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A new species of poison frog, genus *Ameerega* (Anura: Dendrobatidae), from the southern Amazonian rain forest

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Abstract. A new species of poison frog in the *Ameerega picta* group is described from Amazonia, states of Pará and Mato Grosso. It inhabits rock outcrops in open areas near streams of the central Teles Pires River system. The new species differs from congeners by the combination of four characters: 24.87–28.59 mm adult SVL, black immaculate dorsum, white ventral side with black vermicular blotches, and an orange stripe in the axillar region. Genetically, the new species is most closely related to *A. flavopicta*.

Key words. Amphibia, Anura, Dendrobatidae, *Ameerega* sp. n., *A. picta* group, systematics, taxonomy, Brazil.

Introduction

The Amazonian rain forest is one of the most diversified ecoregions in the world (Mittermeier et al. 2003). New species are still discovered in this region even within well-known groups of animals, such as large mammals (Cozzuol et al. 2013, Hrbek et al. 2014) and birds (Pivetta 2013). The same applies to amphibians, and many new species have been described in recent years (e.g., Angulo & Icochea 2010, Simões et al. 2010, Kok et al. 2013, Melo-Sampaio et al. 2013, Lima et al. 2014, 2015, Fouquet et al. 2015, 2016, Vaz-Silva et al. 2015). Amongst them, several studies have used integrative taxonomy approaches to diagnose species, which facilitates the recognition of the cryptic diversity (Bickford et al. 2006, Fouquet et al. 2007, Padial et al. 2010, Funk et al. 2011).

The family Dendrobatidae currently is composed of almost 200 described species. The genus *Ameerega* Bauer, 1986 is the most species-rich group in it and contains more than 32 species (Frost et al. 2016). This genus has a mainly cis-Andean distribution, with most species being distributed in the Amazon basin and adjacent areas (Grant et al. 2006, Frost 2016). The taxonomy of *Ameerega* is historically poorly understood, and the actual number of members in this group remains far from being fixed (Lötters et al. 2005). One reason for this is that interspecific variation is limited. Using integrative taxonomy some sibling species have been detected hidden in nominal species and several taxonomic misinterpretations have been resolved in recent years (e.g., Lötters et al. 2005, Grant et al. 2006, Brown & Twomey 2009).

In this paper, we use an integrative approach to describe a new species of this genus from southern Amazonia in the northern parts of the states of Mato Grosso and Pará, Brazil.

Materials and methods

Fieldwork

We collected specimens by visual searches in February and May of 2016 at two sites: municipality of Paranaíta, state of Mato Grosso; and municipality of Jacareacanga, state of Pará. Both sites are located near the hydroelectric power plant São Manoel (9°11'20.85" S, 57°3'11.64" W, ~140 m above sea level). The collected frogs were euthanised using a liquid solution of 2% lidocaine chlorhydrate, fixed in 10% formalin, and transferred to permanent storage in 70% ethanol. We also collected tissue samples (muscle and liver) before specimen fixation and stored them in 100%

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ethanol. Vouchers have been deposited in the Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul (ZUFMS-AMP), Campo Grande, MS, Museu de Zoologia João Moojen (MZ[illegible]) of the Universidade Federal de Viçosa, Viçosa, MS, and in the Museu de Zoologia da Universidade Federal da Bahia, Salvador da Bahia, BA (UFBA).

(MS) ?

Bioacoustics

We recorded the vocalizations of one male paratype (ZUFMS-AMP 05143) at the type locality and analysed a total of 33 advertisement calls. A Tascam DR-44 digital recorder was used. Recordings were made between 11:00 and 12:00 h (air temperature 29°C; air humidity 80%) and digitalized at 44.1 kHz with a 16-bit resolution. We analysed all calls with Raven Pro v. 1.5, 64-bit version (Bioacoustics Research Program 2014).

Morphology

We used the following characters of Grant et al. (2006) and Lötters et al. (2007): colour in life (compared to other species based on literature); presence/absence of: bright signal spot in the calf region; light dorsolateral stripe; dark lateral stripe; collar pattern; neopalatinae; maxillary teeth; median lingual process; dorsal skin texture; toe webbing; and dermal flap. In addition, we examined the shape of the end of finger IV and the relative sizes of the fingers.

We measured morphometric characters of adult specimens using digital callipers (precision = 0.01 mm), following Haddad & Martins (1994): snout to vent length (SVL); head length (HL); head width (HW); internarinal distance (IND); eye to nostril distance (END); eye diameter (ED); tympanum diameter (TD); hand length (HAL); thigh length (THL); tibia length (TBL); and foot length (FL) (from the tip of the longest toe to the articulation of tibiofibula and tarsus). We identified the sexes of specimens by the presence/absence of vocal slits.

Material studied in addition to the new species is listed in Appendix 1.

Molecular data

Whole genomic DNA was extracted from muscle tissue using the QIAGEN DNeasy Blood and Tissue Kit (QIAGEN). PCR amplification and sequencing was performed with the primers 16Sa (5-CGC CTG TTT ATC AAA AAC AT-3) and 16Sb (5-CCG GTC TGA ACT CAG ATC ACG T-3) of Palumbi et al. (2002) to amplify a section of the mitochondrial 16S ribosomal RNA gene. PCR conditions followed those described by Costa el al. (2016). PCR products were purified with ethanol/sodium acetate and sequenced with an ABI 3730 XL DNA Analyzer (Applied Biosystems, Foster City, California/CA). Sequences were edited by aligning forward and reverse reads using Geneious v. 8.1.7. Final sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Mitochondrial sequences were aligned with those of other *Ameerega* species available from GenBank (n = 186, Supplementary document 1). The final sequence length used for further phylogenetic analyses was 476 bp. Sequence divergences (uncorrected p-values) were calculated using Mega v. 6.06 (Tamura et al. 2013).

Nomenclatural acts?

Results

Ameerega munduruku sp. n.

(Figs. 1–3, Table 1)

ZooBank LSID: XXXXXXXXXXXXXXXXXXXXX.
Ameerega picta (non Tschudi): Prates et al. (2012)

Holotype: ZUFMS-AMP 03762, adult male, from the Jacareacanga Municipality, state of Pará, Brazil (09°19'1.00" S, 56°46'35.76" W, 200 m a.s.l.), collected by L.A. da Silva, 25 May 2016.

Paratypes: Five adult males (ZUFMS-AMP 03747–03748; MZUFV 17134–17135; UFBA 14250) and five adult females (ZUFMS-AMP 03749; MZUFV 17136; UFBA 14249, 14251, 14254), collected along with the holotype by the same collector; two adult females (MZUFV 17132; ZUFMS-AMP 03761); one adult male (ZUFMS-AMP 05143) from the type locality, collected by L.A. da Silva, 17 January 2017; and one adult male (MZUFV 17133) from Paranaíta Municipality, state of Mato Grosso, Brazil (09°18'57.96" S, 56°47'33.53" W), collected by M.O. Neves, 25 February 2016.

Diagnosis: The following characters diagnose the new species as a member of the genus *Ameerega* sensu Grant et al. (2006): colour in life aposematic; bright signal spot in the calf region present; light dorsolateral stripe present; light lateral stripe absent (the line visible from the lower lip to groin is an extension of the ventral coloration); presence of the lateral stripe dark; neopalatinae present; dorsal skin slightly granular; finger I > finger II when adpressed; webbing between toes absent; presence of dermal flap.

The new species is diagnosed and distinguished from all other described *Ameerega* species by the combination of the following characters: (1) medium size (SVL of adult males 24.87–27.33 mm, of adult females 20.42–28.59 mm); (2) snout truncate in dorsal view (Fig. 2A); (3) snout protruding in lateral view (Fig. 2B); (4) hand small (HAL/SVL = 0.21) (Fig. 2C); (5) dorsum black and granular in life (Fig. 3A); (6) in life, an orange spot is present in the axillar region, an orange stripe runs from the inguinal region to the dorsal face of the thigh, and another orange spot is present in the concealed region of the tibia (Figs 3A–B); (7) dorsolateral stripes cream to pale yellow in life (Figs 3A–B); (8) venter and flank white with black vermicular blotches, and gular region with black blotches

Table 1. Measurements (in mm) of the holotype and entire type series of *Ameerega munduruku* sp. n. (the mean is followed by the standard deviation and ranges in parentheses). Abbreviations are specified in Material and methods.

	Holotype (male)	Females (n=7)	Males (n=7)
SVL	26.00	25.30 ± 3.55 (20.42–28.59)	26.03 ± 0.79 (24.87–27.33)
HL	5.91	5.92 ± 0.82 (4.7–7.08)	6.28 ± 0.42 (5.59–6.78)
HW	7.88	7.54 ± 0.84 (6.54–8.59)	7.80 ± 0.32 (7.1–8.11)
IND	3.26	2.71 ± 0.27 (2.29–3.07)	2.88 ± 0.26 (2.57–3.26)
END	2.74	2.43 ± 0.30 (2.2–2.89)	2.70 ± 0.15 (2.49–2.94)
ED	3.01	2.91 ± 0.43 (2.52–3.64)	2.86 ± 0.15 (2.64–3.01)
TD	1.70	1.63 ± 0.26 (1.36–1.97)	1.67 ± 0.18 (1.42–2.01)
HAL	6.04	5.37 ± 0.73 (4.37–6.25)	5.67 ± 0.49 (4.66–6.13)
THL	13.41	11.99 ± 0.79 (10.98–13.29)	12.77 ± 0.66 (11.65–13.45)
TBL	12.67	12.21 ± 1.07 (10.31–13.38)	12.81 ± 0.50 (12.13–13.60)
FL	10.22	9.46 ± 1.65 (6.66–11.71)	10.25 ± 0.43 (9.66–10.57)

(Figs 2B, 3B); (9) tympanic annulus well visible only in its lower portion, supratympanic fold absent; (10) discs of the fingers well developed (Fig. 2C).

Ameerega munduruku sp. n. can be distinguished from *A. berohoka*, *A. flavopicta* and *A. braccata* by its black immaculate dorsum (irregular cream spots in *A. berohoka*, bright yellow spots in *A. flavopicta,* golden, yellow or white spots in *A. braccata*). The new species is distinguishable from *A. berohoka*, *A. picta, A. hahneli, A. trivittata* and *A. pulchripecta* by having a white venter with black vermicular blotches (black and bluish marbling in *A. berohoka* and *A. pulchripecta,* bluish with dark gular region in *A. hahneli,* dark gular region *A. picta*, entirely black in *A. trivittata*). *Ameerega munduruku* sp. n. (mean SVL 26.03 mm) is larger than *A. berohoka* (mean SVL 21.9 mm) and *A. braccata* (mean SVL 21.3 mm) and smaller than *A. trivittata* (mean SVL 35.0 mm) and *A. silverstonei* (mean 42.8 mm). Furthermore, *A. munduruku* sp. n. is distinguished from *A. picta* by its smaller hands (HAL/SVL = 0.21 vs. 0.3). The ventral thigh coloration of the new taxon described herein (white with black vermicular blotches) differs from that of *A. berohoka* (pale immaculate), and its slightly granular dorsal skin distinguishes it from *A. macero* (strongly granular dorsal skin). Moreover, *A. munduruku* sp. n. differs from *A. picta* and *A. trivittata* by the presence of a complete cream labial stripe (incomplete).

Ameerega munduruku sp. n. differs form some species of the genus by the combination of: (1) black immaculate dorsum (dark greyish brown in *A. boliviana*, and spotted or striped patterns in *A. bassleri*, *A. berohoka*, *A. boeh-*

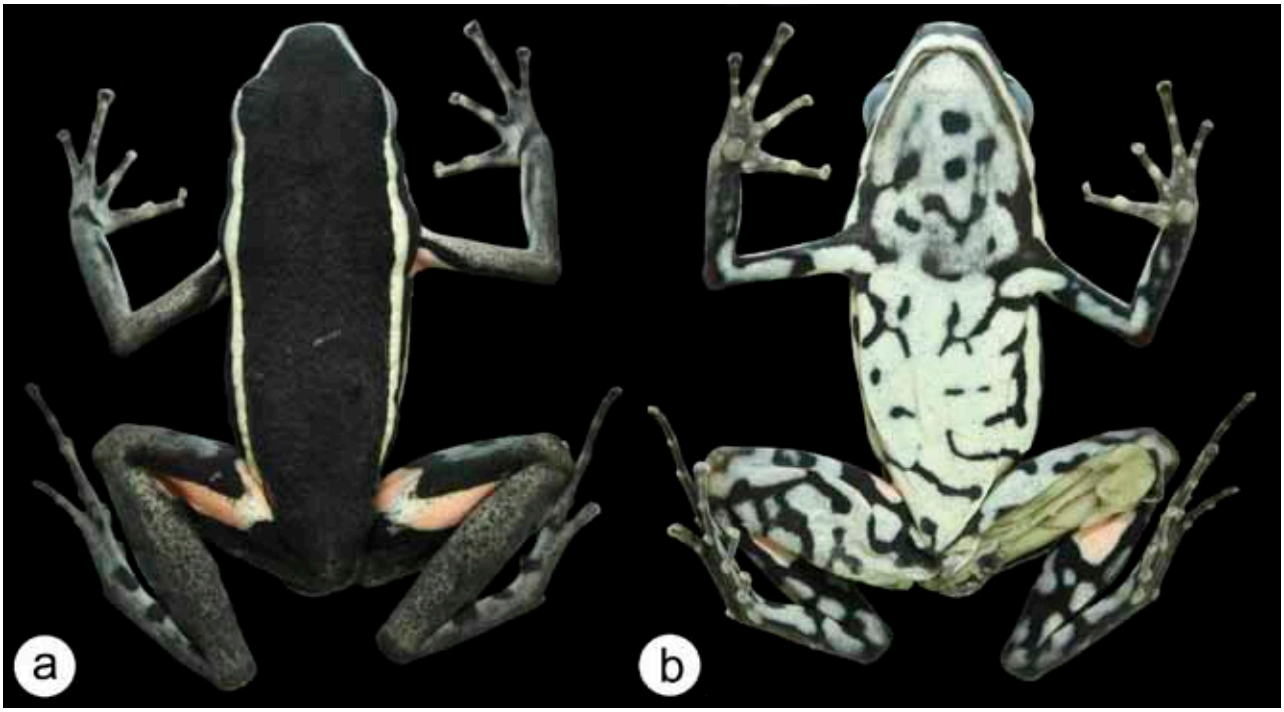

Figure 1. Holotype of *Ameerega munduruku* sp. n.(ZUFMS-AMP 03762) in (a) dorsal and (b) ventral views.

mei, *A. braccata*, *A. flavopicta*, *A. pepperi*, *A. silverstonei*, *A. yoshina* and *A. yungicola*); (2) ventral region white with black vermicular blotches in life (blue, bluish or bluish white with black spots in *A. altamazonica*, *A. cainarachi*, *A. hahneli*, *A. ignipedis*, *A. macero* and *A. petersi*, and yellow in *A. rubriventris*); and (3) an orange stripe in the axillar region (yellow in *A. andina*, *A. hahneli* and *A. ingeri*).

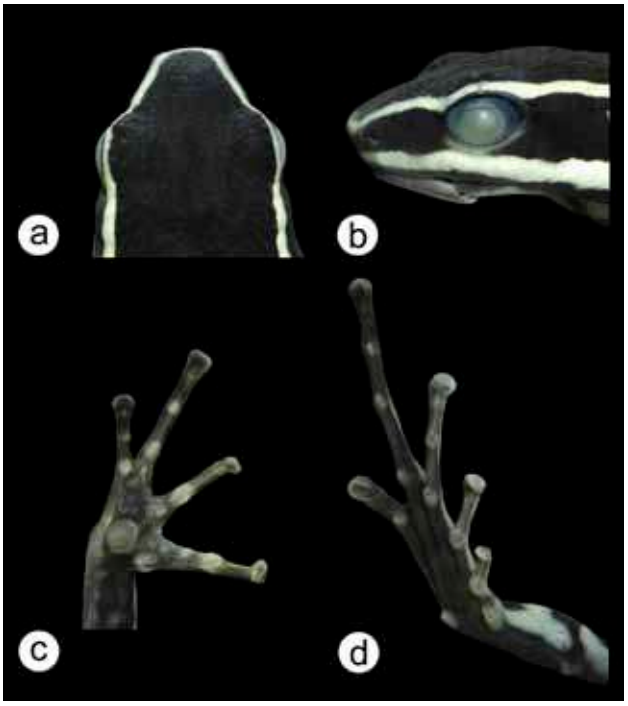

Figure 2. Holotype of *Ameerega munduruku* sp. n. (ZUFMS-AMP 03762): (a) head in dorsal and (b) lateral views, (c) hand, and (d) foot.

Description of holotype: Adult male, SVL 26.0 mm (Fig. 1). Dorsal skin of body granular and dorsal surface of hind limbs slightly granular, dorsal skin of head, forelimbs and ventral surfaces smooth (Fig. 1A). Dorsal surface black (Fig. 1A). In life, a cream dorsolateral stripe (faded in preservative) runs from the tip of the snout, passing over the eyelid towards the inguinal region, and a cream (faded in preservative) labial stripe extends from the tip of the upper jaw to the fore limb (Fig. 1A). Flank sectioned by a black upper stripe with the lower region white with black vermicular blotches extending to the ventral region of the body and onto the limbs (Figs 1A–B). Dorsal face of limbs brown with a few black vermicular blotches on the hands. Gular region with black spots (Fig. 1B). In life, an orange spot (faded in preservative) is present in the axillar region. An orange stripe (faded in preservative) extends from the inguinal region to the dorsal face of the thigh. Another orange spot (faded in preservative) is present in the concealed area of the tibia. In life, iris metallic golden (grey in preservative). Tongue with a large base, narrowing in the anterior portion, with the tip more acuminated. Premaxillary and maxillary teeth absent. Vocal slits present. Snout protruding in lateral view (Fig. 2B), truncate in ventral and dorsal views (Fig. 2A). Nares situated and directed posterolaterally at the tip of the snout; nares visible in frontal and in ventral views, but not in dorsal view. Canthus rostralis sloped, slightly concave; loreal region nearly vertical. Eye

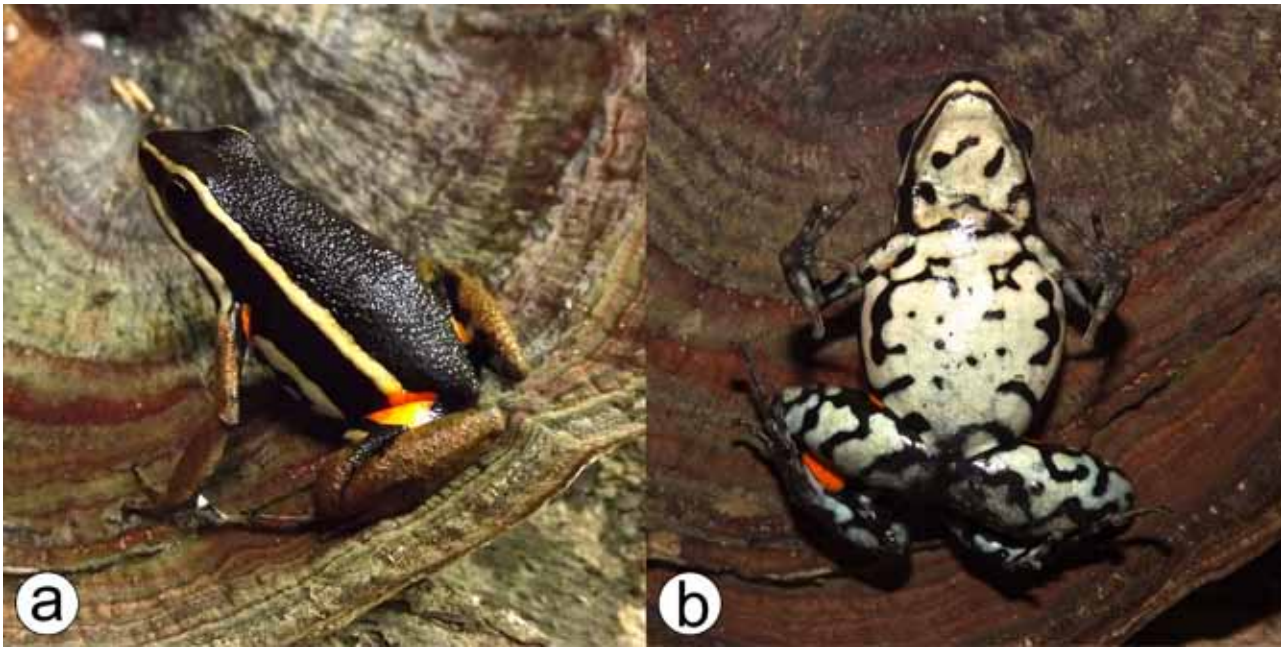

Figure 3. Individual of *Ameerega munduruku* sp. n. in life from the type locality (non-collected specimen) in (a) dorsal and (b) ventral views.

large and prominent with a diameter of 15% of the SVL; pupil rounded, horizontally elliptical. Tympanum circular, posterodorsally indistinct, tympanic annulus well visible in lower portion; diameter less than 56% of ED; supratympanic fold absent. Hand length 23% of SVL (Fig. 2C). Relative lengths of adpressed fingers: IV < II < III = I (Fig. 2C). Finger discs moderately expanded (Fig. 2C). A large, oval outer metacarpal tubercle on median base of palm; a smaller inner metacarpal tubercle on base of finger I; two well-developed subarticular tubercles on fingers III and IV, one prominent and well-developed subarticular tubercle on fingers I and II, and one intercalar tubercle on finger I (Fig. 2C). Tibia length 49% of SVL. Relative lengths of adpressed toes: I < II < V < III < IV (Fig. 2D); outer metatarsal tubercle circular and well developed; inner metatarsal tubercle ovoid and larger than outer one (Fig. 2D). One subarticular tubercle on toes I and II each, two subarticular tubercles on toes III and V each, and three subarticular tubercles on toe IV (Fig. 2D). Hands and feet lacking lateral fringes and webbing. Tarsal tubercles absent.

Variation: The paratypes resemble the holotype in their general morphology save for females not having vocal slits. Measurements of the type specimens are provided in Table 1.

Advertisement call: The advertisement call (Fig. 4) of *Ameerega munduruku* sp. n. consists of a single harmonic note with a duration of 0.113–0.116 s (mean 0.136 ± 0.012 s) and an intercall-interval of 0.096–0.639 s (mean 0.132 ± 0.093 s). The dominant frequency ranges from 3,445.3 to 3,617.6 Hz (mean 3,591.5 ± 62.7). The single harmonic note of the advertisement call of the new species distinguishes it from *A. berohoka*, *A. braccata*, and *A. flavopicta*, which all have pulsed notes, and from *A. boehmei* that has indistinctly pulsed notes (Lötters et al. 2009, Martins & Giaretta 2012, Andrade et al. 2014). The dominant frequency of the advertisement call of the new species is distinct from that of *A. altamazonica* (4,300.0 to 5,140.0 Hz), *A. berohoka* (3,919.0 to 4,478.9 Hz), *A. boehmei* (2,650.0 to 3,220.0 Hz), *A. braccata* (3,740.0 to 4,210.0 Hz) and *A. picta* (3,400.0 to 4,300.0 Hz) (Haddad & Martins, 1994, Twomey & Brown 2008, Lötters et al. 2009, Andrade et al. 2014). The call duration (i.e., the single harmonic note) in *A. munduruku* sp. n. differs from that of *A. braccata* (0.065 s), *A. hahneli* (0.015 s) and *A. picta* (0.05 s) (Haddad & Martins 1994, Twomey & Brown 2008, Lötters et al. 2009).

Molecular genetics: Sequences of the mitochondrial 16S ribosomal RNA gene of two paratypes (UFBA 14252, ZUFMS-AMP 03752) of *Ameerega munduruku* sp. n. have been deposited at GenBank (KX666099, KX666098). Average sequence divergences between the new species and congeners range from 3.0 to 10.2% (Supplementary document 2). Considering those *Ameerega* species for which genetic information is available, *A. munduruku* sp. n. is most similar to *A. flavopicta*.

Life History: We found specimens of *Ameerega munduruku* sp. n. in Mato Grosso Seasonal Forest and Madeira-Tapajós Forest of the Amazon forest domain. Individuals inhabited rock outcrops near water bodies in glades within forested areas, using rocks on the floor as shelter. Additional individuals were observed inside the forest habitat. The new species is apparently common in the area, and is easily found on fallen trunks, in leaf litter and on rocks. Males were calling at 09:30 h perched on the herbaceous vegetation, ~ 30 cm above the ground. We found one male (not collected) carrying nine tadpoles on its dorsum during the wet season (November 2016). *Allobates tapajos* and *Adenomera* sp. were found sympatrically with *A. munduruku* sp. n.

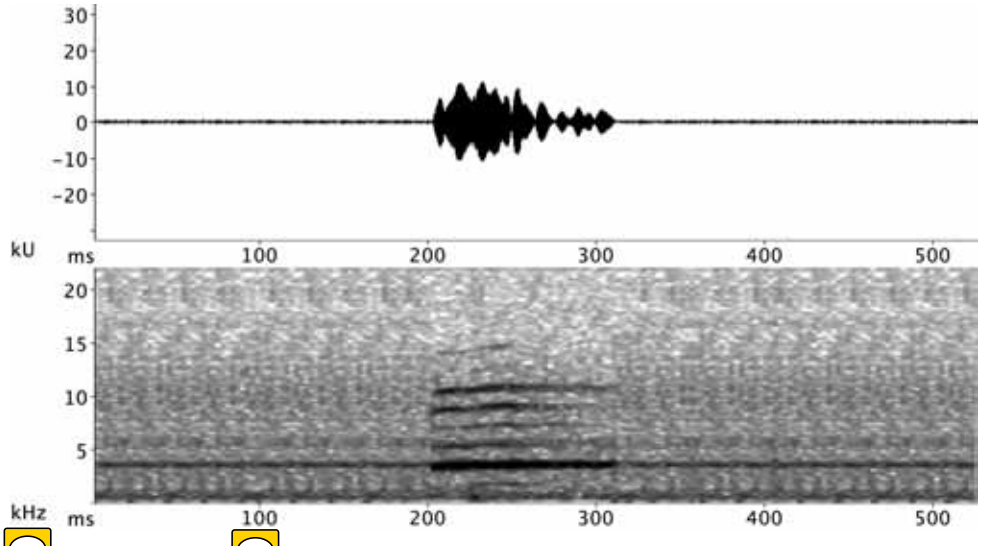

Figure 4. An (a) oscillogram and an (b) audiospectrogram of the advertisement call of a male paratype of *Ameerega munduruku* sp. n. (ZUFMS-AMP 05143) from the type locality (air temperature 29°C; relative humidity 80%).

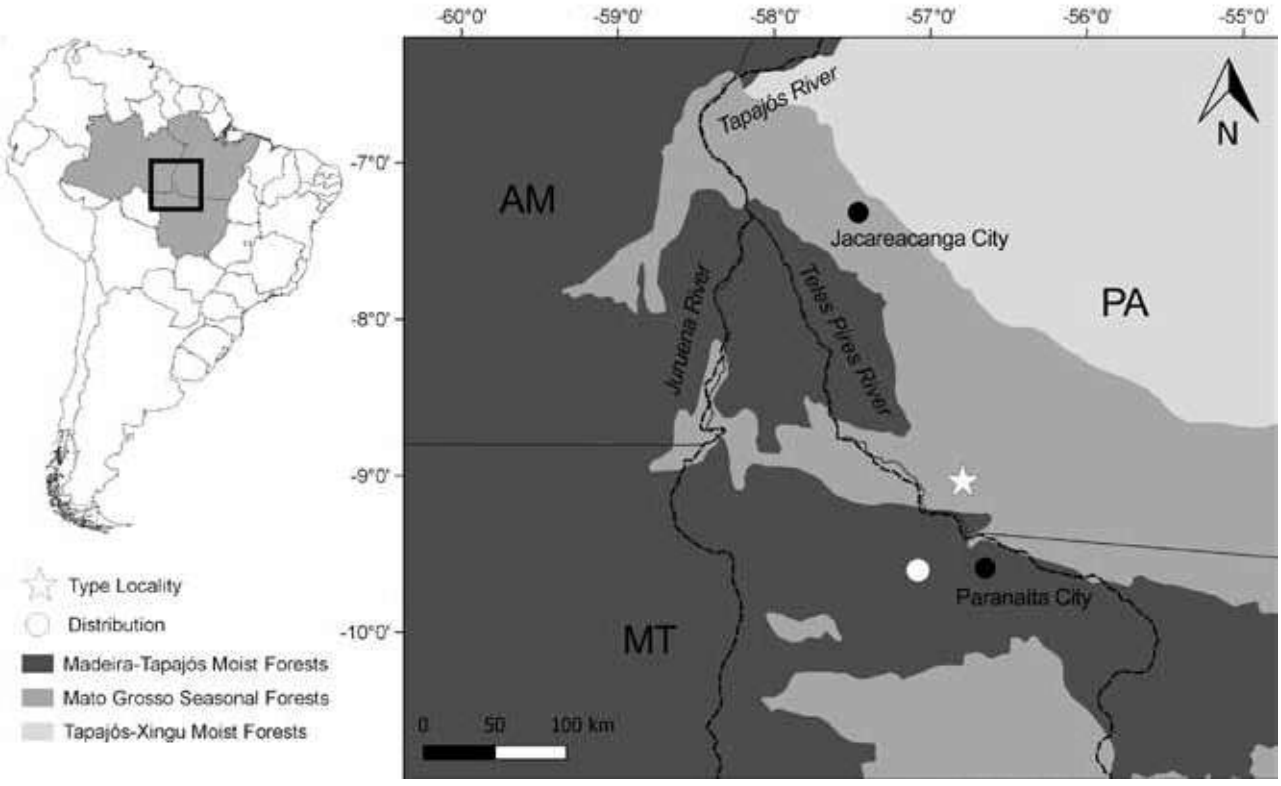

Figure 5. Known geographic range of *Ameerega munduruku* sp. n. in Amazonian Brazil. Abbreviations: AM = Amazonas State, MT = Mato Grosso State, PA = Pará State.

Distribution: The new species is only known from the type locality (Jacareacanga Municipality, state of Pará) and one site ~ 70 km away on the other side of the Teles Pires River (Paranaíta Municipality, Mato Grosso State) (Fig. 5).

Etymology: The specific epithet *munduruku* is a noun in apposition referring to the Munduruku ethnic group, which inhabits the southwestern parts of the state of Pará and the northern region of the state of Mato Grosso, Brazil.

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Appendix 1

Material examined in addition to the new species.

Ameerega berohoka: Rio do Peixe, Rio Negro Municipality, Mato Grosso do Sul State, Brazil: ZUFMS-AMP 03763–03786. *Ameerega braccata*: Manso, Chapada dos Guimarães Municipality, Mato Grosso State, Brazil: CZV-UFMT 1478, 1479. *Ameerega flavopicta*:

Serra da Canastra, São Roque de Minas Municipality, Minas Gerais State, Brazil: MZUFV 4293, 10325, 10489. *Ameerega hahneli*: Primavera Municipality, Pará State, Brazil: CZV–UFMT 16063, 16068, 16071. *Ameerega macero*: CFBH 26637 Reserva Extrativista Riozinho da Liberdade, Cruzeiro do Sul, Acre. *Ameerega picta*: Estância Mimosa Ecoturismo, Bonito Municipality, Mato Grosso do Sul State, Brazil: ZUFMS-AMP 03787–03808. *Ameerega trivittata*: Rorainópolis municipality, Roraima state, Brazil: MZUFV 12030–12032, 12657, 12658.

Acronyms: CFBH: Celio F. B. Haddad Collection, Universidade Estadual Paulista, Rio Claro/SP, Brazil; CZV-UFMT: Coleção Zoológica de Vertebrados da Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil; MZUFV: Museu de Zoologia João Moojen, Viçosa, MG, Brazil; UFBA: Museu de Zoologia, Universidade Federal da Bahia, Salvador, BA, Brazil; ZUFMS-AMP: Coleção Zoológica da Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.

Supplementary material

Supplementary document 1. List of sequences from GenBank used in this study (16S rRNA).

Supplementary document 2. Uncorrected p-distances for a 318-bp aligned sequence of the 16S rRNA gene of the new species and 186 samples of other *Ameerega* species taken from GenBank (see Supplementary document 1).