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The description of a cryptic species related to the pan-Amazonian frog *Allobates femoralis* (Boulenger 1883) (Anura: Aromobatidae)

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Abstract

We describe a new species of litter frog from western Brazilian Amazon previously referred to as *Allobates femoralis* (Boulenger 1883). The new species is allopatric to *A. femoralis* and its known occurrence is restricted to terra-firme forests on the left bank of the upper Madeira River and southeastern State of Acre. This species is distinguished from *A. femoralis* and from other species in the *A. femoralis* group by presenting two-note advertisement calls and conspicuous reddish-orange color on ventral surfaces of hind limbs and posterior abdomen. Phylogenetic analyses based on a fragment of the 16S rRNA mitochondrial gene suggest the new species is the sister group to a clade referred to as *A. femoralis* occurring in southern State of Acre, from which it is distinguished by six unambiguous nucleotide substitutions, in addition to exclusive advertisement calls and color patterns. The new species is more distantly related to *A. femoralis* sensu stricto occurring near the *A. femoralis* type locality in the Peruvian Amazon. Summarizing evidence from molecular phylogenetic analysis, genetic distances and available data on advertisement calls, we identify one possible case of genetic introgression between lineages in this group and highlight the potential for the description of more species within the *A. femoralis* complex.

Key words: Amazonia, *Allobates hodli* **sp. nov.** , Brazil, Dendrobatoidea, *femoralis*, new species, species complex, taxonomy

Resumo

Nós descrevemos uma nova espécie de rã de folhiço para a Amazônia Brasileira ocidental, a qual foi previamente tratada como *Allobates femoralis* (Boulenger 1883). A nova espécie é alopátrica em relação a *A. femoralis* e sua ocorrência conhecida é restita a florestas de terra-firme na margem esquerda do alto rio Madeira e sudeste do Estado do Acre. Esta espécie se distingue de *A. femoralis* e de outras espécies do grupo *A. femoralis* por possuir cantos de anúncio constituídos por duas notas e coloração laranja-avermelhada na superfície ventral dos membros posteriores e abdôme posterior. Análises filogenéticas baseadas em um fragmento do gene mitocondrial 16S rRNA sugerem que a nova espécie é o grupo-irmão de um clado reconhecido como *A. femoralis* que ocorre no sul do Estado do Acre, do qual se distingue por seis substituições nucleotídicas não-ambíguas, além de padrões exclusivos de vocalizações de anúncio e de coloração. A nova espécie é evolutivamente mais distante de *A. femoralis* sensu stricto, que ocorrem próximos à localidade-tipo de *A. femoralis* na Amazônia peruana. Sumarizando evidências obtidas através de análise filogenética molecular, distâncias genéticas e dados disponíveis sobre vocalizações de anúncio, nós identificamos um possível caso de introgressão genética entre linhagens deste grupo e enfatizamos o potencial para a descrição de mais espécies dentro do complexo *A. femoralis*.

Palavras-chave: Amazônia, *Allobates hodli* **sp. nov.**, Brasil, Dendrobatoidea, *femoralis*, nova espécie, complexo de espécies, taxonomia

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Introduction

For some time researchers have pointed out that the existence of cryptic species within widespread anuran taxa could be frequent in the Amazon basin (Wynn and Heyer 2001; Azevedo-Ramos & Galatti 2002). Such suggestions now receive great support from recent work providing evidence for the existence of cryptic lineages within different families of frogs (Fouquet *et al.* 2007; Twomey & Brown 2008; Brown & Twomey 2009; Lötters *et al*. 2009; Padial & De la Riva 2009). Adding to the conservative nature of some morphological characters frequently used in taxonomic studies, the lack of extensive behavioral databases and very long distances between sampling sites compromise the diagnosis of cryptic lineages and the accurate determination of their distributions.

Allobates Zimmermann & Zimmermann (1988) is the most species-rich and widespread genus within the family Aromobatidae (Grant *et al.* 2006). Forty-four *Allobates* species are currently recognized, distributed in lowland forests from the eastern slope of the Andes, across the Amazonian lowlands of Bolivia, Colombia, Ecuador, Peru and Brazil, and reaching the Guyana Shield and Atlantic forests of Brazil (Lötters *et al.* 2007; Frost 2009). New species of *Allobates* are regularly found in the Amazonian lowlands (Lima & Caldwell 2001; Caldwell & Lima 2003; Lima *et al*. 2007) and recent species redescriptions that include behavioral, reproductive mode and larval morphology data from type locality populations (Caldwell *et al.* 2002; Lima *et al.* 2009) will likely increase the rate of species discoveries in this region. Although sampling efforts are still deficient, many of the recently described species apparently have limited distributions, and revisionary studies of currently widely distributed taxa will probably result in the discovery of many new species.

Recently, comprehensive studies of the phylogenetic relationships and evolution of the Amazonian poison-frogs (Grant *et al*. 2006; Santos *et al*. 2009) have indicated the existence of elevated genetic divergence between lineages of a ground-dwelling frog, *Allobates femoralis*, and proposed that this taxon consists in a complex of cryptic species. *Allobates femoralis* is widely distributed throughout primary, non-flooded forest areas in the Amazon Basin. During the last 30 years, several populations belonging to this taxon have been the subject of numerous studies, ranging from acoustic and visual communication (Hödl 1987; Narins *et al*. 2003; Hödl *et al.* 2004; Amézquita *et al.* 2005; 2006; Göd *et al.* 2007) to territorial and reproductive behavior (Roithmair 1992; 1994; Ringler *et al.* 2009), with numerous authors pointing out the existence of conspicuous population variation in morphology, acoustic signal detection, advertisement call characteristics, color and genetic traits (Lutz & Kloss 1952; Hödl 1987; Lougheed *et al.* 1999; Amézquita *et al.* 2006; 2009; Simões *et al*. 2008).

In this study, we aim to add to the findings reported in Simões *et al.* (2008) and Amézquita *et al*. (2009) on the acoustic, morphological and genetic differentiation of a geographically restricted group found in southwestern Brazilian Amazon that presents a two-note advertisement call, previously referred to as *Allobates femoralis*. This group is allopatric (and in two instances, parapatric) to populations of *Allobates femoralis* that resemble that from type locality in call characteristics, color pattern and morphology. The locations of two contact zones between these lineages are provided herein. We describe this group as a new species, presenting detailed information on morphology, behavioral traits, geographic distribution, as well as phylogenetic and genetic differentiation data based on mitochondrial DNA. Additionally, we use available mtDNA sequences and records of advertisement calls to explore the relationships between the new species and other populations referred to as *A. femoralis*, identifying cryptic lineages that might be potential subjects for future taxonomic investigation.

Material and methods

Specimens described here were deposited in the herpetology section of the zoological collection of Instituto Nacional de Pesquisas da Amazônia (INPA-H), in Manaus, Brazil, coming from field work carried out in four localities in the extreme southeast of the State of Acre (in January 2003) and along the left bank of the upper Madeira River (from November 2004 to February 2005) in northern state of Rondônia (Fig. 1). Specimens were collected as part of studies addressing the geographic variation in populations of the group *Allobates* *femoralis*. Complementary information on field procedures and more comprehensive data on the study area can be found in Simões *et al*. (2008) and Amézquita *et al*. (2009).

We examined and measured all specimens in the laboratory using a digital caliper or a micrometer on a dissecting microscope to the nearest 0.01 mm. Measurements and terminology, as well as diagnostic characters, followed Lima *et al.* (2007). Some diagnostic characters were included following Grant *et al.* (2006) and Lötters *et al.* (2007). Measurements were: snout to vent length (SVL), head length from tip of snout to posterior edge of maxilla articulation (HL), head width at the level of maxilla articulation (HW), snout length (SL), eye-to-nostril distance from anterior corner of the eye to the center of nostril (EN), internarial distance (IN), eye length from anterior to posterior corner (EL), interorbital distance (IO), maximum diameter of tympanum (TYM), forearm length from proximal edge of palmar tubercle to outer edge of flexed elbow (FAL), lengths from proximal edge of palmar tubercle to tips of fingers I, II and III (HAND I, HAND II, HAND III), width of disk on Finger III (WFD), thigh length from the posterior extremity of the coccyx to the outer edge of flexed knee (THL), tibia length from outer edge of flexed knee to heel (TIL), foot length from proximal edge of outer metatarsal tubercle to tip of Toe IV (FL), width of disk on Toe IV (WTD). Additionally, we measured arm length from anterior corner of arm insertion to the outer edge of flexed elbow (AL), the length from proximal edge of palmar tubercle to tip of Finger IV (HAND IV) and tarsus length from heel to the distal edge of inner metatarsal tubercle (TAR).

Descriptions of color in life were based in direct observation of specimens during field work and photographs by A. P. Lima and Walter Hödl.

Four tadpoles were used for description. These tadpoles were obtained from a clutch collected in the locality of Abunã on 15 January, 2005. Tadpoles were raised in laboratory until stage 36 of Gosner (1960), anesthetized in a solution of lidocaine and preserved in 10% formalin on 28 January, 2005. Measurements and terminology for description of tadpoles follow McDiarmid and Altig (1999). Measurements were: 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 spiracle level (BW), body height at spiracle level (BH), tail muscle maximum width (TMW), tail muscle maximum height (TMH), tail maximum height (TH), head width at the level of the eyes (HWLE), interorbital distance (IOD) and internostril distance (IND).

We recorded advertisement (n = 60) and courtship (n = 4) calls of males collected in the localities along the upper Madeira River (Cachoeira do Jirau, Mutum-Paraná and Abunã) using a Sony WM-D6C tape recorder (2004, Sony Corr., Japan) and AKG 568 EB directional microphone (2003, AKG acoustics GMBH, Austria), positioned approximately 1 m away from the calling individual. All recordings were made at 06:30-18:00 h and air temperature at the moment of recording was registered. Recordings were digitized from tapes using Raven 1.2 software (Charif *et al.* 2004) at a sample rate of 22050 Hz and 16 bits sample format.

From the recording of each individual, we sampled three advertisement calls from which we measured spectral and temporal parameters, according to procedures described in Simões *et al.* (2008). Measurements were: silent interval between calls (SIC), silent interval between first and second note (SIN), duration of call (DC), duration of first note (D1), duration of second note (D2), maximum frequency of call (MFC), highest frequency of call (HFC), lowest frequency of call (LFC), maximum frequency of first note (MFN1), highest frequency of first note (HFN1), lowest frequency of first note (LFN1), maximum frequency of second note (MFN2), highest frequency of second note (HFN2), lowest frequency of second note (LFN2). Courtship calls were recorded opportunistically during the recording of advertisement calls, and the number of calls obtained from a total four individuals varied. Therefore, measurements (DC, MFC, and number of pulses) were obtained from a single call or from all available calls. In the latter case, values presented are the averages among all available calls.

Samples of muscle and liver tissue preserved in 95% ethanol were obtained from individuals collected in the three localities along the upper Madeira River (Cachoeira do Jirau, Mutum-Paraná, Abunã, Fig. 1) and were housed at Coleção de Tecidos de Genética Animal at Universidade Federal do Amazonas (CTGA – ICB/UFAM), Manaus, Brazil. Additional tissue samples were obtained from populations referred to as *A. femoralis* in 10 other localities in Brazilian Amazonia (Fig. 1). Two of these populations (Monte Alegre, Lower Jirau, Fig. 1) are located immediately outside contact zones with the species described herein.

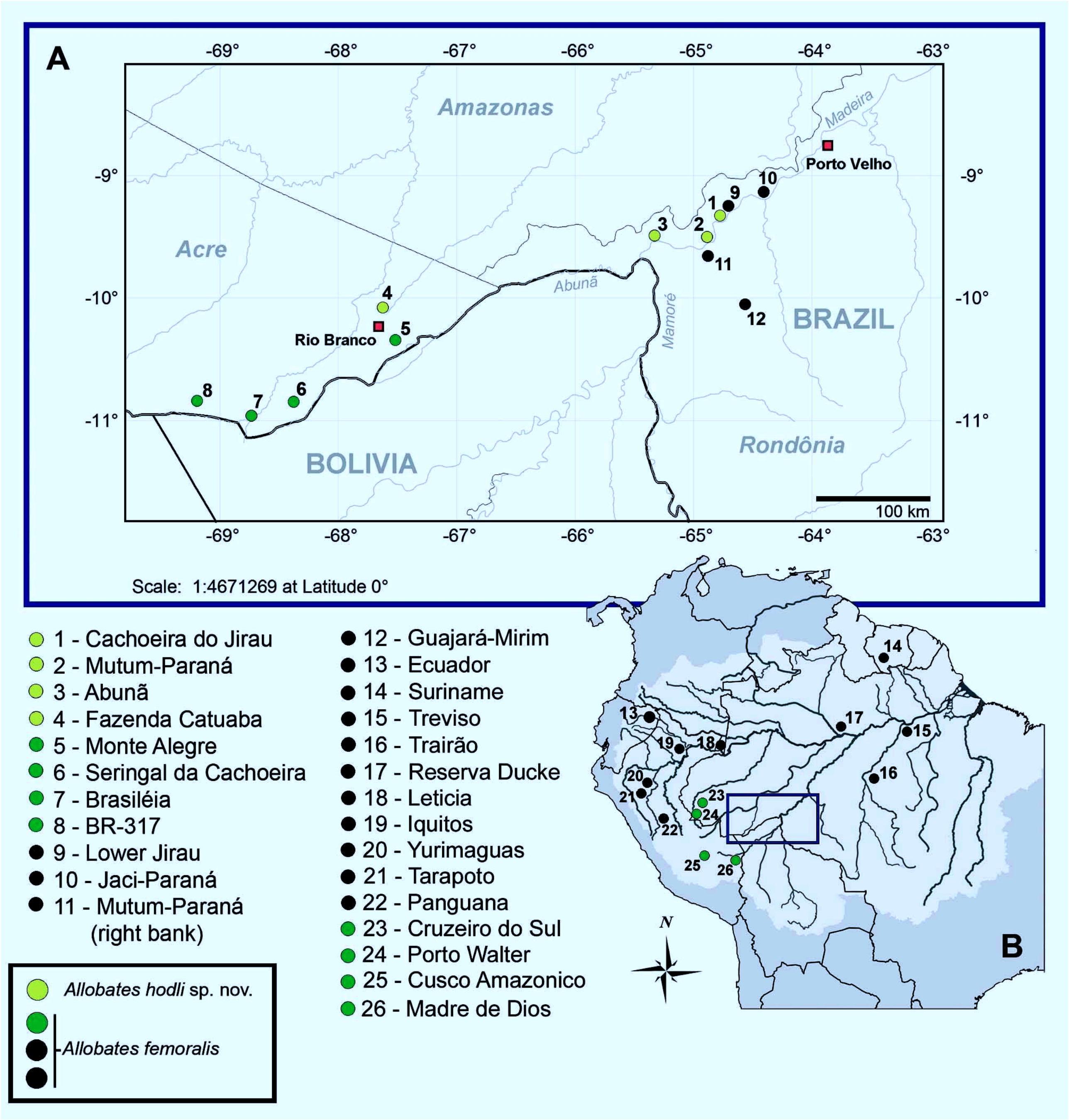

FIGURE 1. Relative location and denomination of (A) sampling sites in the Brazilian States of Acre and Rondônia, and (B) sampling sites and locations from where *Allobates femoralis* 16S rRNA mtDNA reference sequences were available in the Amazon Basin (shaded in paler gray). Yellow dots represent the distribution of *Allobates hodli* **sp. nov.** Light-green, dark-green, and black dots represent localities of samples referred to as *A. femoralis*, including two sites (5 and 9) where *A. hodli* reaches contact zones with these populations. Dot colors stand for major lineages recovered by phylogenetic analysis of a partial sequence of the 16S rRNA mitochondrial gene (see text and Fig. 7). Site 20, Yurimaguas, is considered the closest to *A. femoralis* type locality.

Total genomic DNA extraction was carried out from samples using cetyl trimethyl ammonium bromide (CTAB) protocol (modified from Doyle & Doyle 1987). We used primers 16Sar and 16Sbr (Palumbi 1996) to amplify a 518 b.p. partial sequence of the 16S rRNA mitochondrial gene via polymerase chain reaction (PCR) from total genomic DNA. PCR reactions used a final volume of 16 µL and contained 6.7 µL ddH$_2$O, 2.0 µL of 25 mM MgCl$_2$, 1.5 µL of 10 mM dNTPs (2.5mM each dNTP), 1.5 µL of 10X amplification buffer (75 mM

Tris HCl, 50 mM KCl, 20 mM $(NH_4)2SO_4$), 1.5 µL of a 2 µM solution of each primer, 0.3 µL of Taq DNA Polymerase 5 U/µL (Biotools, Spain) and 1 µL of DNA (about 30 ng/µL). PCR conditions had a pre-heating step of 92°C for 60 s, followed by 35 cycles of denaturation at 92° for 60 s, primer annealing at 50°C for 50 s and primer extension at 72°C for 90 s. A final extension step occurred at 72°C for 5 min. Sequencing reactions were performed according to manufacturer's recommended ABI BigDye Terminator Cycle Sequencing protocol, using primer 16Sbr and an annealing temperature of 50°C. Sequencing was performed in an automatic ABI 3130xl Sequencer.

Sequences were aligned using the ClustalW algorithm (Thompson *et al.* 1994) implemented in BioEdit (Hall 1999) and checked by eye. Final data set included 72 sequences of the new species (27 of topotypic individuals from Cachoeira do Jirau), plus 96 additional sequences of *A. femoralis* from the additional 10 sampling sites, as well as 28 sequences from reference *A. femoralis* populations (Fig. 1, Table 5). Reference sequences included one sequence from a locality close to Yurimaguas, Loreto (collected at Shucshuyacu, 20 km from *A. femoralis* type-locality), one sequence from Tarapoto, San Martin, (130 km from *A. femoralis* type-locality) and one sequence from Panguana (400 km from *A. femoralis* type-locality), all in Peru. To date, Sucshuyacu (site 12 in Fig. 1B), near Yurimaguas, is considered the site closest to the *A. femoralis* type-locality from where DNA sequences were made available. Other reference sequences include individuals sampled in Ecuador, Colombia, Suriname and other sites in Peruvian and Brazilian Amazon. *Aromobates nocturnus*, *Anomaloglossus stepheni*, *Allobates talamancae*, *Allobates nidicola* and *Allobates zaparo* were used as outgroups. The first four taxa are considered basal to *A. femoralis*, and *A. zaparo* is considered its sister species (Grant *et al.* 2006; Santos *et al.* 2009). Reference and outgroup sequences were all obtained from GenBank (Table 5). Uncorrected pairwise genetic distances between groups were calculated in MEGA (Tamura *et al.* 2007). Data set was reduced to unique haplotypes (including outgroups) for phylogenetic analysis. Phylogenetic analysis was performed in Treefinder (Jobb 2008) under the Maximum Likelihood criterion with GTR+I+G model of substitution, selected via Akaike information criterion as implemented in Modeltest 3.7 (Posada & Crandall 1998).

Natural history observations were made opportunistically during field work by P. I. Simões and A. P. Lima. Additionally, stomachs of 81 preserved individuals from the three localities along the upper Madeira River were dissected under stereoscopic microscope for a brief analysis of diet. Prey items were identified to order and quantified as simple frequencies (number of stomachs containing item / total non-empty stomachs examined).

***Allobates hodli* sp. nov**

Figures 2–5.

Epipedobates femoralis Hödl *et al.* 2004 p. 823, Catuaba, Acre population (partim).

Allobates femoralis Amézquita *et al.* 2006 p. 1877, Catuaba, Acre population (partim); Lötters *et al.* 2007 p. 307, Fig. 379; Simões *et al.* 2008 p. 610, Fig. 2B. (partim); Amézquita *et al.* 2009, Fig. 1, Catuaba pattern (partim).

Holotype. INPA–H 16555 (original field number APL 2014). Adult male, collected by P. I. Simões and A. P. Lima after recording of advertisement calls at 07:55 h, 25th of November 2004, at Cachoeira do Jirau, on the left bank of the upper Madeira River (09.3347° S, 64.7375° W), approximately 125 km upstream from the city of Porto Velho, Estado de Rondônia, Brazil.

Paratopotypes. INPA-H 16541–16554, INPA-H 16556–16569 (original field numbers APL 2000–2013, 2015–2018, 2022–2030, 2032), 6 females, 22 males. Collected in the same locality as holotype, 23-25 November 2004 by P. I. Simões and A. P. Lima.

Paratypes. All from Brazil. **Acre:** INPA-H 11621–11640, , 4 females, 17 males, Fazenda Catuaba, Municipality of Rio Branco, 10.0742° S, 67.6249° W, collected in February 2004 by A. P. Lima. **Rondônia:** 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, 13 females, 26 males, collected on the left bank of the upper Madeira River, across the river from the village of

Fortaleza do Abunã, 160 km upstream from the city of Porto Velho, 72 km upstream from Cachoeira do Jirau, 9.5160° S, 65.3249° W, collected 05-08 January 2005 by P.I. Simões and A.P. Lima. INPA-H 16596, 16730, 16739, 16756, 16758, 16767, 16771, 16777–16778, 16788, 16805, 16818–16819, 2 females, 11 males, collected on the left bank of the upper Madeira River, across the river from the village of Mutum-Paraná, 121 km upstream from the city of Porto Velho, 34 km upstream from Cachoeira do Jirau, 9.5732° S, 64.9211° W, collected 10–13 January 2005 by P. I. Simões and A. P. Lima.

Etymology. The specific epithet is a patronym for Dr. Walter Hödl, an Austrian biologist and professor who pioneered research on behavior and acoustic communication in anurans. For the past two decades, Walter and his students have dedicated special attention to the *Allobates femoralis* complex.

Diagnosis. The new species is assigned to the genus *Allobates* by the combination of the following characters: presence of a pale dorsolateral stripe, dorsal skin texture granular posteriorly, basal webbing present only between Toes III and IV, Finger I longer than Finger II, finger discs generally weakly expanded (moderately expanded on Finger I), median lingual process absent, testes not pigmented, dark collar absent on throat, oral disc of tadpoles emarginate, not umbelliform. *Allobates hodli* is distinguished in life from all other species of *Allobates* (except *Allobates femoralis*, *Allobates myersi* and *Allobates zaparo*) for presenting relatively large body-size (average SVL = 24.76 ± 1.08 mm, males and females pooled), by the lack of brown or light-brown colors or patterning on dorsum and lateral surface of body, and by presenting dark and white marbling on anterior ventral surface of body, replaced by solid reddish-orange color on the ventral surface of hind limbs.

Allobates hodli is distinguished from other taxa and morphotypes that form the *A. femoralis* complex by presenting advertisement calls consisting of groups of two notes repeated in series or bouts (instead of groups of one, three or four notes), and by presenting a conspicuous reddish-orange coloration on the ventral surface of legs, instead of an exclusively black and white reticulated pattern, observed in *A. femoralis*. *Allobates hodli* also has diffuse reddish-orange and black patches on dorsal surface of thighs, as opposed to regular, pale (yellowish to red) longitudinal flash marks extending onto the entire dorsal surface of thighs, generally margined by dark patches, observed in typical *A. femoralis*.

A. hodli is distinguished from *A. zaparo* and *A. myersi* by the color of dorsum, which is uniformly black/dark-brown in *A. hodli* (Fig.2), but reddish in *A. zaparo* and brown to light-brown in *A. myersi*. *Allobates myersi* also lacks a pale dorsolateral stripe.

A. hodli is largely sympatric to *Ameerega picta*, a dendrobatid frog that presents similar body size and color pattern. However, *A. hodli* can be distinguished from *Ameerega picta* by lacking a bright (orange to red) flash mark on calf region.

Description of holotype. Morphological measurements of holotype are presented in Table 1. Body robust, head slightly wider than long (HL/HW = 0.94) (Fig. 3A). Eye diameter slightly larger than distance from nostril to anterior corner of the eye. Nares located posterolaterally to tip of snout, directed posterolaterally, visible in ventral and anterior view. Center of nostril not visible dorsally. Canthus rostralis convex from tip of snout to nostril, straight from nostril to anterior corner of the eye. Loreal region vertical. Tympanum well visible, with maximum diameter horizontal, corresponding to 44% the maximum diameter of the eye. Maxillary teeth present. Tongue length twice as large as wide, attached anteriorly on first third. Median lingual process absent. Choanae round. A single vocal sac is present, corresponding to most of the area of the medial and posterior subgular region. Vocal sac round when expanded. When retracted, vocal sac forms two lateral slits at the level of maxilla articulation (Fig. 3, B).

Skin granular on dorsum and dorsal surface of legs. Granules round, more developed on dorsal surface of urostyle region and shanks. Skin smooth ventrally and laterally. Dermal flap above cloaca absent.

Palmar tubercle slightly triangular. Thenar tubercle well-developed, oval to elliptic, maximum diameter 1.28 times smaller than maximum diameter of palmar tubercle. Subarticular tubercles of Fingers II, III and IV are round, small, never exceeding the width of phalanges. Subarticular tubercle of Finger I elliptic, 1.21 times larger than thenar tubercle in maximum diameter. Supernumerary tubercles absent. Carpal pad and metacarpal ridges absent on hands. No fringes or webbing on fingers. A distal tubercle on finger IV is weakly developed (Fig. 3C). Finger I is slightly (1.08 times) longer than Finger II. Length of finger IV does not reach distal subarticular tubercle of finger III when fingers are pressed against each other. Relative lengths of fingers: IV <

II < I < III. Finger III not swollen. Disc of Finger I moderately expanded, edges of disk corresponding approximately to width of digital shaft, disc width 1.37 times the width of adjacent phalange. Discs of Fingers II, III and IV weakly expanded, edges of discs corresponding approximately to half or less than half width of digital shafts, 1.26, 1.32 and 1.39 times the width of adjacent phalanges, respectively.

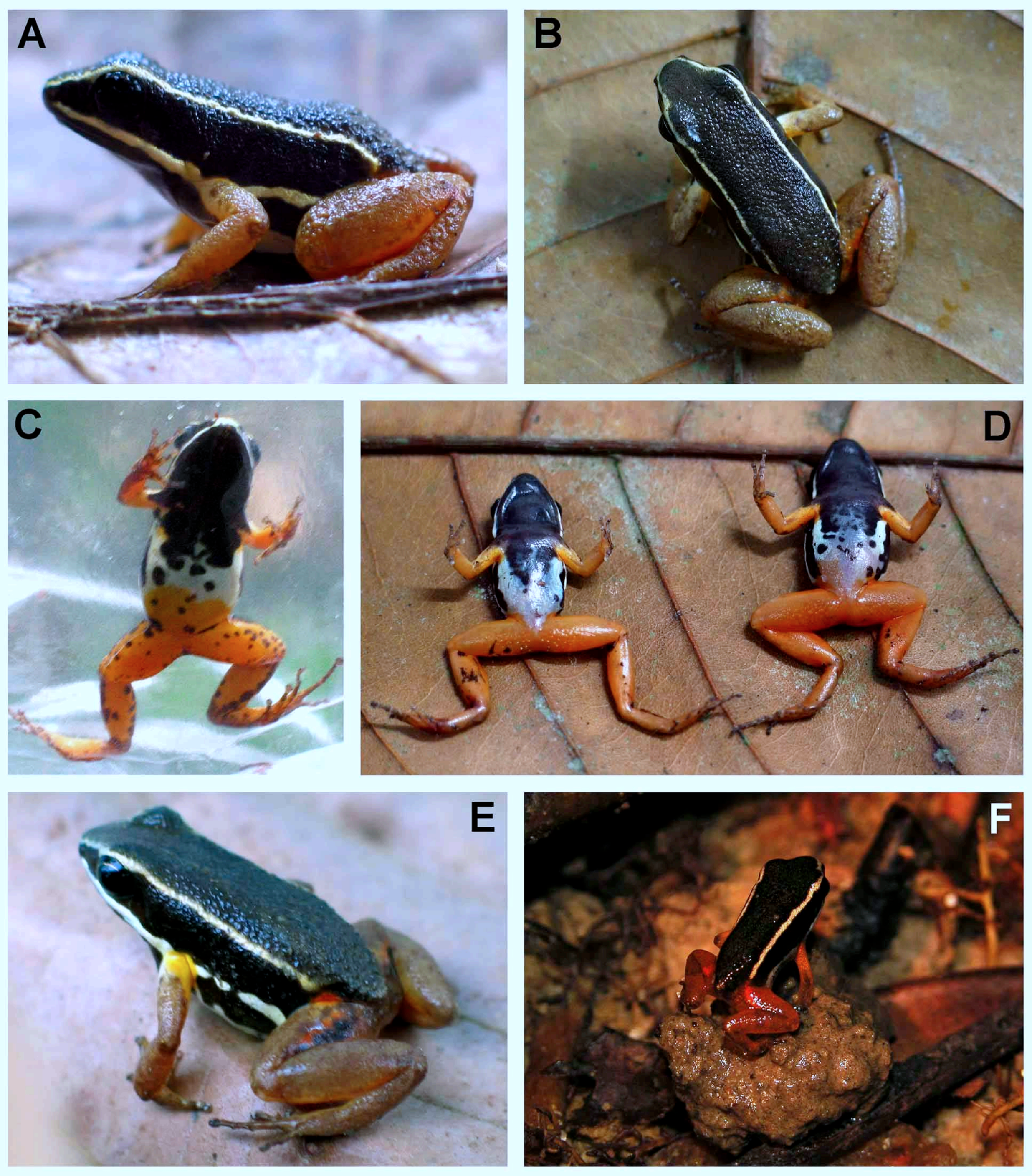

FIGURE 2. Color in life of *Allobates hodli* **sp. nov.** (A) Lateral view of and adult male from Abunã, in Rondônia. (B) Dorsal view of a male from Cachoeira do Jirau, Rondônia. (C) Ventral view of an adult male from Fazenda Catuaba, in Acre, photographed through a transparent plastic bag. Note bright reddish–orange color of posterior abdomen and ventral surface of legs. (D) Ventral view of a male (left) and a female (right) from Cachoeira do Jirau. (E) Dorsolateral view of a male from Cachoeira do Jirau. Note irregular reddish-orange and black blotches and spots on dorsal surface of thighs and bright yellow flash marks on upper arms. (E) Juvenile from Abunã, photographed in laboratory after completion of metamorphosis. Photos A–C taken under natural light conditions. Photos A, B, D and E taken in July 2004; C in January 2003; A–E by Walter Hödl. F taken in February 2005 by A.P. Lima.

Length of shank corresponding to 48% of snout-to-vent length (Table 1). Tarsal keel is tubercle-like, strongly curved at its proximal end, flattening towards the metatarsal tubercle. Metatarsal fold evident (but not folding over itself) running from the base of Toe V towards metatarsal tubercle, but not reaching it. Preaxial edge of tarsus smooth, with no fringe. Basal webbing present only between Toes III and IV, and II and III. Relative lengths of toes: I < II < V < III < IV (Fig. 3D). Disc of Toe I weakly expanded, edges of disc corresponding to less than half the width of digital shaft, disk width 1.25 times the width of adjacent phalange. Discs of toes II, III, IV and V moderately expanded, edges of disks corresponding approximately to width of their respective digital shafts, width of discs 1.54, 1.44, 1.52 and 1.46 times the width of adjacent phalanges, respectively.

TABLE 1. Measurements (in mm) and proportions of *Allobates hodli* holotype (INPA-H 16555) and type series. Males and females present size dimorphism, females generally larger than males. Values in type series columns represent mean ± standard deviation (minimum value observed in the series – maximum value observed in the series).

		Type series	
Measurements	Holotype	Males (*n* = 76)	Females (*n* = 25)
SVL	23.99	24.41 ± 1.13 (22.2–27.3)	25.54 ± 1.05 (23.6–28.1)
HL	7.52	8.19 ± 0.49 (7.3 – 9.7)	8.54 ± 0.34 (7.9–9.4)
HW	8.01	7.84 ± 0.56 (4.5–9.1)	8.11 ± 0.31 (7.5–8.6)
SL	4.00	4.16 ± 0.50 (2.0–5.0)	4.49 ± 0.50 (3.2–5.5)
EN	2.30	2.36 ± 0.35 (1.9–3.1)	2.51 ± 0.28 (1.9–3.1)
IN	3.90	3.74 ± 0.22 (3.1–4.2)	3.89 ± 0.19 (3.5–4.2)
EL	2.70	2.94 ± 0.24 (2.0–3.4)	3.07 ± 0.23 (2.4–3.6)
IO	7.30	7.70 ± 0.38 (7.0–8.6)	7.96 ± 0.37 (7.2–8.8)
TYM	1.20	1.49 ± 0.15 (1.1–1.9)	1.60 ± 0.18 (1.2–2.0)
AL	5.23	5.26 ± 0.61 (4.11–6.83)	5.25 ± 0.93 (4.4–9.0)
FAL	6.46	6.23 ± 0.48 (5.0–7.1)	6.30 ± 0.46 (4.9–7.1)
H1	5.33	5.16 ± 0.32 (4.4–6.0)	5.24 ± 0.36 (4.5–6.0)
H2	4.91	4.61 ± 0.31 (3.7–5.7)	4.60 ± 0.28 (4.0–5.3)
H3	6.12	6.08 ± 0.31 (5.3–6.8)	6.10 ± 0.30 (5.6–6.9)
H4	4.54	4.21 ± 0.29 (3.6–4.9)	4.14 ± 0.35 (3.5–4.9)
WFD	0.80	0.79 ± 0.08 (0.6–0.9)	0.77 ± 0.07 (0.6–0.9)
THL	11.02	10.78 ± 0.67 (7.5–12.6)	10.47 ± 1.29 (5.5–12.1)
TIL	11.46	11.22 ± 0.52 (8.2–12.0)	11.29 ± 0.62 (9.2–12.3)
TAR	7.51	6.73 ± 0.63 (4.3–10.0)	6.74 ± 0.40 (5.9–7.6)
FL	9.95	10.33 ± 0.79 (7.3–11.5)	10.42 ± 0.65 (8.4–11.4)
WTD	1.10	1.05 ± 0.10 (0.8–1.2)	1.05 ± 0.09 (0.8–1.3)
HL/SVL	0.31	0.34 ± 0.02 (0.29–0.39)	0.33 ± 0.01 (0.30–0.36)
HW/SVL	0.33	0.32 ± 0.02 (0.18–0.36)	0.32 ± 0.01 (0.30–0.34)
TL/SVL	0.48	0.46 ± 0.02 (0.34–0.50)	0.44 ± 0.02 (0.37–0.48)
TYM/EL	0.44	0.51 ± 0.06 (0.39–0.65)	0.52 ± 0.06 (0.41–0.64)
ENA/EL	0.85	0.81 ± 0.12 (0.63–1.35)	0.82 ± 0.12 (0.72–1.17)

Variation in type series. Morphological measurements of individuals constituting type series are presented in Table 1. Morphological characters described for the holotype apply to all individuals in type series, except for the following: Males slightly smaller (4.42%, in average) than females. Head slightly longer than wide in males (HL/HW = 1.04) and females (HL/HW = 1.05) in average. Maximum diameter of tympanum corresponding to approximately half the maximum diameter of the eye in males and females (Table 1). Vocal sac and slits absent in females.

Palmar tubercle round to slightly triangular. A distal tubercle on finger IV is present in 28 of a total 83 (34.1%) inspected specimens, but is absent or weakly developed in the remaining 54 specimens (65.9%).

Color in life. Males and females do not present dimorphism in relation to color and color pattern. Dorsal surface of body solid black to solid dark-brown (Fig. 2B). Lateral surface of body solid black. Dorsolateral line white, thinner than lateral line (Fig. 2A, 2E). When continuous with flash marks on thighs, dorsolateral line becomes reddish-orange on groin. Lateral line white. Gular region solid black to dark bluish-gray in males and females (Fig. 2D). In males, vocal sacs usually with a paler bluish-gray color when inflated. Mid abdomen white with irregular black to dark-gray blotches or speckling, merging with solid dark color of gular region. Abdomen bright reddish-orange posteriorly, with dark irregular spots appearing marginally from lateral edges. Ventral surfaces of hind limbs also bright reddish-orange, sometimes with small marginal dark spots (Fig. 2C, 2D). Plantar surface of feet brown. Ventral surfaces of arms bright reddish-orange, with bright yellow flash marks extending from dorsal surface of upper arms. Black to dark-gray spot ventrally on upper arm, at the point of body insertion, continuous with gular region pattern. Dorsal surfaces of posterior and anterior limbs reddish to brick-brown (Fig. 2B, 2E). Dorsal and rear surfaces of thighs with irregular bright reddish-orange flash marks or patterning, same color as ventral surfaces of legs, with irregular black or dark-brown blotches or spots. Granules on dorsal surface of shanks usually darker than overall color of shanks. A yellow flash mark is present dorsally on upper arms, at the point of body insertion (Fig. 2B, 2E). The iris is evident, with metallic yellowish-brown pigmentation.

Color in life of juveniles. Color of juveniles after metamorphosis is the same of adults. Dorsum and flanks are solid black to dark-brown, with dorsolateral and lateral lines white and conspicuous. Limbs generally reddish-brown. Bright yellow flash marks are present dorsally on the upper arms, and may reach the elbow. Dorsal surface of thighs with conspicuous longitudinal bright reddish-orange flash marks, lacking black or dark-brown blotches or spots (Fig. 2F).

Color in alcohol of holotype. Dorsum is solid black to dark-brown. A thin, pale white dorsolateral line is present, continuous from groin at hind limb insertion, over the orbit and nostril, to the tip of snout (Fig. 3A). A pale white lateral line is present, broader than dorsolateral stripe, running from groin, over the insertion of arm, below nostril, to tip of upper lip. Lateral stripe is continuous on both sides of the body. Lateral surface between dorsolateral and lateral stripe solid black. Color of gular region and throat is solid black to dark-brown. Abdomen color is white with irregular black blotches or speckling. Abdomen color becomes solid black/dark-brown from chest towards the gular region. The black/dark-brown speckling over white background pattern is replaced posteriorly by a solid pale-tan pattern, continuous with the ventral color pattern of hind limbs (Fig. 3B).

Arms uniformly very pale-brown in dorsal view, paler on the axilla and carpal/metacarpal regions. Irregular dark blotches appear on dorsal surfaces of tarsus and fingers. Arms uniformly pale white to pale tan in ventral view, with a black patch (continuous with color pattern of gular region) on anterior surface of the arm. Surface of outer lateral edge of forearm and metacarpal region same color as dorsal surface of arms, extending laterally from elbow and reaching the palm of hands and ventral surface of fingers (Fig. 3C). Legs are pale brown in dorsal view. Irregular pale, unpigmented patches, as well as irregular black blotches and spots are present on dorsal and rear surfaces of thighs (Fig. 3A). The area immediately around vent is darker than the overall surface of thighs. Dorsal surfaces of shanks with darker granules. Inner dorsal surface of tarsal region is lighter than overall pattern of legs. Toes are generally darker than tarsal region in dorsal view. Ventral surface of legs is uniformly pale-tan with small brown spots appearing marginally from outer edges. Ventral surfaces of tarsal region and toes darker, same color as dorsal surface of legs (Fig. 3B, 3D). Tongue is cream-colored; large intestine (removed for the analysis of diet) is unpigmented. Testes are unpigmented.

Color variation in type series. Color in alcohol described for the holotype apply to all individuals in type series, except for the following: Lateral stripe is usually continuous, but can be interrupted in some individuals, on one or on both sides of body. Gular region and throat solid black in females, solid black to dark-brown in males. Mature oocytes are pigmented, with black pigment concentrated on animal pole.

Description of tadpoles. Tadpole measurements were obtained from four tadpoles in developmental stage 36 (Table 2). Tadpoles correspond to a lot under the same collection number, INPA-H 23693. The largest tadpole (TL = 24.7 mm, Fig. 4) was used for detailed description.

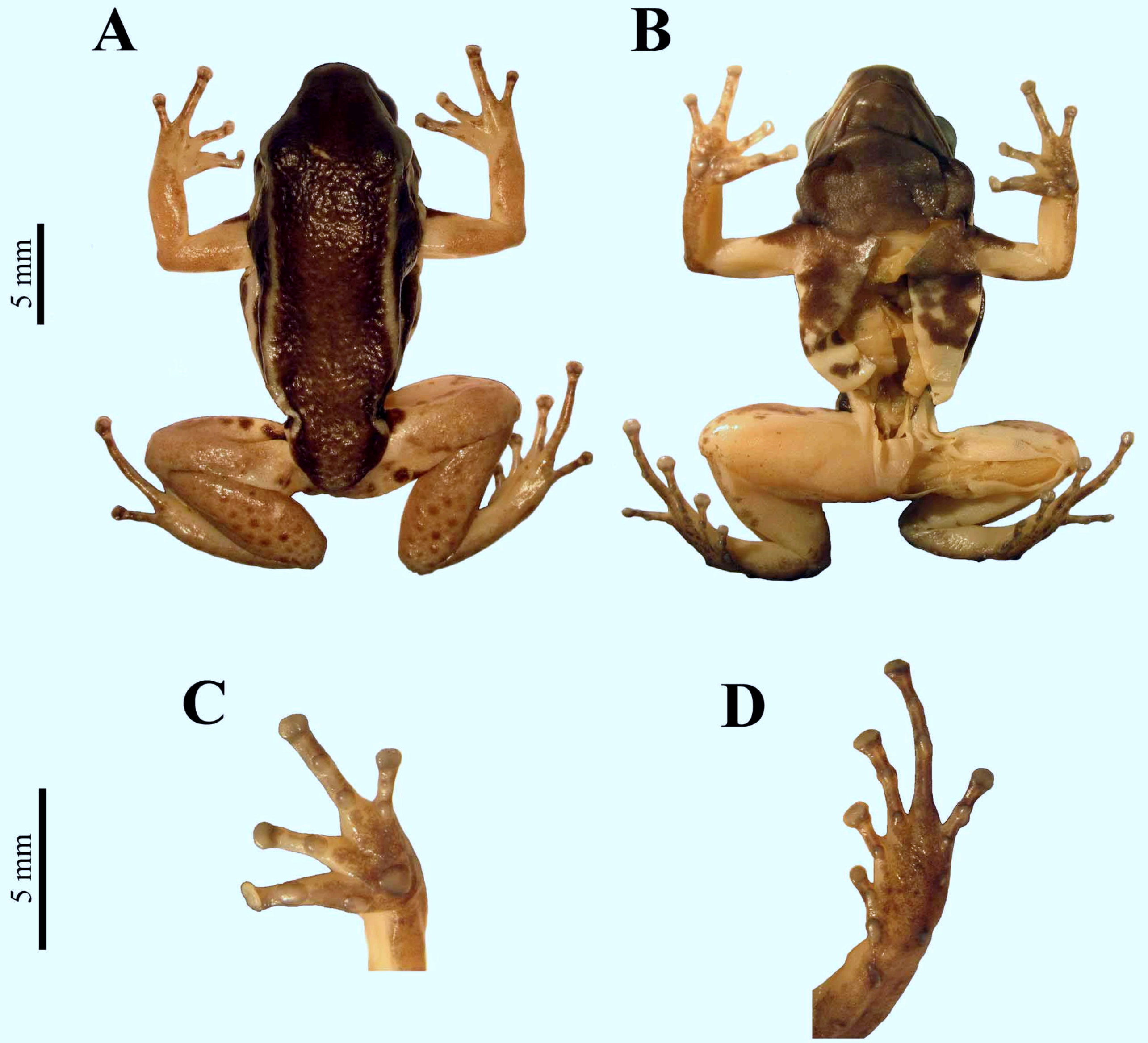

FIGURE 3. (A) Dorsal and (B) ventral views of *Allobates hodli* holotype (INPA-H 16555), a male collected at Cachoeira do Jirau in November, 2004. This individual lacks the disgestive tract and liver, removed for diet and genetic analyses respectively. (C) and (D) Hand and foot of *A. hodli* holotype.

Body is depressed, body width (6.0 mm) larger than body depth (4.8 mm), body length 16.1 mm. Snout nearly round, flattened anterodorsally in lateral view (Fig. 4C). Tip of snout flattened anteriorly in dorsal view (Fig. 4A). Nares small, directed anterolaterally, located 0.8 mm anterior to the eye, and 1.0 mm posterior to tip of snout. Nostrils narrowly spaced, distance between nostrils 0.9 mm. Eyes dorsal, directed dorsolaterally, 0.9 mm in maximum length, located 1.8 mm posterior to tip of snout. Distance between medial margins of the eyes is 1.4 mm. Spiracle single, sinistral, forming a free tube opening posterodorsally below body axis in lateral view, 5.0 mm posterior from tip of snout (Fig. 4C). Vent tube medial, free, 0.9 mm in length, opening dextrally.

FIGURE 4. (A) Dorsal view of preserved *Allobates hodli* tadpole in developmental stage 36 collected at Abunã, on the left bank of the upper Madeira River, in Rondônia, Brazil, on January 2005 (INPA-H 23693). (B) and (C) Ventral and lateral views of the same tadpole, respectively.

FIGURE 5. Oral disk of *Allobates hodli* tadpole from Abunã (INPA-H 23693).

Tail musculature reaches maximum depth (2.4 mm) approximately at the end of first third of tail length, and maximum width at body-tail insertion (2.6 mm). Ventral tail fin originates at body-tail insertion. Dorsal tail fin originates slightly posterior (0.8 mm) to body-tail insertion, and reaches maximum high 14.5 mm from tip of snout, corresponding to the region of maximum tail depth. At maximum depth of tail, depth of musculature is 1.4 mm, dorsal fin 1.5 mm and ventral fin 1.1 mm.

Oral disc is positioned anteroventrally, emarginate laterally, transversely elliptical, 2.6 mm in transverse width. Anterior labium continuous with snout, 2.6 mm in length. Marginal papillae absent dorsally on anterior labium (gap 1.7 mm, 74% of total anterior labium length), present only laterally, on its outer margins. Posterior labium free from body wall, 2.4 mm in length, with a single row of marginal papillae. All papillae with rounded tips (Fig. 5).

Labial tooth row formula is 2(2)/3(1). Rows A-1 and A-2 with same length (2.1 mm), A-2 with a large medial gap (0.6 mm). Rows P-1, P-2 and P-3 with same length (2.0 mm), P-1 presenting a very narrow medial gap (< 0.1 mm), best evidenced by a break between subjacent tooth ridges. Upper jaw sheath arch-shaped, 1.1 mm in length (42 % of oral disk width), 0.1 mm in width. Cutting edge serrate, with serrations not extending to lateral process of the upper jaw. Lower jaw sheath deeper than upper jaw, V-shaped, 0.8 mm in length, with serrate cutting edge (Fig. 5).

Color in preservative is dark to light tan. Body is darker than tail, with scattered brown melanophores in higher densities on anterior dorsum. High concentrations of melanophores also appear posteriorly on dorsum at the top and on flanking regions of tail muscle insertion (Fig. 4A). Melanophores are evenly distributed on anterior ventral surface of body. Posterior ventral surface of body is transparent, not pigmented, and intestines are clearly visible through skin (Fig. 4B). Tail musculature is light tan, tail fins transparent, with scattered brown melanophores forming irregular blotches on tail surface (Fig. 4C).

Advertisement and courtship calls description and variation. Advertisement calls of *Allobates hodli* consist in trills of calls formed by two whistle-like notes with ascending frequency modulation (Fig. 6A, 6C). Measurements of advertisement call characteristics of holotype and average values for 60 males from type series are presented on Table 3. The average maximum frequency of calls within type series is 3425.0 ± 184.7 Hz, and average duration of calls (summed durations of first and second notes, and inter-note silent interval) 0.164 ± 0.011 s. First note is less modulated (average difference between lower and higher frequency = 470.7 ± 94.8 Hz) than the second note (average difference between lower and higher frequency = 740.7 ± 115.0 Hz) and shorter in duration (0.033 ± 0.004 s, in comparison to second note, 0.056 ± 0.007 s).

Courtship calls of *A. hodli* are quite distinct from their advertisement calls and are constituted by a continuous pulsed tone (Fig. 6D), emitted only in the presence of females near the male’s calling perch. Average maximum frequency and duration of courtship calls obtained from calls of four individuals were 3190.2 Hz and 0.571 s, respectively (Table 4). The average pulse emission rate between calls was 125.2 pulses per second.

TABLE 2. Measurements (in mm) of four *A. hodli* tadpoles (INPA-H 23693) in developmental stage 36 of Gosner (1960) raised in laboratory from an egg clutch collected in Abunã, on the left bank of the upper Madeira River, in Rondônia, Brazil.

	Individuals				
Measurements	I	II	III	IV	X ± *s.d.*
TL	24.7	23.3	21.4	21.4	22.7 ± 1.6
BL	16.1	14.6	13.2	13.1	14.3 ± 1.4
TAL	16.1	14.6	13.2	13.1	14.3 ± 1.4
BW	6.0	6.3	6.2	5.5	6.0 ± 0.4
BH	4.8	4.1	4.5	3.9	4.3 ± 0.4
TMW	2.6	2.5	2.4	2.1	2.4 ± 0.2
TMH	2.4	2.1	2.1	2.2	2.2 ± 0.1
TH	4.5	4.0	4.0	3.6	4.0 ± 0.4
HWLE	5.6	5.3	5.4	5.2	5.4 ± 0.2
IOD	1.4	1.5	1.5	1.4	1.5 ± 0.1
IND	0.9	0.9	0.8	0.8	0.9 ± 0.1

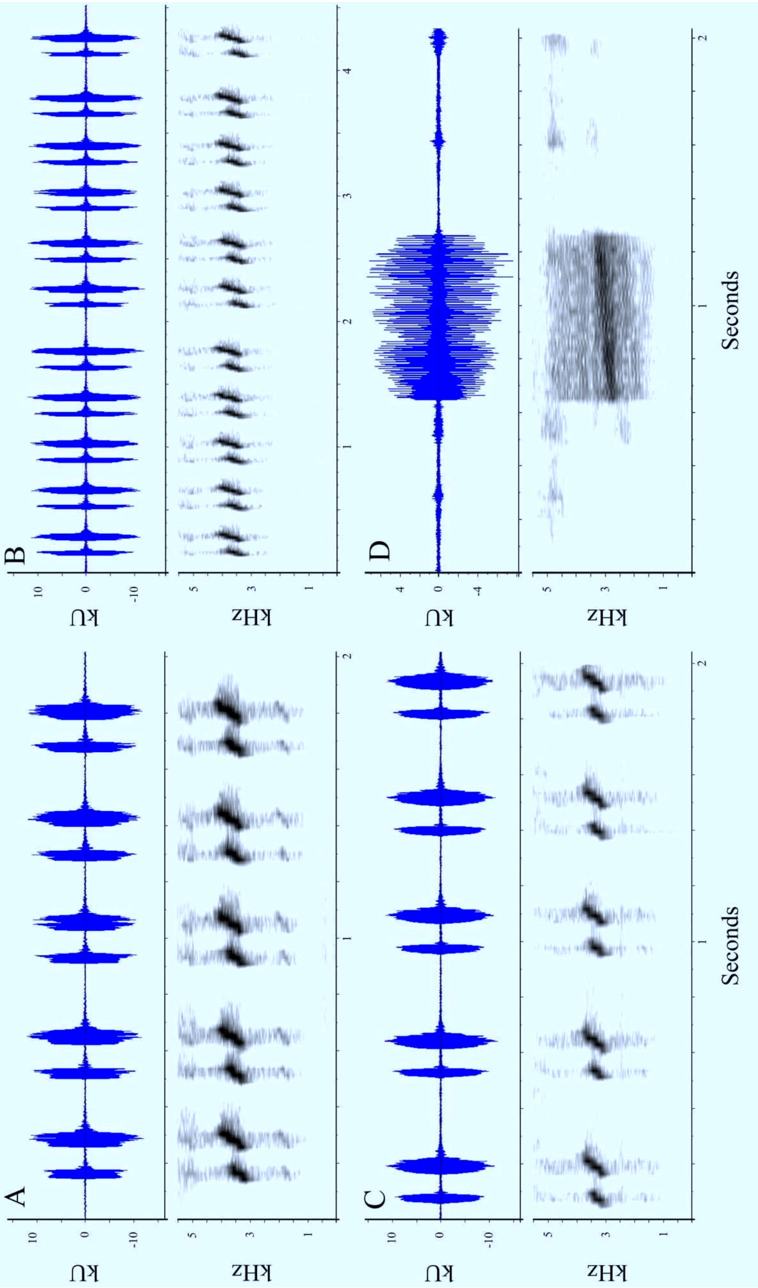

FIGURE 6. The advertisement calls of *Allobates hodli* are constituted by trills of two notes repeated in series. (A) Waveform and sonogram of advertisement calls of *A. hodli* holotype (INPA–H 16555) recorded at Cachoeira do Jirau, Rondônia, at 07:55 h., in November 2004, air temperature 25.3°C, scaled to evidence ascending frequency modulation of notes. (B) Advertisement call of holotype in a larger scale, evidencing continuous repetition of two-note calls. (C) Advertisement call of an *A. hodli* male paratype (SVL = 24.62 mm) from Abunã, Rondônia, recorded at 24.7°C. (D) Courtship call of one *A. hodli* male (INPA-H 16553, SVL = 23.84 mm) recorded at type locality at 09:00 h, in November 2004, air temperature 26.3°C.

Molecular phylogeny and genetic distances. From an initial sequence database containing 203 16S rDNA sequences (including outgroups), a total of 93 unique haplotypes were used in the phylogenetic analysis (Table 5). Phylogenetic reconstructions support the existence of two basal clades within the *Allobates femoralis* group, both forming the sister clade to *Allobates zaparo* (Fig. 7A). One of the basal clades contains *Allobates hodli* and the second contains samples from areas nearby *Allobates femoralis* type-locality, which we refer to as *Allobates femoralis* sensu stricto (clade femo 04, Fig. 7B). *A. hodli* is marginally paraphyletic to populations that occur in the southern reaches of the Brazilian State of Acre (clade Acre 01, Fig. 7B), which present advertisement calls constituted by four notes and color pattern more similar to that of *A. femoralis* than to that of *A. hodli*. Samples from the locality Monte Alegre were not clearly positioned within *A. hodli* or Acre 01 clade. This locality probably corresponds to a relictual contact zone between these clades. *Allobates hodli* and clade Acre 01 form the sister group to a third clade occurring in the northern and western forests of the State of Acre, in Brazil, and along the Madre de Dios River, in Peru (clade Acre 2, Fig. 7B). Despite the clear differentiation in advertisement calls and color pattern, average uncorrected pairwise genetic distance between *A. hodli* and clade Acre 01 does not exceed 1.5%, while distance between *A. hodli* and clade Acre 02 exceeds 2.0% (Table 6). Despite the low levels of genetic divergence from clade Acre 01, *A. hodli* is differentiated from this clade by six unambiguous character state changes in the 16S rDNA fragment analyzed. The observed high levels of genetic similarity between *A. hodli* and clade Acre 01 and the relatively restricted distribution of the latter clade brings up the possibility of that clade Acre 01 originated from past genetic introgression from the widely distributed clade Acre 02 into *A. hodli* along the western portion of its geographic distribution (see Discussion below).

The basal clade containing *A. hodli* and the Acre 01 and Acre 02 clades is the sister group to the clade including *A. femoralis* sensu stricto (clade femo 04, Fig. 7B) and other populations of *A. femoralis* from Peru, Ecuador, Colombia, Suriname, and Brazil. Within this clade, samples from Ecuador form a highly supported clade (clade femo 01, Fig. 7B), which is weakly supported as the sister group to all the remaining *A. femoralis* populations included in this basal clade. Samples from the upper Madeira River basin (clade femo 02, Fig. 7B) and from the Brazilian state of Pará (clade femo 03, Fig. 7B) formed well supported clades, which together are the sister group to a weakly supported clade including *A. femoralis* sensu stricto and additional samples from Iquitos, Panguana (both in Peru), Reserva Ducke (Brazil), and Leticia (Colombia). Average pairwise genetic distances between samples in this basal clade and *A. hodli* ranged from approximately 3.9% (between *A. hodli* and *A. femoralis* from Suriname) to 4.9% (between *A. hodli* and clade femo 04, which contains *A. femoralis* sensu stricto). *Allobates hodli* is distinguished from clades femo 01–04 by at least 23 unambiguous character state changes in the 16S rDNA fragment analyzed. The lack of support and the existence of highly divergent sequences found within clade femo 04 suggest elevated levels of genetic variability between populations across the Peruvian Amazon, and additional sampling is necessary in order to clarify their phylogenetic relationships.

TABLE 3. Advertisement call measurements of *Allobates hodli* holotype (INPA-H 16555) and type series collected in three localities along the upper Madeira River, in Rondônia, Brazil. Values in type series column represent mean ± standard deviation (minimum value observed in the series – maximum value observed in the series). Holotype was recorded at 07:55 h, air temperature during recording was 25.3°C. Average snout to vent length among 60 recorded males was 24.39mm ± 1.11mm (22.26mm-27.31mm). and average air temperature at the time of recording 26.52°C ± 1.46°C (23.3°C–29.8°C).

Measurements	Holotype	Type series (*n* = 60)
SIC (s)	0.207	0.218 ± 0.044 (0.128–0.357)
SIN (s)	0.082	0.074 ± 0.007 (0.062–0.099)
DC (s)	0.170	0.164 ± 0.011 (0.140–0.198)
D1 (s)	0.035	0.033 ± 0.004 (0.020–0.047)
D2 (s)	0.053	0.056 ± 0.007 (0.039–0.079)
MFC (Hz)	3565.53	3425.0 ± 184.7 (2991.3–3897.5)
HFC (Hz)	4002.40	3831.3 ± 174.6 (3262.1–4223.5)
LFC (Hz)	3186.73	3029.6 ± 124.5 (2713.3–3240.8)
MFN1 (Hz)	3488.37	3319.6 ± 141.5 (2971.6–3610.4)
HFN1 (Hz)	3702.83	3552.2 ± 157.5 (3087.1–3964.5)
LFN1 (Hz)	3226.47	3082.3 ± 130.7 (2779.5–3287.8)
MFN2 (Hz)	3637.30	3482.7 ± 193.8 (2977.0–3895.7)
HFN2 (Hz)	4011.43	3838.2 ± 175.1 (3262.1–4254.2)
LFN2 (Hz)	3260.73	3099.8 ± 121.4 (2787.3–3333.4)

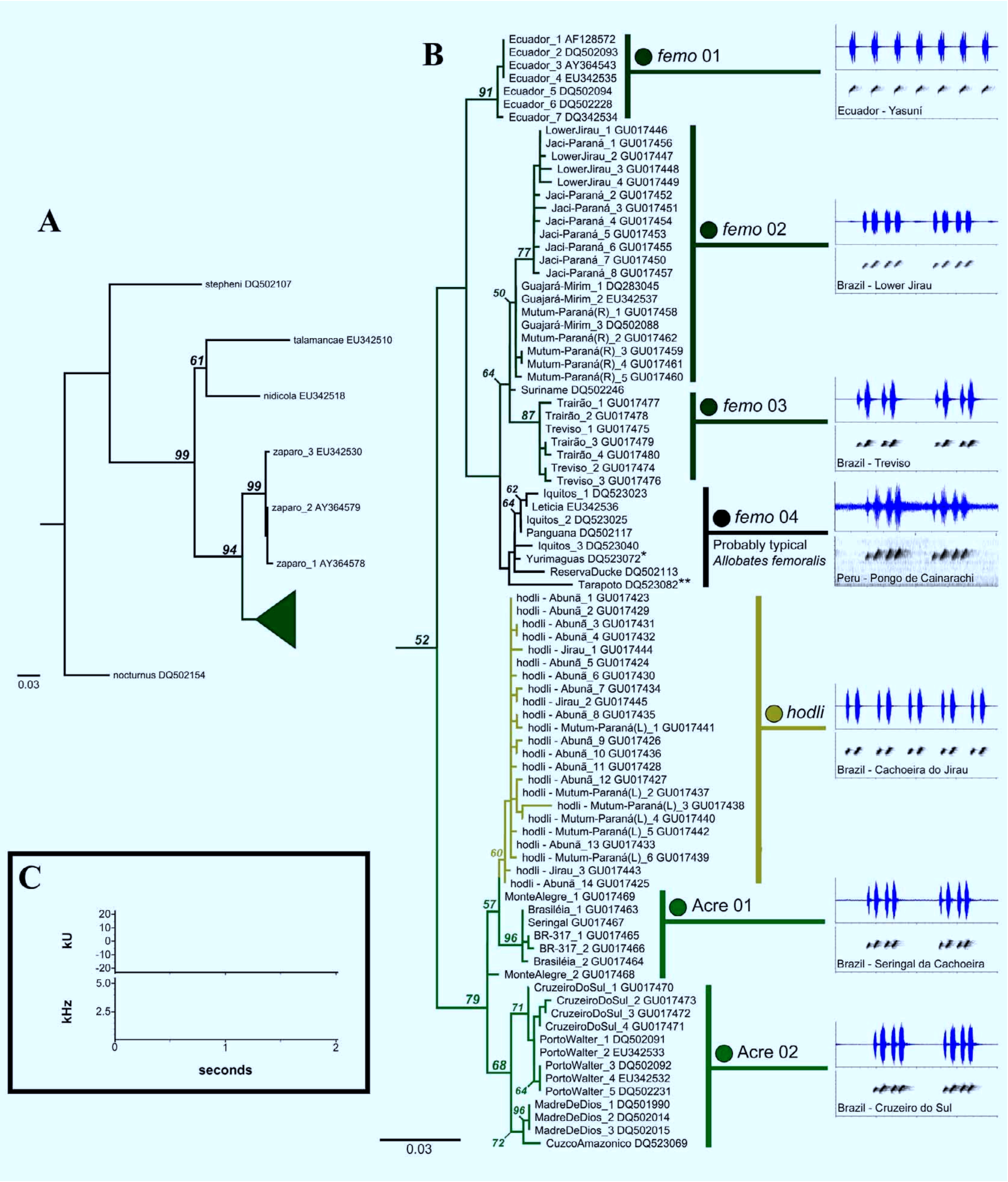

FIGURE 7. Maximum Likelihood phylogenetic tree reconstructed from unique haplotypes of a 518 b.p. fragment of the mitochondrial gene 16S rRNA of *Allobates femoralis* and *Allobates hodli* **sp. nov.** sampled in 13 localities in Brazilian Amazon. Data set included reference sequences from Peru, Colombia, Ecuador, Suriname and other localities in Brazil obtained from GenBank. (A) Phylogenetic position of the clade including *A. hodli* **sp. nov.** and *A. femoralis* in relation to outgroups supports *Allobates zaparo* as their sister group. Clade labels represent support values from 5000 bootstrap replicates (only values above 50 are shown). (B) Relative phylogenetic placement of clades within the ingroup. Sample oscillograms and sonograms of advertisement calls from populations within clades display natural variation in number of notes. *A. hodli* is closely related to populations of *A. femoralis* from the southern Brazilian state of Acre (clade Acre 01), which present a four-note advertisement call and color pattern characteristic of *A. femoralis*. Both form the sister group to samples from northwestern Acre and from the Madre de Dios River basin (clade Acre 02). The basal clade containing *A. hodli* and Acre 01 and Acre 02 clades is the sister group to a basal clade containing *A. femoralis* sensu stricto (placed in the weakly supported clade femo 04) and the other reference sequences from populations referred to as *A. femoralis* (clades femo 01, femo 02, femo 03, femo 04). Samples from Ecuador form a divergent and well supported clade, with advertisement calls formed by a single note. Individuals from Panguana (placed in clade femo 04) present a distinctive 3-note advertisement call (not shown). All remaining populations have advertisement calls constituted by four notes. Calls from Yasuní, Ecuador, published by Read (2000). Calls from Pongo de Cainarachi, Peru (about 45 km south from *A. femoralis* type-locality in Yurimaguas) provided by Jason L. Brown. (C) Corresponding values on axes of oscillograms and sonograms of advertisement call samples.

Natural history notes. *Reproduction and behavior.* Observations were made during the rainy season, when males were found calling during the day from sunrise (time of earlier recording 07:15, INPA-H 16602) to sunset (time of later recording 18:15 h, INPA-H 16592). The number of individuals calling generally decreased around mid-day. Males called from sites slightly elevated from the forest floor, such as logs or perches among fallen branches. Individuals were also frequently observed on the bases of small palm trees and on rocks. Males are territorial, approaching portable amplifiers when we executed playback recordings of their own calls, calls of other males from the same population or calls of *A. femoralis* males from the upper Madeira River near the calling site of the focal male. Courtship calls were emitted only in the presence of females in male's territory, but further courtship, oviposition or larvae relocation behaviors were not observed. One tadpole clutch was collected at Abunã on 15 January 2005. The clutch was found on the ground, over a dead leaf, less than 1 m from a male's calling site. The clutch was transported to Manaus, and tadpoles were raised until developmental stage 36 for tadpole description or until complete metamorphosis for observations of color pattern ontogeny (see above).

In the localities of Abunã and Cachoeira do Jirau, juveniles were frequently found close to small streams inside the forest. Although tadpoles were not found in those streams, there is a possibility that this species uses such water bodies or temporary ponds created by their sporadic overflow as sites for tadpole deposition.

Diet. From 81 dissected stomachs, 24 (29.6%) were empty. Considering only stomachs that contained prey, ants (Formicidae) and adult coleopterans were the most frequent items found, each found in 25 (43.8%) stomachs. Spiders were found in 12 (21.0%) and dipterans in 9 (15.8%) stomachs. Other less frequent items found were isopterans (5 stomachs, 8.8%), miriapods (3 stomachs, 5.26%), coleopteran larvae (3 stomachs, 5.26%), hemipterans (2 stomachs, 3.5%), other hymenopterans (2 stomachs, 3.5%) and terrestrial dipteran larvae (2 stomachs, 3.5%). Collembolans, orthopterans, blattarians and acari were found each in a single stomach.

Distribution. Known distribution of *Allobates hodli* is restricted to southwestern Brazilian Amazonia (coordinates are given in Table 5), from the locality of Cachoeira do Jirau (09.3347° S, 64.7375° W), in the Municipality of Porto Velho, to the eastern reaches of the Municipality of Rio Branco, in the state of Acre (10.0742° S, 67.6249° W). The eastern boundary of the species' distribution is well known, as it reaches a contact zone with a population of *Allobates femoralis* (clade femo 02, Fig. 7B) on the left bank of the upper Madeira River, about 1 km downstream of the Jirau rapids (9.3206° S, 64.7225° W). The westernmost site of occurrence of *A. hodli* is located in the vicinity of the city of Rio Branco, in Fazenda Catuaba (site 4, Fig. 1A). South of Rio Branco, in a district known as Monte Alegre (site 5, Fig. 1A), *A. hodli* is replaced by another population (clade Acre 01, Fig. 7B) which presents typical *A. femoralis* coloration and 4-note advertisement calls.

TABLE 4. Courtship call measurements of *Allobates hodli* from type locality at Cachoeira do Jirau (INPA-H 16553 and 16567) and from Abunã (INPA-H 16606 and 16621), on the left bank of the upper Madeira River, in Rondônia, Brazil. More than one courtship call was available for INPA-H 16567 and INPA-H 16621, and values represent the averages between all available calls.

	Individual sampled (INPA-H #)				
	16553	16567	16606	16621	X ± *s.d.*
Nº of calls analysed	1	6	1	2	
Temperature (°C)	26.3	26.3	28.2	26.0	26.7 ± 1.0
SVL (mm)	23.84	24.75	25.47	23.94	24.5 ± 0.7
DC (s)	0.624	0.800	0.402	0.457	0.571 ± 179
MFC (Hz)	2960.8	2865.7	3488.4	3446.0	3190.2 ± 322.6
Nº of pulses	73	90	61	55	70 ± 15
Nº of pulses/second	117.0	113.0	151.7	119.0	125.2 ± 17.8

TABLE 5. Sample names and available sample information for sequences of *Allobates hodli* sp nov., reference *Allobates femoralis* sequences and outgroup sequences included in the molecular phylogenetic analysis. Clades correspond to monophyletic groups presented in Figure 7B).

Sample name	Clade	Locality	Coordinates	16S	Reference
Ecuador 1	femo 01	Cuyabeno, Sucumbios, Ecuador	-	AF128572	Clough & Summers, 2000
Ecuador 2	femo 01	Cuyabeno, Sucumbios, Ecuador	0°0' S, 76°10'W	DQ502093	Grant *et al.* 2006
Ecuador 3	femo 01	Ecuador	-	AY364543	Santos *et al.* 2003
Ecuador 4	femo 01	Parque Nac. Yasuni, Ecuador	-	EU342535	Santos *et al*. 2009
Ecuador 5	femo 01	Cuyabeno, Sucumbios, Ecuador	0°0' S, 76°10'W	DQ502093	Grant *et al.* 2006
Ecuador 6	femo 01	Cuyabeno, Sucumbios, Ecuador	0°0' S, 76°10'W	DQ502228	Grant *et al.* 2006
Ecuador 7	femo 01	Cuyabeno, Sucumbios, Ecuador	-	DQ342534	Santos *et al*. 2009
LowerJirau 1	femo 02	Lower Jirau, Rondônia, Brazil	9.311°S, 64.717°W	GU017446	this study
LowerJirau 2	femo 02	Lower Jirau, Rondônia, Brazil	9.311°S, 64.717°W	GU017447	this study
LowerJirau 3	femo 02	Lower Jirau, Rondônia, Brazil	9.311°S, 64.717°W	GU017448	this study
LowerJirau 4	femo 02	Lower Jirau, Rondônia, Brazil	9.311°S, 64.717°W	GU017449	this study
Jaci-Paraná 1	femo 02	Jaci-Paraná, Rondônia, Brazil	9.169°S, 64.429°W	GU017456	this study
Jaci-Paraná 2	femo 02	Jaci-Paraná, Rondônia, Brazil	9.169°S, 64.429°W	GU017452	this study
Jaci-Paraná 3	femo 02	Jaci-Paraná, Rondônia, Brazil	9.169°S, 64.429°W	GU017451	this study
Jaci-Paraná 4	femo 02	Jaci-Paraná, Rondônia, Brazil	9.169°S, 64.429°W	GU017454	this study
Jaci-Paraná 5	femo 02	Jaci-Paraná, Rondônia, Brazil	9.169°S, 64.429°W	GU017453	this study
Jaci-Paraná 6	*femo* 02	Jaci-Paraná, Rondônia, Brazil	9.169°S, 64.429°W	GU017455	this study
Jaci-Paraná 7	*femo* 02	Jaci-Paraná, Rondônia, Brazil	9.169°S, 64.429°W	GU017450	this study

continued next page

TABLE 5. (continued)

Sample name	Clade	Locality	Coordinates	16S	Reference
Jaci-Paraná 8	*femo* 02	Jaci-Paraná, Rondônia, Brazil	9.169°S, 64.429°W	GU017457	this study
Guajará-Mirim 1	*femo* 02	Guajará-Mirim, Rondônia, Brazil	10°19'S, 64°33'W	DQ283045	Frost *et al*. 2006
Guajará-Mirim 2	*femo* 02	Guajará-Mirim, Rondônia, Brazil	10°19'S, 64°33'W	EU342537	Santos *et al*. 2009
Guajará-Mirim 3	*femo* 02	Guajará-Mirim, Rondônia, Brazil	10°19'S, 64°33'W	DQ502088	Grant *et al*. 2006
Mutum-Paraná(R) 1	*femo* 02	Mutum-Paraná (right bank), Rondônia, Brazil	9.641°S, 64.886°W	GU017458	this study
Mutum-Paraná(R) 2	*femo* 02	Mutum-Paraná (right bank), Rondônia, Brazil	9.641°S, 64.886°W	GU017462	this study
Mutum-Paraná(R) 3	*femo* 02	Mutum-Paraná (right bank), Rondônia, Brazil	9.641°S, 64.886°W	GU017459	this study
Mutum-Paraná(R) 4	*femo* 02	Mutum-Paraná (right bank), Rondônia, Brazil	9.641°S, 64.886°W	GU017461	this study
Mutum-Paraná(R) 5	*femo* 02	Mutum-Paraná (right bank), Rondônia, Brazil	9.641°S, 64.886°W	GU017460	this study
Suriname	-	Sipaliwini, Suriname	3°5.7'N, 56°28.3'W	DQ502246	Grant *et al*. 2006
Trairão 1	*femo* 03	Trairão, Pará, Brazil	4.683°S, 56.022°W	GU017477	this study
Trairão 2	*femo* 03	Trairão, Pará, Brazil	4.683°S, 56.022°W	GU017478	this study
Trairão 3	*femo* 03	Trairão, Pará, Brazil	4.683°S, 56.022°W	GU017479	this study
Trairão 4	*femo* 03	Trairão, Pará, Brazil	4.683°S, 56.022°W	GU017480	this study
Treviso 1	*femo* 03	Fazenda Treviso, Pará, Brazil	3.158°S, 54.859°W	GU017475	this study
Treviso 2	*femo* 03	Fazenda Treviso, Pará, Brazil	3.158°S, 54.859°W	GU017474	this study
Treviso 3	*femo* 03	Fazenda Treviso, Pará, Brazil	3.158°S, 54.859°W	GU017476	this study
Iquitos 1	*femo* 04	Iquitos, Loreto, Peru	-	DQ523023	Roberts *et al*. 2006
Iquitos 2	*femo* 04	Iquitos, Loreto, Peru	-	DQ523025	Roberts *et al*. 2006

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TABLE 5. (continued)

Sample name	Clade	Locality	Coordinates	16S	Reference
Iquitos 3	*femo* 04	Iquitos, Loreto, Peru	-	DQ523040	Roberts *et al*. 2006
Leticia	*femo* 04	Cerca Viva, Leticia, Amazonas, Colombia	-	EU342536	Santos *et al*. 2009
Panguana	*femo* 04	Panguana, Peru	-	DQ502117	Grant *et al*. 2006
Yurimaguas	*femo* 04	Shucshuyacu, Yurimaguas, Loreto, Peru	6.032°S, 75.857°W	DQ523072	Roberts *et al*. 2006
ReservaDucke	*femo* 04	Reserva Ducke, Amazonas, Brazil	-	DQ502113	Grant *et al*. 2006
Tarapoto	*femo* 04	Saposoa, Tarapoto, San Martin, Peru	6.771°S, 76.941°W	DQ523082	Roberts *et al*. 2006
hodli – Abunã 1	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017423	this study
hodli – Abunã 2	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017429	this study
hodli – Abunã 3	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017431	this study
hodli – Abunã 4	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017432	this study
hodli – Abunã 5	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017424	this study
hodli – Abunã 6	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017430	this study
hodli – Abunã 7	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017434	this study
hodli – Abunã 8	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017435	this study
hodli – Abunã 9	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017426	this study
hodli – Abunã 10	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017436	this study
hodli – Abunã 11	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017428	this study
hodli – Abunã 12	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017427	this study

continued next page

TABLE 5. (continued)

Sample name	Clade	Locality	Coordinates	16S	Reference
hodli – Abunã 13	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017433	this study
hodli – Abunã 14	*hodli*	Abunã, Rondônia, Brazil	9.516°S, 65.324°W	GU017425	this study
hodli – Jirau 1	*hodli*	Cachoira do Jirau, Rondônia, Brazil	9.335°S, 64.737°W	GU017444	this study
hodli – Jirau 2	*hodli*	Cachoira do Jirau, Rondônia, Brazil	9.335°S, 64.737°W	GU017445	this study
hodli – Jirau 3	*hodli*	Cachoira do Jirau, Rondônia, Brazil	9.335°S, 64.737°W	GU017443	this study
hodli – Mutum-Paraná(L) 1	*hodli*	Mutum-Paraná (left bank), Rondônia, Brazil	9.573°S, 64.921°W	GU017441	this study
hodli – Mutum-Paraná(L) 2	*hodli*	Mutum-Paraná (left bank), Rondônia, Brazil	9.573°S, 64.921°W	GU017437	this study
hodli – Mutum-Paraná(L) 3	*hodli*	Mutum-Paraná (left bank), Rondônia, Brazil	9.573°S, 64.921°W	GU017438	this study
hodli – Mutum-Paraná(L) 4	*hodli*	Mutum-Paraná (left bank), Rondônia, Brazil	9.573°S, 64.921°W	GU017440	this study
hodli – Mutum-Paraná(L) 5	*hodli*	Mutum-Paraná (left bank), Rondônia, Brazil	9.573°S, 64.921°W	GU017442	this study
hodli – Mutum-Paraná(L) 6	*hodli*	Mutum-Paraná (left bank), Rondônia, Brazil	9.573°S, 64.921°W	GU017439	this study
MonteAlegre 1	-	Monte Alegre, Acre, Brazil	10.346°S, 67.518°W	GU017469	this study
MonteAlegre 2	-	Monte Alegre, Acre, Brazil	10.346°S, 67.518°W	GU017468	this study
Brasiléia 1	Acre 01	Brasiléia, Acre, Brazil	10.965°S, 68.733°W	GU017463	this study
Brasiléia 2	Acre 01	Brasiléia, Acre, Brazil	10.965°S, 68.733°W	GU017464	this study
Seringal	Acre 01	Seringal da Cachoeira, Acre, Brazil	10.833°S, 69.381°W	GU017467	this study
BR-317 1	Acre 01	BR-317, Acre, Brazil	10.820°S, 69.192°W	GU017465	this study
BR-317 2	Acre 01	BR-317, Acre, Brazil	10.820°S, 69.192°W	GU017466	this study
CruzeiroDoSul 1	Acre 02	Cruzeiro do Sul, Acre, Brazil	7.956°S, 72.077°W	GU017470	this study

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TABLE 5. (continued)

Sample name	Clade	Locality	Coordinates	16S	Reference
CruzeiroDoSul 2	Acre 02	Cruzeiro do Sul, Acre, Brazil	7.956°S, 72.077°W	GU017473	this study
CruzeiroDoSul 3	Acre 02	Cruzeiro do Sul, Acre, Brazil	7.956°S, 72.077°W	GU017472	this study
CruzeiroDoSul 4	Acre 02	Cruzeiro do Sul, Acre, Brazil	7.956°S, 72.077°W	GU017471	this study
PortoWalter 1	Acre 02	Porto Walter, Acre, Brazil	8°15’S, 72°46’W	DQ502091	Grant *et al.* 2006
PortoWalter 2	Acre 02	Porto Walter, Acre, Brazil	-	EU342533	Santos *et al.* 2009
PortoWalter 3	Acre 02	Porto Walter, Acre, Brazil	8°15’S, 72°46’W	DQ502092	Grant *et al.* 2006
PortoWalter 4	Acre 02	Porto Walter, Acre, Brazil	-	EU342532	Santos *et al.* 2009
PortoWalter 5	Acre 02	Porto Walter, Acre, Brazil	9°34’S, 72°46’W	DQ502231	Grant *et al.* 2006
MadreDeDios 1	Acre 02	Puerto Maldonado, Cusco Amazônico, Peru	-	DQ501990	Grant *et al.* 2006
MadreDeDios 2	Acre 02	Puerto Maldonado, Cusco Amazônico, Peru	-	DQ502014	Grant *et al.* 2006
MadreDeDios 3	Acre 02	Puerto Maldonado, Cusco Amazônico, Peru	-	DQ502015	Grant *et al.* 2006
CuzcoAmazonico	Acre 02	Boca Manu, Cuzco, Peru	-	DQ523069	Roberts *et al.* 2006
nidicola	-	Castanho, Amazonas, Brasil	-	EU342518	Santos *et al.* 2009
nocturnus	-	Trujillo, Venezuela	-	DQ502154	Grant *et al.* 2006
stepheni	-	Reserva Ducke, Amazonas, Brazil	-	DQ502107	Grant *et al.* 2006
talamancae	-	Quibdo, Choco, Colombia	-	EU342510	Santos *et al.* 2009
zaparo 1	-	Ecuador	-	AY364578	Santos *et al.* 2003
zaparo 2	-	Ecuador	-	AY364579	Santos *et al.* 2003
zaparo 3	-	Pastaza, Ecuador	-	EU342530	Santos *et al.* 2009

The species is not known to occur on the right bank of the Madeira and Mamoré Rivers (it is possible that these rivers represent barriers to the distribution of this species), thus its southernmost record is also Fazenda Catuaba, probably reaching forest remnants south of the city of Rio Branco. The northern distribution limit for the species is unknown.

Discussion. The taxon *Allobates femoralis* has already been considered a complex of closely related species by many authors (e.g. Grant *et al.* 2006; Lötters *et al.* 2007; Santos *et al.* 2009). Recent studies considered the phylogenetic relationships of this group in a higher taxonomic context (Grant *et al.* 2006; Santos *et al.* 2009), and agreed in relation to the existence of cryptic species under this taxon. Grant *et al.* (2006) argue that, in spite of forming a monophyletic group, pronounced genetic distances between sampling sites (3.9–14.6%, cytocrome *b*) are indicative of multiple (at least eight) species. In a more recent approach, Santos *et al.* (2009) estimated *A. femoralis* comprised nine distinct species that diversified within the Amazon Basin 5.4–8.7 million years ago. However, these studies only circumstantially addressed phylogenetic relationships within the *A. femoralis* clade, using samples from localities separated by hundreds of kilometers. A detailed description of phenotypes, as well as the distribution of each group/species was beyond the scope of these works. No sequences from the known distribution range of *A. hodli* were included in these studies, and *Allobates hodli* represents a new taxon, additive to the number of cryptic species presumed by the studies of Grant *et al.* (2006) and Santos *et al.* (2009).

The existence of conspicuous genetic differences between the individuals from Catuaba and other *A. femoralis* populations (including reference populations from Reserva Ducke, Treviso, Leticia and Panguana) was observed by Amézquita *et al.* (2009), based on a 306 b.p. fragment of the cytochrome *b* mitochondrial gene. Despite the pronounced geographic distances between most populations sampled, authors argue that genetic distances observed between Fazenda Catuaba and other populations were larger than expected to be explained by geographic distance alone, and are largely correlated to phenotypic distances, considering combined data on morphometrics, acoustic properties of calls, and color pattern.

TABLE 6. Mean uncorrected pairwise genetic distances between major clades of the Maximum Likelihood phylogenetic tree obtained from 518 b.p. fragment of the 16S rRNA gene of *Allobates hodli* **sp. nov.** and reference populations of *Allobates femoralis*. Denominations in first column correspond to those in Fig. 7. Samples from Suriname and Monte Alegre were not placed within any major clade and their relative genetic distances are calculated separately.

	n	1	2	3	4	5	6	7	8	9	10	11	12	13
1 *Allobates hodli* **sp. nov.**	72													
2 Acre 01	13	0.014												
3 Acre 02	17	0.022	0.021											
4 Monte Alegre	2	0.010	0.012	0.015										
5 femo 01	7	0.040	0.040	0.036	0.035									
6 femo 02	41	0.044	0.051	0.045	0.043	0.029								
7 femo 03	19	0.040	0.046	0.041	0.039	0.024	0.016							
8 femo 04	8	0.049	0.055	0.051	0.047	0.028	0.021	0.023						
9 Suriname	1	0.039	0.046	0.040	0.038	0.024	0.007	0.012	0.016					
10 *Allobates zaparo*	3	0.062	0.064	0.056	0.055	0.049	0.052	0.049	0.050	0.048				
11 *Allobates nidicola*	1	0.090	0.098	0.091	0.089	0.098	0.098	0.098	0.095	0.095	0.093			
12 *Allobates talamancae*	1	0.101	0.111	0.108	0.102	0.103	0.108	0.107	0.101	0.103	0.108	0.107		
13 *Anomaloglossus stepheni*	1	0.135	0.140	0.132	0.129	0.140	0.148	0.141	0.146	0.145	0.137	0.131	0.149	
14 *Aromobates nocturnus*	1	0.130	0.137	0.128	0.129	0.135	0.130	0.13	0.137	0.131	0.133	0.141	0.149	0.119

Allobates hodli is the first species of this complex to be described since the description of *A. myersi* by Pyburn (1981). It has a relatively well-known distribution and is characterized by unambiguous molecular, morphological and behavioral characters that make this taxon distinguishable from all other clades included in the *A. femoralis* complex and their close relatives. To our knowledge *A. hodli* is the only species in the

Allobates femoralis complex (sensu Lötters *et al.* 2007) on Brazilian territory to present advertisement calls constituted by the repetition of groups of two frequency-modulated notes. Similar 2-note advertisement calls have been noted for *A. myersi* in Colombia (Pyburn 1981) and *A. zaparo* in Ecuador (Read 2000).

Detailed morphological and acoustic comparisons between 2-note call populations from the left bank of the upper Madeira River (herein described as *A. hodli*) and 4-note call populations distributed in other localities in this area were presented in Simões *et al.* (2008). The study also highlighted the coincidence between the distribution of both groups and the boundaries between distinct geomorphological domains. Although relationships between habitat variation and underlying geomorphology is largely unknown in this area, summary of evidence of phenotypic differentiation and restricted distribution point to the rejection of the hypothesis of current ecological exchangeability (sensu Crandall *et al.* 2000) between individuals of these two groups, but this issue deserves further testing using niche-modeling approaches. The reciprocal monophyly between basal clades containing *A. hodli* and *A. femoralis* from the upper Madeira River (clade femo 02, Fig. 7B) points to past genetic isolation that remains in recent time, despite of their occurrence in sympatry across a narrow contact zone downstream of Cachoeira do Jirau (Simões *et al.* 2008).

Advertisement calls are considered the most conspicuous sexual signals in frogs and the first pre-mating signals perceived by a distant female, playing a crucial role in female attraction and sexual selection by females, besides mediating territorial male-to-male interactions. All other signals, including courtship calls, are usually emitted once the female is already within a male's territory (Gerhardt & Huber 2002; Wells 2007). Our results highlight the existence of clear differentiation in the number of notes in advertisement calls of *A. hodli* and other populations referred to as *A. femoralis*. Despite this difference, playback trials using calls of *A. femoralis* and *A. hodli* (performed in populations of the upper Madeira River) triggered aggressive phonotactic behavior in males of both species. However, the existence and strength of call differentiation effects on sexual selection by females belonging to the *A. femoralis*-*A. hodli* complex are yet to be tested.

Genetic introgression is not uncommon among amphibians (Hofman, S. & Szymura 2007; Vogel & Johnson 2008; Brown & Twomey 2009). In cases of relaxed selection on signal recognition, hybridization would likely take place at suture zones, allowing for genetic introgression, and thus rendering polyphyletic or paraphyletic molecular phylogenies (Funk & Omland 2003). In addition to a paraphyletic mtDNA phylogeny, the restricted geographic distribution of clade Acre 01 (which present 4-note advertisement calls and color patterns similar to those of clade Acre 02) suggests that it could have arisen from past genetic introgression from clade Acre 02 into *A. hodli* along the western distribution of the latter (McGuire et al. 2007; Brown & Twomey 2009). This hypothesis remains to be tested with nuclear DNA markers and experiments on female sexual selection. The current range of extant primary forest in this region (reduced to very small patches) makes it difficult to sample more individuals in the area between Fazenda Catuaba and Monte Alegre. Ongoing deforestation across this area will likely increase the geographic isolation between *A. hodli* and populations of clades femo 01 and femo 02 occurring in southern and western State of Acre.

Although apparently allowing for some hybridization along contact zones (at least in the past), differences in advertisement calls between *A. hodli* and other clades referred to as *A. femoralis* are geographically fixed, and are maintained along the remaining areas of sympatry. The 2-note advertisement calls of *A. hodli* are also clearly distinguished from the 4-note calls of individuals sampled in areas south of *A. femoralis* type locality in Yurimaguas (individuals were recorded in Chazuta, 70 km, and Pongo de Cainarachi, 45 km, Fig. 8), which are highly allopatric to *A. hodli*. In addition to fixed differences in such bioacoustic characters, we describe differentiation in morphological traits that are not variable among reference populations of *A. femoralis* and *A. hodli*, such as the reddish-orange color on ventral surface of posterior abdomen and hind limbs, and diffuse flash marks on thighs in *A. hodli*. These characters are also clearly distinguished from those observed in populations inhabiting localities close to *A. femoralis* type locality (Barranquita, 36 km south of Yurimaguas, Fig. 9), and as such can be treated as diagnostic characters. When combined with generally high levels of genetic differentiation in 16S rDNA relative to reference *A. femoralis* populations, our results match the criteria proposed by Vieites *et al.* (2009) for validation of a candidate taxon, according to which *A. hodli* should be regarded as a distinct species.

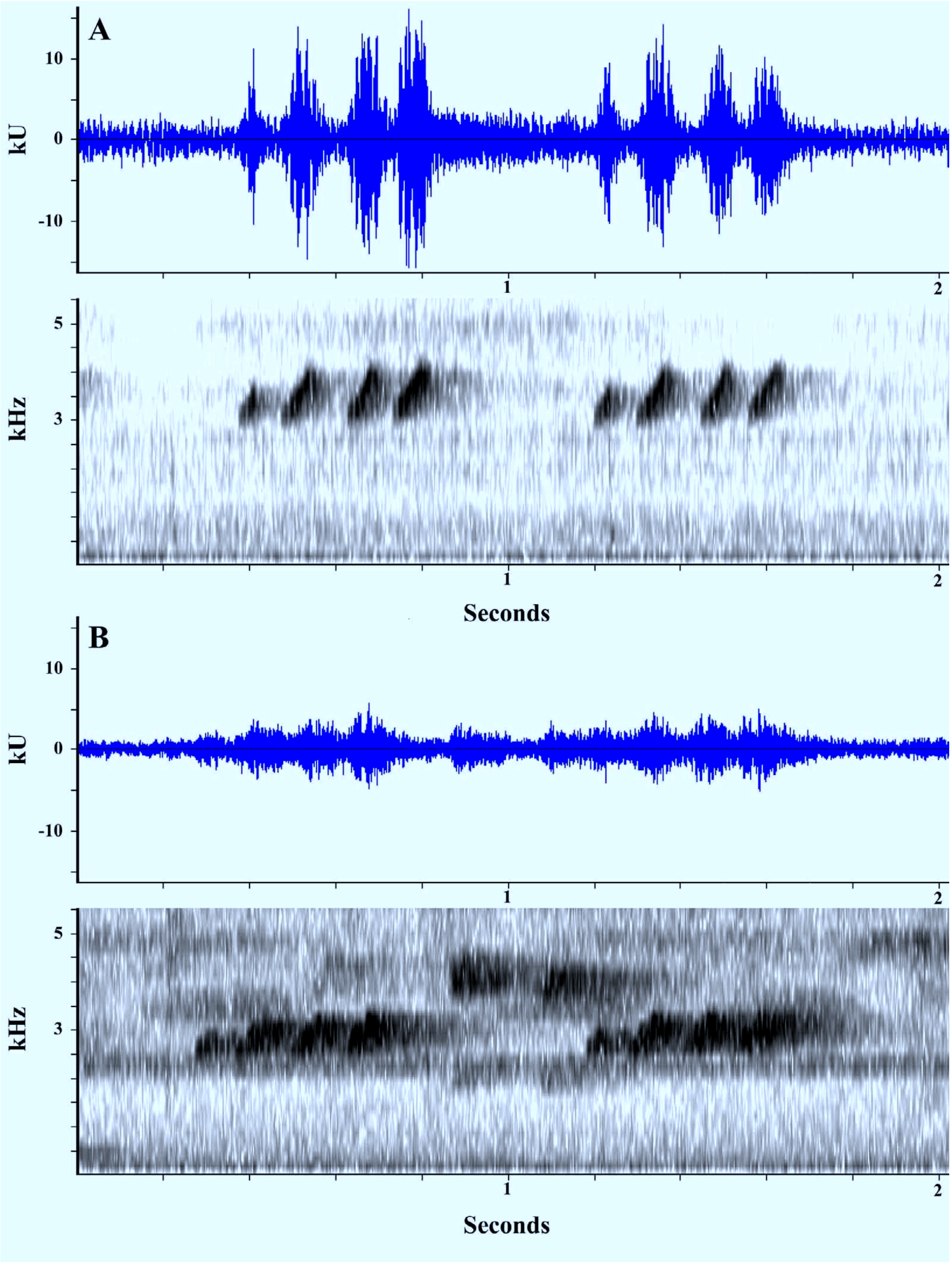

FIGURE 8. Advertisement calls of *Allobates femoralis* recorded in (A) Chazuta (6.5419°S, 76.1083°W) and (B) Pongo de Cainarachi (6.2974°S, 76.2343°W), both localities in San Martin, south of *A. femoralis* type-locality in Yurimaguas. In both sites, calls are constituted by groups of four frequency-modulated notes. Sounds appearing with peak frequency at approximately 4.0 kHz in B are background noise. Recordings are courtesy of Jason L. Brown.

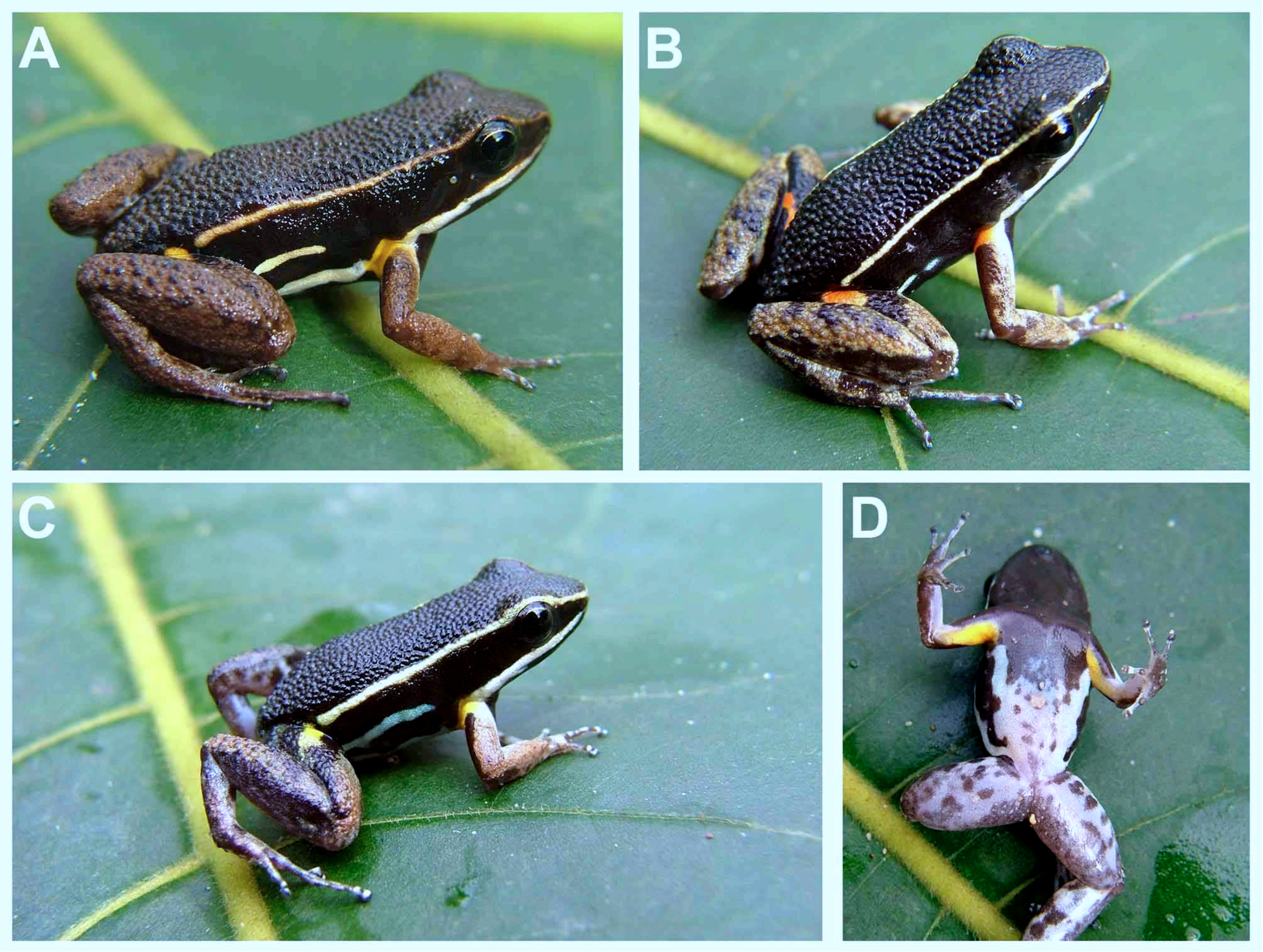

FIGURE 9. (A), (B) and (C) Dorsolateral color pattern of three specimens of *Allobates femoralis* photographed near Barranquita (6.2653°S, 76.0434°W), 36 km from *Allobates femoralis* type locality in Yurimaguas, Loreto, Peru. (D) Ventral view of same individual B, showing exclusively black and white color patterning on belly and ventral surface of thighs. Photos are courtesy of Jason L. Brown.

Summarizing information from the mtDNA phylogeny and available records of advertisement calls, we suggest that there is potential for taxonomic reappraisal of other geographically restricted populations which are currently recognized under the name *Allobates femoralis*. Namely, populations from Ecuador (clade femo 01, Fig. 7B) and southwestern Amazon Basin (clade Acre 02, Fig. 7B) represent putative new taxa, with characteristic phenotypes and relatively well known geographic distribution. Although presenting lower between-clade genetic distances, populations from the Madeira and Tapajós River basins (clades femo 02 and 03) represent geographically structured monophyletic lineages, and further population genetics studies should address the existence of current gene flow between them.

Samples from Colombian and northern Peruvian Amazon that constitute clade femo 04 (Fig. 7B) probably represent populations of nominal *A. femoralis*. Silvertone (1976) designated a male individual collected in Yurimaguas, in the Huallaga River, Peru, as the *A. femoralis* lectotype, as the same individual was used in the original description by Boulenger in 1883. Although samples from the immediate vicinity of Yurimaguas were not available for this study, calls (Fig. 8, also see Amézquita 2009), photographs (Fig. 8, also see Pyburn 1981), and DNA sequences (Table 5) obtained in localities near the type locality suggest that populations of *A. femoralis* distributed across Departamento Loreto, in Peru, and Departamento Amazonas, in Colombia, present similar advertisement calls and color pattern, and thus we propose represent *A. femoralis* sensu stricto. In the future, increased sampling across northwestern Colombian Amazon, southern Peruvian Amazon, and Bolivia will possibly reveal a wider geographic distribution for this clade. However, at least two advertisement call phenotypes are known to exist across this region, and our DNA sequence analyses pointed to the existence of high levels of genetic divergence between samples collected in this area. Mapping the boundaries between these distinct acoustic morphotypes, and including more samples from each population in new phylogenies will allow us testing the hypothesis of reciprocal monophyly and current gene-flow between them, in addition to elucidating their evolutionary relationships in relation to the remaining species that form the *Allobates femoralis* complex.

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