, fig. 1.
Rhinella cf. *proboscidea* 2 – Fouquet *et al.* 2024: 12, fig. 2.

Diagnosis

A member of the *Rhinella margaritifera* group, nested within the *R. proboscidea* clade of Fouquet *et al*. (2024), *Rhinella guianensis* sp. nov. is diagnosed by the following combination of characters: (1) snout-vent length range 30.5–45.9 mm in males (n = 44), 38.3–55.1 mm in females (n = 15); (2) snout rounded in dorsal view, acute in lateral view; (3) cranial crests absent, except for a low supratympanic crest; (4) dorsolateral row of tubercles present, tuberculated, extending from the edge of parotoid gland to groin; (5) bony protrusion at angle of jaws absent; (6) tympanum barely distinct; (7) vertebral apophyses absent; (8) toes half-webbed; (9) iris bluish to greenish in life; (10) advertisement call characterized by 7–10 pulsed notes with a dominant frequency of 1730–2229 Hz.

Etymology

The specific epithet is a reference to the species distribution in the eastern Guiana Shield.

Type material

Holotype

BRAZIL – **Amapá** • ♂ (SVL 33.3 mm); Calçoene Municipality; 2.52066° N, 50.95535° W (SAD-69); 11 m a.s.l.; 10 Feb. 2023; R.W. Avila and A.R.L. Ramos leg.; CHUFC-A 11010.

Paratypes

BRAZIL – **Amapá** • 3 ♂♂, 4 ♀♀; same data as for holotype; CHUFC-A 11020-22, 11025, 11027-28, 11038 – **Serra do Navio Municipality** • 1 ♂; collected near ICOMI mining company; 0.92333° N, 52.00444° W (SAD-69); 140 m a.s.l.; 15–17 Aug. 1989; R. Benevides and R. Otoch leg.; CHUFC-A 8756; • 1 ♂, same data as for preceding; Jun. 1966; E. Dente leg.; MZUSP 76284 • 17 ♂♂, 6 ♀♀, same data as for preceding; Jan. 1957; A. Machado and F.S. Pereira leg.; MZUSP 97842–45, 97846, 97849–51, 97853–56, 97858–62, 97865–69, 97872 • 2 ♂, 1 ♀, same data as for preceding; MNRJ 28557, 28655, 35230 – **Mazagão municipality** • 1 ♀; Igarapé do Breu, near Preto River; 16–17 Aug. 1989;

R.B. Marques and R. Otoch leg.; CHUFC-A 2510 • 1 ♂; Amapari River; 10 Jul. 1959; J. Lane leg.; MZUSP 95689 • 1 ♂; Igarapé Camaipi, Maracá River; 13–18 Jun. 2001; M.T. Rodrigues and D. Pavan leg.; MZUSP 100273 – **Porto Grande municipality** • 1 ♂, 1 ♀; MNRJ 80522, 80524. – **Pracuúba municipality** • 4 ♂, 2 ♀; right margin of Itaubal river; 1.584361° N, 52.901242° W (SAD-69); 19 m a.s.l; 13 Feb. 2023; R.W. Avila and A.R.L. Ramos leg.; CHUFC-A 11060-65 – **Tartarugalzinho**



Fig. 5. A–E. *Rhinella guianensis* sp. nov., holotype, ♂ (CHUFC A11010), Calçoene municipality, Amapá state, Brazil. **F–J**. *Rhinella latebrosa* sp. nov., holotype, ♂ (MNHN-RA-2022.0001), collected near the base camp on Mont Itoupé, French Guiana. Scale bars = 10 mm.

municipality • 1 ♂; 1.528281° N, 50.915257° W (SAD-69); 32 m a.s.l.; 14 Feb. 2023; R.W. Avila and A.R.L. Ramos; CHUFC-A 11104.

Description of holotype

SVL 33.28 mm; head as long (12.8 mm) as wide (12.7 mm) at the angle of jaws. Snout rounded in dorsal view, acute in lateral view. Cephalic crests weakly developed, only the supratympanic crest is present; canthus rostralis without crests, concave; nostrils protuberant, directed dorsolaterally; eye–nostril

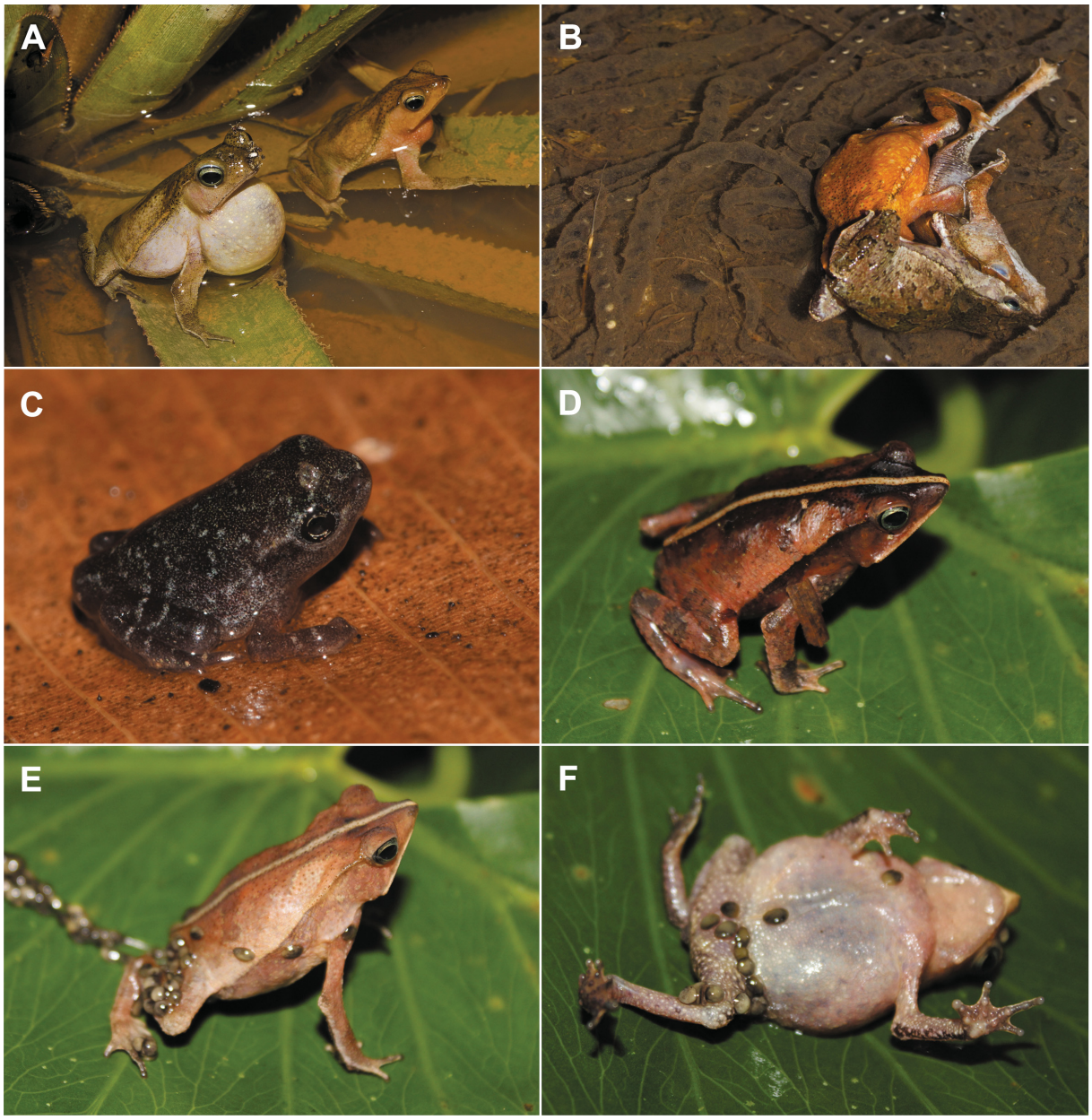


Fig. 6. Specimens of *Rhinella guianensis* sp. nov. in life. **A**. Unvouchered male specimens calling (note the dipterans around the nostrils of the calling individual) Serra do Navio, Amapa, Brazil. **B**. Two males trying to mate with a dead female (necrophilia) in a shallow pond with strings of eggs, Nouragues, French Guiana. **C**. Imago of *R. guianensis* (unvouchered). **D**. Holotype, ♂ (CHUFC A11010). **E–F**. Paratype, ♀ (CHUFC-A 11022), Calçoene municipality, Amapá state, Brazil.

distance equal to upper eyelid width distance, twice as long as internarial distance. Tympanum barely distinct, large, rounded; its diameter equal to internarial distance. Corner of mouth angular, without bony knobs at lower angle of jaws. Vocal slits present, laterally to the tongue; choanae small, ovoid, lateral, widely separated; tongue free, not notched behind. Parotoid glands poorly visible, small, triangular. Weak dorsolateral line of small rounded tubercles extending from anterior border of the parotoid gland almost to groin. Vertebral apophyses absent. Skin on dorsal and ventral surfaces granulated, with scattered rounded tubercles more concentrated around the parotoid gland and in the chest. Forearms and forelimbs robust, granulated. Fingers free, with tips slightly enlarged; relative length of fingers 4 < 2 < 1 < 3. Palmar tubercle oval, large, smooth; thenar tubercle oval, about one third of the palmar tubercle; subarticular and supernumerary tubercles present, rounded. Hindlimbs robust, thighs longer than tibia, thigh + tibia length approximately 85% of SVL; foot length 89% of tibia length, tarsal length 73% of foot length. Inner metatarsal tubercle oval, twice as long as the outer metatarsal tubercle; subarticular and supernumerary tubercles present, rounded, small, round. Relative length of toes 1 < 2 < 5 < 3 < 4; toes half-webbed, webbing formula: I 1–2 II 1–2 ½ III 1–3 IV 3–1V.

Measurement (mm)

SVL 33.3; HL 12.8; HW 12.7; IND 2.6; END 4.2; ED 3.9; UEW 3.9; POCH 1.7; POCL 3; HTD 3.2; VTD 3.3; PGL 4.8; HAL 9.2; FAL 10.2; THL 14.4; TL 13.9; TAL 9.1; FL 12.4.

Coloration

In life, dorsum light brown, with dark brown bands on arms and legs (Fig. 6). Creamy vertebral line from the tip of the snout to the cloaca, surrounded by dark brown bands. Dark brown stripe extending from the orbit to the groin, right below the dorsolateral line of tubercles. Venter light brown, immaculate. Throat light brown, without blotches. Iris greenish, with black reticulations. In preservative, general color similar to that in life, although slightly faded mainly in dark brown blotches and the iris grey (Fig. 5A–E).

Intraspecific variation

We examined 80 specimens (44 males, 15 females and 21 juveniles). Females are slightly larger than males and a summary of the variation in measurements between sexes can be found in Table 1. Both sexes lack cranial crests, except for low supratympanic crest. The dorsal skin texture changes with the breeding period, from tuberculated to smooth. The dorsal coloration is extremely variable, from dark brown to cream, sometimes with dark brown spots on the midbody (Fig. 6). A creamy colored vertebral line is present in some individuals, including in the holotype. Ventral coloration is also variable from almost immaculate light brown to reddish brown mottled with light brown blotches.

Advertisement call

The advertisement call of *Rhinella guianensis* sp. nov. (n = 5 males and 25 calls) is composed of 7–10 pulsed notes (mean 8.1 ± 0.8 SD) emitted at irregular intervals from 143.2–479.4 ms (Table 2, Fig. 7A–C). The duration of each call varies from 205.4 to 302.0 ms (mean 255.4 ± 36.4 ms SD) and the rate of notes per minute is 1641–2489 (mean 1930 ± 211 SD). The duration of each note with two pulses varies from 5 to 26 ms (mean 15 ± 7 SD) emitted at irregular intervals of 4–26 ms (mean 13 ± 4 SD). The dominant frequency varies from 1730–2229 Hz (mean 2026 ± 200 SD) and the bandwidth varies from 306.3 Hz to 768.3 Hz (mean 476.9 ± 193.0 SD).

Tadpole

Not known.

Table 1. Morphometric measurements (mm) of males and females of the type series of *Rhinella guianensis* sp. nov. and *R. latebrosa* sp. nov. including the holotypes. Mean ± standard deviation ranges in the parentheses.

	***Rhinella guianensis* sp. nov.**		***Rhinella latebrosa* sp. nov.**	
	Males (n = 44)	Females (n = 15)	Males (n = 6)	Females (n = 7)
SVL	37.6 ± 3.9 (30.5–45.9)	43.6 ± 4.4 (38.1–55.1)	37.2 ± 1.8 (35.3–40.0)	53.7 ± 2.2 (50.5–56.2)
HL	13.3 ± 1.2 (10.7–16.6)	15.3 ± 1.3 (13.4–16.7)	12.3 ± 0.5 (11.6–12.9)	16.6 ± 0.6 (15.8–17.3)
HW	13.3 ± 1.3 (10.5–16.8)	15.5 ± 1.9 (12.7–20.4)	13.9 ± 1.0 (12.7–15.3)	19.6 ± 0.5 (18.9–20.3)
IND	2.2 ± 0.4 (1.3–3.1)	2.5 ± 0.5 (1.6–3.4)	2.6 ± 0.2 (2.3–2.8)	3.7 ± 0.3 (3.3–4.1)
END	3.3 ± 0.4 (2.7–4.3)	3.7 ± 0.5 (3.2–4.6)	4.5 ± 0.2 (4.3–4.7)	5.8 ± 0.3 (5.3–6.1)
ED	3.2 ± 0.4 (2.2–4.0)	3.7 ± 0.5 (3.1–4.5)	3.4 ± 0.2 (3.1–3.8)	4.2 ± 0.2 (4.1–4.5)
UEW	3.1 ± 0.3 (2.5–3.8)	3.5 ± 0.5 (2.7–4.4)	3.3 ± 0.3 (3.0–3.6)	4.3 ± 0.2 (3.9–4.5)
POCH	1.3 ± 0.5 (0.5–2.2)	1.7 ± 0.5 (1.0–2.6)	0.7 ± 0.1 (0.5–0.9)	0.9 ± 0.2 (0.7–1.2)
POCL	3.4 ± 0.6 (1.9–4.7)	4.2 ± 0.6 (3.3–5.2)	3.4 ± 0.4 (2.9–3.9)	4.8 ± 0.2 (4.6–5.0)
HTD	2.7 ± 0.6 (1.7–5.7)	3.0 ± 0.8 (2.0–4.4)	1.9 ± 0.2 (1.6–2.3)	2.8 ± 0.1 (2.6–3.0)
VTD	3.0 ± 0.4 (2.0–3.7)	3.4 ± 0.6 (2.6–4.4)	2.2 ± 0.3 (1.8–2.7)	3.3 ± 0.4 (3.0–4.0)
PGL	4.8 ± 0.6 (3.5–6.9	5.3 ± 1.0 (3.9–7.3)	5.7 ± 1.2 (4.4–7.2)	7.7 ± 0.8 (6.4–8.5)
HAL	8.9 ± 1.0 (6.7–11.0)	9.7 ± 1.4 (6.7–11.9)	10.2 ± 1.0 (9.3–11.6)	14.7 ± 0.7 (13.5–15.8)
FAL	10.3 ± 0.9 (8.3–12.4)	11.6 ± 1.6 (10.3–16.6)	10.5 ± 1.1 (9.4–12.0)	14.4 ± 0.3 (14.0–14.8)
THL	15.4 ± 1.6 (11.7–18.6)	17.0 ± 3.0 (12.4–25.3)	15.0 ± 1.5 (13.6–16.5)	20.9 ± 1.2 (19.4–22.7)
TL	14.4 ± 1.9 (10.7–17.0)	16.5 ± 2.3 (14.4–22.8)	13.9 ± 1.4 (12.2–15.7)	19.6 ± 0.7 (19.0–21.0)
TAL	9.4 ± 0.9 (7.2–12.7)	10.2 ± 0.8 (9.2–11.3)	8.6 ± 1.3 (7.4–10.5)	12.0 ± 0.6 (11.1–12.7)
FL	12.7 ± 1.2 (9.7–15.0)	13.6 ± 1.6 (11.9–14.7)	12.6 ± 1.0 (11.2–13.7)	18.2 ± 0.7 (17.2–19.2)

Table 2. Acoustic variables of *Rhinella guianensis* sp. nov. and *R. latebrosa* sp. nov.

	***Rhinella guianensis* sp. nov. (n = 5)**	***Rhinella latebrosa* sp. nov. (n = 2)**
MaxCS	20–52 (28.4 ± 13.7)	3
CD (ms)	205.4–302 (255.4 ± 36.4)	338.6–335
IC (ms)	143.2–479.4 (294 ± 142.5)	304.3–421.5
NN	7–10 (8.8 ± 1.2)	6.8–7.6
SND (ms)	5.8–10.4 (7.6 ± 2.0)	6.2–11
LND (ms)	25.3–41.6 (33.7 ± 6.7)	18.2–24
SI (ms)	7.6–20 (12.3 ± 4.7)	29–34.2
LI (ms)	18.3–23.5 (21.2 ± 2.2)	48.3–54
NP1	1.4–2 (1.9 ± 0.3)	1
NP2	2.8–4.8 (3.8 ± 1.0)	1.3–1.8
DF (Hz)	1730–2229 (2026.4 ± 200.4)	1073–1582
B90 (Hz)	306.3–768.3 (476.9 ± 193.0)	274–291.3

Differential diagnosis

Rhinella guianensis sp. nov. is distinguished from other members of the *proboscidea* clade (characters of these species in parentheses unless stated otherwise): from *R. parecis* by its smaller size, with a SVL of 30.5–45.9 mm in males, and 38.3–55.1 mm in females (37.3–49.0 mm in males, 44.9–50.6 mm in females), the absence of supraorbital and parietal crests (present) and its shorter call with a length of 205.4–302.0 ms and a higher dominant frequency of 1730–2229 Hz (356.8–389.4 ms and 1576–1640 Hz); from the holotype of *R. proboscidea*, by its snout lacking a developed proboscis (snout with a proboscis), its acute snout in lateral view (protruding), its dorsolateral line of tubercles extending through the border of parotoid gland (dorsolateral line of tubercles not extending to the parotoid), its call with a higher dominant frequency of 1730–2229 Hz by the absence of bony knobs (rudimentary), dorsolateral line of tubercles extending through the border of the parotoid gland (dorsolateral line of tubercles not extending to the parotoid) and its snout shape, rounded in dorsal view and acute in lateral view (triangular and protruding, respectively), its call with a lower dominant frequency of 1730–2229 Hz (2153–2239 Hz); finally, from *R. castaneotica* by the lack of preocular crest (present), its dorsolateral line of tubercles extending through the border of parotoid gland (dorsolateral line of tubercles not extending to the parotoid), its acute snout in lateral view (snout protruding), its longer call with a duration of 205–302 ms (133–186 ms) and a lower dominant frequency of 1730–2229 Hz (2239–2928 Hz).

From the members of the *Rhinella margaritifera* clade (*R. cristinae*, *R. exostosica*, *R. hoogmoedi*, *R. margaritifera*, *R. martyi*, *R. sebbeni* and *R. stanlaii*), *Rhinella guianensis* sp. nov. differs by the absence of developed cranial crests in females, except from *R. dapsilis* and *R. lescurei*. *Rhinella guianensis* sp. nov. is differentiated from *R. dapsilis* by its smaller size (SVL 52.1–56.2 mm in males, 66.4–79.8 mm in females), its snout lacking a developed proboscis (proboscis well-developed), the

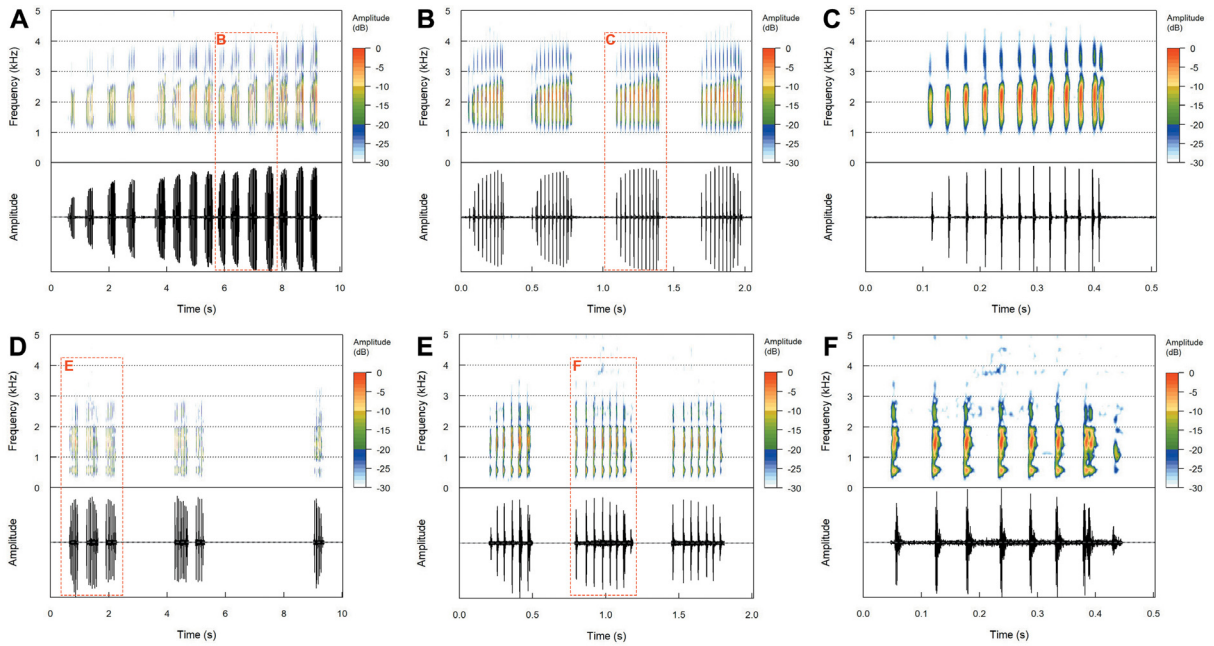


Fig. 7. Sonograms of the advertisement calls (spectrogram above and oscillogram below). **A**–**C**. *Rhinella guianensis* sp. nov., holotype, ♂ (CHUFC-A 11010), Calçoene municipality, Amapá state, Brazil. **A**. Call series. **B**. Region enlarged from dashed red box in A. **C**. Region enlarged from dashed red box in B. **D**–**F**. *Rhinella latebrosa* sp. nov. (MNHN-SO-2023-3183), Mont Itoupé, French Guiana. **D**. Call series. **E**. Region enlarged from dashed red box in D. **F**. Region enlarged from dashed red box in E.

absence of parietal and supraocular crests and neural spines (presence of parietal and supraocular crests and neural spines). From the sympatric *R. lescurei*, *R. guianensis* sp. nov. can be distinguished by the absence of canthal, supraocular and parietal crests (low cranial crests), its rounded snout in dorsal view (mucronate) and small apical ridge in lateral view (protruding with large apical ridge), its bluish to green iris (yellow); its dorsum granulated or smooth when breeding (spiculate), and dorsolateral line of tubercles extending through the border of the parotoid gland (dorsolateral line of tubercles not extending to the parotoid).

From species in the *Rhinella acutirostris* clade, *Rhinella guianensis* sp. nov. is distinguished from *R. roqueana* and *R. sclerocephala* by the absence of developed cranial crests (present); from *R. acutirostris* by its rounded snout in dorsal view (snout pointed), absence of bony protrusions at the angle of the jaws (presence of bony knobs at the angle of the jaws) and by its tuberculated dorsolateral line (spinose dorsolateral line); from *R. alata* differs by the snout shape, rounded in dorsal view and acute in lateral view (subacuminate in dorsal view and rounded to protruding in lateral view), its dorsolateral line of tubercles extending through the border of the parotoid gland (dorsolateral line of tubercles not extending to the parotoid), and triangular parotoid glands (elongate).

From species of the *Rhinella ocellata* clade, *Rhinella guianensis* sp. nov. differs from *R. ocellata* by the absence of a dorsal ocelli color pattern (present) and from *R. iserni* by the absence of canthal, preocular, supraorbital, postocular and parietal cranial crests (present) and its acute snout in lateral view (protruding).

From the *Rhinella magnussoni* clade, the new species is distinguished from *R. angeli* by the absence of a fleshy proboscis (long fleshy proboscis present); from *R. magnussoni* by its rounded snout in dorsal view (snout pointed in dorsal view) and absence of bony knobs (presence of bony protrusion at angle of jaws); from *R. unapensis* by the absence of supraorbital and parietal crests (present) and bony knobs (presence of bony protrusion at the angle of jaws).

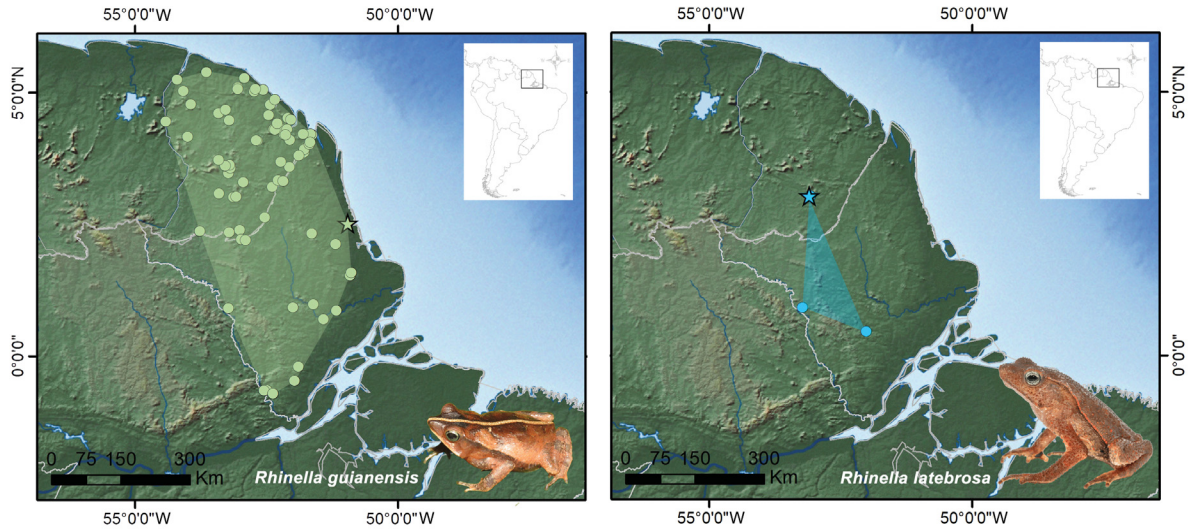


Fig. 8. Geographic distribution map of *Rhinella guianensis* sp. nov. (green circles) and *Rhinella latebrosa* sp. nov. (light blue circles), with stars indicating their respective type localities in the municipality of Calçoene, Amapá state, Brazil, and near the base camp on Mont Itoupé, French Guiana.

From *Rhinella sternosignata*, *Rhinella guianensis* sp. nov. differs by the absence of developed cranial crests and its rounded snout in dorsal view (developed supraorbital and supratympanic crests and snout truncate in dorsal view).

Distribution and natural history

Rhinella guianensis sp. nov. is distributed in French Guiana and the Brazilian state of Amapá from 0 to 700 m a.s.l (Fig. 8). This species displays a distribution pattern circumscribed to the eastern part of the Guiana shield sensu Vacher *et al*. (2020). Other anuran species (*Engystomops* sp. *Amazophrynella teko* Rojas-Zamora, Fouquet, Ron, Hernández-Ruz, Melo-Sampaio, Chaparro, Vogt, Carvalho, Pinheiro, Ávila, Farias, Gordo, & Hrbek, 2018, *Pristimantis gutturalis* (Hoogmoed, Lynch, and Lescure, 1977), *P. crepitaculus* Fouquet, Peloso, Jairam, Lima, Mônico, Ernst, and Kok, 2022, *Boana dentei* (Bokermann, 1967), *Boana diabolica* (Fouquet, Martinez, Zeidler, Courtois, Gaucher, Blanc, Lima, Souza, Rodrigues, and Kok, 2016), *Synapturanus zombie* Fouquet, Leblanc, Fabre, Rodrigues, Menin, Courtois, Dewynter, Hölting, Ernst, Peloso, and Kok, 2021 display very similar ranges.

In French Guiana and Amapá, *Rhinella guianensis* sp. nov. usually breeds in stalks of dead palm leaves full of water or small holes in fallen trunks (Fouquet *et al*. 2007b). We also observed reproduction occurring in small temporary puddles and terrestrial bromeliads. It is therefore rather ubiquitous. It is noteworthy that necrophilia has been observed (Fig. 6B), like in *R. proboscidea* (Izzo *et al.* 2012), although we ignore if the eggs were expelled from the female postmortem or before. The activity is largely diurnal and individuals are often found perched sleeping on small plants and twigs at night.

Figueiredo *et al*. (2022) reported 31 prey categories, mainly arthropods in the diet of *Rhinella guianensis* sp. nov., and a clutch size of 199–720 eggs from Amapá state, Brazil.

Rhinella latebrosa sp. nov.
urn:lsid:zoobank.org:act:9CADF89F-27B8-4142-9264-130B4F692127
(Figs 5F–J; 9A)

Rhinella sp. Itoupé Vacher *et al*. 2020: supp. file 16. – Fouquet *et al*. 2024: 12, figs 1–2.

Diagnosis

A member of the *Rhinella margaritifera* group, nested within the *magnussoni* clade of Fouquet *et al*. (2024). *Rhinella latebrosa* sp. nov. is diagnosed by the following combination of characters: (1) snout-vent length range of 35.3–40.0 mm in males (n = 6), 50.6–56.2 mm in females (n = 7); (2) snout truncate in dorsal view, acute in lateral view; (3) cranial crests small; canthal, parietal, supraocular and supratympanic present; (4) dorsolateral row of tubercles present, from the anterior border of the parotoid gland to the groin; (5) bony protrusion at the angle of jaws absent; (6) tympanum barely distinct (7) vertebral apophyses absent; (8) toes half-webbed; (9) iris silver; (10) spiculate skin on dorsum, granulated on venter; (11) advertisement call characterized by 5–10 pulsed notes with a dominant frequency of 1073–1582.

Etymology

The specific epithet '*latebrosa*' is a Latin adjective meaning 'hidden, secret', refers to the isolated range of the species in the interior of the eastern Guiana Shield and the fact that only three populations are currently known to exist of this species that was only discovered in 2010 for the first time.

Type material

Holotype

FRENCH GUIANA • ♂ (SVL 36.8 mm); near base camp on Mont Itoupé; 3.02300° N, 53.09550° W (WGS-84); ca 600 m a.s.l.; 11 Jan. 2016; A. Fouquet, M. Dewynter, E. Courtois and B. Villette leg.; MNHN MNHN-RA-2022.0001 (field no. AF 3652)

Paratypes

FRENCH GUIANA • 5 ♂♂, 7 ♀♀, same data as for holotype; MNHN-RA-2022.0002–2022.0013.

Refered material (unvouchered)

BRAZIL – **Amapá state** • Calçoene municipality FLOTA III, 2.12711° N, 51.19133° W, (WGS-84); FTA 171 • Montanhas do Tumucumaque National Park, Serra Macacoara, 0.90912° N, 53.22848° W, (WGS-84); ST281 • Mazagão municipality, Rio Vila Nova, 0.44934° N, 52.02214° W (WGS-84); 12 Sep. 2012; FTA89.

Description of the holotype

SVL 36.8 mm; head wider (14.0 mm) than long (12.4 mm) at angle of jaws. Snout truncate in dorsal view, acute in lateral view. Cephalic crests weakly developed; canthal, supraorbital, parietal and supratympanic crest present, small; canthus rostralis concave; nostrils protuberant directed dorsolaterally; eye to nostril



Fig. 9. Specimens of *Rhinella latebrosa* sp. nov. in life. **A–C**. Holotype, ♂ (MNHN-RA-2022.0001), collected near the base camp on Mont Itoupé, French Guiana. **D–F**. Paratype, ♀ (MNHN-RA-2022.0004). **G–I**. Paratype, ♀ (MNHN-RA-2022.0011).

distance larger than eye diameter and upper eyelid width, which are almost equal in size. Tympanum barely distinct, small, ovoid; its vertical diameter equal to internarial distance. Corner of mouth angular, without bony knobs at lower angle of jaws. Vocal slits present, lateral to the tongue; choanae small, ovoid, lateral, widely separated; tongue free, not notched behind. Parotoid glands scarcely perceptible, small, triangular. Dorsolateral line of small tubercles extending from anterior border of parotoid gland to groin. Vertebral apophyses absent. Skin on dorsal and ventral surfaces spiculate. Forearms and forelimbs robust, spiculated. Fingers basally webbed, with tips slightly enlarged; relative length of fingers 3 < 4 < 2 < 1; webbing formula: I 1–1 II 1–2 III 2–1 IV. Palmar tubercle oval, large, smooth; thenar tubercle oval, about one third of the palmar tubercle; subarticular and supernumerary tubercles present. Hindlimbs robust, thigh length larger than tibia length, thigh+tibia length approximately 83% of SVL; foot length 92% of tibia length, tarsal length 82% of foot length. Inner metatarsal tubercle oval, its size almost equal to outer metatarsal tubercle; subarticular and supernumerary tubercles present, small, round. Relative length of toes 1 < 2 < 5 < 3 < 4; toes half-webbed, webbing formula: I–12 II ½–1 III 1–3 IV 3–1V.

Measurement (mm)

SVL 36.8; HL 12.4; HW 14; IND 2.8; END 4.6; ED 3.3; UEW 3.5; POCH 0.8; POCL 3.9; HTD 1.6; VTD 2.7; PGL 6.7; HAL 9.8; FAL 8.1; THL 16.2; TL 14.2; TAL 10.6; FL 13.

Coloration

In life (Fig. 9A–C), reddish brown dorsum, with red tubercles from posterior corner of eye to groin. Dark brown bands present in tarsus. Venter light brown with scattered black blotches and white and red tubercles. Throat light brown, without blotches. Iris silver. In preservative (Fig. 5F–J), general color similar to that in life, although slightly faded with reddish brown turn to light brown and iris grey.

Intraspecific variation

We examined 13 specimens (6 males and 7 females). Females are larger than males and a summary of the variation in measurements between sexes can be found in Table 1. Both sexes present low cranial crests, except for preocular and postocular crests, which are absent. The dorsal skin texture changes with the breeding period, from tuberculated to smooth. The dorsal coloration is variable, from reddish brown to dark brown in life (Fig. 9), sometimes with dark brown spots on midbody. A thin creamy colored vertebral line is present in some individuals. Ventral coloration is also variable from almost immaculate light brown to light brown mottled with dark brown blotches.

Advertisement call

The advertisement call of *Rhinella latebrosa* sp. nov. (n = 2 males and 2 calls) is composed of 6–7 notes emitted at irregular intervals from 304 to 421 ms (Table 2, Fig. 7D–F). The duration of each call varies from 336.8 to 338.6 ms. Each note has a single pulse which duration varies from 6 to 24 ms (mean 18 ± 3 SD) emitted at irregular intervals from 29 to 54 ms (mean 31 ± 9 SD). The dominant frequency varies from 1073 to 1582 Hz and the bandwidth ranges from 274 to 291 Hz.

Tadpole

Undescribed. A crevice in a tree buttress and a large bromeliad were found containing late developmental stage tadpoles and imagos (Fig. 10). These developmental stages were not considered relevant for tadpole description.

Differential diagnosis

Rhinella latebrosa sp. nov. can be distinguished from the other species of the *R. magnussoni* clade (*R. angeli*, *R. magnussoni* and *R. unapensis*): from *R. angeli* by the absence of a fleshy proboscis (long fleshy proboscis present), a larger body size in females of 50.6–56.2 mm (40.1–48.6 mm) and presence

of a canthal crest (absent); from *R. magnussoni* by having a truncate snout in dorsal view (pointed), absence of bony knobs (present), and the advertisement call composed of 5–10 notes (single note); from *R. unapensis* by having larger (50.6–56.2 mm) females (36.3–46.1 mm in adult females in *R. unapensis*), the absence of bony knobs (bony knobs at the angle of the jaws present) and presence of a canthal crest (absent).

From the members of the *Rhinella margaritifera* clade (*R. exostosica*, *R. hoogmoedi*, *R. margaritifera*, *R. martyi*, *R. sebbeni* and *R. stanlaii*) the new species can be distinguished by its small cranial crests (supratympanic crest developed/hypertrophied in these species). *Rhinella latebrosa* sp. nov. can be further distinguished from *R. lescurei* by lacking a developed proboscis in the snout (proboscis well developed), by a truncate snout in dorsal view (acuminate), and by a triangular parotoid gland

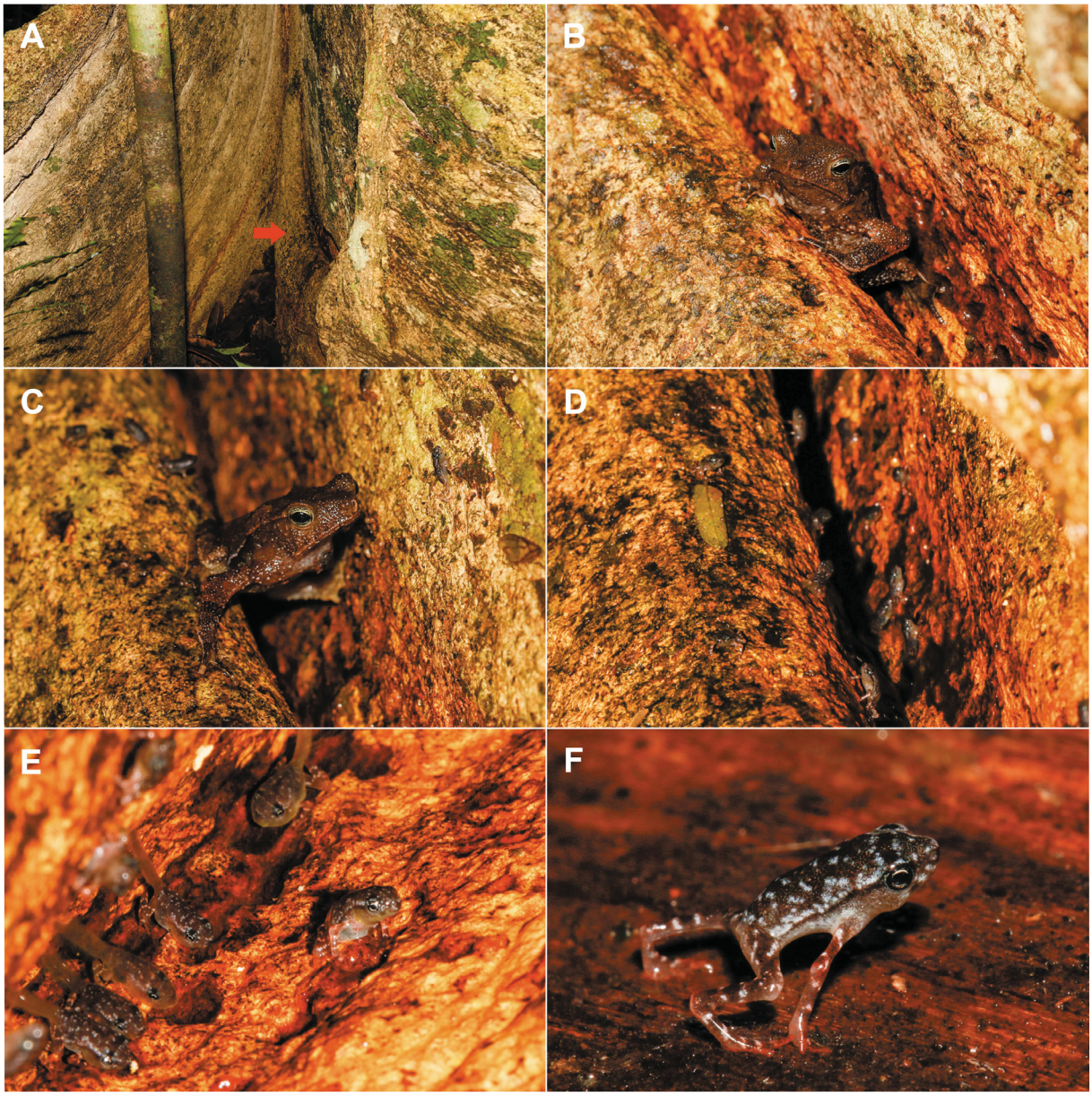


Fig. 10. Breeding ecology of *Rhinella latebrosa* sp. nov. **A**. Oviposition site at the base of a tree root. **B–C**. Oviposition site with an adult (male) at the entrance which was calling before disturbance. **D–F**. Presence of tadpoles and recently metamorphosed juveniles.

(elongated); from *R. cristinae* bythe evident tympanum; and the absence of preocular and postocular crests (tympanum concealed and preocular and postocular crests present); and from *R. dapsilis* by its smaller body size of 35.3–40.0 mm in males and 50.6–56.2 mm in females (SVL 52.1–56.2 mm in males, 66.4–79.8 mm in females).

From the species of the *Rhinella acutirostris* clade (*R. acutirostris*, *R. alata*, *R. roqueana* and *R. sclerocephala*), *Rhinella latebrosa* sp. nov. differs from *R. roqueana* by having poorly developed cranial crests (more developed crests); from *R. acutirostris* by the truncate snout in dorsal view and the absence of bony protrusions at the angle of the jaws (snout pointed and bony knobs at angle of jaws); from *R. alata* by the truncate and acute snout in dorsal and lateral views (subacuminate and rounded to protruding).

From species of *Rhinella ocellata* clade (*R. ocellata* and *R. iserni*), the new species is distinguished by the absence of a dorsal ocelli color pattern (present in *R. ocellata*) and by the acute snout in lateral view (protruding in *R. iserni*) and absence of preocular and postocular crests (present in *R. iserni*).

Rhinella latebrosa sp. nov. differs from the species of the *R. proboscidea* clade (*R. castaneotica*, *R. guianensis* sp. nov., *Rhinella parecis*, *R. proboscidea* and *R. teotoniensis*): from *R. castaneotica* by the presence of a dorsolateral line of tubercles (absent), a truncate snout in dorsal view (snout pointed in dorsal view) and by having larger females (SVL 34.8–41.6 mm in females); from *R. guianensis* sp. nov. by smaller size in males (SVL 30.5–45.9 mm), presence of canthal, supraocular and parietal crests (absent), snout truncate in dorsal view (rounded), and by the marked dorsolateral row of tubercles (weak). From *Rhinella parecis*, the new species differs by having smaller males (SVL 37.3–49.0 mm) and by truncate snout in dorsal view (rounded). The absence of a developed proboscis in the snout (snout with a proboscis); from *R. teotoniensis* by its larger males and smaller females (SVL 35.7–44.6 mm in males and 38.3–52.1 mm in females), the absence of bony knobs (rudimentary) and by the snout truncate and acute in dorsal and lateral views (triangular and protruding, respectively).

From *Rhinella sternosignata*, *Rhinella latebrosa* sp. nov. differs by its smaller males and the absence of a preocular crest (mean SVL of 49.5 mm and preocular crest present).

Distribution and natural history

This species is only known from three populations (Fig. 8), one on Mont Itoupé in French Guiana and two in Amapá, Brazil (Rio Vila Nova in Mazacão municipality and Serra Macacoara in Calçoene municipality). However, an additional population from Amapá (Rio Amapá Grande) forms a related but distinct mtDNA-based OTU which may in fact be conspecific.

Rhinella latebrosa sp. nov. is a diurnal species associated with mature forest on uplands. Males form groups of a few individuals in small collections of water, especially in cavities formed by the buttresses of trees and terrestrial bromeliads (Fig. 10). It is abundant between 300 and 800 m altitude on Mount Itoupé but seems absent at lower altitudes.

Discussion

Amphibians are extremely diverse in the Neotropics, notably in Amazonia, where over 2000 species probably exist (Giam *et al*. 2012; Vacher *et al*. 2020) but with less than 700 being actually recognized (Godinho & da Silva 2018). However, this number is constantly growing with many species notably described from the eastern Guiana Shield in the last 10 years (e.g. *Allobates ripicolus* Fouquet, Ferrão, and Jairam, 2023 and *A. vicinus* Fouquet, Ferrão, and Jairam, 2023*,* Fouquet *et al*. 2023; *Pristimantis guianensis* Mônico, Ferrão, Chaparro, Fouquet, and Lima, 2022, Monico *et al*. 2022; *Synapturanus zombie* Fouquet, Leblanc, Fabre, Rodrigues, Menin, Courtois, Dewynter, Hölting, Ernst, Peloso, and

Kok, 2021, Fouquet *et al*. 2021). From these descriptions, and an overall better understanding of the distribution of amphibians in the region, a striking pattern of endemism emerges, the Guiana Shield representing a distinct biogeographic region itself structured into three subregions (Vacher *et al*. 2020). The two new species in the present study strengthen this pattern adding two species endemic to the easternmost of these subregions i.e., Amapá and French Guiana. The new species possibly occur in nearby Suriname and the Brazilian Pará state, but it is unlikely that they occur much further within the Guiana Shield. In fact, the distribution of *Rhinella guianensis* possibly overlaps with that of *Rhinella proboscidea* somewhere in the interior of the Guiana Shield where a transition towards another subregion occurs (Vacher *et al*. 2020). In contrast, *Rhinella latebrosa* is the only member of the *magnussoni* clade in the Guiana Shield and thus has no counterparts.

Our study is based on the most used short fragment of the mitochondrial 16S gene, which implies limitations in the inferences of phylogenetic relationships and biogeographic scenarios (Chan *et al.* 2022). Bearing in mind these limitations, we can nevertheless hypothesize that, *Rhinella guianensis* sp. nov. has close relatives on the opposite bank of the Amazon River (*R. castaneotica*). Which suggests relatively recent transriverine dispersal. The closest relative of *R. latebrosa,* however, is *R. unapensis* from western Amazonia (Fouquet *et al*. 2024). Therefore, *R. latebrosa* seems to have a different evolutionary history with a western origin and subsequent isolation. Moreover, the isolation of *R. latebrosa* in fragmented populations on massifs of the interior of the Guiana Shield suggest particular thermal and humidity requirements that also possibly explain the large spatial distance with relatives in Western Amazonia, a pattern that has similarities with that of *Atelopus* Duméril and Bibron, 1841 for example which was explained by past colder conditions that have favoured dispersal and then isolation (Lotters *et al.* 2010; Fouquet *et al*. 2012). By contrast, *R. guianensis* is found in most forest habitats in the lowlands, even those disturbed by human activities, and its distribution is much more continuous and extensive. It can breed in ruttings on roads, small temporary puddles, dead palm leaves full of water or small holes in fallen trunks and these ubiquitous habits at least partly explain its distribution.

The *Rhinella margaritifera* species group has such a complex taxonomic history that it has represented a challenge for taxonomists for many decades (Ávila *et al*. 2018). This situation arose from very succinct descriptions with vague type localities (as is the case of *R. margaritifera*), combined with an overall morphological similarity between sometimes more than four co-occurring species and extensive intraspecific variation, notably ontogenic and sexual (Ávila *et al*. 2020). In recent years, however, the integration of molecular, acoustic and morphological data has allowed some of the problems to be resolved and some species to be described (Ferrão *et al*. 2022; Fouquet *et al*. 2024). However, crucial issues remain, notably the assignment of the oldest taxa such *Rana margaritifera* (Laurenti, 1768) and *Bufo proboscideus* (Spix, 1824) to modern populations. Museomics, i.e. study of ancient or historic DNA from specimens in museum collections, has the potential to solve such taxonomic problems associated with imprecise type localities and dubious identities of modern populations (Grant *et al*. 2025).

Another issue is the completeness of the sampling and its limitation to mostly mitochondrial data. Even though Fouquet *et al*. (2024) used a large database, the addition of a few sequences in the present work notably revealed a new lineage ('*proboscidea* 13' from the Uatumã River at Presidente Figueiredo and Urucará municipalities, Central Amazonia, a previous sampling gap). Thus, even with the description of two new species, bringing the group to a total of 25 recognized species, the group's taxonomy still requires extensive work, as some estimates indicate more than 50 species (Fouquet *et al*. 2024). Previous works identified incongruences between mitochondrial and nuclear DNA, but also between mtDNA and morphology and bioacoustics, leading to the hypothesis of several instances of hybridization. Genomics is thus required in this group to understand the actual species boundaries (Pereyra *et al*. 2021; Fouquet *et al*. 2024). Nevertheless, in the case of our two new species the possibility of large admixture with nearby relatives seems unlikely, since mitochondrial, nuclear, morphological and acoustic data separates these species from conspecifics.

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Author contribution

Conceptualization: RWA, AF. Data curation: RWA, MD, AF. Formal analysis: RWA, MD, ARLR, AF. Investigation & Validation: RWA, AF, ARLR. Methodology: ARLR, RWA, AF, MD. Writing - original draft: RWA, ARLR, MD, AF. Writing - review and editing: RWA, ARLR, MD, AF

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