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A new nurse frog of *Allobates masniger-nidicola* complex (Anura, Aromobatidae) from the east bank of Tapajós River, eastern Amazonia

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Abstract

The small nurse frogs of the genus *Allobates* (Anura, Aromobatidae) represent one of the most challenging taxonomic issues of the Neotropics. During several amphibian surveys in the Middle Tapajós River region, state of Pará, Brazil, we collected phenotypic, ecological, and molecular data on species of this genus, leading to the identification of a new species included in the *Allobates masniger-nidicola* complex. The new species is characterized by a large body size (snout-vent length 19.2–21.7 mm in males and 19.3–22.0 mm in females), finger III not swollen in adult males; cryptic external coloration, with dorsum uniformly ochre; a dark brown lateral stripe and a pale cream ventrolateral stripe; limbs ranging from ochre to orange; throat and chest violaceous in males and yellowish in females. The advertisement call is usually arranged in bouts of four closely spaced notes, which we term 4-pulsed units of repetition (UR), 0.317 s long on average, followed by silent intervals, and an average dominant frequency of 4.163 kHz. The new species also has exotrophic tadpoles with a unique fin morphology, which begins after the body-tail insertion and is deeper posteriorly to half of the caudal length. Sequencing of the *16S* and *COI* regions of the mitochondrial DNA show a genetic *p*-distance of approximately 6–10% compared to closely related congeners. We discuss the biogeography of the new species based on phylogenetic relationships of the species within the *Allobates masniger-nidicola* complex and the allopatric geographic distribution in relation to sister taxa. Functional characteristics and geographic restrictions make this species particularly sensitive to the increasing human impact in eastern Amazonia.

Key words: Allopatry, Brazil, bioacoustics, conservation, cryptic diversity, mtDNA, taxonomy, exotrophic tadpole

Resumo

As pequenas rãs cuidadoras do gênero *Allobates* (Anura, Aromobatidae) representam uma das questões taxonômicas mais desafiadoras do Neotrópico. Durante vários levantamentos de anfíbios na região do Médio Rio Tapajós, Pará, Brasil, coletamos dados fenotípicos, ecológicos e moleculares de espécies desse gênero, levando à identificação de uma nova espécies incluída no complexo *Allobates masniger-nidicola*. A nova espécie é caracterizada por um grande tamanho corporal (comprimento rostro-cloacal de 19.2 a 21.7 mm nos machos e 19.3 a 22.0 mm nas fêmeas), dedo III não inchado nos machos adultos; coloração externa críptica, com o dorso uniformemente ocre; uma faixa lateral marrom-escura e uma faixa ventrolateral creme; membros variando de ocre a laranja; garganta e peito violáceos nos machos e amarelados nas fêmeas. O canto de anúncio é geralmente organizado em sessões de quatro notas espaçadas, que chamamos de unidades de repetição de 4 pulsos (UR), com média de 0.317 s, seguidos por intervalos silenciosos e uma frequência dominante média de 4,163 kHz. A nova espécie também possui girinos exotróficos com uma morfologia única da nadadeira, que começa após a inserção corpo-cauda mas é mais alta somente após a metade do comprimento caudal. O sequenciamento das regiões *16S* e *COI* do DNA mitocondrial mostra uma distância genética *p* de aproximadamente 6 a 10% comparado a congêneres proximamente relacionados. Discutimos sobre a biogeografia das novas espécies com base nas relações filogenéticas das espécies dentro do complexo *Allobates masniger-nidicola* e a distribuição geográfica alopátrica em relação aos táxons irmãos. Características funcionais e restrições geográficas tornam essa espécie particularmente sensível ao crescente impacto humano na Amazônia oriental.

Palavras-chave: Alopatria, Brazil, bioacústica, conservação, diversidade críptica, DNA mitocondrial, taxonomia, girino exotrófico

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Introduction

The genus *Allobates* (family Aromobatidae) comprises small terrestrial frogs abundant in the leaf litter of neotropical forests, from Central America to the Amazon and Atlantic Forest, also occurring in gallery forests of open habitats (Grant *et al.* 2006, 2017; Frost 2018). The genus includes 54 species, over 60% described in the last two decades (Frost 2018), but several other taxa remain undescribed (Grant *et al.* 2017; Melo-Sampaio *et al.* 2018). In a recent revision of Aromobatidae, Grant *et al.* (2017) recognized four main sub-groups within *Allobates*: (1) cryptically colored (cryptic) species from the Amazon (22-chromosome group), (2) cryptic species west of the Andes (trans-Andean group), (3) cryptic species from the Atlantic Forest (Atlantic Forest group), and (4) brightly colored Amazonian species (*Allobates femoralis* group). However, several species remained unassigned to any group.

The high degree of morphological similarity among *Allobates* species, combined with high intraspecific polymorphism, has led to a confusing taxonomic history that has been gradually clarified (Simões *et al.* 2013b). The recent elucidation of the taxonomy of this genus results from an integrative approach in taxonomic studies (e.g., Simões *et al.* 2013a, b; Lima *et al.* 2014, 2015; Simões 2016). Under this approach, taxonomic boundaries are based on integrative analyses from distinct evolutionary sources, such as morphological, ecological, behavioral, bioacoustic, ontogenetic, physiological, and molecular data (Padial *et al.* 2010; Ortega-Andrade *et al.* 2015).

In addition to this integrative approach, the continuous exploration of Amazonian areas, some with difficult access, has increased our knowledge of *Allobates*, as this biome still harbors significant knowledge gaps regarding biodiversity levels (Azevedo-Ramos & Gallati 2001; Oliveira *et al.* 2016). For example, recent expeditions to the Middle Tapajós River region, eastern Amazon basin, led to a recent description of two new *Allobates* species (Lima *et al.*, 2014, 2015). With more field surveys and data collection, other species from Madeira, Purus (Simões *et al.* 2010, 2013a; Melo-Sampaio *et al.* 2013; Simões *et al.* 2016; Melo-Sampaio *et al.* 2018), and Japurá River basins were described (Simões *et al.* 2018).

Based on extensive herpetofauna surveys in the Middle Tapajós River region, state of Pará, Brazil, we obtained phenotypic, ecological, and molecular data for different taxa of the genus *Allobates*, which enabled us to recognize and describe here a new species closely related to *A. masniger* (Morales, 2002) and *A. nidicola* (Caldwell & Lima, 2003).

Material and methods

We collected specimens during field expeditions to the Middle Tapajós River region from 2012 to 2018. The individuals were euthanized with a topical solution of 10% xylocaine, fixed with 10% formalin, and preserved in 70% ethanol. Before fixing and preserving specimens, we removed samples of liver and muscle tissue and stored them in absolute ethanol. Voucher specimens were deposited in the Collection of Amphibians and Reptiles (INPA-H) of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, state of Amazonas, Brazil (accession numbers INPA-H 40305, 40307, 40308, 40320, 40324, 40474–40489).

We identified the new species using an integrative approach, comprising morphological, ecological, bioacoustic, and molecular data. To describe the coloration patterns in living individuals, we used photographs taken at the field laboratory and field notes. Morphological terminology followed Grant *et al.* (2017) and measurements followed Lima *et al.* (2007). Sex was determined by calling activity or the presence of vocal slits. We took the following morphological measurements of adults using a caliper (to the nearest 0.1 mm): snout—vent length (SVL); forearm length, from distal edge of hand to the outer edge of flexed elbow (FA); hand length, from distal edge of hand to the tip of finger III (HA); tibia length, from proximal edge of flexed knee to heel (TL); foot length, from proximal edge of inner metatarsal tubercle to the tip of toe IV (FL); head width at level of angle of jaw (HW); head length from angle of jaw to tip of snout (HL); eye diameter (ED); eye–nostril distance (EN); internarial distance (IN); snout length, from proximal edge of eye to tip of snout (SL); interorbital distance (IO); tympanum diameter (TD); width of finger III disc (WFD); width of toe disc IV (WTD); thigh length (THL); and first finger length (1Fil). Fingers and toes were numbered preaxially to postaxially from I–IV and I–V, respectively.

Aiming to detect differences in body proportions regarding adult sexual dimorphism, we included the morphometric data SVL and 15 morphometric ratios (measurement/SVL) in a Principal Component Analysis (PCA) and visually checked for overlapping in the multivariate space. We also used the same process to detect morphometric differences between the new species and its sister taxa *A. masniger* and *A. nidicola*, considering the SVL and 13 morphometric ratios. We used a Multivariate Analysis of Variance (MANOVA) to test for significant differences on PCA scores, with sex or species as the factor and the first three Principal Components (PCs) as dependent variables. These analyses were carried out in the R platform (R Core Team 2018).

We used 12 specimens for tadpole description, collected in gelatinous clutches at the municipality of Trairão on 18 February 2018 (lot INPA-H 40487). These tadpoles were raised to three developmental stages (following the categories described by Gosner 1960), euthanized in a diluted solution of benzocaine, and preserved in 10% formalin. Morphological terminology, measurements, and diagnostic characters followed Altig & McDiarmid (1999). We took the following morphological measurements of tadpoles (to the nearest 0.01 mm) under a stereomicroscope: total length, from tip of snout to tip of tail (TL); body length, from tip of snout to body-tail insertion (BL); tail length, from body-tail insertion to tip of tail (TAL); body width at level of spiracle (BW); body height at level of spiracle (BH); head width at level of eyes (HWLE); maximum tail height (MTH); tail muscle maximum height (TMH); tail muscle maximum width (TMW); interorbital distance (IOD); internarial distance (IND); eye-naris distance (END); naris-snout distance (NSD); eye diameter (ED); vent tube length (VTL), spiracle tube length (STL), oral disc width (ODW); oral disc length (OD); posterior labium width (PL); anterior labium width (AL); upper jaw sheath length (UJ); length of first anterior tooth row (A1); length of second anterior tooth row (A2); length of median gap in second anterior tooth row 2 (GAP); marginal papilla length (MP); length of lateral process in upper jaw sheath (LP); lower jaw sheath length (LJ); length of first posterior tooth row (P1); length of second posterior tooth row (P2); length of third posterior tooth row (P3); and mouth length (M).

We recorded advertisement calls of nine adult males from a distance of 1–2 m from the emitter, with a digital Zoom H4n Handy Recorder (Zoom Corporation, Tokyo, Japan) and a Tascam DR-40 Audio Recorder (TASCAM Corporation, California, USA), equipped with internal microphones, and stored them as uncompressed WAV files. We analyzed spectral and temporal parameters of calls in Raven Pro 1.5 (Cornell Laboratory of Ornithology, USA), at a sampling frequency of 44 kHz and 16-bit resolution. We measured the following quantitative parameters, considering standardization within Dendrobatoidea (Santos *et al.* 2014) and anuran taxonomy (Köhler *et al.* 2017): call duration (s), inter-call interval (s), note duration (s), inter-note duration (s), note emission rate (notes/s), and dominant frequency (kHz). The data were summarized as mean values ± standard deviation and range (min–max) and compared to *Allobates* calls obtained in our sampling or described in the literature (e.g., Simões *et al.* 2010, 2013a, b; Lima *et al.* 2014, 2015; Melo-Sampaio *et al.* 2013; Simões *et al.* 2016; Melo-Sampaio *et al.* 2018).

For molecular analyses, we extracted total genomic DNA from collected tissues using a commercial kit (Wizard, Promega Corp., Madison, WI). We amplified two mitochondrial genes (*16S* and *COI*), considered standard markers for amphibians (Vences *et al.* 2012; Lyra *et al.* 2017), and *Allobates* taxonomy (see Simões *et al.* 2013b; Lima *et al.* 2014, 2015; Simões 2016), via Polymerase Chain Reaction (PCR), using primers 16Sar/16Sbr (Palumbi *et al.* 1991) for *16S* gene and CHmR4/CHmL4 (Che *et al.* 2012) for *COI* gene. PCR products were purified with ExoSAP-IT (USB Corporation, Ohio, USA) and sequenced with the Big Dye Terminator Kit (Applied Biosystems, Waltham, USA) in an automated sequencer ABI PRISM 3500 (Applied Biosystems, Waltham, USA). Newly generated sequences were deposited in GenBank (Appendix 3).

We used the MEGA 6.06 software (Tamura *et al.* 2013) to align the sequences through the MUSCLE algorithm and calculate interspecific uncorrected pairwise genetic distances. To assess the relationships of the new species with described and undescribed taxa within *Allobates*, we downloaded additional sequences from GenBank, preferably from type localities. We concatenated the mtDNA dataset (*16S*+*COI*; 1199 bp) and selected substitution models and partition schemes with Partition-Finder 2 (Lanfear *et al.* 2016) under the Akaike Information Criterion. Bayesian phylogenetic inference was performed in MrBayes 3.2.2 (Ronquist *et al.* 2012), under two independent runs of 10^7 generations and four Markov chain Monte Carlo (MCMC) chains, with the best partition schemes of GTR+I+G for *16S* and first codon position of *COI*, and GTR+G for second and third codon positions of *COI*. We assessed the convergence of posterior distributions with TRACER 1.6 (Rambaut *et al.* 2018).

Results

Phylogenetic relationships. The tree topology of our mtDNA phylogenetic analyses corroborates with high nodal support (Bayesian posterior probability = 1) the genetic distinction of a monophyletic new species compared to 32

Allobates species or species complexes (Fig. 1). The new species is closely related to *A. masniger* and *A. nidicola*. Genetic distances between the new species samples and other *Allobates* range from 6% to 17% in the *16S* mitochondrial gene and from 15% to 22% in the *COI* mitochondrial gene. The minimum genetic distances between the new species and the congeners is 6–10% in the *16S* gene and occurs in relation to the *A. masniger* and *A. nidicola*. The phylogenetic analyses recovered, as in Grant *et al.* 2017 (comparisons with their results in parentheses), some main sub-clades within *Allobates:* (1) brightly colored species (*Allobates femoralis* group, plus the species unassigned to groups *A. hodli* Simões, Lima, & Farias, 2010), (2) cryptically colored species from the Atlantic Forest (Atlantic Forest group), (3) cryptically colored species from the Amazonia (22-chromosome group, plus species unassigned to groups *A. fratisenescus* (Morales, 2002) and *A. kingsburyi* (Boulenger, 1918)), (4) cryptically colored species from west of Andes (trans-Andean group), (5) cryptically colored species from Guiana Shield Highlands (only *A. undulatus,* Myers & Donnelly 2001, unassigned to groups). Minor divergences between the recognition of main sub-clades within *Allobates* could be an artifact of a less comprehensive molecular scope in our study. Below we describe the new species related to *A. masniger* and *A. nidicola.*

Allobates nunciatus sp. nov.

(Figs. 2–7)
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Allobates cf. *crombiei* Moraes *et al.* 2016

Holotype. Adult male (INPA-H 40486, field number DT 3955, 21.4 mm SVL, Figs. 2–4) collected by D. Pavan on 15 December 2012 on the east bank of Middle Tapajós River, municipality of Itaituba (05°09'07.1" S 56°27'35.2" W, 96 m asl), state of Pará, Brazil.

Paratypes. Ten specimens, three adult males (INPA-H 40305, 40307, 40482), six adult females (INPA-H 40324, 40475, 40476, 40480, 40484, 40489), and one young female (INPA-H 40485) collected by D. Pavan, L.J.C.L. Moraes, T.F.D. Rodrigues, J. Gomes and L.F. Storti between July 2012 and October 2013 at east bank of Middle Tapajós River, municipalities of Itaituba and Trairão, Pará state, Brazil (Fig. 5). Of these, eight were collected in the west bank of Jamanxim River, inside the municipality of Itaituba (INPA-H 40305, 40307, 40475, 40480, 40485, 40489 at 5°13'49.1" S 56°55'32.7" W, 82 m asl; and INPA-H 40324, 40476 at 4°44'47.6" S 56°36'57.3" W, 92 m asl) and two in the east bank of this river, inside the municipality of Trairão (INPA-H 40482, 40484 at 5°06'12.6" S 56°26'35.5" W, 91 m asl). Another eight adult males and one female were collected by A.P. Lima in 16 January 2014 and 18 February 2018 in three additional localities at banks of Jamanxim River: inside the municipality of Itaituba (INPA-H 40320, 40474, 40477, 40478, 40481, 40483 at 06°16' S 55°39' W, 218 m asl; and INPA-H 40308, 40479 at 06°17'42.7" S 55°49'19.6" W, 221 m asl) and Trairão (INPA 40488 at 04°45'25.1" S 55°57'56.4" W, 167 m asl). We designate the adult female specimen INPA-H 40489 (Figs. 2–4) as the species allotype.

Etymology. The specific epithet '*nunciatus*' (Latin verb: announce, report; derived from 'nuntiatus'), refers to the great conspicuity of the loud advertisement call of the species, allowing it to be readily recognized by the human ear wherever it occurs.

Generic placement. The new species was allocated to the genus *Allobates* based on its external morphology similar to other species of the genus (see comparisons), behavior (diurnal activity), and molecular phylogenetic relationships.

Definition. A large species of cryptically-colored *Allobates*, which can be characterized by the following combination of characters: (1) mean SVL of adult males 20.8 mm (range 19.2–21.7 mm), mean SVL of adult females 20.9 mm (range 19.3–22.0 mm) (Table 1); (2) skin texture of dorsum mostly smooth, with some granules posteriorly; (3) finger III not swollen in adult males; (4) absence of lateral fringes and basal webbing on fingers; (5) paired dorsal digital scutes present; (6) fingers and toes discs expanded, larger in toes II, III, and IV; (7) basal webbing between toes II–III and III–IV; (8) finger II longer than or with a similar size as finger IV; (9) toe III length reaches or surpasses the middle of the central sub-articular tubercle of toe IV; (10) metatarsal fold absent; (11) tympanum conspicuous and small; (12) in life, background color of dorsum uniformly ochre; cream or pale brown dorsolateral stripe absent; dark brown lateral stripe from the tip of the snout to the cloacal region; short oblique lateral stripe diffuse, conspicuous pale cream ventrolateral stripe, which may be diffuse at the lower margin; cream paracloacal marks present; (13) in life, dorsal surface of arms orange; dorsal surface of legs ranging from ochre to orange; (14) in life, throat and chest violaceus in non-vocally active males, becoming darker when excited, and yellowish in females; abdomen pale violaceus in males and yellowish in females; (15) in life, iris golden with black vermiculations and pupil ring gold; (16) in preservative, dorsum pale brown; pale brown dorsolateral stripe weakly defined; dark brown lateral stripe from the tip of the snout to the cloacal region; short oblique lateral stripe diffuse, whitish ventrolateral stripe; cream paracloacal marks present (17) in preservative, dorsal surface of arms cream; dorsal surface of legs pale brown; (18) in preservative, throat, chest and abdomen greyish in males and cream in females; (19) advertisement call in general with a continuous emission of 4-pulsed units of repetition, 0.317 s long, on average, followed by silent intervals, and an average dominant frequency of 4.163 kHz; (20) reproductive cycle with the presence of exotrophic tadpoles carried to water bodies, with an unique fin morphology, which begins after the body-tail insertion and is deeper posteriorly to half of the caudal length.

Comparisons with other species. We limited our comparisons to Brazilian congeners (see Appendix 1 for specimens examined) as *Allobates nunciatus* **sp. nov.** has a geographical distribution range restricted to the Tapajós River Basin, eastern Amazonia, approximately 700 km from the nearest country border. We also detail the differences of the new species with respect to most closely related species, based on results of our phylogenetic analysis.

Allobates nunciatus **sp. nov.** occurs syntopically with other three nominal *Allobates* species: *A. femoralis* (Boulenger, 1884), *A. magnussoni* Lima, Simões & Kaefer, 2014, and *A. tapajos* Lima, Simões & Kaefer, 2015. From those species *A. nunciatus* **sp. nov.** can be distinguished as follows: from *A. femoralis*, by a cryptic external coloration in life and preservative, with color spectrum ranging between brown, ochre and cream (Fig. 6) (bright coloration, with white dorsolateral stripes on a black body, and bright red, orange, or yellow marks on thighs in *A. femoralis*); from *A. tapajos*, by a larger size (minimum SVL 19.2 mm in *A. nunciatus* **sp. nov.**, maximum SVL 16.9 mm in *A. tapajos*), a conspicuous pale ventrolateral stripe (absent in *A. tapajos*), and a uniformly colored dorsum, with no distinct contrasting brown markings (irregular brown blotches in *A. tapajos*); from *A. magnussoni*, by a conspicuous pale cream ventrolateral stripe (absent in *A. magnussoni*), a uniformly colored dorsum, with no distinct contrasting brown markings (brown hourglass-shaped marking in *A. magnussoni*), and a continuous well-marked brown lateral stripe (discontinuous brown lateral stripe in *A. magnussoni*).

TABLE 1. Morphometric measurements of adult specimens of *Allobates nunciatus* **sp. nov.** type series, from Middle Tapajós River region, state of Pará, Brazil. Values represent mean ± standard deviation, with range shown in parentheses. See text for measurement abbreviations.

Measurements (mm)	**Holotype (male)**	**Males (*n* = 11)**	**Females (*n* = 7)**
SVL	21.4	20.8 ± 0.8 (19.2–21.7)	20.9 ± 0.9 (19.3–22.0)
FA	5.0	4.9 ± 0.1 (4.5–5.0)	4.5 ± 0.4 (4.0–5.0)
HA	4.9	4.8 ± 0.2 (4.4–5.0)	4.9 ± 0.3 (4.6–5.5)
TL	9.8	9.8 ± 0.4 (8.8–10.4)	9.6 ± 0.3 (9.3–10.4)
FL	9.0	8.9 ± 0.5 (7.7–9.7)	8.8 ± 0.8 (7.6–10.0)
HW	7.1	6.8 ± 0.4 (5.8–7.5)	6.9 ± 0.4 (6.2–7.4)
HL	7.2	6.9 ± 0.4 (6.3–7.6)	6.9 ± 0.4 (6.4–7.6)
ED	2.6	2.7 ± 0.2 (2.4–2.9)	2.7 ± 0.2 (2.4–3.0)
EM	2.0	1.9 ± 0.1 (1.7–2.1)	1.9 ± 0.1 (1.8–2.0)
IN	2.8	2.6 ± 0.2 (2.3–3.0)	2.5 ± 0.2 (2.4–2.8)
SL	3.1	3.3 ± 0.1 (3.1–3.5)	3.3 ± 0.1 (3.0–3.5)
IO	4.0	4.1 ± 0.2 (3.8–4.4)	3.8 ± 0.3 (3.5–4.3)
TD	1.0	0.8 ± 0.1 (0.7–1.0)	0.8 ± 0.1 (0.7–1.0)
WFD	0.6	0.5 ± 0.1 (0.4–0.6)	0.4 ± 0.0 (0.4–0.5)
WTD	0.9	0.7 ± 0.1 (0.5–0.9)	0.6 ± 0.1 (0.5–0.7)
THL	10.0	9.9 ± 0.4 (9.0–10.3)	9.3 ± 0.5 (8.6–10.0)
1Fil	3.6	3.7 ± 0.3 (3.1–4.1)	3.4 ± 0.3 (3.1–4.0)
AL	5.0	5.1 ± 0.1 (5.0–5.3)	5.2 ± 0.3 (5.0–5.7)

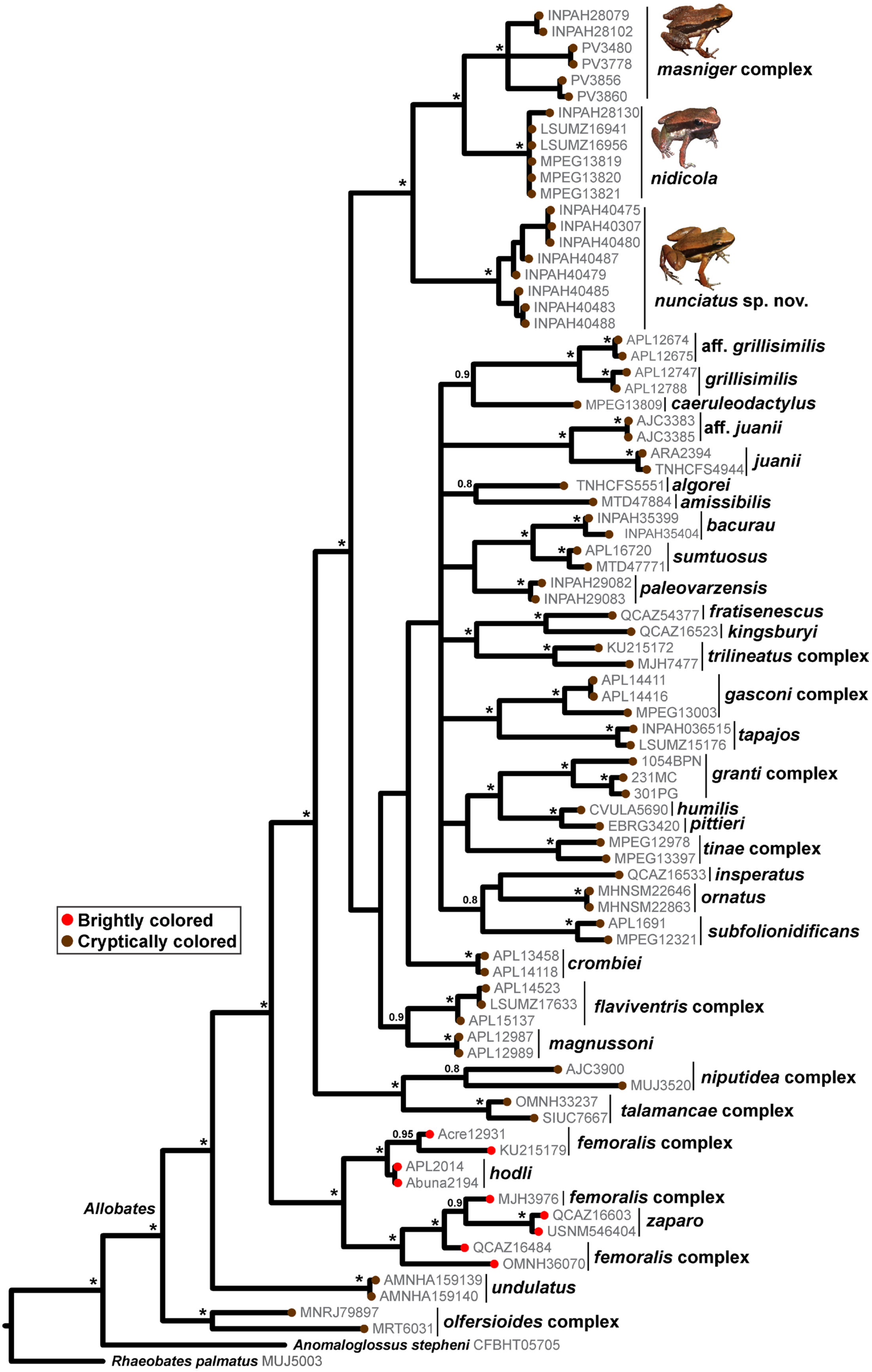

FIGURE 1. Bayesian phylogenetic tree of *Allobates* based on mtDNA (*16S*+*COI*) relationships, showing the close relationship of the new species *Allobates nunciatus* **sp. nov.** from Middle Tapajós River region, state of Pará, Brazil, with *Allobates masniger* and *Allobates nidicola*. Asterisks represent posterior probability = 1.0. Photographs by J. Cassimiro, J. Gomes, and A.P. Lima.

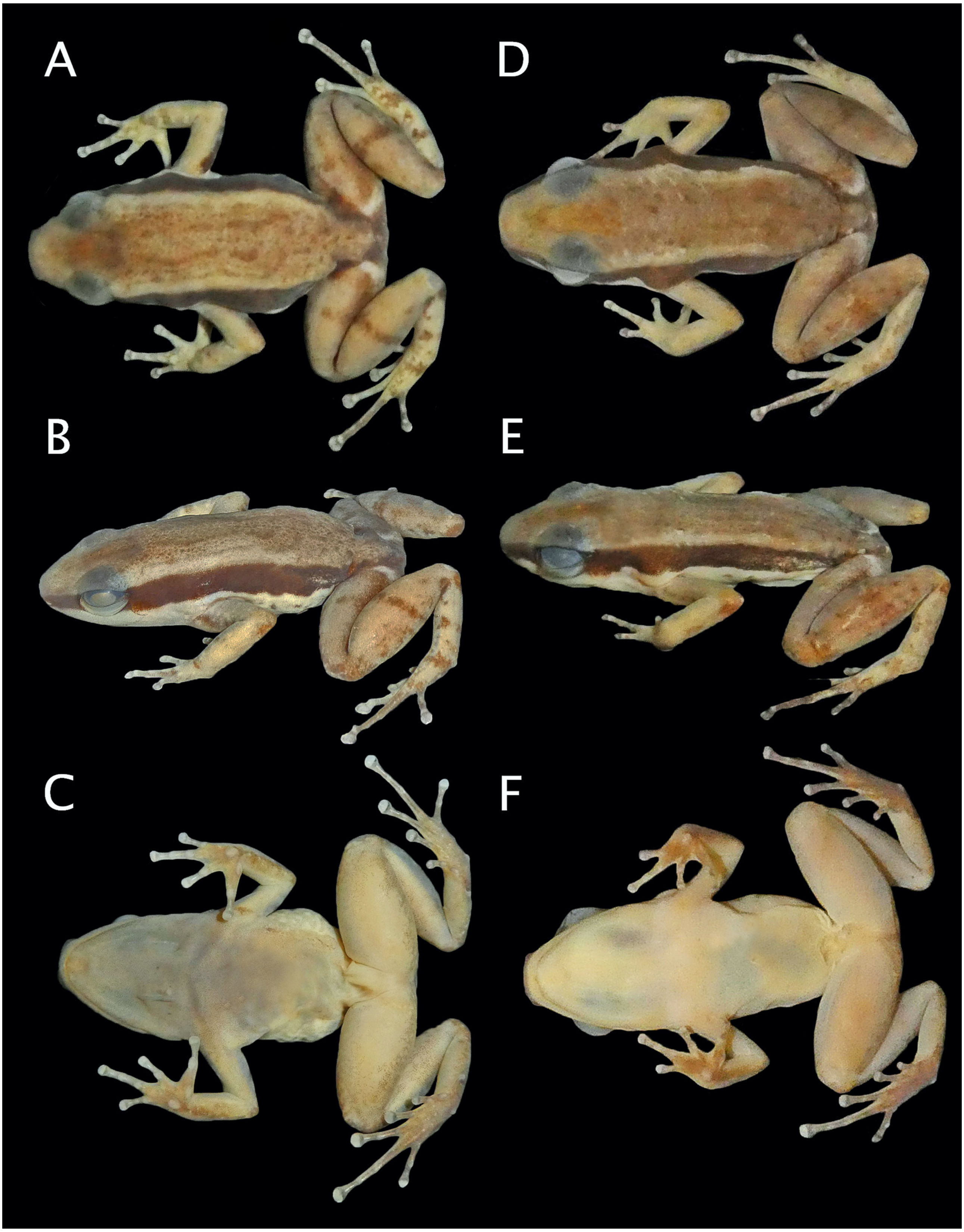

FIGURE 2. (A, B, C) Dorsal, lateral, and ventral views of the preserved adult male holotype of *Allobates nunciatus* **sp. nov.** (INPA-H 40486); (D, E, F) Dorsal, lateral, and ventral views of the preserved adult female paratype of *A. nunciatus* **sp. nov.** (INPA-H 40489). Not to scale. Photographs by J. Magnusson.

Twenty-two cryptically colored *Allobates* species are known to occur in Brazil: *A. bacurau* Simões, 2016, *A. brunneus* (Cope, 1887), *A. caeruleodactylus* (Lima & Caldwell, 2001), *A. carajas* Simões, Rojas & Lima 2019, *A. crombiei* (Morales, 2002), *A. conspicuus* (Morales, 2002), *A. flaviventris* Melo-Sampaio, Souza & Peloso, 2013, *A. fuscellus* (Morales, 2002), *A. gasconi* (Morales, 2002), *A. goianus* (Bokermann, 1975), *A. grillisimilis* Simões, Sturaro, Peloso & Lima, 2013, *A. juami* Simões, Gagliardi-Urrutia, Rojas-Runjaic & Castroviejo-Fisher, 2018, *A. masniger*, *A. marchesianus* (Melin, 1941), *A. nidicola*, *A. paleovarzensis* Lima, Caldwell, Biavati & Montanarin, 2010, *A. olfersioides* (Lutz, 1925), *A. subfolionidificans* (Lima, Sanchez & Souza, 2007), *A. sumtuosus* (Morales, 2002), *A. trilineatus* (Boulenger, 1884), *A. tinae* Melo-Sampaio, Oliveira & Prates, 2018, and *A. vanzolinius* (Morales, 2002). The body size of *A. nunciatus* **sp. nov.**, with minimum SVL 19.2 mm is larger than 13 of these species (maximum SVL in mm: 14.9 in *A. bacurau*, 16.0 in *A. grillisimilis* and *A. sumtuosus*, 16.8 in *A. caeruleodactylus,* 17.0 in *A. subfolionidificans* and *A. marchesianus*, 17.2 in *A. conspicuus* and *A. tinae*, 17.3 in *A. gasconi*, 17.4 in *A. goianus*, 18.0 in *A. trilineatus*, 18.5 in *A. juamii*, and 19.1 in *A. carajas*).

Besides the size, *A. nunciatus* **sp. nov.** is also distinguished by the absence of a pale cream to pale brown conspicuous dorsolateral stripe in living individuals (present in *A. bacurau*, *A. conspicuus*, *A. gasconi*, *A. goianus*, *A. grillisimilis*, *A. tinae*, *A. juamii*, and *A. trilineatus*), a dorsum with no distinct contrasting brown markings in life and preservative (brown dorsal hourglass-shaped markings in *A. carajas*, *A. gasconi*, and *A. goianus*), living males with white abdominal surfaces and violaceous throats (Fig. 7) (white abdomen and yellow throat in *A. sumtuosus*, grey abdomen and throat in *A. bacurau*, yellow abdomen and throat in *A. juamii* and *A. tinae*, yellow abdomen and pinkish throat in *A. carajas*, dark grey throat in *A. marchesianus,* white throat in *A. caeruleodactylus* and *A. subfolionidificans*), finger III not swollen in adult males (swollen in *A. gasconi* and *A. trilineatus*), and absence of blue dots on fingers of living individuals (present in *A. caeruleodactylus*).

FIGURE 3. Detail of ventral surface of hands of a male holotype (INPA-H 40486) (A) and female paratype of *Allobates nunciatus* **sp. nov.** (B) (INPA-H 40489). Scale bar = 2.0 mm. Photographs by J. Magnusson.

Allobates nunciatus **sp. nov.** is distinguished from similarly sized species (*A. brunneus*, *A. crombiei*, *A. flaviventris*, *A. fuscellus*, *A. olfersioides*, *A. paleovarzensis*, and *A. vanzolinius*) by a uniformly ochre dorsal coloration (uniformly brown in *A. fuscellus* and *A. vanzolinius*; brown hourglass-shaped markings in *A. brunneus*, *A. crombiei*, and *A. flaviventris*; brown with a darker brown marking on the head in *A. paleovarzensis*; and brown X-shaped markings in *A. olfersioides*), absence of a distinct pale cream to pale brown continuous dorsolateral line (distinct pale dorsolateral line present in *A. fuscellus*, *A. paleovarzensis*, and *A. vanzolinius*), white abdominal surfaces in living individuals (greenish-yellow abdomen in *A. brunneus*; yellow in *A. flaviventris,* males of *A. crombiei*, and females of *A. paleovarzensis*), violaceus throat in living males (yellowish throat in *A. crombiei* and *A. flaviventris* and white throat in *A. olfersioides*) and dorsal surface of thighs ranging from beige to orange in living individuals, similar to dorsum color (greyish dorsal surface of thighs in *A. crombiei*, different from its brown dorsum).

FIGURE 4. Detail of the ventral surface of feet of a male holotype (INPA-H 40486) (A) and female paratype of *Allobates nunciatus* **sp. nov.** (B) (INPA-H 40489). Scale bar = 3.0 mm. Arrows indicate basal webbing between toes III and IV. Photographs by J. Magnusson.

Two other similarly sized species are closely related and morphologically similar to *A. nunciatus* **sp. nov.**: *A. masniger* and *A. nidicola.* The new species differs from these by a combination of morphological, acoustic, molecular, reproductive, and distributional data. The external morphology of *A. nunciatus* **sp. nov.** differs from theirs by a paler coloration on dorsum of living adult males, ranging from ochre to beige, and ochre-orange limbs (dark brown dorsum and pale brown limbs in *A. masniger* and *A. nidicola*), a conspicuous pale cream ventrolateral stripe (inconspicuous or absent in *A. masniger* and *A. nidicola*), a violaceus throat in living males, which only becomes darker in vocally active individuals (dark grey throat in *A. masniger* and *A. nidicola* which does not change in color during non-reproductive period), a weaker sexual dimorphism in dorsal coloration of living specimens (adult males of *A. masniger* and *A. nidicola* are darker brown than females), finger II longer than or with a similar size to finger IV (finger II longer than finger IV in *A. nidicola*), and toe III length reaches or surpasses the middle of the central sub-articular tubercle of toe IV (toe III length only reaches the posterior margin of the same tubercle in *A. nidicola*).

In addition to these subtle morphological differences, *A. nunciatus* sp. nov differs from *A. masniger* and *A. nidicola* by a distinct advertisement call, which has a continuous emission of bouts of four closely spaced notes, which we term 4-pulsed units of repetition (UR) (single-pulsed UR in those species; see call section below), approximately 6–10% of genetic distance based on mtDNA genes *16S* and *COI* (see phylogenetic relationships section below), and exotrophic tadpoles carried to water bodies (endotrophic tadpoles developing in terrestrial nests, see tadpole section below). Furthermore, these three species are allopatrically distributed along banks of the Madeira and Tapajós rivers, some of the largest southern tributaries of the Amazon River (*A. nidicola* on the west bank of Madeira River, *A. masniger* in the Madeira-Tapajós interfluve and *A. nunciatus* **sp. nov.** on the east bank of Tapajós River; see geographical distribution below).

A segregation pattern between clusters and significant differences were detected when we compared body proportions of adult males (Pillai trace = 0.666, df = 216, $p < 0.001$) and females (Pillai trace = 0.924, df = 110, $p < 0.001$) of the new species with proportions of its sister taxa *A. masniger* and *A. nidicola* (Fig. 8, D–I). In the morphometric multivariate space, *A. nunciatus* **sp. nov.** males differed from *A. nidicola* males when we compared PC1 to PC2 and PC2 to PC3 (Fig. 8, D–F). Likewise, *A. nunciatus* **sp. nov.** females differed from *A. nidicola* (comparison between PC1 and PC2) and both sister taxa (when comparing PC1 and PC2 to PC3) (Fig. 8, G–I). The first three axis summarized 24.77%, 17.75%, and 15.54% of the variation in sister taxa for males and 24.3%, 19.45%, and 15.78% for females. The highest loading values of sister taxa variation regarding males came from snout-vent length (PC1) and the morphometric ratios of hand length (PC2), tibia length (PC1, PC2), head length (PC3), foot length (PC1), tympanum diameter (PC2), head width (PC3) and toe disc width (PC3). The highest loading values of sister taxa variation for females came from snout-vent length (PC1) and the morphometric ratios of hand length (PC2), head length (PC1), foot length (PC3), head width (PC1), finger disc width (PC2, PC3), and toe disc width (PC2, PC3). Despite these significant differences, highest influenced morphometric characters in loading values showed some degree of measurement overlap or very subtle dissimilarities, indicating that morphometric measurements and ratios used as diagnostic characters between sexes and sister species should be treated with caution. See Appendix 2 for complete loading values.

Furthermore, the advertisement call of *A. nunciatus* **sp. nov.** (with a continuous emission of mostly 4-pulsed units of repetition (UR), followed by silent intervals (Fig. 9, 10)) distinguishes this species from most Brazilian cryptically colored *Allobates* (Table 3), which have advertisement calls in series of trills (*A. bacurau*, *A. crombiei*, *A. gasconi*, *A. grillisimilis*, *A. marchesianus*, *A. paleovarzensis*, *A. juami*, *A. sumtuosus*, *A. olfersioides* from the state of Alagoas), double-pulsed UR or trills (*A. flaviventris*, *A. tapajos*), or single-pulsed UR (*A. brunneus*, *A. caeruleodactylus*, *A. goianus*, *A. marchesianus, A. magnussoni*, *A. subfolionidificans, A. trilineatus, A. tinae, A. olfersioides* from the states of Bahia and Rio de Janeiro; Caldwell *et al.* 2002; Carvalho *et al.* 2016; Forti *et al.* 2017; Grant & Rodriguez 2001; Kaefer & Lima 2012; Lima & Caldwell 2001; Lima *et al.* 2007, 2009, 2010, 2012, 2014, 2015; Melo-Sampaio *et al.* 2018; Simões 2016; Simões *et al.* 2013a, b, 2018; Tsuji-Nishikido *et al.* 2012). Despite the differences in call structure between the related species *A. masniger* and *A. nidicola*, *A. nunciatus* **sp. nov.** calls at a similar range and dominant frequency (Table 3), and they share an ascending frequency modulation of notes. In relation to Brazilian brightly colored *Allobates*, the call structure (emission of 4-pulsed UR) distinguishes *A. nunciatus* **sp. nov.** from the allopatrically distributed *A. hodli*, which emits 2-pulsed UR (Simões *et al.* 2010) and *A. myersi* (Pyburn, 1981), which emits 6- or 8-pulsed UR (Simões & Lima 2011), but not from the sympatric *A. femoralis*. However, the call of *A. nunciatus* **sp. nov.** is much faster (mean of 5.16 notes/s) and emitted at a higher dominant frequency of 4.16 ± 0.18 (3.84–4.44) kHz compared to syntopic *A. femoralis* calls, which has a mean rate of 4.19 notes/s and frequency of 3.21 ± 0.08 (3.10–3.33) kHz (Table 3). The three remaining Brazilian *Allobates* species (*A. conspicuus, A. fuscellus,* and *A. vanzolinius*) have no advertisement call description (Guerra *et al.* 2018).

Description of the holotype. Adult male (Figs. 2–4), 21.4 mm SVL. Dorsum mostly smooth, with two rows of small granules from the distal margin of eye to urostyle, granules more abundant in the distal region of dorsum; throat and abdomen smooth (Fig. 2). Head slightly longer than wide (HW: 98% of HL; HL: 33% of SVL); snout truncated in dorsal view and rounded in lateral view, slightly shorter than eye diameter; *canthus rostralis* indistinct; loreal region obtuse; tympanum distinct, tympanum diameter < half of eye diameter; eye diameter: 36% of HL; tongue longer than wide, not adhered to floor of mouth posteriorly; choanae relatively small, round; vomerine odontophores absent. Upper arm and forearm with approximately the same size; hand slightly shorter than upper arm; relative length of fingers IV = II < I < III; fingers with moderately expanded, rounded tips with distinct dorsal scutes; metacarpal tubercles present, inner metacarpal tubercles small and oval and outer metacarpal tubercles large and

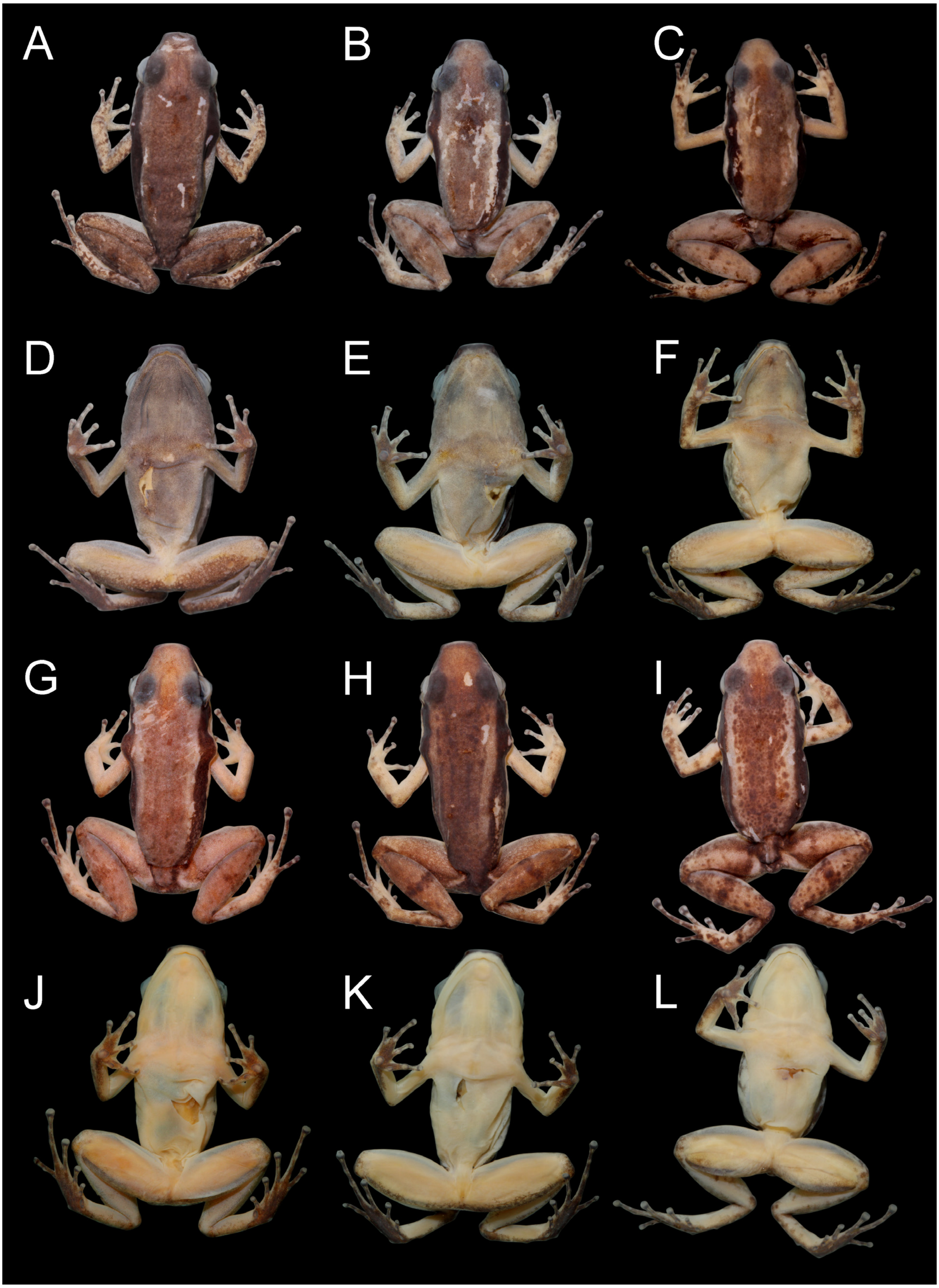

FIGURE 5. Dorsal and ventral views of preserved adult paratypes of *Allobates nunciatus* **sp. nov.** showing morphological variation. (A–F) Males, from left to right: INPA-H 40305 (19.2 mm SVL), INPA-H 40482 (20.0 mm SVL), and INPA-H 40307 (20.5 mm SVL); (G–L) females, from left to right: INPA-H 40489 (21.4 mm SVL), INPA-H 40480 (20.6 mm SVL), and INPA-H 40484 (19.3 mm SVL). Some specimens show preservation scratches on dorsum. Not to scale. Photographs by L. Moraes.

rounded; sub-articular tubercles present and rounded, one in fingers I, II, and IV and two in finger III; finger III not swollen; basal webbing and lateral fringes absent on fingers (Fig. 3). Thigh slightly longer than tibia, foot shorter than thigh and tibia; relative length of toes: I < II < V< III < IV; toe III surpassing the mid-level of the central subarticular tubercle of toe IV; basal webbing present between toes III and IV, and rudimentary webbing between toes II and III; toes II, III, and IV with expanded rounded tips; metatarsal tubercles present, oval, inner metatarsal tubercle slightly larger than outer ones; metatarsal fold absent; tarsal keel in form of a curved tubercle, not connected with metatarsal fold; sub-articular tubercles present, rounded; one sub-articular tubercle on toes I and II, two on toes III and V, and three on toe IV, with proximal and distal tubercles less evident (Fig. 4). For holotype measurements see Table 1.

FIGURE 6. Dorsolateral views of live specimens of *Allobates nunciatus* **sp. nov.**, including one adult male (A, B, INPA-H 40307), one adult female (C, INPA-H 40475), and one juvenile male (D, unvouchered). For comparison of overall color, (E) and (F) are adult males of closely related *A. masniger* and *A. nidicola*, respectively. Note the darker brown dorsum and limbs in *A. masniger* and *A. nidicola*. Photographs by J. Cassimiro (A, E), J. Gomes (B), L. Moraes (C, D), and A.P. Lima (F).

Coloration of the holotype in life. Ochre on the dorsal surface of the snout, anteriorly to the interorbital region and close to the dark brown lateral stripes, and a darker ochre, rectangular area at the center of dorsum, with some darker small dots scattered, following granule rows. Pair of cream paracloacal marks present. A continuous dark brown stripe extends from the tip of snout to flanks and reaches the cloacal region. A cream ventrolateral stripe runs below this dark line, with diffuse limits ventrally. Dorsal surfaces of arms orange, paler than the dorsum color; dorsal surfaces of legs ochre, with a similar color to the dorsum. Weakly marked single transversal brown bar on the central dorsal surface of tibia and thigh. Dorsal surfaces of hands and feet with punctuate white dots. Throat and chest violaceus, and abdomen pale violaceus to slightly transparent, revealing a white peritoneum. Ventral surfaces of forearms, palms of hands, and soles of feet brown. Iris golden with black vermiculations and pupil black, circled by a golden ring.

Coloration of holotype in preservative. Dorsal background surface of body and legs pale brown, with a darker brown rectangular region at the center of the dorsum (Fig. 2). Paler cream markings at the dorsal paracloacal region. Dorsal surfaces of arms cream, paler than the dorsum. A dark brown stripe from the tip of snout to cloacal region. White dots at dorsal surfaces of hands and feet. Weakly marked transversal brown bars above brown dorsal surfaces of thighs and tibia. On ventral surface, brown pigmentation on the throat, chest, and anterior abdomen; posterior abdomen, thighs, and tibia cream. Palmar surface of hands and plantar surface of feet uniformly brown (Figs. 3, 4).

Variation. Adults show a weak sexual dimorphism in dorsal coloration (Fig. 6), but on the ventral surface, the differences can be prominent, with males presenting violaceus throats and females white-yellowish (Figs. 5, 7). Some individuals have a narrow golden stripe on the snout region, above the lateral dark brown stripe, anteriorly to the eye-level. The definition of the pale ventrolateral stripe towards the ventral area varies between individuals but is always present. A weak ontogenetic variation occurs, with some color details more pronounced in juveniles, such as dark transversal bars in legs and a darker region at the center of dorsum, contrasting with adjacent colors (Fig. 6).

The sexual dimorphism based on body proportions (morphometric ratios) showed a degree of segregation of both sexes in the morphometric multivariate space (Fig. 8, A–C) when PC1 was compared to PC2 and PC2 to PC3, showing significant differences between males and females (Pillai trace = 0.723, df = 15, $p < 0.001$). The first three PCA axis summarized 25.11%, 20.05%, and 12.58% of the variation. The highest loading values represent snout-vent length (PC3) and the morphometric ratios (measurement/SVL) of hand length (PC1), tibia length (PC1), first finger length (PC1), thigh length (PC2); eye diameter (PC3); interorbital distance (IO); finger disc width (PC2) and toe disc width (PC2).

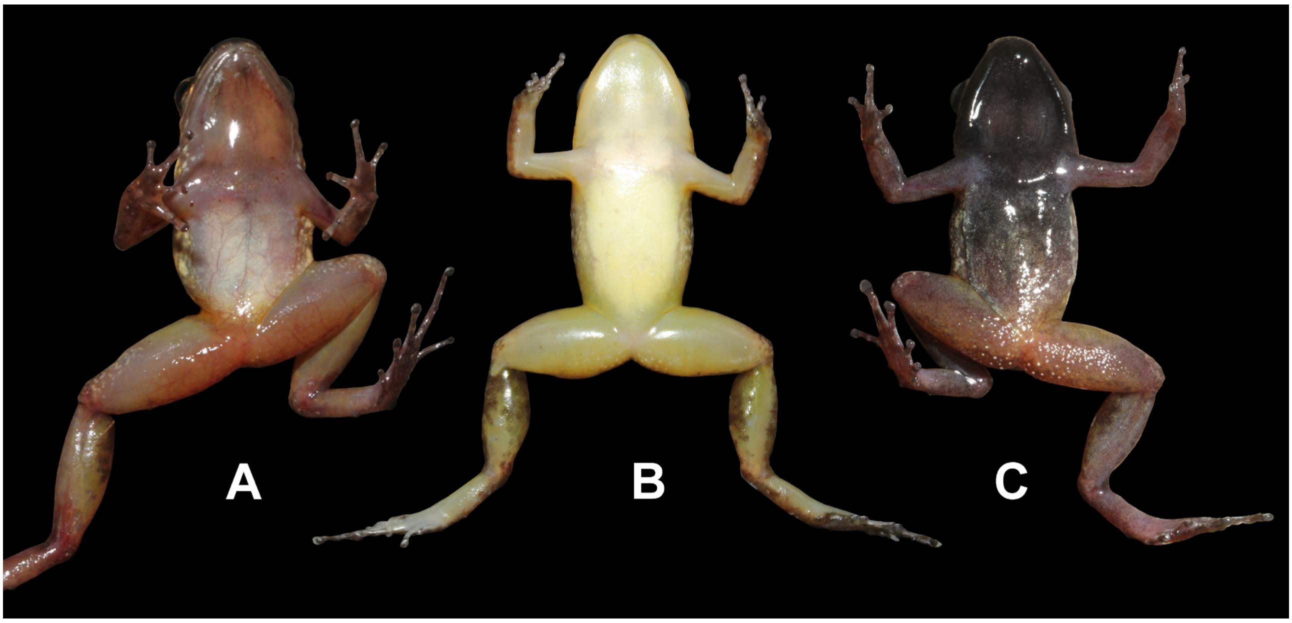

FIGURE 7. Ventral views of two living specimens of *Allobates nunciatus* **sp. nov.** (A, B), including one adult male (A, INPA-H 40307) and one adult female (B, INPA-H 40475), and *Allobates masniger* (C, adult male, unvouchered), showing a darker blackish throat in the latter species. Photographs by J. Cassimiro (A, C) and L. Moraes (B).

TABLE 2. Morphometric measurements of tadpoles of *Allobates nunciatus* **sp. nov.** from Middle Tapajós River region, state of Pará, Brazil. Values represent mean ± standard deviation, with range shown in parentheses. See text for measurement abbreviations.

Measurements (mm)	**Stage 36 (*n* = 3)**	**Stage 37 (*n* = 6)**	**Stage 39 (*n* = 3)**
TL	17.30 ± 0.26 (17.00–17.50)	18.86 ± 0.82 (18.00–20.00)	17.73 ± 0.66 (17.00–18.30)
BL	5.26 ± 0.25 (5.00–5.50)	5.98 ± 0.11 (5.80–6.10)	5.73 ± 0.30 (5.40–6.00)
TAL	12.03 ± 0.05 (12.00–12.10)	12.96 ± 0.74 (12.00–14.00)	12.00 ± 0.86 (11.00–12.50)
BW	4.20 ± 0.17 (4.00–4.30)	4.40 ± 0.51 (3.50–4.90)	4.53 ± 0.15 (4.40–4.70)
BH	3.03 ± 0.35 (2.70–3.40)	3.11 ± 0.34 (2.50–3.50)	2.96 ± 0.05 (2.90–3.00)
HWLE	3.16 ± 0.30 (2.90–3.50)	3.48 ± 0.30 (3.00–3.80)	3.53 ± 0.25 (3.30–3.80)
TMW	2.06 ± 0.07 (2.00–2.15)	2.18 ± 0.12 (2.05–2.35)	1.96 ± 0.18 (1.75–2.10)
MTH	2.43 ± 0.45 (2.00–2.90)	2.78 ± 0.28 (2.30–3.00)	2.08 ± 0.14 (2.00–2.25)
TMH	2.48 ± 0.28 (2.15–2.65)	2.56 ± 0.39 (2.10–3.25)	2.28 ± 0.05 (2.25–2.35)
IOD	2.13 ± 0.12 (2.00–2.25)	2.18 ± 0.06 (2.10–2.30)	2.18 ± 0.18 (2.05–2.40)
IND	1.52 ± 0.22 (1.28–1.71)	1.54 ± 0.09 (1.37–1.62)	1.57 ± 0.10 (1.46–1.68)
END	1.11 ± 0.03 (1.09–1.15)	0.94 ± 0.24 (0.62–1.18)	0.87 ± 0.46 (0.56–1.40)
NSD	0.94 ± 0.31 (0.62–1.25)	0.92 ± 0.19 (0.68–1.15)	0.91 ± 0.10 (0.84–1.03)
ED	0.83 ± 0.09 (0.78–0.93)	0.96 ± 0.06 (0.90–1.09)	1.07 ± 0.03 (1.03–1.09)
VTL	1.01 ± 0.11 (0.93–1.09)	0.95 ± 0.10 (0.84–1.09)	0.57 ± 0.15 (0.46–0.68)
STL	0.14 ± 0.02 (0.12–0.15)	0.21 ± 0.07 (0.12–0.31)	0.31
ODW	1.59 ± 0.05 (1.56–1.65)	1.53 ± 0.16 (1.31–1.78)	1.80 ± 0.33 (1.56–2.18)
OD	1.56 ± 0.05 (1.50–1.60)	1.58 ± 0.20 (1.40–1.90)	1.53 ± 0.11 (1.40–1.60)
PL	0.36 ± 0.11 (0.24–0.46)	0.39 ± 0.04 (0.34–0.46)	0.34 ± 0.09 (0.24–0.40)
AL	0.42 ± 0.07 (0.36–0.50)	0.49 ± 0.12 (0.38–0.70)	0.41 ± 0.08 (0.34–0.50)
UJ	0.61 ± 0.13 (0.50–0.76)	0.68 ± 0.17 (0.44–0.94)	0.72 ± 0.25 (0.54–0.90)
A1	1.38 ± 0.03 (1.34–1.40)	1.54 ± 0.17 (1.40–1.88)	1.43 ± 0.15 (1.30–1.60)
A2	0.58 ± 0.02 (0.56–0.60)	0.59 ± 0.07 (0.52–0.70)	0.61 ± 0.01 (0.60–0.62)
GAP	0.37 ± 0.04 (0.32–0.40)	0.33 ± 0.06 (0.26–0.44)	0.40 ± 0.05 (0.36–0.44)
MP	0.19 ± 0.01 (0.18–0.20)	0.21 ± 0.04 (0.16–0.30)	0.20 ± 0.03 (0.18–0.24)
LP	0.94 ± 0.05 (0.90–0.98)	0.59 ± 0.39 (0.10–1.00)	0.62 ± 0.53 (0.24–1.00)
LJ	0.40	0.46 ± 0.25 (0.20–0.74)	0.45 ± 0.07 (0.40–0.50)
P1	1.42 ± 0.07 (1.36–1.50)	1.44 ± 0.20 (1.10–1.64)	1.38 ± 0.07 (1.30–1.44)
P2	1.47 ± 0.06 (1.40–1.52)	1.52 ± 0.15 (1.30–1.68)	1.43 ± 0.04 (1.40–1.46)
P3	1.20	1.32 ± 0.10 (1.14–1.40)	1.34 ± 0.08 (1.28–1.40)
M	0.24 ± 0.08 (0.18–0.30)	0.37 ± 0.23 (0.12–0.74)	0.26 ± 0.16 (0.14–0.38)

Tadpole description. Qualitative characters based on 12 tadpoles raised to developmental stages 36, 37, and 39 (lot INPA-H 40487, field number APL 22371). Quantitative characters based exclusively on larvae at stage 37 (*n* = 6; Table 2). Body ellipsoid, longer than wide, acuminate anteriorly and rounded posteriorly in dorsal view, flattened in lateral view (Fig. 11). Body length 32% of TL and tail length 68% of TL; HWLE 80% of BW; BH 52% of BW; snout rounded in dorsal view and truncate in lateral view; eyes dorsal and directed laterally; END 97% of ED; mean ED 0.96 ± 0.06 mm; IOD 62% of HWLE. Small naris located dorsolaterally and directed laterally; mean internarial distance 1.54 ± 0.09 mm. Fleshy ring present on the inner margin of nostrils, round and straight, not ornamented. Sinistral spiracle as a free tube at mid-body, measuring 0.21 ± 0.07 mm. Average length of dextral vent tube 0.95 ± 0.10 mm. Dorsal fin begins after the body-tail insertion, with dorsal edge shallow and straight along approximately half of the caudal length, deeper posteriorly. Lower fin similar to the dorsal fin in structure and depth. Tail tip acuminate. Caudal musculature bifurcated dorsally, reaching half of the body. Anteroventral oral apparatus (Fig. 12), slightly emarginated laterally, transversely elliptical, 1.53 ± 0.16 mm wide. Anterior labium with groups of eight to nine short and round papillae distributed in a single row on each lateral margin. Posterior labium with a single row of short marginal papillae with similar length. Upper jaw sheath arch-shaped, slightly curved anteriorly in the middle, longer than the lower jaw sheath. Lower jaw sheath V-shaped, as deep as the upper jaw sheath. Cutting edge of upper and lower jaw sheaths serrated along the entire length. Labial tooth row formula 2(2)/3(1); tooth row A-1 complete, measuring 1.54 ± 0.17 mm in length; tooth row A-2 interrupted medially, consisting of two widely separated rows at the level of upper jaw sheath, segments measuring 0.59 ± 0.07 mm in length and a gap measuring 0.33 ± 0.06 mm. Posterior tooth rows P-1 and P-2 with approximately the same length as row A-1 (means: 1.44 ± 0.20 and 1.52 ± 0.15 mm, respectively), both slightly longer than the complete P-3 (1.32 ± 0.10 mm). Such a structure of oral disc (Fig. 12) was constant throughout all developmental stages examined.

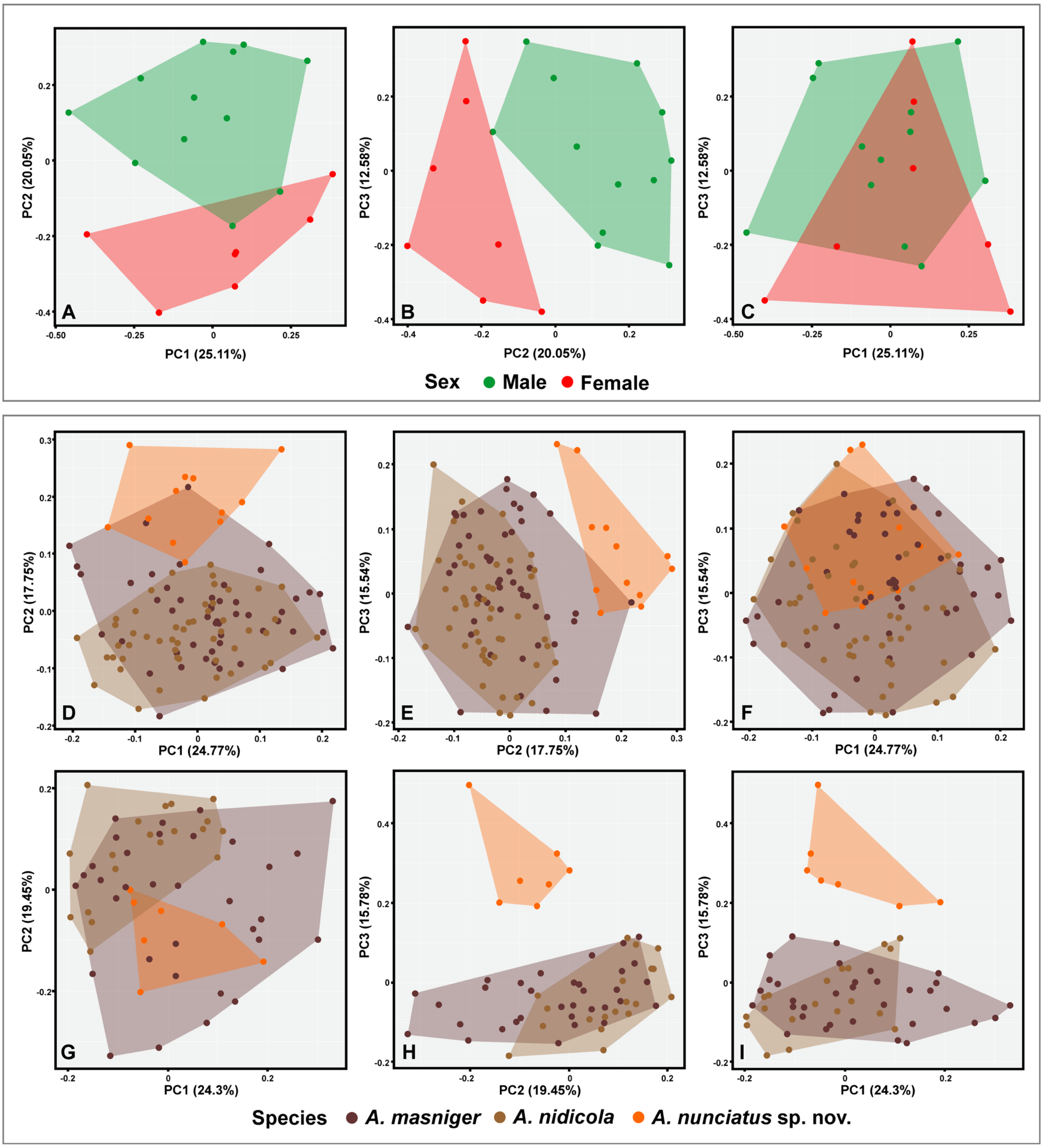

FIGURE 8. Principal Component Analysis of sex-related morphology (A–C) of *Allobates nunciatus* **sp. nov.**, and sister taxa-related morphology, comparing male (D–F) and female (G–I) adult specimens. The morphological variation was based on the SVL and body ratios (measurements/SVL).

TABLE 3. Advertisement call parameters given by mean ± standard deviation, with range shown in parentheses, of the new species of *Allobates* from the east bank of Middle Tapajós River, state of Pará, Brazil, compared to sympatric taxa (*A. tapajos*, *A. femoralis*, and *A. magnussoni*) and allopatric sister taxa *A. masniger* and *A. nidicola.* Parameters are based on field records of calling males ($n = 9$ for *Allobates nunciatus* sp. nov.) and literature data. (UR) Units of Repetition.

Parameters	***A. nunciatus* sp. nov.**	***A. nidicola***	***A. masniger***	***A. femoralis***	***A. tapajos***	***A. magnussoni***
Structure	4-pulsed UR	single-pulsed UR	single-pulsed UR	4-pulsed UR	double-pulsed UR	single-pulsed UR
Note duration (s)	0.031 ± 0.006 (0.025–0.045)	0.047 ± 0.005 (0.034–0.060)	0.051 ± 0.021 (0.031–0.089)	0.062 ± 0.011 (0.041–0.079)	0.042 ± 0.004 (0.035–0.047)	0.080 ± 0.011 (0.065–0.104)
Inter-note duration (s)	short 0.040 ± 0.005 (0.029–0.047) long 0.076 ± 0.007 (0.064–0.085)	0.282 ± 0.050 (0.216–0.448)	0.290 ± 0.103 (0.154–0.518)	short 0.069 ± 0.017 (0.051–0.099) long 0.110 ± 0.029 (0.079–0.140)	0.138 ± 0.015 (0.112–0.162)	0.451 ± 0.062 (0.347–0.535)
Call duration (s)	0.317 ± 0.034 (0.256–0.357)	0.047 ± 0.005 (0.034–0.060)	0.051 ± 0.021 (0.031–0.089)	0.492 ± 0.031 (0.044–0.053)	0.222 (± 0.023)	0.080 ± 0.011 (0.065–0.104)
Inter-call interval (s)	0.360 ± 0.078 (0.257–0.581)	0.282 ± 0.050 (0.216–0.448)	0.290 ± 0.103 (0.154–0.518)	0.512 ± 0.108 (0.360–0.653)	0.293 ± 0.037 (0.211–0.349)	0.451 ± 0.062 (0.347–0.535)
Note/s	5.162 ± 0.995 (2.700–6.533)	3.063 ± 0.496 (2.033–4.016)	3.000 ± 0.953 (2.100–4.000)	4.194 ± 0.498 (3.633–4.583)	2.939 ± 0.147 (2.730–3.230)	1.900 ± 0.280 (1.630–2.400)
Dominant frequency (kHz)	4.163 ± 0.178 (3.843–4.312)	4.196 ± 0.236 (3.759–4.689)	4.294 ± 0.144 (4.031–4.406)	3.218 ± 0.080 (3.100–3.375)	5.619 ± 0.209 (5.379–5.949)	4.462 ± 0.141 (4.273–4.704)

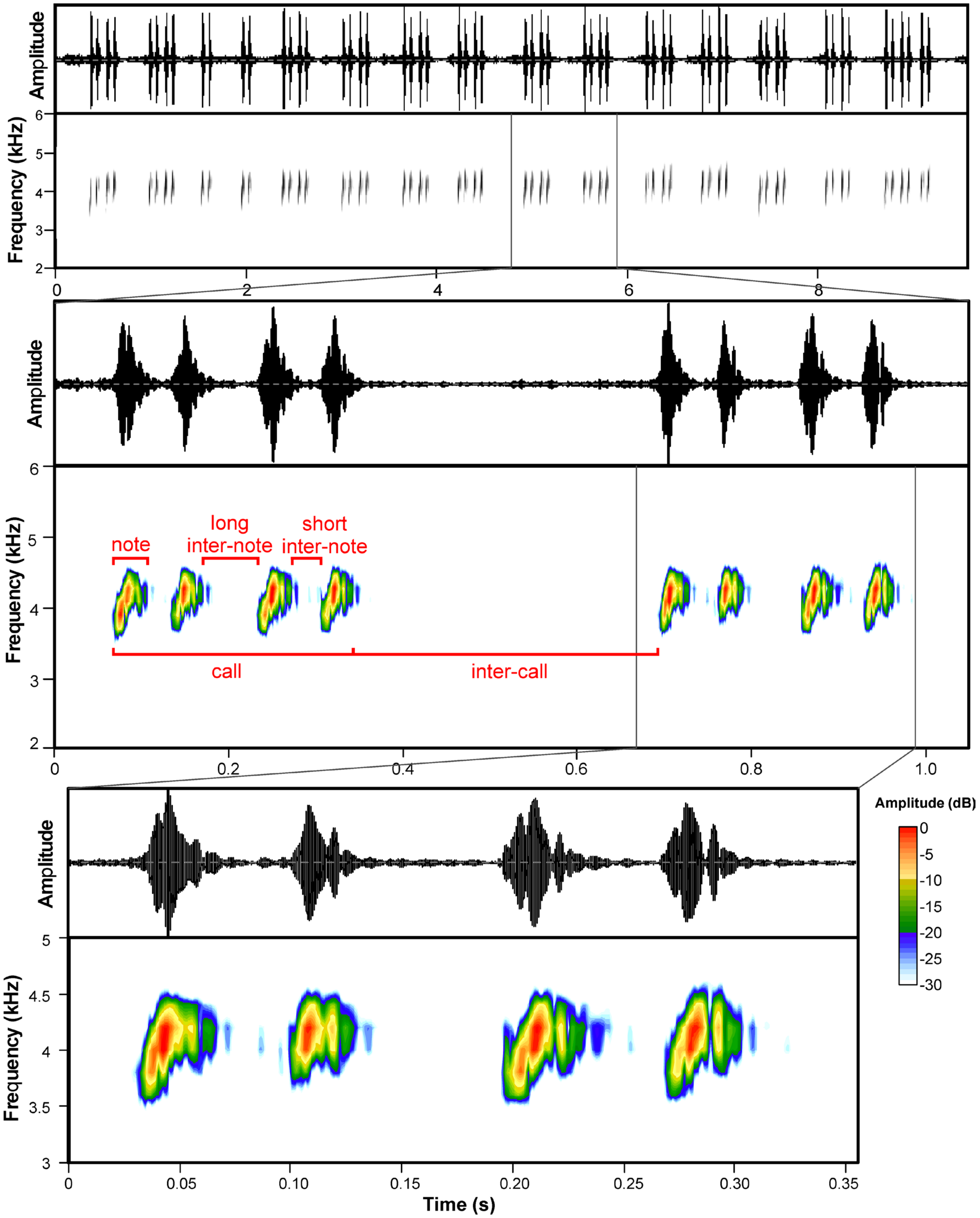

FIGURE 9. Waveform (above) and spectrogram (below) views of the structure of advertisement call of *Allobates nunciatus* **sp. nov.** (paratype INPA-H 40305, 19.2 mm SVL) emphasizing the most frequent arrangement of 4-pulsed units of repetition (UR) followed by silent intervals, and call parameters measured.

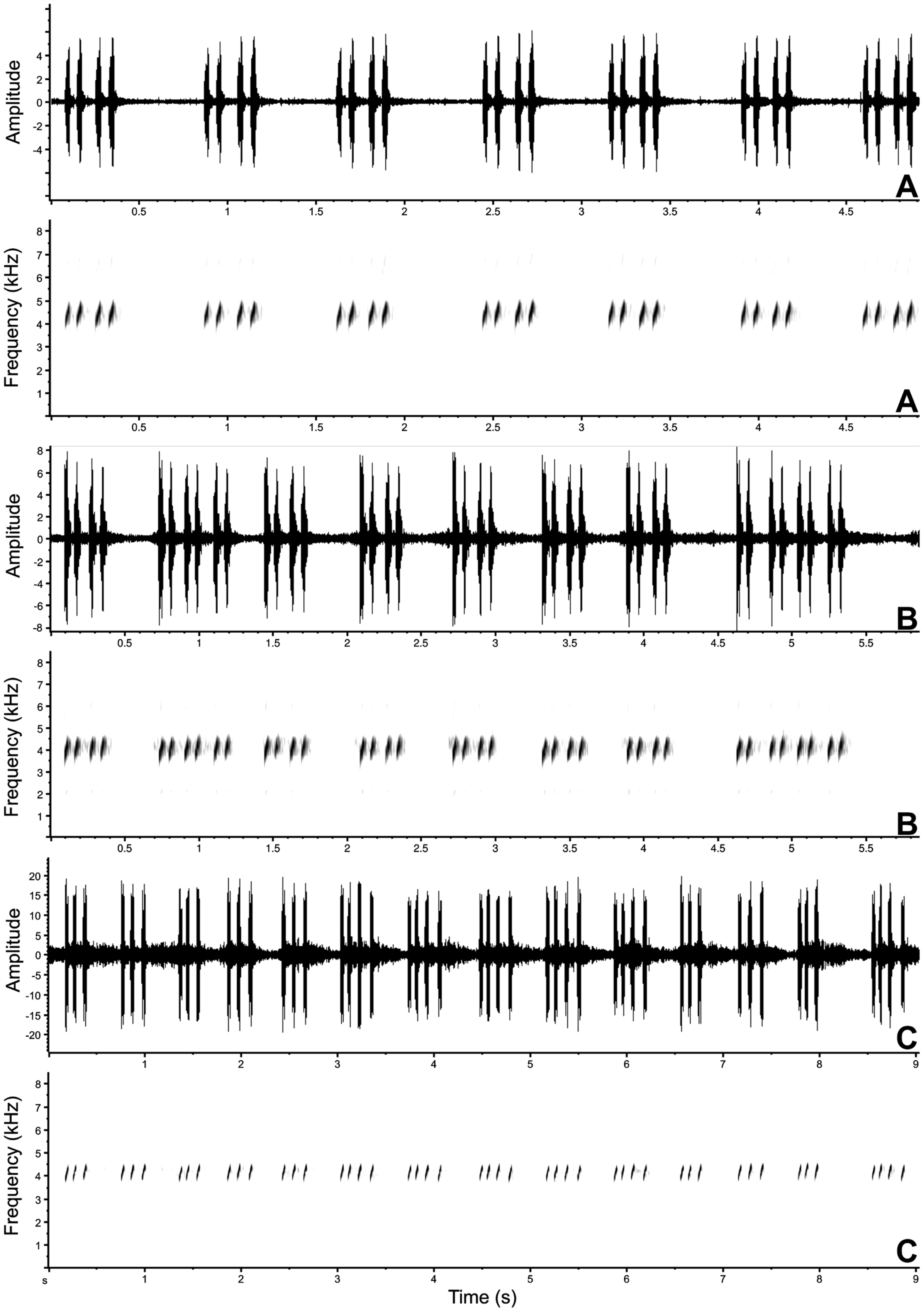

FIGURE 10. Waveform (above) and spectrogram (below) views of structure and variation in advertisement call of *Allobates nunciatus* **sp. nov.**, showing the variation between 3-, 4-, 6-, and 8-pulsed units of repetition (UR) followed by silent intervals. The 4-pulsed UR is the most frequent arrangement. (A) Call of the paratype INPA-H 40305, 19.2 mm SVL; (B) call of the paratype INPA-H 40307, 20.5 mm SVL; (C) call of the paratype INPA-H 40477, 21.5 mm SVL.

Coloration of preserved tadpoles. Dorsum, anterior part of the ventral area, and lateral surfaces with a pale cream background color, densely covered with dark brownish-grey melanophores (Fig. 11). Background color appears as irregular pale cream blotches or spots, more frequent on tail surface. Posterior part of the ventral area translucent, with intestines visible through the skin. Tail musculature cream. Tail fins translucent, with few irregular brown blotches.

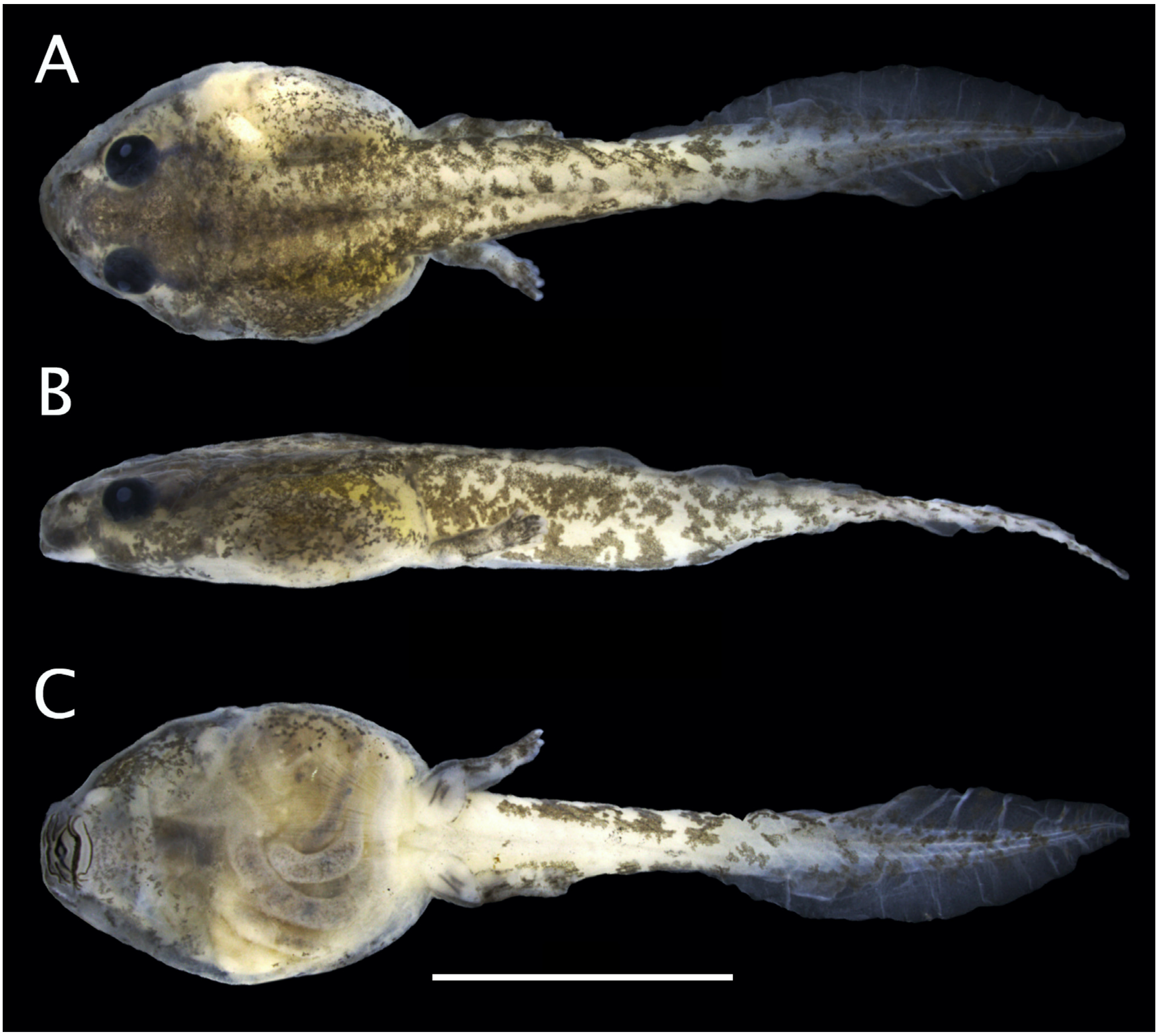

FIGURE 11. (A, B, C) Dorsal, lateral, and ventral views of a preserved tadpole of *Allobates nunciatus* **sp. nov.** in developmental stage 36 (from lot INPA-H 40487). Scale bar = 5.0 mm. Photographs by J. Magnusson.

Comparison with tadpoles of other species. The presence of an oral disc and a spiracle (Figs. 11, 12) clearly distinguishes the exotrophic tadpoles of *A. nunciatus* **sp. nov.** from endotrophic tadpoles of closely related species, such as *A. nidicola* and *A. masniger*, which lack those structures and develop entirely in terrestrial nests. *Allobates nunciatus* **sp. nov.** differs from all described *Allobates* tadpoles: from *A. caeruleodactylus*, *A. marchesianus*, *A. grillisimilis*, and *A. tapajos* by a posterior labium with short papillae of similar size (those species have a posterior labium partially surrounded by long marginal papillae, variable in length; Caldwell *et al.* 2002; Simões *et al.* 2013a; Lima *et al.* 2015); from *A. granti* (Kok, MacCulloch, Gaucher, Poelman, Bourne, Lathrop, & Lenglet, 2006), *A. hodli*, *A. subfolionidificans*, *A. brunneus*, *A. paleovarzensis*, *A. sumtuosus*, and *A. magnussoni* (Lima *et al.* 2007, 2009, 2010, 2014; Simões & Lima, 2012) by the presence of labial tooth row P-3 (absent in *A. granti*), the absence of a distinct brown longitudinal bar from the snout to the mid-body (present in *A. sumtuosus*), the presence of similar-sized papillae on anterior and posterior labium (posterior papillae slightly larger in *A. subfolionidificans*), almost subequal tooth rows P-1, P-2, and P-3 (P-3 distinctly shorter than others in *A. brunneus*, *A. magnussoni*, and *A.*

paleovarzensis), and the presence of pale cream blotches or spots along the body and tail (uniform brownish-yellow coloration in *A. hodli*). Furthermore, the fin morphology, beginning after the body-tail insertion, but deeper posteriorly to half of the caudal length is not shared by any other known *Allobates* larvae (fins increase in size gradually after the body-tail insertion).

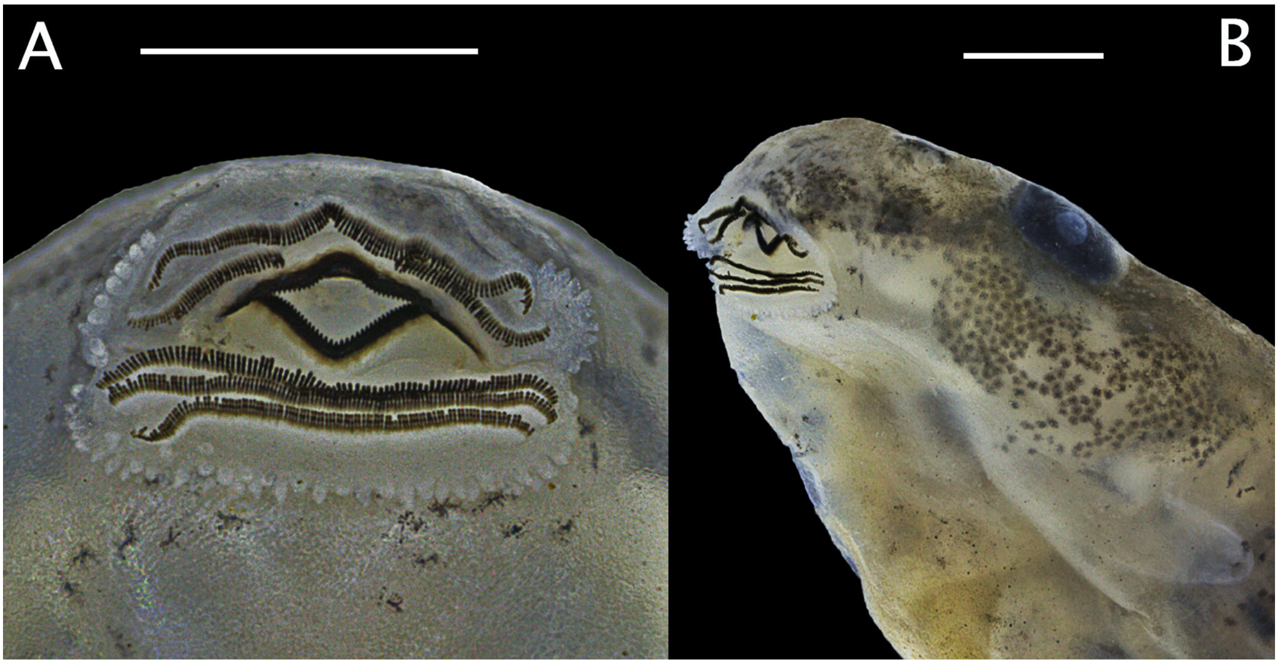

FIGURE 12. Ventral (A) and oblique (B) views of the oral disc of a preserved tadpole of *Allobates nunciatus* **sp. nov.** in developmental stage 36 (from lot INPA-H 40487). Scale bar = 1.0 mm. Photographs by J. Magnusson.

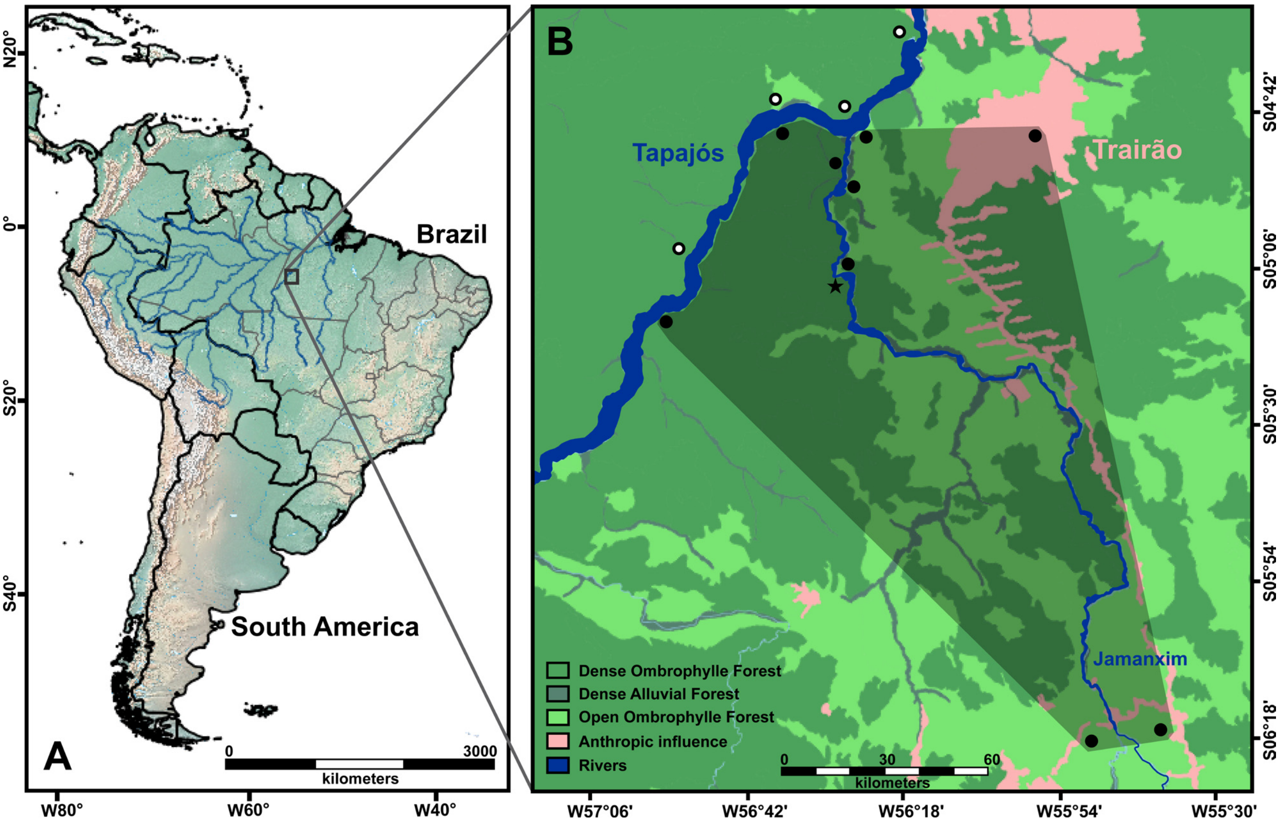

FIGURE 13. Geographical distribution of *Allobates nunciatus* **sp. nov.** in South America (A) and zoomed at Middle Tapajós River region, state of Pará, Brazil, on a vegetational background (B). Black dots and star (type locality) represent occurrence localities and white dots represent standardized sampling sites where the species was not recorded (see Moraes et al. 2016 to additional information on these sampling sites).

Advertisement call and variation. *Allobates nunciatus* **sp. nov.** has a complex advertisement call, with a continuous emission of short tonal notes, usually arranged in bouts of four closely spaced notes, which we term 4-pulsed units of repetition (UR), followed by silent intervals (Figs. 9, 10). Such an arrangement in four notes is the most frequently emitted by excited males, corresponding to 356 of 527 (67.5%) arrangements analyzed in nine distinct recordings. The second most frequent arrangement was the emission of 3-pulsed UR (14.2%). The species also emits arrangements in 2- (6.6%), 5- (3.4%), 6- (6%), 8- (1.5%), and 10-pulsed UR (the rarest arrangement, 0.6%) (Fig. 10). The 2-pulsed UR is the most frequent arrangement when males start to call and can be considered a warming call. Disturbed males interrupt the main series of 3- to 10-pulsed UR and restart emitting 2-pulsed UR. Despite the note arrangement variation, the spectral and temporal characteristics of notes are constant in all analyzed advertisement calls. Note duration lasts 0.031 ± 0.006 (0.025–0.045) s, with a lower frequency of 3.60 ± 0.34 (3.21–4.06) kHz and higher frequency of 4.60 ± 0.13 (4.30–4.79) kHz. Four-pulsed UR are 0.317 ± 0.034 (0.256–0.357) s long, and with two types of inter-note intervals, two short with 0.040 ± 0.005 (0.029–0.047) s, interpolated with a long one lasting 0.076 ± 0.007 (0.064–0.085) s. Silent intervals between UR lasts 0.360 ± 0.078 (0.257–0.581) s. This pattern is observable in all paired arrangements (Figs. 9, 10).

Geographical distribution and natural history. *Allobates nunciatus* **sp. nov.** is the most abundant *Allobates* species in non-flooded (*terra firme*) forests on the east bank of Middle Tapajós River (Figs. 13, 14), and might be geographically restricted to this bank and river basin. It also inhabits riparian habitats of small streams but is rarely recorded in *igapó* forests, periodically flooded by large rivers. The species occurs on both banks of the large tributary Jamanxim River (Figs. 13, 14), with no evident morphological, acoustic or molecular distinction between these two separate populations.

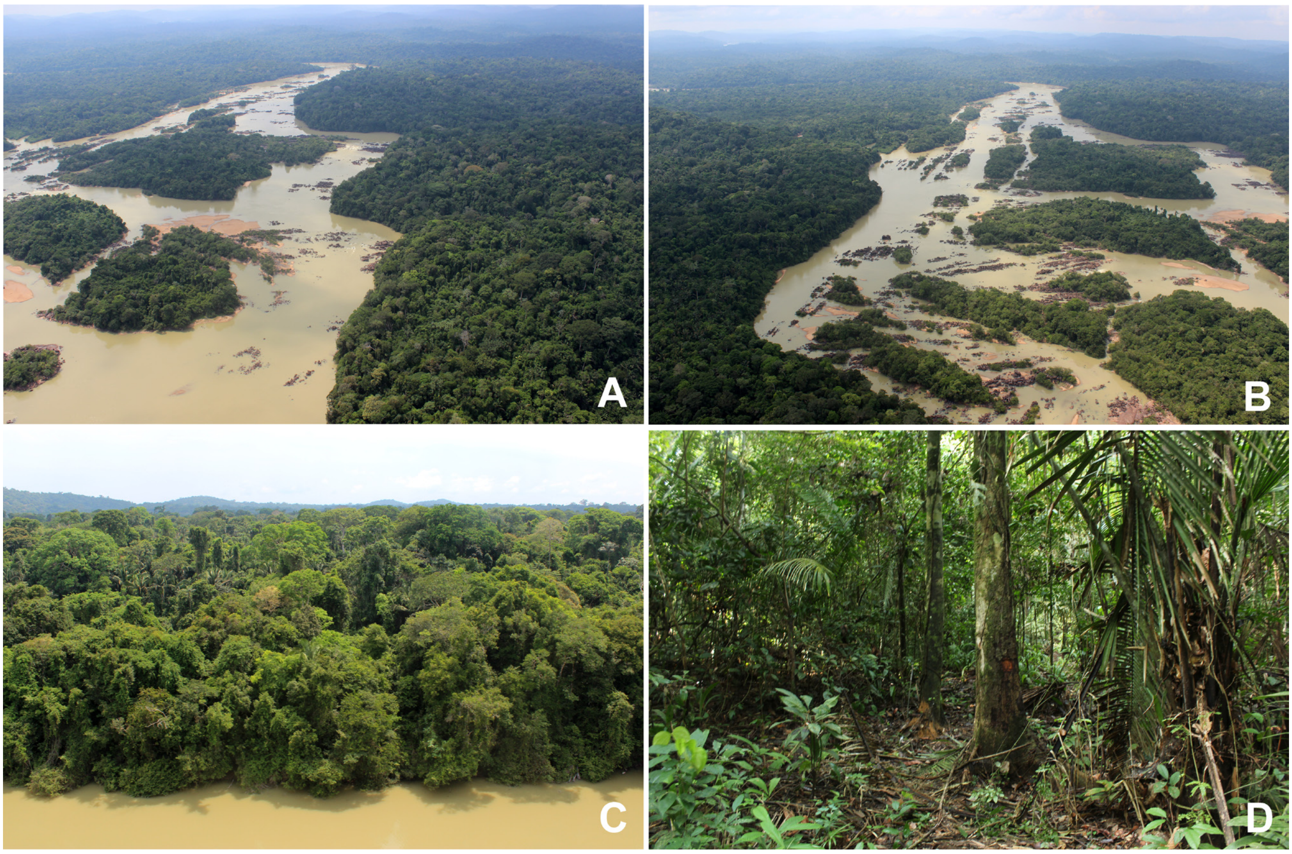

FIGURE 14. Photographs from the occurrence area of *Allobates nunciatus* **sp. nov.** in Middle Tapajós River region, state of Pará, Brazil. (A–C) Aerial view of the Jamanxim River and its forested banks; (B) Primary dense non-flooded forest (*terra firme*), the main habitat of the species. Photographs by D. Pavan (A–C) and L. Moraes (D).

Alobates nunciatus **sp. nov.** is diurnal and breeds (Fig. 15) during the rainy season (from November to March), mostly in the beginning of rains (November and December). However, a higher number of individuals, comprising adults and juveniles, were spotted during the dry season (September and October), which indicates a high spatial movement in this period. Males call during the day, mostly in the morning and late afternoon. Most of the visually recorded individuals were in the leaf litter (Fig. 15). Eggs were deposited in a gelatinous mass inside nests made of dry fallen leaves on the leaf litter (Fig. 15). The eggs developed into exotrophic tadpoles, which were then carried by the males to nearby water bodies. This reproductive strategy differs from that of the closely related *A. nidicola* and *A. masniger,* whose entire development of endotrophic tadpoles takes place in forest floor nests (Caldwell & Lima 2003; Tsuji-Nishikido *et al.* 2012).

FIGURE 15. Reproductive behavior of *Allobates nunciatus* **sp. nov.** (A) Calling male on large dry *Cecropia* spp. leaves on leaf litter, INPA-H 40479; (B, C) twisted dry fallen leaves on leaf litter, used as nests; (D) gelatinous mass with tadpoles on a twisted dry fallen leave. Photographs by A. P. Lima.

Discussion

With the description of *A. nunciatus* **sp. nov.**, the Middle Tapajós River region harbors five nominal species of *Allobates*. The landscape changes in the Tapajós River basin plays an essential role in the evolutionary history of *Allobates* (Maia *et al.* 2017; Moraes *et al.* 2016), as some species are geographically restricted to the Tapajós River basin, such as *A. magnussoni* and *A. tapajos* or even to a single river bank, such as *A. nunciatus* **sp. nov.** and *A. masniger* (Lima *et al.* 2014, 2015; Maia *et al.* 2017).

Based on morphological, acoustic, ecological, and molecular similarities, *A. nunciatus* **sp. nov.** is closely related to *A. masniger* and *A. nidicola*. These phylogenetic relationships highlight this complex of species as a good model for future studies on the evolution of the landscape in Southern Amazonia, as these three taxa are allopatrically distributed in Tapajós-Xingu, Madeira-Tapajós, and Purus-Madeira interfluves, respectively (Tsuji-Nishikido *et al.* 2012). This case is one of the few known examples of anuran groups with geographical distribution patterns coincident with the Amazon areas of endemism proposed for bird and primate distributions (Cracraft 1985; Silva *et al.* 2005; Ribas *et al.* 2012) and may reflect similar evolutionary histories with these species.

It is interesting to note that the unique nidicolous reproductive mode of the species pair *A. masniger-nidicola* (Tsuji-Nishikido *et al.* 2012) is not shared by the sister taxa *A. nunciatus* **sp. nov.** This fact allied with mtDNA phylogenetic relationships indicates a possible East to West polarity in the evolution of nidicolous reproductive mode in this group. However, due to the absence of additional data, this hypothesis remains speculative.

A degree of sub-structuring can be recognized within the molecular variation of *A. masniger* and *A. nunciatus* **sp. nov.** There is no evidence that this subdivision reflects distinct taxonomic entities, as no significant differences were detected in phenotypic or bioacoustic variation of these populations. Such a pattern may reflect the fact that this species group is particularly sensitive to diversification due to low dispersal ability, high territoriality, specialization in habitat, and reproductive mode (Moraes *et al.* 2016). This natural potential for diversification may increase in the face of any dispersal impediment, e.g., the emergence of geographical barriers such as large rivers (Maia *et al.* 2017; Moraes *et al.* 2016). These events can increase molecular divergence between populations despite morphological, acoustic or ecological conservatism, a well-known pattern within *Allobates* (e.g., *A. femoralis* complex—Simões *et al.* 2010, *A. magnussoni*—Lima *et al.* 2014, *A. masniger-nidicola*—Tsuji-Nishikido *et al.* 2012, *A. tapajos*—Maia *et al.* 2017). Such functional characteristics also affect diversification rates of other vertebrate groups in the Amazon (Smith *et al.* 2014) and, in some cases, even small rivers can represent geographical barriers to distinct intra- and interspecific lineages (e.g., Fernandes *et al.* 2014; Dias *et al.* 2018), which may also occur in *A. masniger* and *A. nunciatus* **sp. nov.** diversification.

According to IUCN criteria to determine species' conservation status (IUCN 2014), *Allobates nunciatus* **sp. nov.** may be attributed to the Data Deficient (DD) category as there is not enough information to make a direct or indirect assessment of its conservation status. Although the geographic distribution range of *A. nunciatus* **sp. nov.** is within the limits of Federal Protected Areas (National Forests of Itaituba I, II, and Trairão and Jamanxim National Park), most of these areas have a permissive character, and the anthropic influence inside them has been growing rapidly, which places protected areas of this region among the most affected by deforestation in recent years (Araújo *et al.* 2017). Additionally, the ecological preference of these small frogs for primary forests, and their geographic restriction to the east bank of Tapajós River makes them sensitive to anthropic impacts, such as large hydropower plants and waterways and the increasing deforestation in this region (Barreto *et al.* 2014; Moraes *et al.* 2016). Further studies are needed to address this impact thoroughly and the species conservation status.

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APPENDIX 1. Specimens examined.

Allobates bacurau (*n* = 13). Brazil: Amazonas: Estrada do Miriti, Manicoré. INPA-H 35398 (**holotype**), 35397, 35399–35409 (**paratypes**).

Allobates brunneus (*n* = 38). Brazil: Mato Grosso: NE of Chapada dos Guimarães. INPA-H 10111–48 (**topotypes**). Tadpoles (*n* = 15). Brazil: Mato Grosso: NE of Chapada dos Guimarães. INPA-H 10025–10027, 10029–10030, 10032–10037, 10039, 10041, 10043, 10044 (**topotypes**).

Allobates caeruleodactylus (*n* = 17). Brazil: Amazonas: Km 12 on the road to Autazes. INPA-H 7238 (**holotype**), 7229–7232, 7234–7237 (**paratypes**). Tadpoles (*n* = 11). Brazil: Amazonas: Km 12 on the road to Autazes. INPA-H 8037–8046, INPA-H 8085.

Allobates crombiei (*n* = 11). Brazil: Pará: Cachoeira do Espelho. INPA-H 30457–30477 (**topotypes**).

Allobates femoralis (*n* = 39). Brazil: Pará: Treviso. INPA-H 11657–11671, 15232, 30769–30778. Brazil: Pará: Itaituba. INPA-H 26342–26354.

Allobates fuscellus (*n* = 6): Brazil: Amazonas: Ipixuna: Penedo, east bank of Juruá river. INPA-H 2532 (**holotype**), 2531 (**paratopotype**). Brazil: Itamarati: Jainu, Juruá River. INPA-H 3114, 3250, 3270, 3514 (**paratypes**).

Allobates gasconi (*n* = 18). Brazil: Amazonas: Itamarati: Jainu, west bank of Juruá River. INPA-H 3082 (**holotype**), 3073, 3079, 3085, 3090, 3150, 3151, 3172, 3249, 3406, 3415, 3483, 3484, 3491, 3494, 3496, 3512, 3513 (**paratypes**).

Allobates grillisimilis (*n* = 45). Brazil: Amazonas: Borba. INPA-H 30779 (**holotype**), 30780–30808 (**paratopotypes**). Brazil: Amazonas: Nova Olinda do Norte. INPA-H 30809–30823 (**paratypes**).

Allobates hodli (*n* = 102). Brazil: Acre: Fazenda Experimental Catuaba. INPA-H 11621–11640 (**paratypes**). Brazil: Rondônia: Cachoeira do Jirau. INPA-H 16555 (**holotype**) 16541–16554, 16556–16569 (**paratopotypes**). Brazil: Rondônia: Near Fortaleza do Abunã. INPA-H 16578, 16584–16587, 16589, 16591, 16592, 16597, 16602, 16603, 16605–16607, 16611–16614, 16620–16624, 16626, 16628, 16631, 16633, 16636, 16637, 16639–16641, 16643, 16645, 16646, 16648. Brazil: Rondônia: Near Mutum- Paraná. INPA-H 16596, 16730, 16739, 16756, 16758, 16767, 16771, 16777, 16778, 16788, 16805, 16818, 16819 (**paratypes**).

Allobates magnussoni (*n* = 36). Brazil: Pará: Parque Nacional da Amazônia. INPA-H 32960 (**holotype**), 32961–32976, 32978–32982 (**paratopotypes**). Brazil: Pará: Treviso. INPA-H 10105–10109, 33930–33934. Brazil: Pará: Jamanxim. INPA-H 33935 (**paratypes**). Tadpoles (*n* = 7). Brazil: Pará: Treviso. INPA-H 10054, 10056, 10058, 10059, 10060. Brazil: Pará: Parque Nacional da Amazônia. INPA-H 32983, 33936.

Allobates marchesianus (*n* = 41). Brazil: Amazonas: Missão Taracuá. INPA-H 7959–7990 (**topotypes**). Brazil: Amazonas: São Gabriel da Cachoeira, 175 km E Missão Taracuá. INPA-H 7991, 7993, 8000–8007). Tadpoles (*n* = 11). Brazil: Amazonas: Missão Taracuá. INPA-H 7943–7950, 7992, 7998, 8084 (**topotypes**).

Allobates masniger (*n* = 96). Brazil: Amazonas: Borba. INPA-H 28075, 28078, 28084, 28089, 28092, 28095, 28098, 28100, 28104, 28105, 28112, 28114, 28119. Brazil: Amazonas: Novo Aripuanã. INPA-H 28054–28056, 28058–28060, 28062–28067, 28069, 28072–28074, 28080, 28083, 28086–28091, 28096, 28097, 28101, 28108, 28117. Brazil: Amazonas: Road to Apuí: INPA-H 28057, 28061, 28068, 28071, 28076, 28079, 28081, 28085, 28102, 28106, 28109, 28116, 28190, 28191. Brazil: Pará: Parque Nacional da Amazônia. INPA-H 28195–28217 (**topotypes**). Brazil: Pará: Jacareacanga. INPA-H 28053, 28070, 28077, 28082, 28093, 28094, 28099, 28103, 28107, 28110, 28111, 28113, 28115, 28118, 28120.

Allobates myersi (*n* = 8). Brazil: Amazonas: São Gabriel da Cachoeira. INPA-H 26369–26372, 26374, 26376, 26377, 26379

Allobates nidicola (*n* = 80). Brazil: Amazonas: Km 12 on road to Autazes. INPA-H 8093 (**holotype**), 7253–7259, 7261, 7262, 8094 (**paratypes**), INPA-H 28122, 28124, 28127, 28129, 28131, 28144, 28159, 28163, 28166, 28169, 28171, 28172, 28174, 28179, 28184, 28185 (**topotypes**). Brazil: Amazonas: BR-319, Km 260. INPA-H 28126, 28133, 28153, 28156, 28168, 28173, 28175, 28178, 28181, 28183, 28186, 28189. Brazil: Amazonas: PPBio Manaquiri. INPA-H 28137, 28140, 28143, 28146, 28148, 28155, 28157, 28160, 28162, 28164, 28167, 28170, 28176, 28182, 28187. Brazil: Amazonas: BR-319 Tupana. INPA-H 28128, 28130, 28134, 28136, 28141, 28142, 28145, 28151, 28152, 28161, 28174, 28177, 28188. Brazil: Amazonas: Vila Gomes. 28121, 28123, 28125, 28132, 28135, 28138, 28139, 28147, 28149, 28150, 28154, 28158, 28165, 28180. Tadpoles (*n* = 16). INPA-H 8021–8033, 8137–8139.

Allobates paleovarzensis (*n* = 45). Brazil: Amazonas: Careiro da Várzea. INPA-H 20904 (**holotype**), 20861–20903, 20905 (**paratypes**).

Allobates subfolionidificans (*n* = 30). Brazil: Acre: Parque Zoobotânico da Universidade Federal do Acre. INPA-H 13760 (**holotype**), 11958–11974, 13749–13754, 13756–13759, 13761, 13762 (**paratypes**). Tadpoles (*n* = 2). Brazil: Acre: Parque Zoobotânico da Universidade Federal do Acre. INPA-H 14822, 14823.

Allobates sumtuosus (*n* = 11). Brazil: Pará: Reserva Biológica do Rio Trombetas. INPA-H 31952–56, INPA-H 31958–60 (**topotypes**). Brazil: Amazonas: Reserva Florestal Adolpho Ducke. INPA-H 31949–31951.

Allobates tapajos (*n* = 24). Brazil: Pará: Parque Nacional da Amazônia. INPA-H 34425 (**holotype**), 34402–34424 (**paratypes**). Tadpoles (*n* = 2) Brazil: Pará: Parque Nacional da Amazônia. Lots INPA-H 34426, 34427.

Allobates tinae (*n* = 6). Brazil: Acre: Boca do Acre. APL 21678, 21679, 21685, 21687.

Allobates trilineatus (*n* = 36). Brazil: Acre: Parque Zoobotânico da Universidade Federal do Acre. INPA-H 11958–11993.

Allobates vanzolinius (*n* = 7). Brazil: Amazonas: Vai-Quem-Quer, Rio Juruá. INPA-H 4896 (**holotype**), 4903, 4904, 4905, 4912 (**paratypes**). Brazil: Amazonas: Jainu, Rio Juruá. INPA-H 3381, 3413 (**paratypes**);

APPENDIX 2. Loadings result of the Principal Component Analysis related to sex of *Allobates nunciatus* **sp. nov.** and sister-taxa morphometry (adult specimens), with highest values of three first axis highlighted. Morphological variation based on the SVL and morphometric ratios. Abbreviations: (PC) Principal Component, (SVL) snout–vent length; (FA) forearm length; (HA) hand length; (TL) tibia length; (FL) foot length; (HW) head width; (HL) head length; (ED) eye diameter; (EN) eye–nostril distance; (IN) internarial distance; (IO) interorbital distance; (TD) tympanum diameter; (WFD) width of finger III disc, (WTD) width of toe IV disc; (THL) thigh length and (1Fil) first finger length.

Morphometric variation related to sex of *Allobates nunciatus* sp. nov.

Morphometric measurements	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**	**PC11**	**PC12**	**PC13**	**PC14**	**PC15**
SVL	0.023	0.075	**0.168**	0.051	0.166	0.012	0.012	0.012	0.065	0.180	0.006	0.049	0.027	0.065	0.093
HA/SVL	**0.127**	0.036	0.013	0.049	0.05	0.102	0.092	0.053	0.109	0.028	0.040	0.186	0.080	0.017	0.110
TL/SVL	**0.129**	0.019	0.059	0.109	0.078	0.011	0.052	0.005	0.085	0.013	0.093	0.071	0.113	0.142	0.104
FL/SVL	0.124	0.021	0.051	0.015	0.044	0.137	0.067	0.024	0.140	0.046	0.002	0.050	0.043	0.165	0.046
HW/SVL	0.033	0.045	0.018	0.100	0.291	0.03	0.094	0.039	0.060	0.100	0.080	0.019	0.03	8E-04	0.030
HL/SVL	0.057	0.026	0.116	0.123	0.004	0.046	0.114	0.231	0.094	0.034	0.050	0.024	0.057	0.011	0.030
ED/SVL	0.008	0.018	**0.199**	0.022	0.068	0.143	0.051	0.037	0.060	0.187	0.050	0.001	0.032	0.008	0.047
EN/SVL	0.114	0.024	0.018	0.099	0.004	0.182	0.053	0.013	0.078	0.079	0.001	0.008	0.146	0.102	0.043
IN/SVL	0.012	0.035	0.025	0.271	0.027	0.060	0.002	0.050	0.011	0.103	0.136	0.095	0.020	0.052	0.004
IO/SVL	0.050	0.114	**0.149**	0.030	0.092	0.014	0.066	0.022	0.023	0.092	0.077	0.025	0.012	0.050	0.198
TD/SVL	0.080	0.070	0.034	0.019	0.039	0.030	0.239	0.154	0.045	0.031	0.027	0.033	0.054	0.096	0.020
WFD/SVL	0.019	**0.163**	0.020	0.054	0.018	0.067	0.022	0.048	0.062	0.049	0.136	0.017	0.145	0.092	0.103
WTD/SVL	0.033	**0.139**	0.036	0.030	0.087	0.042	0.001	0.173	0.081	0.003	0.179	0.022	0.006	0.089	0.039
THL/SVL	0.065	**0.126**	0.072	0.021	0.010	0.122	0.079	0.090	0.034	0.019	0.091	0.148	0.101	0.070	0.102
1Fil/SVL	**0.125**	0.089	0.021	0.008	0.023	0.000	0.053	0.049	0.054	0.036	0.032	0.251	0.135	0.040	0.031
Cumulative proportion of variance	25.1%	45.1%	57.7%	67.5%	74.8%	80.8%	86.2%	91.1%	94.3%	96.4%	97.9%	99%	99.4%	99.7%	100%

APPENDIX 2. (Continued)

Morphometric variation related to female specimens of sister-taxa (*A. masniger*, *A. nidicola* and *A. nunciatus* sp. nov.)

Morphometric measurements	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**	**PC11**	**PC12**	**PC13**	**PC14**
SVL	**0.107**	0.067	0.023	0.019	0.022	0.102	0.053	0.043	0.112	0.185	0.144	0.064	0.000	0.060
FA/SVL	0.099	0.059	0.072	0.038	0.022	0.132	0.003	0.001	0.164	0.127	0.010	0.097	0.110	0.051
HA/SVL	0.061	**0.097**	0.068	0.075	0.002	0.130	0.095	0.165	0.017	0.051	0.133	0.019	0.031	0.068
TL/SVL	**0.100**	**0.089**	0.01	0.087	0.023	0.012	0.012	0.119	0.012	0.134	0.066	0.102	0.127	0.109
FL/SVL	**0.102**	0.088	0.02	0.020	0.112	0.045	0.041	0.115	0.022	0.052	0.017	0.041	0.200	0.094
HW/SVL	0.064	0.061	**0.158**	0.015	0.099	0.045	0.014	0.030	0.017	0.047	0.022	0.108	0.100	0.174
HL/SVL	0.042	0.046	**0.149**	0.136	0.112	0.063	0.006	0.030	0.097	0.000	0.046	0.098	0.145	0.059
ED/SVL	0.100	0.082	0.039	0.013	0.017	0.089	0.088	0.108	0.047	0.124	0.012	0.159	0.054	0.089
EN/SVL	0.070	0.084	0.027	0.121	0.118	0.122	0.138	0.025	0.020	0.001	0.121	0.091	0.073	0.043
IN/SVL	0.076	0.039	0.082	0.183	0.025	0.071	0.139	0.096	0.016	0.002	0.102	0.054	0.002	0.116
TD/SVL	0.020	**0.111**	0.040	0.056	0.114	0.013	0.284	0.019	0.051	0.012	0.079	0.005	0.021	0.062
WFD/SVL	0.024	0.059	0.148	0.056	0.141	0.060	0.087	0.112	0.108	0.133	0.06	0.053	0.027	0.036
WTD/SVL	0.043	0.051	**0.154**	0.044	0.088	0.100	0.000	0.053	0.194	0.118	0.002	0.047	0.079	0.025
FIL/SVL	0.092	0.067	0.01	0.136	0.105	0.017	0.039	0.083	0.124	0.015	0.186	0.063	0.032	0.012
Cumulative proportion of variance	24.7%	42.5%	58%	65.6%	71.3%	76.8%	81.8%	85.6%	88.9%	91.9%	94.6%	97%	98.6%	100%

APPENDIX 2. (Continued)

Morphometric variation related to female specimens of sister-taxa (*A. masniger*, *A. nidicola* and *A. nunciatus* sp. nov.)

Morphometric measurements	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**	**PC11**	**PC12**	**PC13**	**PC14**
SVL	0.103	0.055	0.038	0.054	0.205	0.089	0.026	0.051	0.088	0.078	0.060	0.043	0.097	0.109
FA/SVL	0.059	0.100	0.082	0.075	0.015	0.197	0.031	0.022	0.075	0.068	0.012	0.04	0.074	0.153
HA/SVL	0.008	0.138	0.021	0.019	0.024	0.122	0.148	0.001	0.093	0.063	0.166	0.021	0.008	0.027
TL/SVL	0.078	0.057	0.106	0.133	0.014	0.019	0.046	0.014	0.114	0.174	0.007	0.037	0.05	0.102
FL/SVL	0.088	0.063	0.125	0.052	0.042	0.014	0.033	0.094	0.076	0.118	0.041	0.029	0.037	0.202
HW/SVL	0.107	0.037	0.093	0.057	0.043	0.032	0.122	0.015	0.039	0.145	0.084	0.147	0.081	0.064
HL/SVL	0.130	0.019	0.039	0.022	0.122	0.008	0.072	0.073	0.07	0.048	0.027	0.239	0.061	0.066
ED/SVL	0.098	0.070	0.002	0.100	0.051	0.015	0.113	0.193	0.149	0.052	0.07	0.014	0.065	0.019
EN/SVL	0.103	0.071	0.029	0.102	0.132	0.088	0.013	0.03	0.048	0.071	0.094	0.162	0.120	0.025
IN/SVL	0.066	0.015	0.115	0.144	0.042	0.071	0.157	0.006	0.005	0.045	0.129	0.054	0.049	0.081
TD/SVL	0.067	0.078	0.091	0.033	0.059	0.06	0.044	0.302	0.062	0.045	0.095	0.03	0.005	0.021
WFD/SVL	0.031	0.113	0.122	0.019	0.05	0.006	0.067	0.078	0.13	0.013	0.045	0.024	0.204	0.016
WTD/SVL	0.004	0.109	0.123	0.069	0.003	0.120	0.046	0.002	0.012	0.078	0.112	0.121	0.133	0.081
1Fil/SVL	0.059	0.074	0.013	0.120	0.199	0.159	0.082	0.119	0.040	0.001	0.059	0.039	0.016	0.033
Cumulative proportion of variance	24.3%	43.7%	59.5%	67.4%	73.3%	78.6%	83.3%	87.6%	90.5%	93.1%	95.4%	97.4%	98.8%	100%

APPENDIX 3. Specimens of *Allobates* included in molecular analysis, with respective voucher number, locality of occurrence and Genbank accession numbers. Highlighted are newly generated sequences.

Taxon	Voucher	Locality	16S	COI
Allobates algorei	TNHCFS5551	Road from San Cristobal to. Río Negro, Táchira, Venezuela	HQ290950	_
Allobates amissibilis	MTD47884	Iwokrama, Guyana	KC520680	KC520687
Allobates bacurau	INPA-H35406	Estrada do Miriti, Manicoré, Amazonas, Brazil	KU195698	_
Allobates bacurau	INPA-H35399	Estrada do Miriti, Manicoré, Amazonas, Brazil	KU195703	_
Allobates caeruleodactylus	MPEG13809	Castanho, Amazonas, Brazil	DQ502100	DQ502814
Allobates crombiei	APL13458	Cachoeira do espelho, Altamira, Pará, Brazil	KF250508	_
Allobates crombiei	APL14118	Fazenda Raio-de-Sol, near Pacajá, Pará, Brazil	KJ747345	_
Allobates femoralis	OMNH36070	Porto Walter, Acre, Brazil	DQ502092	DQ502811
Allobates femoralis	QCAZ16484	Yasuni, Ecuador	AY364543	_
Allobates femoralis	KU215179	Cusco Amazônico, Madre de Dios, Peru	DQ501990	DQ502716
Allobates femoralis	ACRE12931	Brasiléia, Acre, Brazil	GU017463	_
Allobates femoralis	MJH3976	Reserva Ducke, Manaus, Amazonas, Brazil	DQ502113	_
Allobates flaviventris	APL14523	Cachoeira do Jirau, Rondônia, Brazil	KJ747336	_
Allobates flaviventris	APL15137	Jaci-Paraná, Rondônia, Brazil	KJ747337	_
Allobates flaviventris	LSUMZ17633	Parque Estadual Guajará-Mirim, Rondônia, Brazil	EU342521	_
Allobates fratisenescus	QCAZ54377	Comunidad Jempekat, Morona Santiago, Ecuador	MF624172	_
Allobates gasconi	APL14411	Eirunepé, Amazonas, Brazil	KJ747334	_
Allobates gasconi	APL14416	Eirunepé, Amazonas, Brazil	KJ747335	_
Allobates gasconi	MPEG13003	Rio Ituxi, Lábrea, Amazonas, Brazil	DQ502052	DQ502777
Allobates granti	MC231	Saul, French Guyana	JN690927	_
Allobates granti	PG301	Haut Marwini, French Guyana	JN690928	_
Allobates granti	BPN1054	Lely Mountain, Suriname	JN690923	_
Allobates grillisimilis	APL12747	Borba, Amazonas, Brazil	KF250504	_
Allobates grillisimilis	APL12788	Borba, Amazonas, Brazil	KF250505	_
Allobates aff. *grillisimilis*	APL12674	Nova Olinda do Norte, Amazonas, Brazil	KF250506	_
Allobates aff. *grillisimilis*	APL12675	Nova Olinda do Norte, Amazonas, Brazil	KF250507	_
Allobates hodli	APL2014	Cachoeira do Jirau, Porto Velho, Rondônia, Brazil	JQ436705	_
Allobates hodli	ABU2194	Abunã, Porto Velho, Rondônia, Brazil	KX044279	_
Allobates humilis	CVULA5690	Road to San Ramon, Calderas, Barinas, Venezuela	KJ940454	_
Allobates insperatus	QCAZ16533	Yasuni, Ecuador	AY364557	_
Allobates juanii	ARA2394	Pozo Azul, Villavicencio, Meta, Colombia	DQ502271	DQ502933

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APPENDIX 3. (Continued)

Taxon	Voucher	Locality	16S	COI
Allobates juanii	TNHCFS4978	Villavicencio-Restrepo road, Villavicencio, Meta, Colombia	HQ290960	–
Allobates aff. *juanii*	AJC3383	Sabanalarga, Casanare, Colombia	KJ130697	KJ130661
Allobates aff. *juanii*	AJC3385	Sabanalarga, Casanare, Colombia	KJ130698	KJ130662
Allobates kingsburyi	QCAZ16523	Rio Chicana, Ecuador	HQ290963	–
Allobates magnussoni	APL12989	Parque Nacional da Amazônia, Itaituba, Pará, Brazil	KJ747328	–
Allobates magnussoni	APL12987	Parque Nacional da Amazônia, Itaituba, Pará, Brazil	KJ747329	–
Allobates masniger	INPA-H28102	Novo Aripuanã, Amazonas, Brazil	JQ966882	–
Allobates masniger	INPA-H28079	Novo Aripuanã, Amazonas, Brazil	JQ966879	–
Allobates masniger	**PV3778**	**West bank of middle Tapajós River, Itaituba, Brazil**	**MK673770**	**MK770341**
Allobates masniger	**PV3480**	**West bank of middle Tapajós River, Itaituba, Brazil**	**MK673769**	–
Allobates masniger	**PV3860**	**West bank of middle Tapajós River, Itaituba, Brazil**	**MK673772**	**MK770340**
Allobates masniger	**PV3856**	**West bank of middle Tapajós River, Itaituba, Brazil**	**MK673771**	–
Allobates nidicola	MPEG13821	Km 12 on road to Autazes, Castanho, Amazonas, Brazil	DQ502101	–
Allobates nidicola	MPEG13820	Km 12 on road to Autazes, Castanho, Amazonas, Brazil	DQ502142	–
Allobates nidicola	INPA-H28130	Careiro, Amazonas, Brazil	JQ966867	–
Allobates nidicola	MPEG13819	Km 12 on road to Autazes, Castanho, Amazonas, Brazil	DQ502210	–
Allobates nidicola	LSUMZ16956	Km 12 on road to Autazes, Castanho, Amazonas, Brazil	EU342519	–
Allobates nidicola	LSUMZ16941	Km 12 on road to Autazes, Castanho, Amazonas, Brazil	EU342518	–
Allobates nunciatus **sp. nov.**	**INPA-H40475**	**East bank of middle Tapajós River, Itaituba, Pará, Brazil**	**MK680962**	**MK761068**
Allobates nunciatus **sp. nov.**	**INPA-H40307**	**East bank of middle Tapajós River, Itaituba, Pará, Brazil**	**MK680963**	**MK761067**
Allobates nunciatus **sp. nov.**	**INPA-H40480**	**East bank of middle Tapajós River, Itaituba, Pará, Brazil**	**MK680964**	–
Allobates nunciatus **sp. nov.**	**INPA-H40485**	**East bank of middle Tapajós River, Itaituba, Pará, Brazil**	**MK680965**	–
Allobates nunciatus **sp. nov.**	**INPA-H40487**	**East bank of middle Tapajós River, Trairão Pará, Brazil**	**MK680966**	–
Allobates nunciatus **sp. nov.**	**INPA-H40483**	**East bank of middle Tapajós River, Trairão Pará, Brazil**	**MK680967**	–
Allobates nunciatus **sp. nov.**	**INPA-H40488**	**East bank of middle Tapajós River, Trairão Pará, Brazil**	**MK680968**	–
Allobates nunciatus **sp. nov.**	**INPA-H40479**	**East bank of middle Tapajós River, Trairão Pará, Brazil**	**MK680969**	–
Allobates niputidea	MUJ3520	Reserva Natural Privada Riomanso, Magdalena, Colombia	DQ502272	DQ502703
Allobates niputidea	AJC3900	Reserva Cabildo Verde, Santander, Colombia	KP149439	KP149233
Allobates olfersioides	MTR6031	São José da Vitória, Bahia, Brazil	DQ502126	DQ502833
Allobates olfersioides	MNRJ79897	Marica, Ponta Negra, Rio de Janeiro, Brazil	MF624178	–
Allobates ornatus	MHNSM22863	17km W from Tarapoto to Moyobamba, Tarapoto, Colombia	EU342550	–

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APPENDIX 3. (Continued)

Taxon	Voucher	Locality	16S	COI
Allobates ornatus	MHNSM22646	17km W from Tarapoto to Moyobamba, Tarapoto, Colombia	EU342549	_
Allobates paleovarzensis	INPA-H29082	Barcelos, Amazonas, Brazil	JQ966862	_
Allobates paleovarzensis	INPA-H29083	Barcelos, Amazonas, Brazil	JQ966863	_
Allobates pittieri	EBRG3420	Parque Nacional Pittier, Venezuela	EU380801	EU380853
Allobates subfolionidificans	APL1691	Parque Zoobotanico, UFAC, Rio Branco, Acre, Brazil	KF250492	_
Allobates subfolionidificans	MPEG12321	Porto Walter, Acre, Brazil	DQ502134	DQ502841
Allobates sumtuosus	MTD47771	Mabura Hill Forest Reserve, Guyana	KC520682	KC520688
Allobates sumtuosus	APL16720	Reserva Biológica do Rio Trombetas, Pará, Brazil	KF250497	_
Allobates talamancae	SIUC7667	El Cope, Cocle, Panama	DQ502166	DQ502868
Allobates talamancae	OMNH33237	Rio San Juan, Nicaragua	DQ502057	_
Allobates tapajos	LSUMZ15176	Near Rio Curuá-Uma, Pará, Brazil	DQ502046	DQ502772
Allobates tapajos	INPA-H36515	Vila Rayol, Itaituba, Pará, Brazil	KX524482	_
Allobates tinae	MPEG13397	Parque Estadual Guajará-Mirim, Rondônia, Brazil	DQ502213	DQ502900
Allobates tinae	MPEG12978	Rio Ituxi, Lábrea, Amazonas, Brazil	DQ502098	_
Allobates trilineatus	KU215172	Cusco Amazônico, Madre de Dios, Peru	DQ501986	DQ502712
Allobates trilineatus	MJH7477	Panguana, Rio Llullapichis, Huanuco, Peru	DQ502118	DQ502826
Allobates undulatus	AMNHA159139	Cerro Yutaje, Amazonas, Venezuela	DQ283044	DQ502756
Allobates undulatus	AMNHA159140	Cerro Yutaje, Amazonas, Venezuela	DQ502029	DQ502757
Allobates zaparo	QCAZ16603	Via Ahuano, Jatun Sacha, Napo, Ecuador	HQ291003	_
Allobates zaparo	USNM546405	130 km S of Nuevo Golandrina, Coca, Pastaza, Ecuador	DQ502026	DQ502752
Rhaeobates palmatus	MUJ5003	Finca Tacarcuna, La Mesa, Cundimarca, Colombia	AH015828	DQ502925
Anomaloglossus stepheni	CFBHT05705	Reserva ZF-2, Manaus, Amazonas, Brazil	KU495128	KU494335

Collections: (INPA-H) Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil; (MPEG) Museu Paraense Emílio Goeldi, Belém, Pará, Brazil; (QCAZ) Museo de Zoología, Pontifica Universidad Católica del Ecuador, Quito, Ecuador; (KU) University of Kansas, Museum of Natural History, Division of Herpetology, Lawrence, Kansas, USA; (LSUMZ) Louisiana State University, Museum of Natural Science, Baton Rouge, Louisiana, USA; (SIUC) Southern Illinois University, Herpetology Collection, Department of Zoology, Illinois, USA; (OMNH) The Sam Noble Oklahoma State Museum of Natural History, Norman, Oklahoma, USA; (CVULA) Coleccion Vertebrados, Facultad de Ciencias, La Hechicera, Universidad de los Andes, Mérida, Venezuela; (MUJ) Lorenzo Uribe Museum of Natural History, Pontificia Universidad Javierana, Bogotá, Colombia; (MNRJ) Museu Nacional, Rio de Janeiro, RJ, Brazil; (MHNSM) Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Avenida Arenas, Lima, Peru; (EBRG) Museo de la Estación Biología de Rancho Grande, Maracay, Aragua, Venezuela. (MTD) Senckenberg Naturhistorische Sammlungen Dresden, Königsbrücker Landstrasse Dresden, Germany; (AMNH) American Museum of Natural History, New York, New York, USA; (UNSM) National Museum of Natural History, Division of Amphibians and Reptiles, Washington, D.C, USA; (TNHCF) Texas Memorial Museum, Texas Natural History Collection, Texas, USA; (BPN) B. P. Noonan's field series; (PG) P. Gaucher's field series; (MC) C. Marty's field series; (CFBHT) C. Haddad's field series; (APL, ABUNA and ACRE) A. P. Lima's field series; (MTR) M. T. Rodrigues' field series; (AJC) A. Crawford's field series; (PV) D. Pavan's field series.