



## Redescription of the Amazonian tiny tree toad *Amazophrynella minuta* (Melin, 1941) (Anura: Bufonidae) from its type locality

ROMMEL R. ROJAS<sup>1,8</sup>, ANTOINE FOUQUET<sup>2</sup>, VINÍCIUS TADEU DE CARVALHO<sup>1,6</sup>,  
SANTIAGO RON<sup>3</sup>, JUAN CARLOS CHAPARRO<sup>4</sup>, RICHARD C. VOGT<sup>5</sup>, ROBSON W. ÁVILA<sup>6</sup>,  
IZENI PIRES FARIAS<sup>1</sup>, MARCELO GORDO<sup>7</sup> & TOMAS HRBEK<sup>1</sup>

<sup>1</sup>Laboratório de Evolução e Genética Animal (LEGAL), Departamento de Genética, Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Av. General Rodrigo Octávio Jordão Ramos, 6200. CEP 69077–000 Manaus, AM, Brazil

<sup>2</sup>USR 3456 LEEISA—Laboratoire Ecologie, Evolution et Interactions des Systèmes Amazoniens, Centre de recherche de Montabo, 275 route de Montabo, BP 70620, 97334 Cayenne, French Guiana.

<sup>3</sup>Museo de Zoología, Escuela de Biología, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre y Roca, Aptdo. 17–01–2184, Quito, Ecuador.

<sup>4</sup>Museo de la biodiversidade-MUBI, Peru.

<sup>5</sup>CEQUA, Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Av. André Araújo, 2936, Aleixo, CEP 69060-001, Manaus, AM, Brazil.

<sup>6</sup>Departamento de Química Biológica, Universidade Regional do Cariri, Campus do Pimenta, Rua Cel. Antônio Luiz, 1161, Bairro do Pimenta, CEP 63105–100, Crato, CE, Brazil.

<sup>7</sup>Departamento de Biologia, Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Av. General Rodrigo Octávio Jordão Ramos, 6200. CEP 69077–000 Manaus, AM, Brazil.

<sup>8</sup>Corresponding author. E-mail: [rrojasmora@gmail.com](mailto:rrojasmora@gmail.com)

### Abstract

The description of *Amazophrynella minuta* was published in 1941 by the Swedish naturalist Douglas Melin based on material from Taracúá (Amazonas state, Brazil). This description was very brief and based on the morphology of few specimens with diagnostic characters and color variation not well defined. Moreover, the type series is currently in poor state of conservation. Consequently, taxonomic ambiguity surrounds the nominal taxon *A. minuta*, which hampers the description of many unnamed congeneric species. Herein, we redescribe *A. minuta* based on recently collected specimens from the type locality, designate a lectotype, formulate a new diagnosis, provide patterns of morphological variation, measurements and body proportions.

**Key words:** Amphibians, Brazil, lectotype, systematics, Taracúá, taxonomy

### Resumen

La descripción de la especie *Amazophrynella minuta* fue realizada en 1941 por el naturalista Sueco Douglas Melin en la localidad de Taracúá, estado de Amazonas, Brasil. La descripción fue muy breve y basada solamente en la morfología de pocos especímenes. Por ese motivo, los caracteres diagnósticos y el padrón de coloración no fueron bien definidos en la descripción original, y en la actualidad la serie tipo se encuentra en malas condiciones de preservación. En este trabajo, realizamos la redescipción de *A. minuta* basados en especímenes recientemente colectados de la localidad tipo, designamos un lectotipo, formulamos una nueva diagnosis y proporcionamos el patrón de variaciones morfológicas, medidas morfológicas y proporciones corporales.

### Resumo

A descrição da espécie *Amazophrynella minuta* foi publicada no ano 1941 pelo naturalista Sueco Douglas Melin baseado em material proveniente da localidade de Taracúá, estado do Amazonas, Brasil. A descrição original foi muito breve e

baseada na morfologia de poucos indivíduos. Assim, os caracteres diagnósticos e o padrão de coloração não foram bem definidos na descrição original, e a série tipo encontra-se em condições precárias. Consequentemente há ambiguidade taxonômica envolvendo o táxon nominal *A. minuta*, o que dificulta a descrição de muitas espécies existentes no gênero *Amazophrynella*. Neste trabalho, realizamos a redescricao de *A. minuta* utilizando espécimes recentemente coletados da localidade tipo, designamos um lectótipo, formulamos uma nova diagnose morfológica, o padrão de variação morfológica, medidas morfométricas e proporções corporais.

## Introduction

The genus *Amazophrynella* Fouquet, Recoder, Teixeira, Cassimiro, Amaro, Camacho, Damasceno, Carnaval, Moritz, & Rodrigues 2012a, comprises a group of small-size bufonids distributed throughout Amazonia (Frost, 2017, Fouquet *et al.*, 2012b). They inhabit primary forest leaf litter, have diurnal activity and reproduce in seasonal ponds (Magnusson & Hero, 1991; Ávila *et al.*, 2012; Rojas *et al.*, 2014, 2015). Currently eleven species are recognized (Rojas *et al.*, 2018).

Douglas Melin described the type species of the genus, *Amazophrynella minuta* in 1941, as *Atelopus minutus*. The description was based on four specimens collected in 1924 in Taracua, municipality of São Gabriel da Cachoeira, Amazonas state, Brazil. The type specimens are syntypes deposited in the Göteborgs Naturhistoriska Museum, Sweden (NHMG 462–465). The specimens are presently in poor state of conservation, thus, difficult to compare morphologically with other species and populations of *Amazophrynella*. In addition, the description is quite brief and lacks many details in morphological diagnosis, variation, measurements and body proportions that are perceived today as crucial given the actual diversity of the group.

During the last decade, several studies demonstrated that the genus *Amazophrynella* represents a complex of cryptic species (Fouquet *et al.*, 2012b; Rojas *et al.*, 2016; Rojas *et al.*, 2018) and that many species have been, and still are, erroneously identified as *A. minuta* (Fouquet *et al.*, 2012b; Rojas *et al.*, 2016). A redescription of this species is, therefore necessary to accurately delimit and describe other species of *Amazophrynella*.

Preliminary characterization of topotypic *A. minuta* carried out by Rojas *et al.* (2014) only provided general morphological characters and did not describe the morphological variation and body measurements or define the species' current distribution. Herein we use extensive morphological, molecular and bioacoustics data to redescribe *A. minuta*. We also provide a new combination of diagnostic characters, and describe morphological variation, reproductive behavior and geographic occurrence.

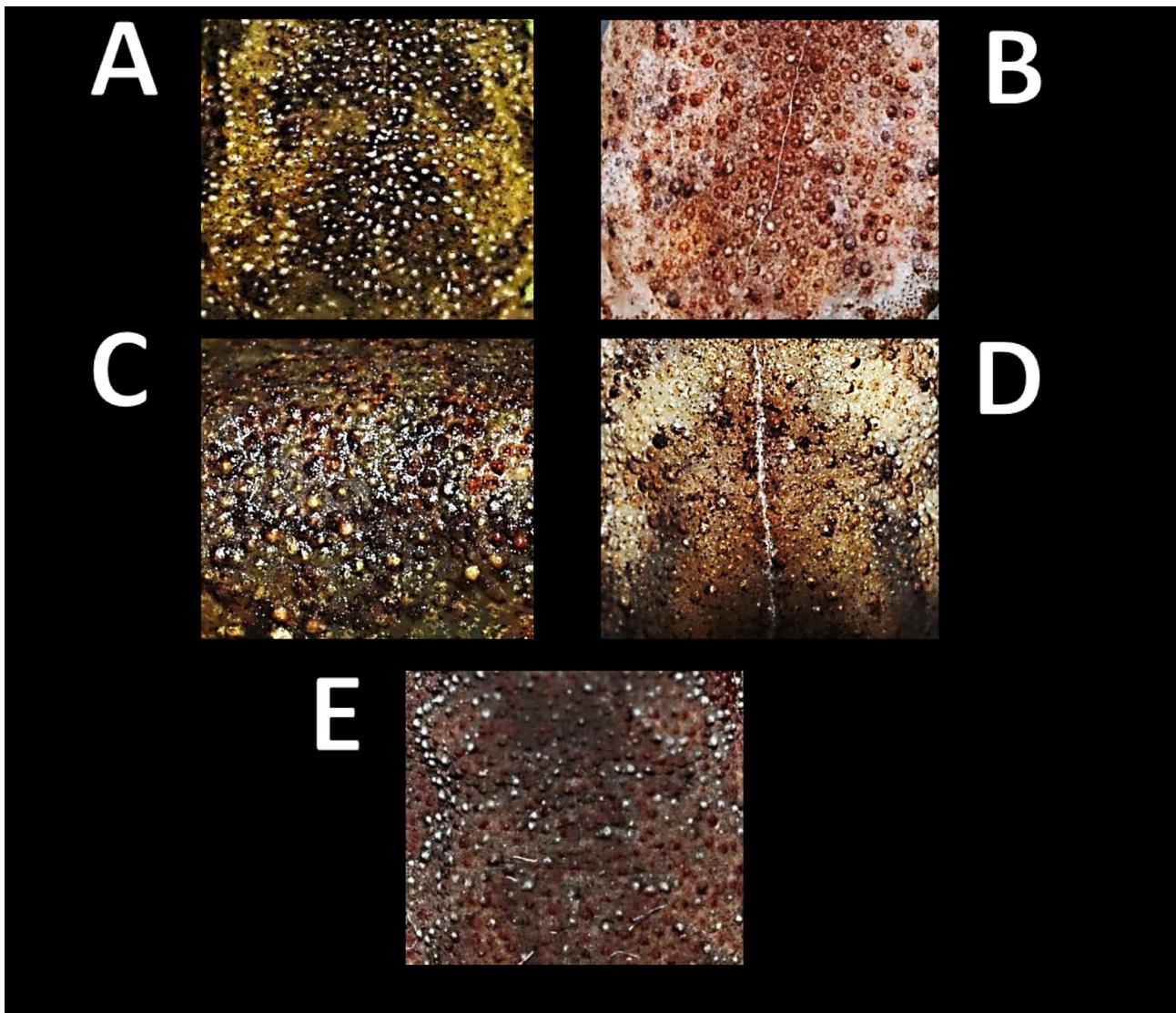
## Material and methods

**Type locality.** The type locality of *Amazophrynella minuta* is “Taracua, Rio Uapés, Brazil” (Melin, 1941) (= Taracua, 0.10°S, 68.46°W, datum = WGS-84, municipality of São Gabriel da Cachoeira, Amazonas state, Alto Rio Negro Amerindian Reserve, Brazil). Topotypical specimens studied herein were collected on August 19 and 20, 2013. See Appendix 1 for examined specimens.

**Measurements.** The following morphological measurements were taken with a Mitotuyo digital calliper (0.1 mm precision) with an ocular micrometer in a Zeiss stereomicroscope: snout-vent length (SVL); head length (HL); head width (HW); upper eyelid width (UEW); eye diameter (ED); snout length (SL); eye-to-nostril distance (END); internarial distance (IND); interorbital distance (IOD); hand length (HAL); upper arm length (UAL); thigh length (THL); tibial length (TL); tarsal length (TAL) and foot length (FL) following Kok & Kalamandeen (2008). Sex was determined by gonadal analysis.

**Definition of morphological characters.** External morphological nomenclature follows Kok & Kalamandeen (2008). Main diagnostic characters within *Amazophrynella* were defined as follows:

**Texture of skin** (Figure 1). Most bufonids species are covered with variable-sized warts (Ford & Cannatella, 1993), defined as, bearing protuberances with keratinized tip (Kok & Kalamandeen, 2008). We defined texture of skin in *Amazophrynella* as follows: tuberculate (Figure 1A), when the species present small sized wart with conical tips. Granular (Figure 1B), when the species present medium sized warts with rounded tips. In order to refine these taxonomic character, we considered skin as “highly granular”, when species present high density of medium sized warts with rounded tips (Figure 1C) and “finely granular”, when species present low density of small sized warts with rounded tips (Figure 1D). Spiculate, when the species present small sized warts with pointed tips (Figure 1E).



**FIGURE 1.** Terminology used in this paper to describe texture of dorsal skin in *Amazophrynella*. A) Tuberculate; B) granular; C) highly granular; D) finely granular; E) Spiculate. See Materials and Methods for character definition.

**Ventral color pattern** (Figure 2). We defined ventral color pattern in *Amazophrynella* as follows (adapted from Kok & Kalamandeen, 2008): Blotches, when the species present small to large, irregular light or black markings contrasting with the background coloration (Figure 2A–D). Dots, when the species present small or minute, more or less regular light or black markings contrasting with the background coloration (Figure 2E–F). Points, when the species present small to medium-sized roughly round black markings in contrast to the background color (Figure 2G).

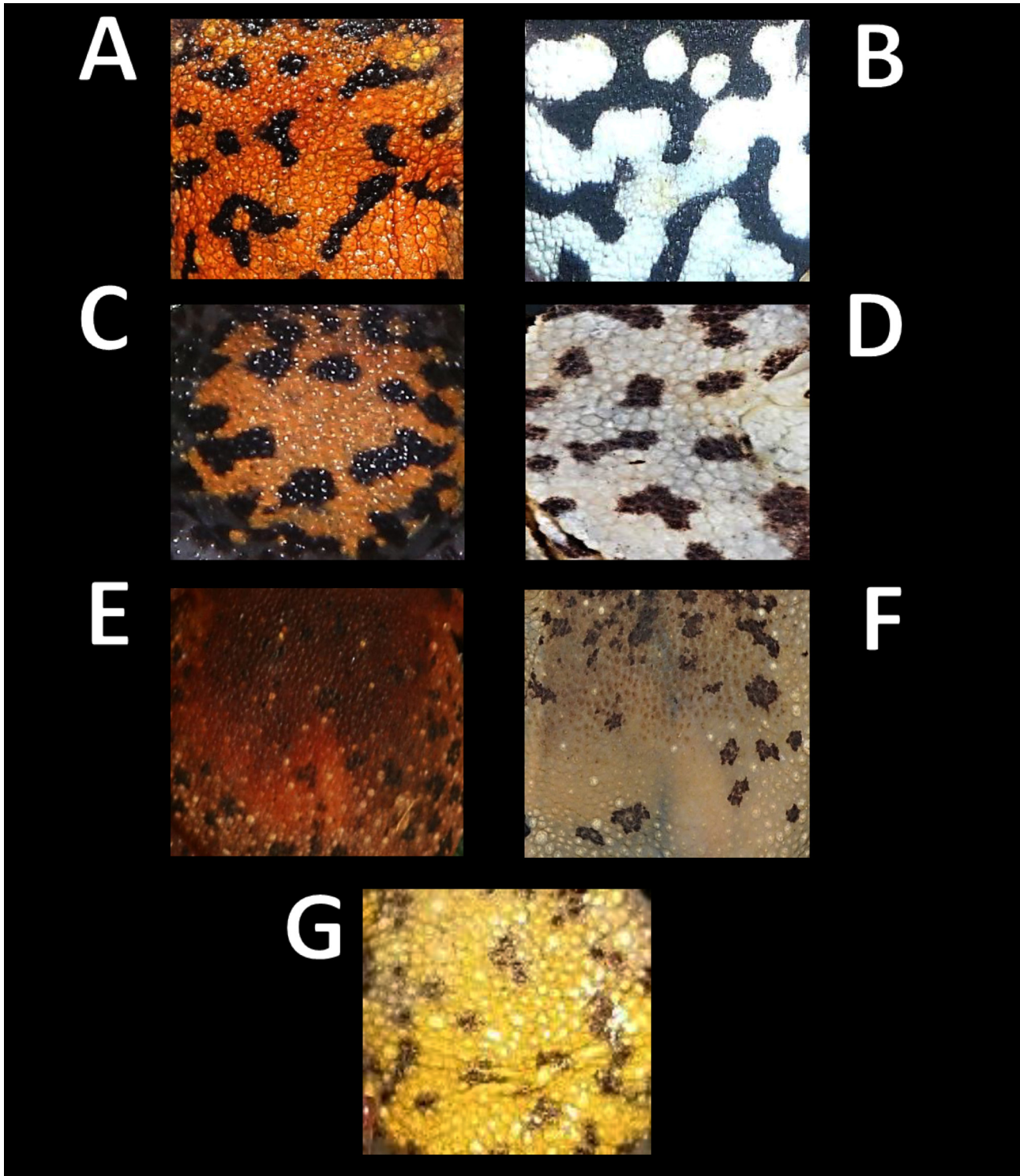
**Shape of head.** We followed the nomenclature proposed by Heyer *et al.* (1990, page 409).

**Shape of palmar and subarticular tubercles** (Figure 3). We identified three main shapes of palmar and subarticular tubercles in species of *Amazophrynella*, that are defined as follows: Rounded, when the palmar and subarticular tubercles are circular shaped, without a distinct point (Figure 3A). Ovoid, when the palmar and subarticular tubercles are oval, inverted egg shape, with a tapering or irregular point (Figure 3B). Elliptical, when the palmar and subarticular tubercles are oval shaped, with a flattened point (Figure 3C).

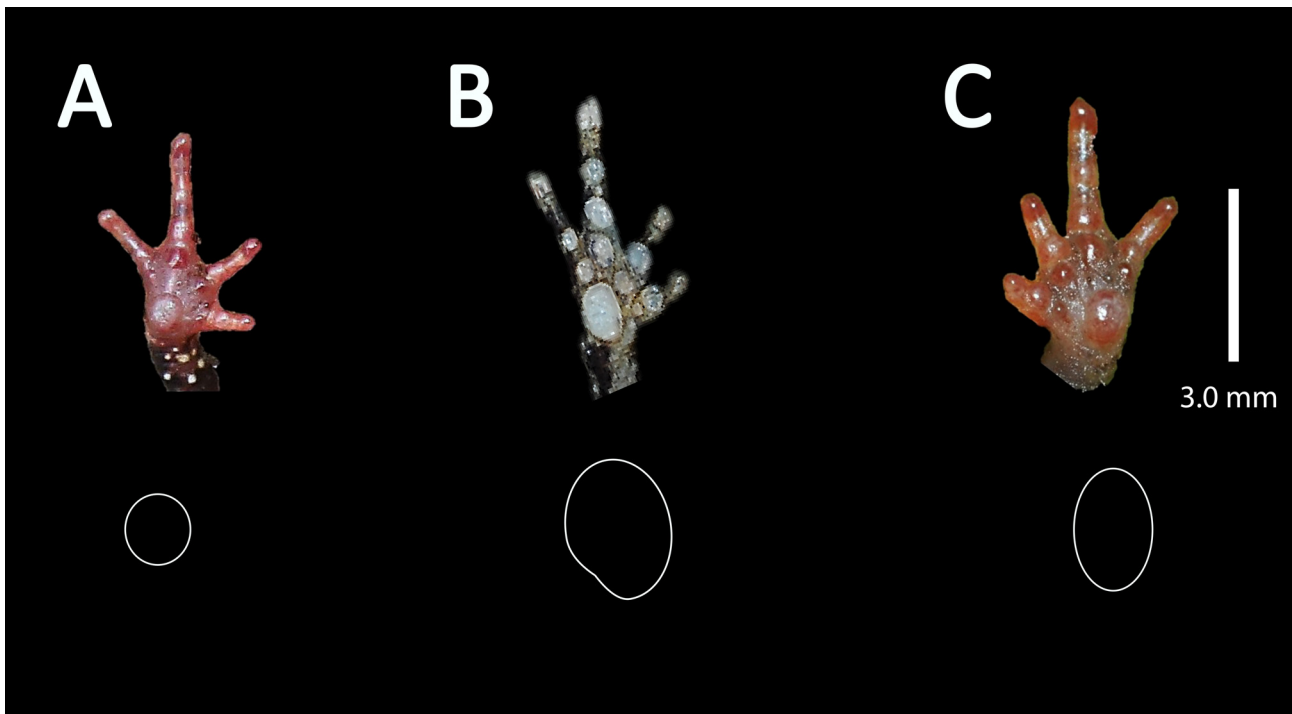
**Advertisement call.** The call was recorded in uncompressed .wav format, with a Zoom H1 Handy Recorder (Zoom Corporations, Tokyo, Japan) equipped with an internal microphone, positioned approximately 1.0–2.5 m from the focal male. All calls were filtered with Audacity 1.2.2 for Windows (Free Software Foundation Inc., 1991). Praat 4.2.22 for Windows (Boersma & Weenick, 2006) was used to generate audiospectrograms and oscillograms at a sampling frequency of 44.0 kHz and 16-bit resolution. Spectral parameters were analyzed



through fast Fourier transformations (FFT) (width 1024 points). Air temperature was measured immediately after each sound recording. Call structures were visually analyzed in the spectrograms subsequent to which we measured the following quantitative parameters considered informative in amphibian taxonomy (Köhler *et al.*, 2017): call duration (s), inter-call interval (s), number of pulses per call, dominant frequency (Hz), fundamental frequency (Hz), pulse rate (pulses/s) and call rise time (s) and numbers of harmonics. Call parameter measurements are presented as range (mean  $\pm$  standard deviation).



**FIGURE 2.** Terminology used in this paper to describe ventral color pattern in *Amazophrynella*. A–B) large black blotches over light background; C–D) small black blotches over light background; E–F) small black dots over light background; G) small black points over light background. See Materials and Methods for character definition



**FIGURE 3.** Terminology used in this paper to describe shape of palmar and subarticular tubercles in *Amazophrynella*. A) Rounded; B) oval; C) elliptical. See Materials and Methods for character definition.

## Results

*Lectotype designation and redescription justification.* Because of the superficiality of the original description, absence of material other than the type series and because the genus *Amazophrynella* is a complex of cryptic and pseudocryptic species (Fouquet *et al.*, 2012b; Rojas *et al.*, 2018) and many similar species are confused with *A. minuta*, a designation of a lectotype will contribute to taxonomic stability. Additionally, the redescription of the type species of the genus *Amazophrynella* will facilitate delimitation of new species and their formal descriptions.

According to the International Code of Zoological Nomenclature (ICZN, 1999) article 74.1.1: “A lectotype may be designated from syntypes to become the unique bearer of the name of a nominal species-group taxon and the standard for its application”. We also follow the recommendations of the article 73C of the ICZN (2017) to provide data on the lectotype.

## Species account

### *Amazophrynella minuta* (Melin, 1941)

#### *Synonymy*

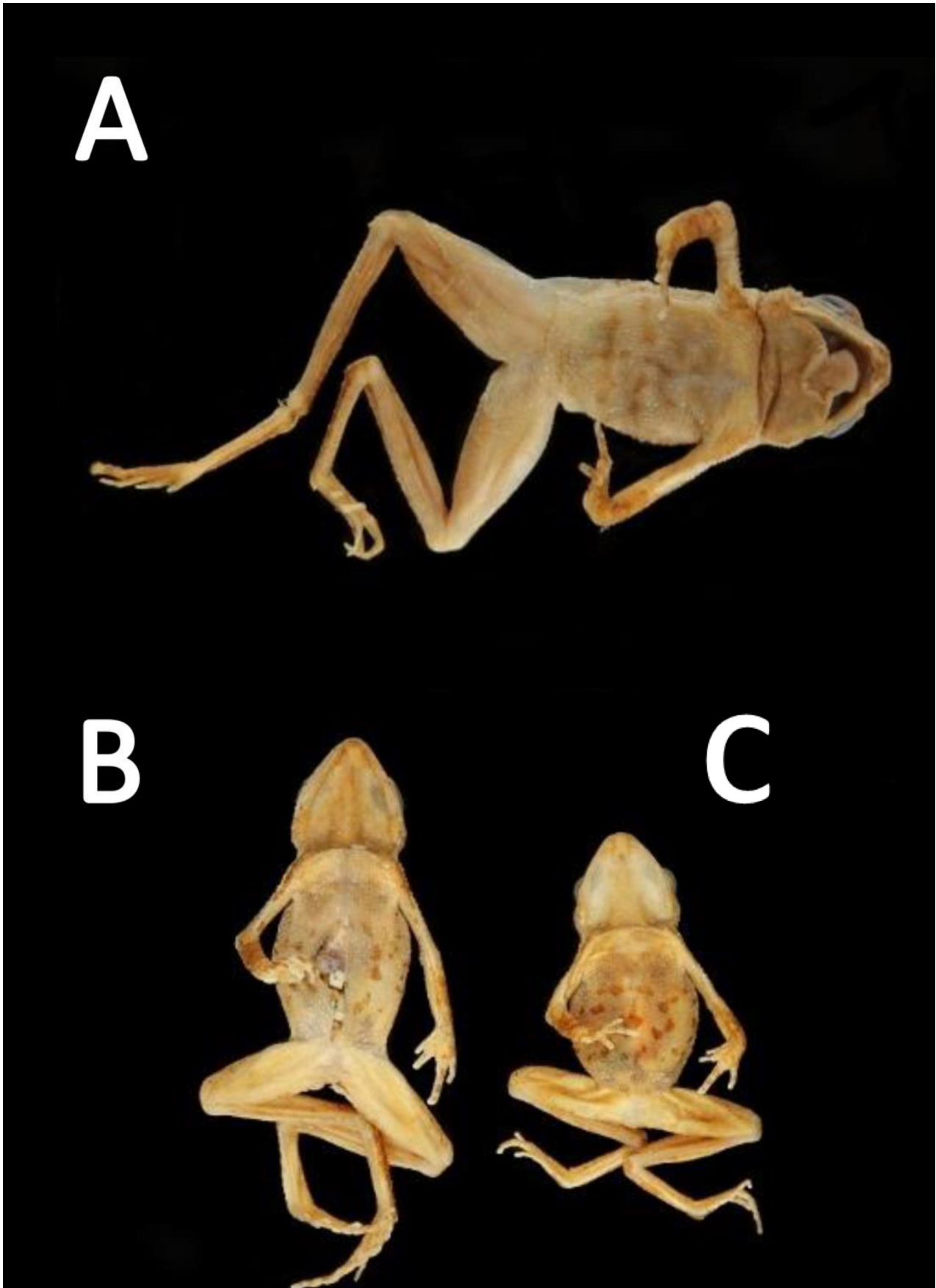
*Atelopus minutus*: Melin (1941: 40, Brazil; Amazonas Taracuá)

*Dendrophryniscus minutus*: McDiarmid (1971: 39, in part)

**Lectotype (designated herewithin).** Naturhistoriska Museet, Göteborg, Sweden, NHMG 462, SVL 13.40, adult male, collected at Taracuá (0.10°S, 68.46°W) 100 m a.s.l., Amazonas state, Brazil by Douglas Melin on 1924.

*Paralectotypes* (Figure 4). NHMG 463, NHMG 465 (adult males), NHMG 464 (adult female) collected at the same place as the lectotype.

**Topotypical specimens.** INPA–H 32721–34, adult males and INPA–H 32735–40, adult females collected by Rommel R. Rojas at the same place as the lectotype on August 19 and 20, 2013.



**FIGURE 4.** Lectotype (A = NHMG 462) and paralectotypes (B = NHMG 465, C = NHMG 463) of *Amazophrynella minuta* (Melin, 1941) from Taracuá, Rio Uaupés, Amazonas State, Brazil deposited at the Naturhistoriska Museet, Göteborg, Sweden. Additional pictures of the types are provided in Figure 3 -page 70 in Ávila *et al.* (2012). Photos by: Anders Larson.

**Diagnosis.** A species of *Amazophrynella* with: (1) Medium body size for the genus (see Table 1), adult females 15.0–19.0 mm SVL (n=11), adult males 12.2–14.2 mm SVL (n=20); (2) snout pointed in lateral view; upper jaw, in lateral view, protruding beyond lower; (3) tympanum, vocal sac, parotid gland and cranial crests inconspicuous; (4) texture of dorsal skin highly granular; (5) abundant small rounded (in part conical) warts present on dorsal surfaces of forelimbs and hindlimbs; (6) texture of ventral skin highly granular; (7) fingers slender, basally webbed; (8) finger I shorter than finger II; (9) finger III relatively long (HAL/SVL 0.2–0.3 mm, n=31); (10) palmar tubercle elliptical; (11) supernumerary tubercles rounded; (12) long hind limbs (TAL/SVL 0.4–0.5, n=31); (13) toes slender, basally webbed; (14) toes lacking lateral fringes; (15) plantar surfaces of feet bearing one metatarsal tubercle, the inner 2.0x larger than the outer, outer subconical; supernumerary plantar tubercles rounded; (16) in life, venter coloration yellow–orange with large to medium size black blotches on venter.

**TABLE 1.** Comparisons of measurements (in mm) among males and females of *Amazophrynella minuta* (for abbreviations of characters see Materials and Methods).

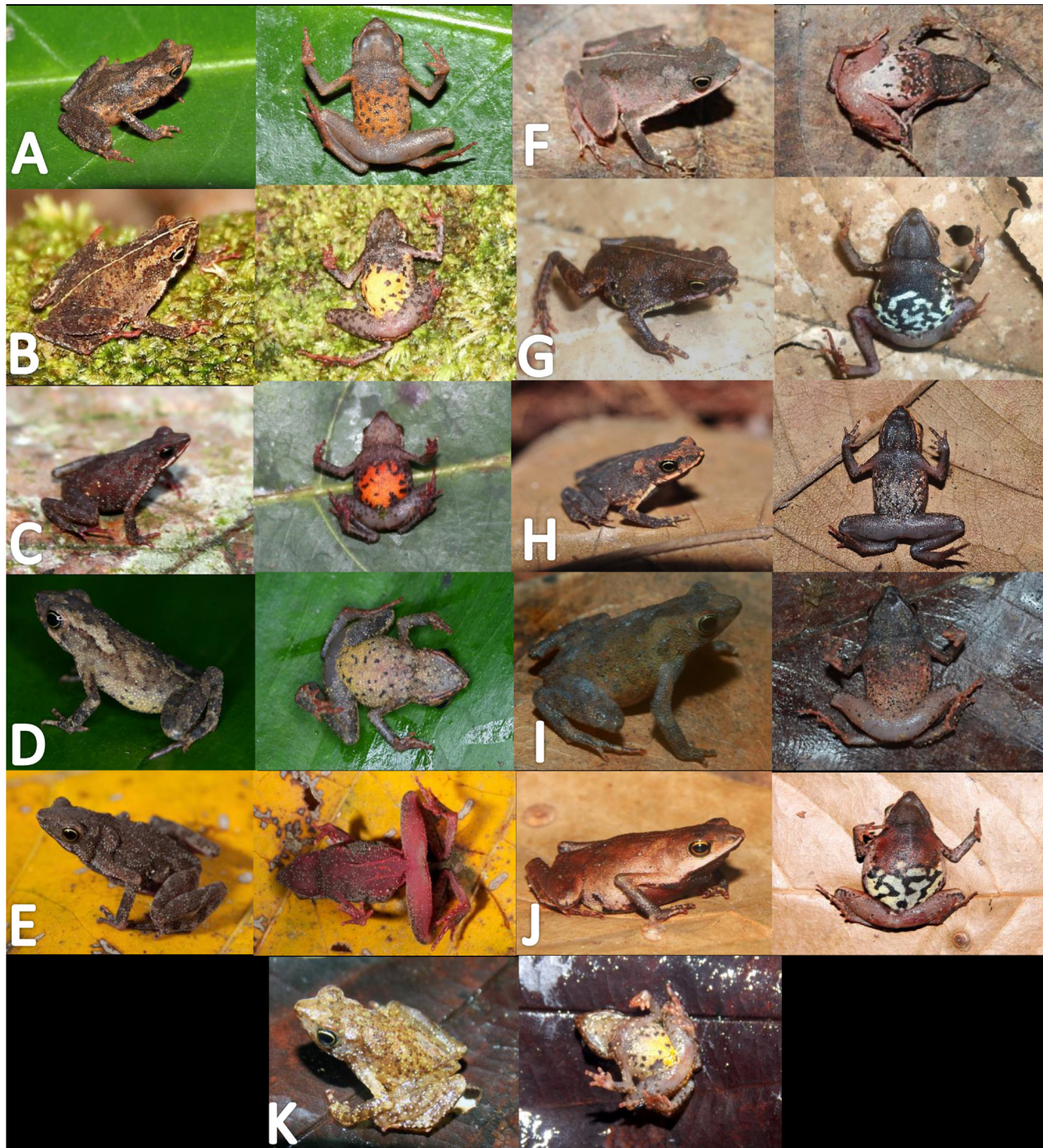
Character/Sex	Male (n=20)	Female (n=11)
SVL	13.6 ± 0.6 (12.2–14.7)	17.4 ± 0.9 (14.5–19.4)
HW	4.3 ± 0.2 (3.8–4.8)	5.1 ± 0.4 (4.6–5.9)
HL	5.1 ± 0.3 (4.6–5.8)	6.0 ± 0.4 (5.1–6.9)
SL	2.3 ± 0.2 (2.1–2.6)	2.7 ± 0.2 (2.4–2.9)
ED	1.5 ± 0.2 (1.2–2.0)	1.7 ± 0.3 (1.5–1.9)
IND	1.2 ± 0.1 (1.0–1.4)	1.4 ± 0.1 (1.2–1.5)
UAL	4.0 ± 0.4 (3.5–5.3)	5.2 ± 0.2 (4.6–5.5)
HAL	2.8 ± 0.2 (2.4–3.2)	3.6 ± 0.3 (3.0–4.4)
THL	7.0 ± 0.4 (6.3–8.1)	8.5 ± 0.9 (6.8–10.1)
TAL	6.8 ± 0.4 (6.1–8.1)	8.4 ± 0.7 (7.2–10.0)
TL	4.4 ± 0.6 (3.7–6.7)	5.4 ± 0.4 (4.6–6.0)
FL	4.9 ± 0.4 (4.2–5.8)	6.4 ± 0.7 (5.3–8.2)

**Comparisons with other species.** *Amazophrynella minuta* is most similar to *A. amazonicola* from which it can be distinguished by (characteristics of compared species in parentheses) absence of small triangular protrusion at the tip of the snout; texture of dorsal skin highly granular (granular); palmar tubercle elliptical (rounded). From *A. matses* by snout pointed in lateral view; texture of dorsal skin highly granular (spiculate); venter coloration yellow–orange (pale yellow; Figure 5A vs. 5C). From *A. javierbustamantei* by texture of dorsal skin highly granular (tuberculate); palmar tubercle elliptical (rounded); throat and chest coloration light brown (grayish cream); large blotches on venter (small points). From *A. siona* by snout profile pointed (acute); warts on dorsum (granules); texture of dorsal skin highly granular (finely granular). From *A. moisesii* by snout profile pointed (acuminate); texture of dorsal skin highly granular (tuberculate); large blotches on venter (tiny points). From *A. bokermanni*, *A. manaos*, *A. vote*, *A. xinguensis* and *A. manaos* the main difference is in venter coloration, yellowish–orange (white in *A. manaos*, *A. bokermanni* and *A. teko*, red brown in *A. vote*, brown in *A. xinguensis*; Figure 5A vs. 5F–J); FI < FII (vs. FI > FII in *A. bokermanni*, and FI ≥ FII in *A. xinguensis*).

**Redescription.** Body slender, slightly enlarged posteriorly. Sexual dimorphism observed in SVL, with 12.2–14.7 mm (13.6 ± 0.6 mm, n=20, Table 4) in adult males and 14.5–19.4 mm (17.5 ± 1.5 mm, n=11) in adult females. Head pointed, longer than wide: HL 4.6–6.8 mm (5.4 ± 0.5 mm), 36.0% of SVL; HW 3.8–5.8 mm (4.6 ± 0.1 mm), 30.0% of SVL. Snout profile pointed in lateral view; SL 2.1–3.2 mm (2.5 ± 0.3 mm), 46.0% of HL. *Canthus rostralis* straight and loreal region vertical. Nostrils protuberant, closer to snout than to eyes. IND 1.0–1.5 mm (1.2 ± 0.1 mm), 27.0% of HW. Interorbital and occipital regions flat. Tympanic membrane and tympanic annulus not apparent. Parotoid glands not visible. Vocal sac not visible in males. Eyes prominent. Eye diameter 1.2–2.0 mm (1.5 ± 0.2 mm), 29.0% of HL. Eyelid with small tubercles on borders. *Choanae* small and circular. Dorsal skin texture highly granular. Small rounded warts (in part conical) on dorsolateral and lateral surfaces posterior to eye, on posterior dorsum, and on dorsal surfaces of entire arm. Texture of gular region, chest and belly highly granular. Forelimbs slender. Forearms robust, especially in males. UAL 3.5–5.5 mm (4.4 ± 0.7 mm); 29.3% of SVL. Fingers

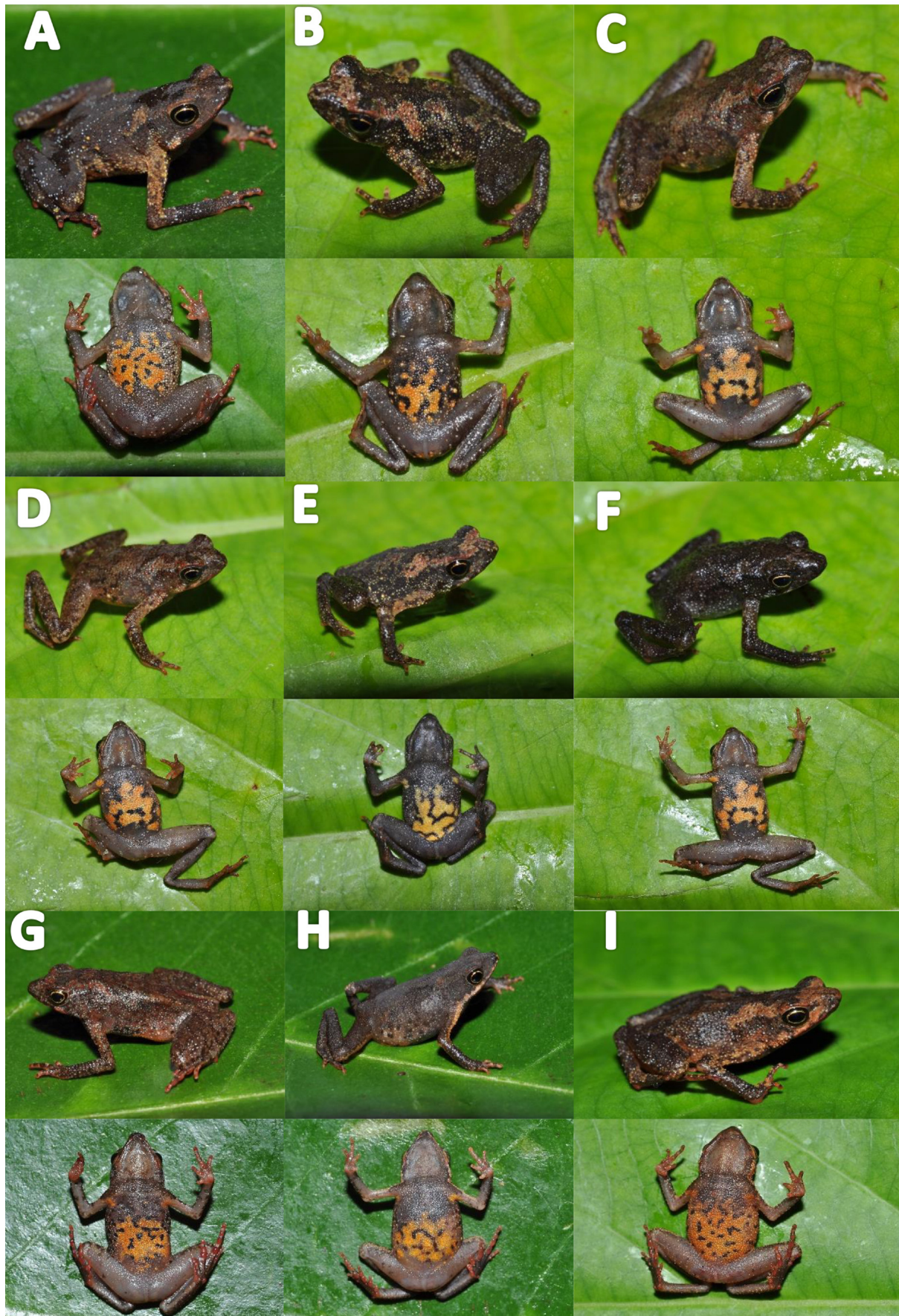


slender. HAL 2.4–4.5 mm ( $3.1 \pm 0.5$  mm); 70.0% of UAL. Edges of forelimbs with scattered granules, in dorsal and ventral view. Fingers basally webbed. Relative length of fingers: I < II < IV < III. First finger short, third two times the size of the second, fourth larger than the first and second. Large elliptical palmar tubercle. Supernumerary tubercles rounded. Thenar tubercle absent. Tip of fingers unexpanded. Nuptial pad not evident. Hind limbs slender. Ventral and lateral surfaces of forelimbs granular. THL 6.3–10.0 mm ( $7.6 \pm 0.1$  mm), 50.0% of SVL. TAL 7.4–10.0 mm ( $7.4 \pm 1.0$  mm); 49.4% of SVL. TL 3.7–6.7 mm ( $4.7 \pm 0.8$  mm); 32.0% of SVL. FL 4.2–8.0 mm ( $5.5 \pm 0.9$  mm); 71.1% of THL. Cloacal opening slightly above mid-level of thighs. Grouping of brownish–yellow granules from the hidden surface of the thighs to the shank. Basal webbing on foot. Toes lacking lateral fringes. Relative length of toes: I < II < III < V < IV. First toe very short; second half the size of the third. Elliptical inner metatarsal tubercle present. Subarticular tubercles visible, more protruding and swollen in females than males. Foot slender. Tips of toes unexpanded.



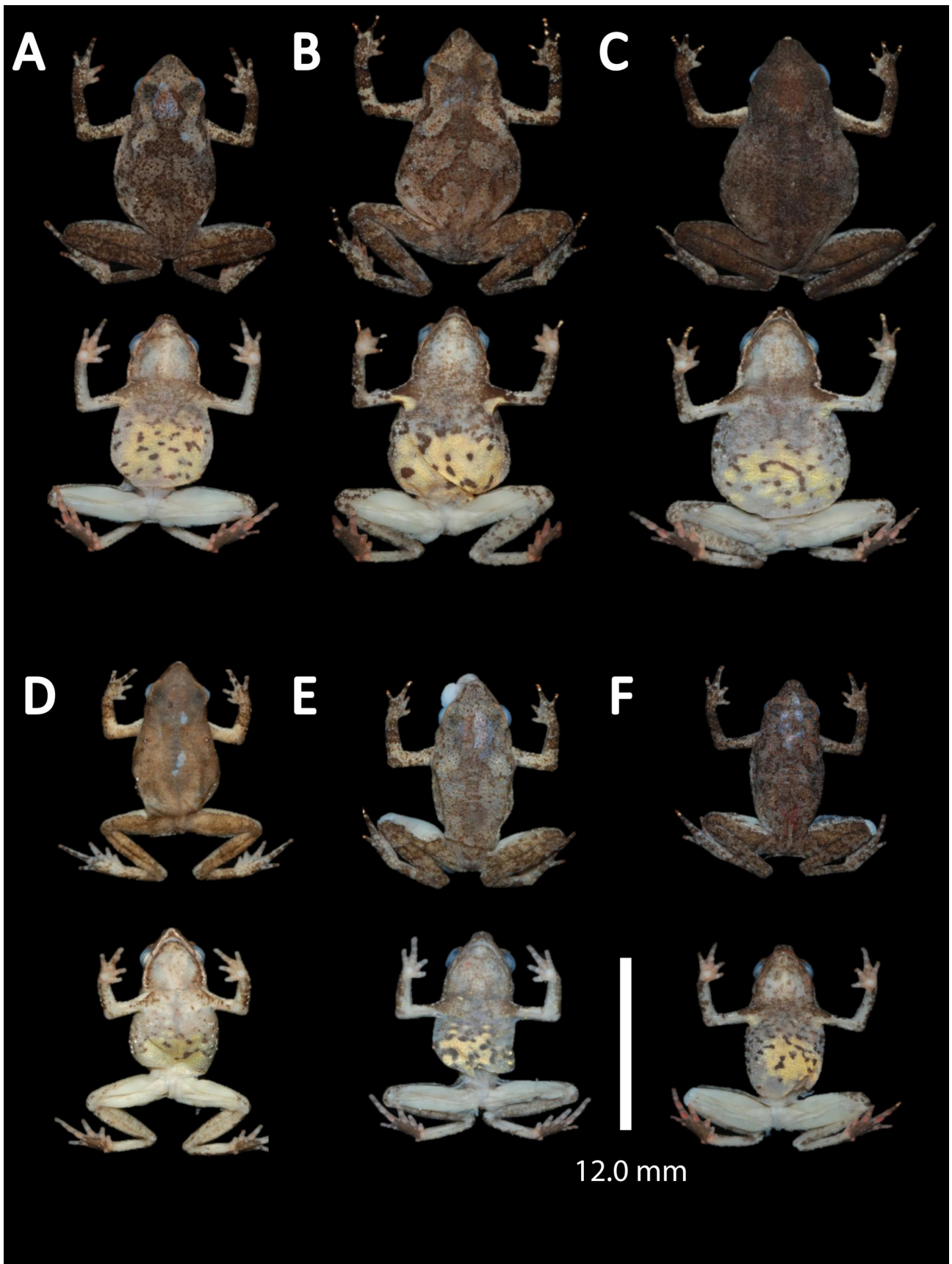
**FIGURE 5.** Species of *Amazophrynella*. A) *A. minuta*; B) *A. matses*; C) *A. amazonicola*; D) *A. javierbustamantei*; E) *A. siona*; F) *A. bokermanni*; G) *A. manaos*; H) *A. vote*; I) *A. xinguensis*; J) *A. teko*; K) *A. moisesii*.



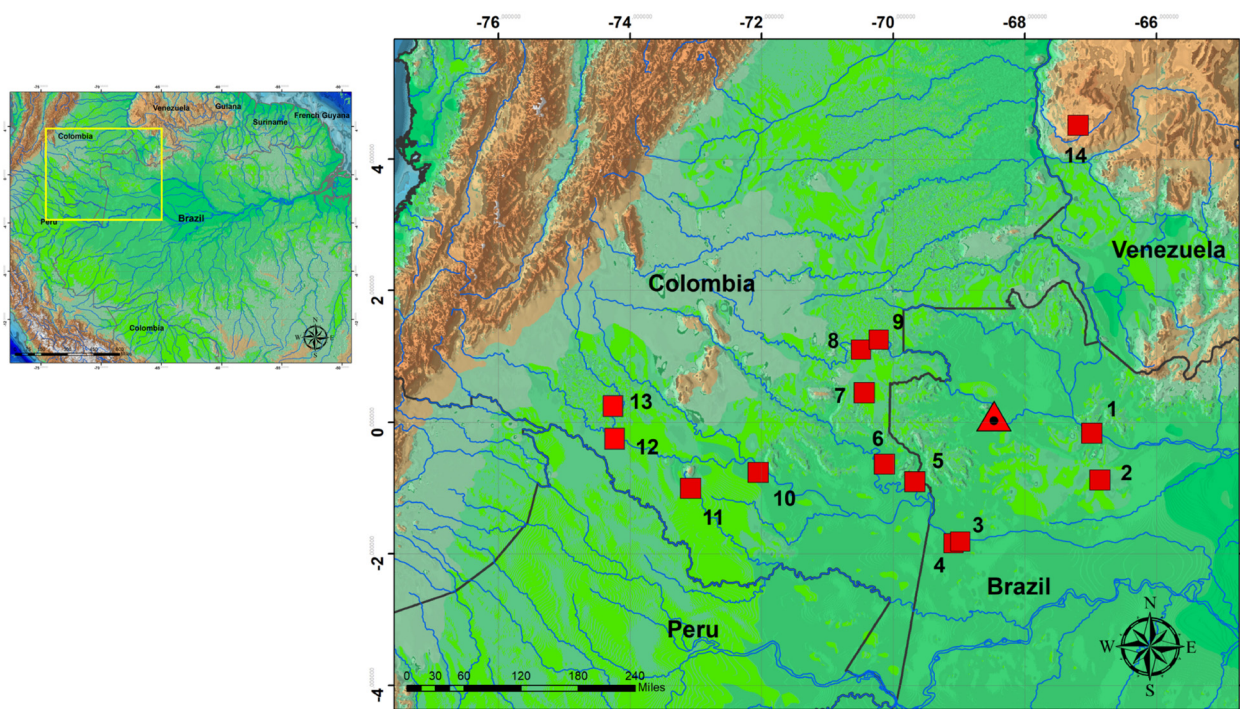


**FIGURE 6.** Morphological variation in life of *Amazophrynella minuta* (Melin, 1941) from type locality. A–F) adult males; G–I) adult females.





**FIGURE 7.** Morphological variation of preserved specimens of *Amazophrynella minuta* (Melin, 1941) from the type locality. A–C) adult females; D–F) adult males.



**FIGURE 8.** Geographic distribution of *Amazophrynella minuta* (Melin, 1941), triangle marks the type locality. Brazil, Amazonas state: 1) São Gabriel da Cachoeira; 2) Lago Jabo-Cucui; 3–4) Vila Bittencourt, Japura River. Colombia, Vaupés department: 5) estación biológica Cacaparú; 6) Santander; 7) Vaupés River; 8) Mitu; Amazonas department: 10) Restinga isla marítima; 11) La chorrera; Department of Caquetá: 12) Cabanachagra; 13) Caqueta River. Venezuela; Amazonas state: 14) Raudal de Danto, Río Cuao.

**Variation.** Variation in measurements is presented in Table 1. Specimens from Japura River (INPA–H 32731, INPA–H 32724, INPA–H 32734) are longer (SL about 1.0 mm) than individuals from the type locality. Some individuals from São Gabriel da Cachoeira present relatively few warts on dorsal surface (INPA–H32731, INPA–H 32724 and INPA–H 32734). Mottled warts disseminated on chest and belly are found in specimens INPA–H32731 (São Gabriel da Cachoeira) and INPA–35496 (Japura River). Some specimens from the type locality (INPA–H 32728, INPA–H 32735) and from the Japura River (INPA–H 35512), present lower abundance of warts on arms.

**Coloration in life** (Figure 6). Head brown. Dorsum reddish–brown. Flanks light brown. Dorsal surfaces of the upper arm and arm reddish–brown. Hands and fingers light brown, in dorsal view. Insertion of the arms present yellow blotches. Dorsal surfaces of the thighs, tibia, tarsus and feet reddish–brown. Ventral surfaces of upper arm and arm cream. Palm light red. Ventral surfaces of thighs mottled cream with small black blotches. Thighs with a transverse lateral black bar. Posterior region of the thigh and cloaca yellow, covered by small black blotches. Tarsus with transverse brown bars. Sole reddish–brown. Gular region and chest grayish–brown. Venter coloration between yellow and yellow–orange. Venter covered by large to medium size black blotches. In ventral view, some specimens present yellow blotches at the insertion of the arms. The specimens INPA–H 32725 (Taracú) and INPA–H 35494 (Japura River), display yellow coloration with small black blotches on posterior region of the thigh near the cloaca. Iris golden and pupil black.

**Color in preservative** (Figure 7). The color in preservative faded. We noted the progressive loss of the ventral coloration that became pale yellow. The yellow coloration on axillary surface disappeared. In ventral view, the color of fingers and toes lost their red color and became cream.

**Distribution** (Figure 8). *Amazophrynella minuta* is distributed throughout the eastern border region of the Amazonas state in Brazil, a region also known as “Cabeça do Cachorro” (“Dog’s head”, English free translation). The species is found in the following localities: Taracú (0.10°N, 68.46°W), São Gabriel da Cachoeira (0.16°S, 66.98°W), Cucuí (1.07°N, 66.88°W) and Japura River south-east of Vila Bittencourt (1.81°S, 68.98°W), at elevations between 90–105 m a.s.l. In Colombia it is reported in the Department of Caquetá (0.92°N, 75.67°W) and Vaupés (0.90°S, 69.67°W) (Lynch, 2006) at elevations between 100–200 m a.s.l. (Acosta–Galvis, 2017). In Venezuela, the species was reported from Raudal de Danto, Río Cuao (4.53°N, 67.18°W) Amazonas state, at elevation of 105 m a.s.l. (Rojas–Runjaic *et al.*, 2013).



**Ecology.** We found all individuals in the morning, near a body of water. Specimens were collected in primary “*terra firme*” forest (“firm land” – non-flooded forest, English free translation) some in the leaf litter and others among tree roots. Specimens from São Gabriel da Cachoeira were collected in a fragment of forest near the airport; the individuals were found around a stream with abundant leaf litter. Amplexus is axillar (Figure 9).

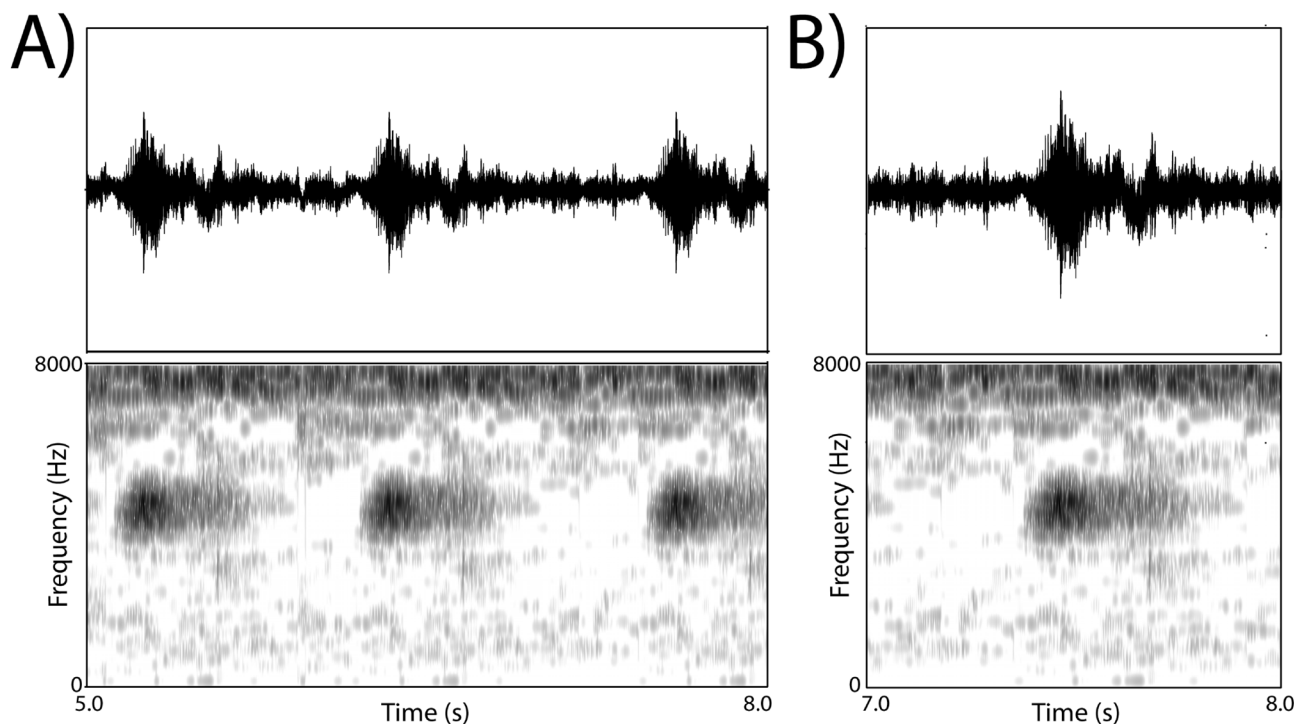
**Phylogenetic analysis.** The complete phylogenetic relationships of *Amazophrynella*, the phylogenetic position of *A. minuta* and genetic distance between species of *Amazophrynella* are reported in Rojas *et al.* (2018, Figure 1 and Table 1).

**Advertisement call** (Figure 10). The characterization of the advertisement call of *Amazophrynella minuta* was based on 14 calls from one individual from São Gabriel da Cachoeira (INPA–H 32730), SVL: 12.2 mm, recorded between 15:30–16:00 h. Air temperature at the time of recording varied between 26.0 to 27.0°C. The call consisted of a trilled note emitted between regular silent intervals. Notes had an upward amplitude modulation, reaching their maximum intensity near the middle of the note. Note duration ranged between 0.310 and 0.108 s ( $0.223 \pm 0.068$  s). Number of pulses per note ranged from 18 to 35 pulses/note ( $26.286 \pm 6.557$  pulses/note). The inter-note interval was 0.663 to 1.050 s ( $0.827 \pm 0.120$  s). The dominant frequency ranged from 4242.790 to 4562.011 Hz ( $4419.213 \pm 125.642$  Hz). The fundamental frequency ranged from 3201.010 to 3788.520 Hz ( $3489.211 \pm 235.791$  Hz). The rise time ranged from 0.045 to 0.083 s ( $0.059 \pm 0.015$  s). Number of harmonics varied from 3 to 5 ( $4.150 \pm 0.587$ ).

**Osteology.** A detailed review and description of the skeleton of *A. minuta* were provided by McDiarmid (1971). In the same publication, McDiarmid (1971) reported characters that could be tentatively considered as putative genus level synapomorphies (see Fouquet *et al.*, 2012b).



**FIGURE 9.** Axillary amplexus between specimens of *Amazophrynella minuta* (Melin, 1941)



**FIGURE 10.** Advertisement call of *Amazophrynella minuta* (Melin, 1941) from São Gabriel da Cachoeira, Amazonas state, Brazil. A) Oscillogram and spectrogram visualizing three notes; B) Oscillogram and spectrogram visualizing one note.

## Discussion

*Amazophrynella minuta* was described by Melin in 1941 from a few specimens collected at Taracuá, municipality of São Gabriel da Cachoeira, Amazonas state, Brazil in 1924. The original description lacked precision in describing morphological characters such as shape of head, in dorsal and lateral view; texture of dorsal and ventral skin; formula of fingers and toes; measurements and proportions of body; and details of coloration of the dorsal and ventral surfaces. Since these morphological characters are important to distinguish and diagnose species of the genus (see Izecksohn, 1993; Ávila *et al.*, 2012; Rojas *et al.*, 2014, Fouquet *et al.*, 2012b), herein, we provided a detailed description of these characters. Furthermore, with the identification of diagnostic characters and definition of character states of *A. minuta*, this publication will facilitate comparison among species of *Amazophrynella* and contribute to future descriptions of the remaining unnamed species of the genus (Rojas *et al.*, 2018).

Morphological traits and phylogenetic relationships suggest that *Amazophrynella minuta* is most closely related to *A. amazonicola*, *A. matses* and *A. javierbustamantei* with which it notably shares coloration and patterns on the belly (see Figure 5); however, *A. minuta* differs from these species by snout profile and texture of dorsal and ventral skin. From the other species of *Amazophrynella*, *A. minuta* differs in SVL, body proportions, size of FI vs. FII; venter coloration, texture of dorsal skin, type of palmar tubercle and webbing type on fingers (see *Comparisons with other species* for details). The morphological crypsis in Amazonian anurans is a taxonomic problem that hinders descriptions based only on morphological data, but can and has been overcome in several groups using an integrated approach to taxonomy (analyses of advertisement calls, morphology, genetics, tadpoles, etc.) (*sensu* Padial *et al.*, 2009; Padial *et al.*, 2012; Caminer & Ron 2014)

The description of the advertisement call of *A. minuta* will contribute to distinguishing nominal populations from others (i.e. Colombia, Venezuela and Brazil) in the aim to delimit new species. As expected, the advertisement call of *A. minuta* is similar to other species of *Amazophrynella* (Rojas *et al.*, 2018). As a genus-level synapomorphic character the advertisement calls of *Amazophrynella* present a simple note that varies between 0.0180 to 0.0146 s; a fundamental frequency between 2354.207 to 3204.169 Hz; a dominant frequency between 3033.86 to 3635.71 Hz; pulse number range from 8 to 52.4 and a rise time from 0.004 to 0.107 s. Unfortunately, we

could not collect tadpoles of *Amazophrynella minuta*, although we made two expeditions to the remote type locality in July 2012 and again in August 2014. Within the genus, tadpoles are scarce and basic ecology remains largely unstudied (Rojas *et al.*, 2016). The description of these (tadpoles and behavior) biological variables in nominal and putative species of *Amazophrynella* may provide important data for future species delimitation within the genus.

Genetic and morphological data from populations of *A. minuta* from Colombia and Venezuela were not analyzed in this study. Given the propensity of hidden diversity in this group a careful examination of material from these populations is needed. Future integrative taxonomic analyses will certainly improve our knowledge of phylogenetic relationships and taxonomy of *Amazophrynella*.

## Acknowledgements

We thank the people of the community of Taracúá, especially Sr. Maximiliano Correia Menezes (representative of the Amerindian village of Taracúá and FOIRN), Sr. Gabriel Correia Menezes, Sr. João Filho Menezes and Sra. Vera Correia for hospitality and logistic support in Taracúá. We thank Junior Menezes, Max Junior Menezes, and Bene Menezes for field support. Federação de Organizações Indígenas do Rio Negro/FOIRN allowed access to Amerindian lands. We thank Göran Nilson and Anders Larsson (Naturhistoriska Museet, Göteborg) for their consideration and for sending color photographs of the syntypes of *A. minuta*, and Marcia Lima de Queiroz and Miss Lana for technical support at INPA. Mario Nunez helped with the molecular analyses. Funding for this work came from CNPq/SISBIOTA Processo No. 563348/2010-0 and SISBIOTA/FAPEAM. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) provided financial support and awarded a PhD fellowship to R. R. R. This work is part of R.R.R.'s PhD Thesis in Genetics, conservation and evolutionary biology program of INPA. AF has benefited from an "Investissement d'Avenir" grant managed by the Agence Nationale de la Recherche (CEBA, ref. ANR-10- LABX-25-01). VTC thanks CAPES for a postdoctoral fellowship (PNPD). RWA thanks CNPq for his productivity research grant (303622/2015-6). SRR thanks SENESCYT and Arca de Noe initiative for laboratory and field funds.

## References

- Acosta-Galvis, A.R. (2017) Lista de los Anfibios de Colombia: Referencia en línea V.07.2017.0 (21 June 2017). Batrachia, Villa de Leyva, Boyacá. Available from: <http://www.batrachia.com> (accessed 13 August 2018)
- Ávila, R.W., Carvalho, V.T., Gordo, M., Kawashita-Ribeiro, R.A. & Morais, D.H. (2012) A new species of *Amazophrynella* (Anura: Bufonidae) from southern Amazonia. *Zootaxa*, 74, 65–74.
- Baum, D.A. & Donoghue, M.J. (1995) Choosing among alternative "phylogenetic" species concepts. *Systematic Botany*, 20, 560–573.  
<https://doi.org/10.2307/2419810>
- Boersma, P. & Weenick, D. (2006) *Program Praat: doing phonetics by computer. Version. 4.5.02*. Institute of Phonetic Sciences. University of Amsterdam, Amsterdam.
- Caldwell, J.P. & Araújo, M.C. (2005) Amphibian faunas of two eastern Amazonian rainforest sites in Pará, Brazil. *Occasional Papers Sam Noble Oklahoma Museum of Natural History*, 16, 1–41.
- Caminer, M. & Ron, S.R. (2014) Systematics of the three frogs *Hybsiboas calcaratus* and *Hybsiboas fasciatus* species complex (Anura: Hylidae) with description of four new species. *Zookeys*, 370, 1–68.  
<https://doi.org/10.3897/zookeys.370.6291>
- Fouquet, A., Gilles, A., Vences, M., Marty, C., Blanc, M. & Gemmell, N.J. (2007) Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PloS one*, 2, e1109.  
<https://doi.org/10.1371/journal.pone.0001109>
- Fouquet, A., Recoder, R., Teixeira Jr., M., Cassimiro, J., Amaro, R.C., Camacho, A., Damasceno, R., Carnaval, A.C., Moritz, C. & Rodrigues, M.T. (2012a) *Amazonella* Fouquet *et al.* 2012 (Anura: Bufonidae) junior homonym of *Amazonella* Lundblad, 1931 (Acari: Unionicolidae): proposed replacement by *Amazophrynella* nom. nov. *Zootaxa*, 3244, 68.
- Ford, L.S. & Cannatella, D.C. (1993) The major clades of frogs. *Herpetological Monographs*, 7, 94–117.  
<https://doi.org/10.2307/1466954>
- Frost, Darrel R. (2017) Amphibian Species of the World: an Online Reference. Version 6.0 (21 June 2017). Electronic Database. American Museum of Natural History, New York, USA. Available from: <http://research.amnh.org/herpetology/amphibia/index.html>.



- Heyer, W. R., Rand, A.S., Cruz, C.A., Peixoto, O.L. & Nelson, C.E. (1990) Frogs of Boracéia. *Arquivos de Zoologia*, 31 (4), 231–410.
- ICZN [International Commission on Zoological Nomenclature] (1999) *International Code of Zoological Nomenclature*. 4<sup>th</sup> Edition. The International Trust for Zoological Nomenclature, London, 106 pp.
- ICZN [International Commission on Zoological Nomenclature] (2017) Declaration 45—Addition of Recommendations to Article 73 and of the term “specimen, preserved” to the Glossary. *Bulletin of Zoological Nomenclature*, 73, 96–97.
- Izecksohn, E. (1993) Nova espécie de *Dendrophryniscus* da região amazônica (Amphibia, Anura, Bufonidae). *Revista Brasileira de Zoologia*, 10, 407–412.  
<https://doi.org/10.1590/S0101-81751993000300006>
- Kok, P.J. & Kalamandeen, M. (2008) *Introduction to the taxonomy of the amphibians of Kaieteur National Park, Guyana*. *Abc Taxa*, 5, 288 pp.
- Lynch, J.D. (2006) The amphibian fauna in the Villavicencio region of eastern Colombia. *Caldasia*, 28, 135–155.
- Melin, D.E. (1941) Contributions to the knowledge of the Amphibia of South America. *Göteborgs Kungl. Vetenskaps- och Vitterhets-samhälles. Handlingar: Serien B, Matematiska och Naturvetenskapliga Skrifter*, 1, 1–71
- McDiarmid, R.W. (1971) Comparative morphology and evolution of frogs of the Neotropical genera *Atelopus*, *Dendrophryniscus*, *Melanophryniscus* and *Oreophrynella*. *Bulletin of the Los Angeles County Museum of Natural History*, 12, 1–66.
- Padial, J.M. & De la Riva, I. (2009) Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura: Strabomantidae). *Zoological Journal of the Linnean Society*, 155, 97–122.  
<https://doi.org/10.1111/j.1096-3642.2008.00424.x>
- Padial, J.M., Chaparro, J.C., Castro Viejo-Fisher, S., Guyasamin, J., Lehr, E., Delgado, A., Vaira, M., Teixeira, J.R., Aguayo, R. & De la Riva, I. (2012) A revision of species diversity in the Neotropical genus *Oreobates* (Anura: Strabomantidae), with the description of three new species from the Amazonian slopes of the Andes. *American Museum Novitates*, 3755, 1–55.  
<https://doi.org/10.1206/3752.2>
- Rojas-Runjaic, F.J.M., Castroviejo-Fisher, S. & Barrio-Amorós, C.L. (2013) First record of the Amazonian tiny tree toad *Amazophrynella minuta* (Melin, 1941) (Anura: Bufonidae), for Venezuela. *Check List*, 9, 1122–1123.  
<https://doi.org/10.15560/9.5.1122>
- Rojas, R.R., Carvalho, V.T., Gordo, M., Ávila, R., Farias, I. & Hrbek, T. (2014) A new species of *Amazophrynella* (Anura: Bufonidae) from the Southwestern part of the Brazilian Guiana Shield. *Zootaxa*, 3753 (1), 79–95.  
<https://doi.org/10.11646/zootaxa.3753.1.7>
- Rojas, R.R., De Carvalho, V.T., Ávila, R.W., Farias, I.P., Gordo, M. & Hrbek, T. (2015) Two new species of *Amazophrynella* (Amphibia: Anura: Bufonidae) from Loreto, Peru. *Zootaxa*, 3946 (1), 79–103.  
<https://doi.org/10.11646/zootaxa.3946.1.3>
- Rojas, R.R., Chaparro, J.C., Carvalho, V.T., De, Ávila, R.W., Farias, I.P., Hrbek, T. & Gordo, M. (2016) Uncovering the diversity inside the *Amazophrynella minuta* complex: integrative taxonomy reveals a new species of *Amazophrynella* (Anura, Bufonidae) from southern Peru. *ZooKeys*, 71, 43–71.  
<https://doi.org/10.3897/zookeys.563.6084>
- Rojas, R.R., Fouquet, A., Ron, S., Hernandez, E., Melo-Sampaio, P., Chaparro, J., Vogt, R., Carvalho, V., Pinheiro, L., Ávila, R., Pires, I., Gordo, M. & Hrbek, T. (2018) A Pan-Amazonian species delimitation: high species diversity within the genus *Amazophrynella* (Anura:Bufonidae). *PeerJ*, 6, e4941.  
<https://doi.org/10.7717/peerj.4941>

## Specimens examined

- Amazophrynella minuta*.—BRAZIL: Amazonas State: Uauapés River, Taracá: INPA–H 32720–23, INPA–H 32725–26, INPA–H 32728–30, INPA–H 32732, INPA–H 32733, INPA–H 32735–40, NHMG 462, NHMG 463, NHMG 464; São Gabriel da Cachoeira: INPA–H32731, INPA–H 32724, INPA–H 32734; Japura River: INPA–H 35507–12, INPA–H 35494–98.
- Amazophrynella bokermanni*.—BRAZIL: Pará State: Juriti (approximately 30 km from the type locality): INPA–H 31861–65; Xingu River: INPA 35473–77; Amazonas State: Autazes: INPA–35529–30.
- Amazophrynella vote*.—BRAZIL: Mato Grosso State: Cotriguaçu, Fazenda São Nicolau: UFMT–A 11138 (Holotype), UFMT 11136, UFMT 11142, UFMT 11145–50, UFMT 11152–55, UFMT 4412; Manicoré, Madeira River: INPA–H 12255–56, 12331, 12342–43, 12366–67 (Paratypes); Parque Estadual do Guariba: INPA–H 21558; Novo Aripuanã, Aripuanã River: INPA–H 12326 (Paratype), INPA–H 35540–50; Amazonas State: Tapauá, Parque Nacional Nascentes do Lago Jari: INPA–H 27412, 27417–19, 27421–23, 27425–26 (Paratypes); Tapauá, Purus River: INPA–H 35551–53; Manaquiri: INPA–H 35535–39; Matupiri: INPA–H 31867, INPA–H 31868, INPA–H 31870–75, INPA–H 31877–80, INPA–H 31882, INPA–H 31883–66.
- Amazophrynella manaos*.—BRAZIL: Amazonas State: Universidade Federal do Amazonas campus: INPA–H 31866 (Holotype), INPA–H 6983–64, INPA–H 6987, INPA–H 7797 (paratypes); Presidente Figueiredo: INPA–H 20986, INPA–H 21217, INPA–H 29568–72, INPA–H 30575–77, INPA–H 30572–73 (Paratypes); Reserva Florestal Adolpho Ducke:

- INPA-H 21028, INPA-H 21170, INPA-H 21060, INPA-H 31866, INPA-H 21007-13, INPA-H 20963-INPA-H 20990.
- Amazophrynella matses*.—PERU: Department Loreto: Requena, Nuevo Salvador: MZUNAP 921(Holotype), MZUNAP 922-23, MZUNAP 925-27, MZUNAP 934, MZUNAP 936, MZUNAP 938, MZUNAP 940, MZUNAP 943-44, MZUNAP 948, MZUNAP 952-53, MZUNAP 955, MZUNAP 958 (paratopotypes); Jenaro Herrera: MZUNAP 928-31, MZUNAP 933, MZUNAP 935, MZUNAP 937, MZUNAP 939, MZUNAP 950, MZUNAP 941-42, MZUNAP 946-47, MZUNAP 949 (Paratypes).
- Amazophrynella amazonicola*.—PERU: Department Loreto: San Juan Bautista, Puerto Almendra: MZUNAP 901 (Holotype), MZUNAP 906-07, MZUNAP 910-11, MZUNAP 913-17, MZUNAP 110, MZUNAP 889 (paratopotypes); km 58 of Iquitos-Nauta highway at Fundo Zamora: MZUNAP 887-88, MZUNAP 900, MZUNAP 902, MZUNAP 886, MZUNAP 905, MZUNAP 908, MZUNAP 919-20, MZUNAP 924 (Paratypes); Maynas, Nauta: MZUNAP 909, MZUNAP 918; Fundo UNAP: MZUNAP 242 (Paratype).
- Amazophrynella javierbustamantei*.—PERU: Madre de Dios Department: Tambopata, Quebrada Guacamayo: MHNC 8331(Holotype), MHNC 8316, MHNC 8238, MHNC 8362-63, MHNC 8245, MHNC 8484; La Pampa: MHNC 1101-04; Nuevo Arequipa: MHNC 8245, MHNC 8331, MHNC 8238, MHNC 8354, MHNC 8484; Rio Tambopata: MHNSM 9633, MHNSM 9635, MHNSM 9640-42, MHNSM 9644, MHNSM 9646-48; Manu, Inambari: MHNSM 17993; La Convencion, Camana: MHNSM 2565; Mapi: MHNC 9939-40; Junin Department: Tambo Poyeni: MHNC 9387; Tsoroja: MHNC 9626, MHNC 9754, MHNC 9756-57, MHNC 9679, MHNC 9680; Cusco Department: Urubamba, Urubamba River: MHNC 9626, MHNC 9686-87.
- Amazophrynella moisesii*.—BRAZIL: Acre State: Parque Nacional da Serra do Divisor, Igarapé Ramon: UFAC-H 2815 (Holotype), UFAC-H 1375, UFAC-H 2772-2773, UFAC-H 2603, UFAC-H 2607, UFAC-H 3573-UFAC-H 3575, UFAC-H 2690, UFAC-H 2692, UFAC-H 2816-2817, UFAC-H 1698; Igarapé Anil: UFAC-H 1337-1343; Zé Luiz lake: UFAC-H 1774-1775; Mõa River: UFAC-H 1493, UFAC-H 2687-2697; Reserva Extrativista Alto do Juruá: UFAC-H 822-823, UFAC-H 878-879, UFAC-H 2606-2611; Gregório Forest Reverse: UFAC-H 5678.
- Amazophrynella teko*.—BRAZIL: Amazonas State: Trombetas River: INPA-H 35513-35530; Amapa state: UFPA AA 604; French Guiana: District of Camopi, Alikéné: MNHN 2015.136 (Holotype); District of Saint Laurent du Maroni, Mitaraka layon: MNHN 2015.137, MNHN 2015.138, MNHN 2015.139, MNHN 2015.140, MNHN 2015.141, MNHN 2015.142, MNHN 2015.143; Pic Coudreau du Sud: MNHN 2015.152, MNHN 2015; Flat de la Waki, INPA-H 36598; District of Camopi, Mitán: INPA-H 36596, MNHN 2015.144, MNHN 2015.145, MNHN 2015.146, MNHN 2015.147, MNHN 2015.148, MNHN 2015.149, MNHN 2015.150; District of Saint Georges, Saint Georges: MNHN 2015.151; Mémora: MNHN 2015.154, MNHN 2015.155; Saut Maripa: INPA-H 36597, INPA-H 36610, INPA-H 36599, INPA-H 36601, INPA-H 36600.
- Amazophrynella xinguensis*.—BRAZIL: Para State: Sustainable Development Project (PDS) Virola Jatobá: INPA-H 35471 (Holotype), INPA-H 35484, INPA-H 35485 INPA-H 35473, INPA-H 35474, INPA-H 35475, INPA-H 35476, INPA-H 35477, INPA-H 35478, INPA-H 35479, INPA-H 354780, INPA-H 35481, INPA-H 35483, INPA-H 35490, INPA-H 35491, INPA-H 3592; Fazenda Paraíso: INPA-H 35493, INPA-H 35472; Ramal dos Cocos: INPA-H 35486, INPA-H 35487, INPA-H 3588, INPA-H 35489.
- Amazophrynella siona*.—ECUADOR: Orellana Province: Yasuni National Park: QCAZ 27790 (Holotype), QCAZ 11981, QCAZ 51068, QCAZ 21425, QCAZ 21431, QCAZ 11973, QCAZ 11979; Tambococha: QCAZ 55345; Garzacocha: QCAZ 20504; Yuriti: QCAZ 10526; Kapawi Lodge: QCAZ 8725, QCAZ 25504, QCAZ 25533; 10 km from Puyo: QCAZ 7135; Morona Santiago Province: Pankints: QCAZ 46430; Sucumbios Province: Reserva de Producción Faunística Cuyabeno: QCAZ 52433-34, QCAZ 37758-59, QCAZ 37761, QCAZ 6071, QCAZ 6091, QCAZ 6095, QCAZ 6097, QCAZ 6105, QCAZ 6111, QCAZ 6113, QCAZ 6118, QCAZ 6127, QCAZ 6128, Santa Cecilia, QCAZ 4469, QCAZ 4472; Tarapoa: QCAZ 36331, QCAZ 36336, QCAZ 36338, QCAZ 36357; Pastaza Province: Kurintza community: QCAZ 38809, QCAZ 54213, QCAZ 56342, QCAZ 56354, QCAZ 56361; Villano community, AGIP oil company: QCAZ 38599, QCAZ 38679, QCAZ 38722, QCAZ 38642; Bataburo Lodge: QCAZ 39408; Lorocachi: QCAZ 8902, QCAZ 56165; Canelos: QCAZ 52819, QCAZ 52823, QCAZ 17391. PERU: Loreto Department: Teniente Lopez: MHNC 7611, MHNC 7685, MHNC 7686, MHNC 7698, MHNC 7699, MHNC 7700; Jibarito: MHNC 7786, MHNC 7809, MHNC 7814; Shiviyacu: MHNC 14730; proximities of Corrientes River: MHNC 6292.