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An integrative appraisal of the diagnosis and distribution of *Allobates sumtuosus* (Morales, 2002) (Anura, Aromobatidae)

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Abstract

We describe the advertisement calls and color in life of *Allobates sumtuosus* (Morales 2002) based on specimens recorded and collected at its type locality in Reserva Biológica do Rio Trombetas, Brazilian Amazonia. We also improve the species diagnosis by adding information on states of characters frequently used in current *Allobates* taxonomy. Finally, we analyze genetic distances and the evolutionary relationships between typical *A. sumtuosus* and other *Allobates* species distributed in Brazil and along the Guiana Shield region using a fragment of the 16S rDNA mitochondrial gene. Based on this integrative analysis, we propose the synonym of *Allobates spumaponens* Kok & Ernst 2007 with *A. sumtuosus* and provide an updated geographic distribution of the species.

Key words: Advertisement calls, *Allobates spumaponens*, Amazonia, Dendrobatoidea, color in life, mtDNA

Resumo

Nós descrevemos as vocalizações de anúncio e a coloração em vida de *Allobates sumtuosus* (Morales 2002) com base em espécimes registrados e coletados na localidade tipo da espécie na Reserva Biológica do Rio Trombetas, Amazônia brasileira. Também aprimoramos a diagnose da espécie ao adicionar informações sobre estados de caracteres frequentemente utilizados na taxonomia de *Allobates*. Por fim, analisamos distâncias genéticas e relações evolutivas entre *A. sumtuosus* típico e outras espécies de *Allobates* distribuídas no Brasil e ao longo da região do Escudo das Guianas usando um fragmento do gene mitocondrial 16S DNAr. Baseados nesta análise integrativa, propomos a sinonímia de *Allobates spumaponens* Kok & Ernst 2007 com *A. sumtuosus* e fornecemos a distribuição geográfica atualizada da espécie.

Palavras-chave: Vocalizações de anúncio, *Allobates spumaponens*, Amazônia, Dendrobatoidea, cor em vida, DNAmt

Introduction

Allobates Zimmermann & Zimmermann 1988 is a species-rich genus of leaf litter frogs widely distributed in tropical forests of South and Central America. More than 45 species of *Allobates* have been described (Frost 2013) and the discovery of new species have progressed at a somewhat steady pace since the start of the 21st century (*e.g.* Lima & Caldwell 2001; Caldwell & Lima 2003; Kok & Ernst 2007; Lima *et al.* 2007; 2010; Barrio-Amorós & Santos 2009; Simões *et al.* 2013). One major reason for the steady rate of detection and description of *Allobates* species is due to increased sampling and the inclusion of additional sources of data in recent publications. In the past, the traditional and limited array of morphological characters applied to *Allobates* taxonomy resulted in unequivocal diagnoses, however newly acquired information related to advertisement calls, color of live specimens, DNA sequences, and larval traits have increased our diagnostic abilities.

Most *Allobates* species are conservative in relation to external morphology and color patterns, and accurate species assignment is usually not trivial when only preserved specimens are available for identification (Kaefer *et*

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al. 2013). Their generalized morphological conservatism is likely the main reason why newly established species of *Allobates* remain undetected in species surveys and why they are often incorrectly classified under older, catch-all names, such as *A. marchesianus, A. brunneus or A. trilineatus.* The issue of diagnosability is further exacerbated by the group's diurnality and because of this, many times, *Allobates* specimens are not detected in amphibian assessments (which are typically conducted at night). Despite these hurdles, taxonomic progress continues, and several revisionary studies have supplemented original species descriptions with new field data (*e.g.* color in life, advertisement calls), which is particularly useful when these data is collected from type localities (Grant & Rodríguez 2001; Caldwell *et al*. 2002; Lima *et al.* 2009). Unfortunately, extended taxonomic descriptions are absent for many *Allobates* species. As a consequence of these information gaps, biologists often fail to recognize previously described species in the field, and conservation planners often risk utilizing outdated databases of species distributions (Azevedo-Ramos & Galatti 2002; Peloso 2011; Verdade *et al.* 2012).

One case of species suffering from the aforementioned issues is that of *Allobates sumtuosus* (Morales 2002). The holotype and seven paratypes of this species were collected in a conservation unit on the Trombetas River (Reserva Biológica do Rio Trombetas—REBio Trombetas), a northern tributary of the Amazon River, in State of Pará, Brazil. Three additional paratypes proceeded from Loreto, Peru. In his highly anticipated paper, Morales (2002) described ten species of Amazonian *Allobates*, providing an identification key and a phylogenetic hypothesis using a set of morphological and color traits obtained exclusively from preserved specimens. Albeit efficient in separating the taxa at hand, the utility of this set of traits in identifying species in the field remains contentious and some of the described species were not reported in subsequent species surveys (*e.g. A. cepedai*, *A. fratisenescus*, *A. fuscellus, A. vanzolinius*). For instance, *A. sumtuosus* was not detected in thorough amphibian assessments conducted along the northern Amazon basin (*e.g*. Menin *et al*. 2008; Ávila-Pires *et al.* 2010), where species such as *A. marchesianus* and *A. spumaponens* have been reported instead (Ávila-Pires *et al.* 2010; Deichmann *et al*. 2010). As a result, *A. sumtuosus* is currently classified as "Data Deficient" and had its taxonomic validity debated (Salas 2004). The relative inaccessibility of the original description likely also contributed to the 'elusiveness' of this species in the past decade.

In this paper, we aim to contribute to a better understanding of the diagnosis and geographic distribution of *Allobates sumtuosus*. Based on specimens recorded and collected at the species type locality, we describe the advertisement calls and color in life of *A. sumtuosus*. We also examine the external morphology and color traits of the same specimens after preservation in ethanol for two years, improving the species diagnosis by adding information on states of characters frequently used in current *Allobates* taxonomy. Finally, we analyze genetic distances and the evolutionary relationships between typical *Allobates sumtuosus* and other *Allobates* species distributed in Brazil and along the Guiana Shield region using a fragment of the 16S rDNA mitochondrial gene. By integrating information on calls, photographic records, and phylogenetic analyses, we provide an updated geographic distribution of *Allobates sumtuosus* and propose its synonym with *Allobates spumaponens* Kok & Ernst 2007.

Material and methods

From 25th to 28th of February 2011 we carried out field work in the surroundings of Tabuleiro Research Station (01°22'12" S, 56°51'08" W) in REBio Trombetas. The research station is located within a mosaic of seasonally flooded (*igapó*) and *terra-firme* primary forests. The latter are notably rich in Brazil-nut trees (*Betholetia excelsa*) and are used by local traditional populations for nut extraction. According to senior staff at the research station, this location corresponds to the area visited by Ronald I. Crombie and Lynn C. Branch in 1979, the collectors of the specimens included in the *Allobates sumtuosus* type series (Morales 2002). The only other species of cryptically colored aromobatid frog found to be sympatric with *A. sumtuosus* in the area is *Anomaloglossus stepheni*.

Over four days we conducted acoustic and visual surveys targeting at diurnal frogs, walking along available trails during daytime, from 06:00h to 18:00h. Vocally active individuals of *A. sumtuosus* were found in areas of *terra-firme* forest on sloped terrain. Field observations of live specimens, photographs, and films made at this occasion were used for the description of color in life.

Advertisement calls of seven *A. sumtuosus* males were recorded using a Marantz PMD 660 digital recorder and a Sennheiser ME 66 directional microphone positioned approximately 1 m from focal males. Most recordings were conducted in the morning (*n*=5), from 08:00 to 08:45h. Two recordings were done in the afternoon, between 17:30–18:10h. Air temperature at time of recording ranged from 23.5 to 25.6°C. Snout-to-vent length (SVL) of recorded males ranged from 14.5 to 16.0 mm.

Calls were analyzed in Raven 1.2 (Charif *et al.* 2004) for the description of temporal and spectral parameters. Because males emit two kinds of advertisement calls (calls formed by regular note trills and continuous calls with irregular note arrangement), different acoustic parameters were measured for each type of call. For calls formed by regular note trills (*n*=15 calls, recorded from four individuals), we measured trill duration (TD), the number of notes emitted in each trill (NT) and duration of the silent interval between trills (TSI). From each call, we randomly sampled five notes (*n* = 75 notes), from which we measured note duration (ND), duration of silent interval between notes (NSI), and note's peak, lowest and highest frequencies (NPF, NLF and NHF, respectively).

From continuous calls (*n*=4 recordings, obtained from four individuals), we sampled 20 notes, uniformly distributed along the entire recording length. This was accomplished by dividing the total number of notes in each recording by 20 and setting the resulting fraction as the sampling interval. From each recording, we measured note duration (ND), duration of silent interval between notes (NSI), and note's peak, lowest and highest frequencies (NPF, NLF and NHF). Spectral analyses of both call types were conducted with frequency resolution of 82 Hz and 2048 points, using Blackman window type (Kaefer & Lima 2012; Simões *et al*. 2013). Lowest and highest frequencies were measured 20 dB below peak frequency, in order to avoid overlap with background noise. One individual (INPA-H 31952) shifted from regular trills to continuous calls during recording time, hence recording sections corresponding to each call type were analyzed separately.

Additional calls were obtained from two locations in Brazil where individuals of *A. sumtuosus* were similar in appearance to nominotypical. Calls from six males were recorded at Reserva Florestal Adolpho Ducke (RFAD), a 10.000 ha forest fragment used as a research station, in Manaus, State of Amazonas (02°55'–03°01'S, 59°53'–59°59'W). Calls were recorded with an AKG 568 EB directional microphone plugged to a Sony WM-D6C tape recorder. Recordings were digitized from tapes with Raven 1.2 at 44.1 kHz and 16 bit resolution. Three males were recorded in a forest fragment near the city of São João da Baliza (SJB), Roraima (00°57'10" N, 59°55'43" W), on July 5th, 2011. Recording equipment and procedures in SJB were the same used in REBio Trombetas. All acoustic analysis followed the same parameters previously described.

Voucher specimens collected in the 2011 field expedition to REBio Trombetas included the seven recorded specimens and one additional male collected opportunistically. All specimens were captured manually, anesthetized and killed with topic solution of 2% benzocaine, fixed in 10% formalin, and preserved in 70% alcohol. A sample of muscle tissue was removed from all individuals before fixation procedures.

In March 2013, specimens were examined under a stereo microscope in order to describe character states for external morphology traits, comparing them with states listed in the original species description (Morales 2002). We provide an updated description of external morphology, including variation in body measurements, body proportions, and additional morphological characters currently applied to *Allobates* taxonomy. Character choice and terminology followed Caldwell & Lima (2003), Grant *et al.* (2006), Lima *et al*. (2007) and Barrio-Amorós & Santos (2009). Body 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), upper arm length from anterior corner of arm insertion to the outer edge of flexed elbow (UAL), lengths from proximal edge of palmar tubercle to tips of fingers I, II, III, and IV (respectively HAND I, HAND II, HAND III, HAND IV), width of disk on Finger III (WFD), width of third phalange of Finger III (WPF), maximum diameter of palmar tubercle (DPT), maximum diameter of thenar tubercle (DTT), leg length from the posterior extremity of the coccyx to the outer edge of flexed knee (LL), tibia length from outer edge of flexed knee to heel (TL), foot length from proximal edge of outer metatarsal tubercle to tip of Toe IV (FL) and width of disk on Toe IV (WTD). Measurements were taken to the nearest 0.1 mm with a digital caliper or graduated lenses. All voucher specimens are housed at the herpetology section of the zoological collection of Instituto Nacional de Pesquisas da Amazônia, in Manaus, Brazil (accession numbers INPA-H 31952–56, INPA-H 31958–60).

Total genomic DNA was extracted from preserved muscle tissues of four voucher specimens from REBio Trombetas (INPA-H 31954–55, 31959–60), three specimens from RFAD (INPA-H 31949–31951), two specimens from SJB (INPA-H 31957, AMNH 20210), and one additional specimen collected north of the city of Manaus, at km 85 of BR 174 road (02°13'23" S, 60°03'55" W - AMNH 20030). DNA extraction used a cetyltrimethylammonium bromide (CTAB) protocol modified from Doyle & Doyle (1987). We used primers 16Sar and 16Sbr (Palumbi, 1996) to amplify a 530 bp fragment of the 16S rRNA mitochondrial gene. DNA amplification via polymerase chain reaction (PCR) used mixes with a final volume of 16 μL, containing 6.7 μL ddH_2O, 2.0 μL of 25mM $MgCl_2$, 1.5 μL of 10 mM dNTPs (2.5mM each dNTP), 1.5 μL of 10X amplification buffer (75mM Tris HCl, 50 mM KCl, 20 mM (NH4)2SO4), 1.5 μL of a 2 μM solution of each primer, 0.3 μL of Taq DNA polimerase 5 U/μL (Biotools, Spain) and 1 μL of DNA (about 30 ng/ μL). Reaction conditions had a pre-heating step at 92°C for 60 s, 35 cycles of denaturation at 92°C for 60 s, primer annealing at 50°C for 50 s, and primer extension at 72°C for 90 s, followed by final extension step of five minutes at 72°C. Sequencing reactions were carried out after PCR product purification with exonuclease and alkaline phosphatase (Fermentas Life Sciences, Canada) and followed ABI BigDye Terminator Cycle Sequencing Kit protocols, as indicated by the manufacturer. The forward primer was used in sequencing reactions, with annealing temperature of 50°C. The resulting single-stranded products were resolved in an ABI 3130xl automatic sequencer.

We applied the same methods described above in order to obtain 16S rDNA sequences from tissue samples of eight *Allobates* specimens belonging to three species (*A. crombiei*, *A. grillisimilis*, *A. subfolionidificans*), proceeding from their type localities (Appendix I). Additional sequences were obtained from GenBank. Sequence selection was restricted to cryptically colored *Allobates* species occurring in Brazil, or distributed in the Guiana Shield region, that were morphologically similar to *A. sumtuosus*. Only sequences proceeding from species' type localities were considered. In three cases (*A. conspicuus*, *A. gasconi*, *A. trilineatus*), we found no published sequences proceeding from vouchers collected at the respective type localities, thus we selected sequences from the closest locations available (Appendix I). We did not include sequences attributed to *A. brunneus* and *A. marchesianus*, as these names have been applied to a large number of distantly related lineages, and available samples were obtained in locations considerably removed from the designated type localities. However, both species are easily distinguished from *A. sumtuosus* by a large set of phenotypic traits (Caldwell *et al*. 2002; Lima *et al.* 2009).

Sequence alignment was carried out in Bioedit (Hall 1999). We used ClustalW algorithm (Thompson *et al.* 1994) to generate preliminary alignments, which were subsequently checked by eye, and corrected manually. Interspecific pairwise and Kimura-2-parameter (K2P—Kimura 1980) genetic distances between clades corresponding to species and sampling locations of putative *A. sumtuosus* were calculated in MEGA 5.05 (Tamura *et al.* 2011). In order to assess the evolutionary relationships between sequence samples we generated a maximum likelihood phylogenetic tree using MEGA 5.05. Clade support was inferred from 5000 bootstrap replicates. A general time reversible model with a portion of invariant sites and gamma distribution of rate variation (GTR+I+G) was selected by both Akaike Information Criterion and Bayesian Information Criterion (AIC and BIC). Model selection was conducted in jMoldeltest 2.1.3 (Darriba *et al*. 2012). The molecular analyses are complementary to other sources of data provided herein, and are applied as a means to assess the geographic distribution of *A. sumtuosus* and the potential occurrence of synonym with morphologically similar taxa. Color in life and parameters of advertisement calls are compared in more detail with those described for *Allobates spumapenens* (Kok & Ernst 2007), which we considered as a potential junior synonym of *A. sumtuosus* upon the conclusion of analyses of genetic distances and evolutionary relationships.

Results

Description of color in life. Dorsal surface of body of *Allobates sumtuosus* is solid tan brown to copper. Small spots of darker brown are visible on dorsum of some individuals. Lateral surface of body is solid dark brown (Fig. 1A–C). On some individuals, a short pale linear mark is visible laterally on dark brown background, extending from inguinal region, but never reaching the lateral mid section of the body (*i.e*. it does not form a conspicuous oblique lateral line *sensu* Morales [2002]). A pale dorsolateral stripe is not evident on live individuals. A white, iridescent ventrolateral stripe is present along the lower margin of the dark brown flanks, from tip of snout to the groin, often interrupted. Pale iridescent mottling, same color as of ventrolateral stripe, is present below ventrolateral stripe, towards the abdomen, over unpigmented background. Small dark brown dots may appear

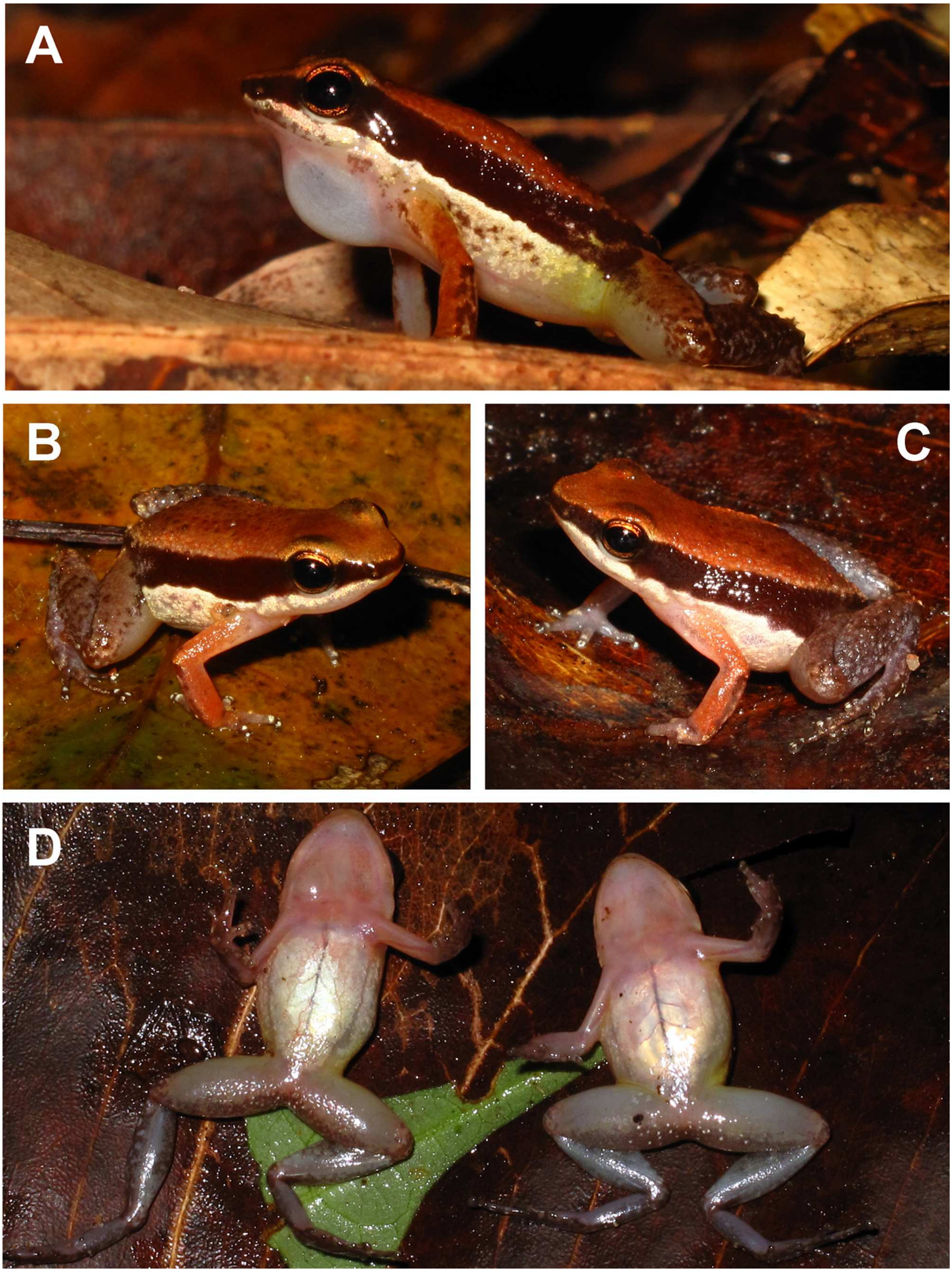

FIGURE 1. Color in life of *Allobates sumtuosus* individuals from the species type locality at Reserva Biológica do Rio Trombetas, State of Pará, Brazil. (A) Lateral view of a calling male. Note white to translucent vocal sac, iridescent white mottling with a few brown blotches from the white lateral line towards the abdomen, and discrete shades of yellow on the inguinal region. (B) and (C) Dorsolateral views of two additional males. (D) From left to right, ventral views of the same males depicted in (B) and (C), respectively. Photos: P.I. Simões.

among white mottling in some individuals. Throat, gular and pectoral regions, and abdomen are uniformly unpigmented, appearing light gray to translucent (Fig. 1D). Vocal sac of males is white to light gray to translucent when inflated (Fig. 1A). Shades of bright yellow are present on inguinal region of most individuals. Iris is golden copper with tiny black flecks (Fig. 1A–C).

Upper and forearm are uniformly light tan brown to orange in dorsal and lateral views. Tegument on arm-body insertion is translucent. Upper arm is white to translucent, in ventral view, same color as chest. Forearm is dark brown ventrally. Forearm, carpal and metacarpal regions are dark brown in ventral view. Fingers are brown in ventral view, brown to translucent in dorsal view. Scutes on finger discs are iridescent white (Fig. 1A–C).

The area immediately adjacent to vent is solid brown, flanked by a light cream paracloacal mark. Distal edge of paracloacal mark is diffuse, merging shortly with color of dorsal surface of thigh. Dorsal surface of thigh is predominantly uniform bluish gray, with a variable number of brown irregular spots, generally distributed dorsolaterally. Dorsal surface of shank same color as that of thigh, with a larger area covered by brown irregular spots and flecks. Ventral surface of thigh and shank generally light gray to translucent, slightly darker than abdomen. Brown marbling is present along outer edges. Tarsal region is gray with brown flecks in dorsal view, uniformly brown in ventral view. Sole is dark brown. Toes have gray and brown patterning. Paired scutes on toe discs are light gray to iridescent white.

The description of color in life of *A. spumaponens* (Kok & Ernst 2007) point two potential differences between color patterns of live male specimens of that species and those of *A. sumtuosus*. A pale dorsolateral stripe was present in the live holotype of *A. spumaponens*, although it has been noted as narrow, diffuse and difficult to distinguish among other individuals in the type series. Color in life of dorsal surface of thighs of the holotype is also noted as brown, with no bluish-gray shades, as observed among typical *A. sumtuosus*. Considering differences in application of color and morphological terminology, the remaining color traits were largely equivalent between descriptions.

Description of advertisement calls. At REBio Trombetas, male *Allobates sumtuosus* produced two forms of advertisement calls. The first consist of a regular trill of short notes (Fig. 2A). Trills were separated by relatively long (22.9 ± 7.0 s) silent intervals, and are formed by 23–35 notes (mode = 32 notes) (Table 1). Average duration of trills were 5.0 ± 0.5 s. Notes that constitute regular trills had an average duration of 0.05 s, with very low intra- and inter-individual variation (standard deviations always < 0.001 s). Silent interval between notes were short, 0.11 ± 0.01 s in average. Notes are tonal, with ascending frequency modulation, with average lowest frequency of 5638.1 ± 129.5 Hz and average highest frequency of 6800.9 ± 120.1 Hz. Average peak frequency of notes produced in regular trills is 6325.4 ± 126.3 Hz (Table1).

The second type of advertisement call consists in the continuous emission of notes, irregularly arranged in time, and separated by silent intervals variable in duration (Fig. 2B). Notes were 0.05 ± 0.01 s long in average, not distinguished in length from those of regular trills. Average duration of silent intervals between notes were 0.31 ± 0.08 s, generally longer and more variable than that of silent intervals between notes in calls emitted in regular trills (Table 2). Notes produced during continuous calls are also tonal, with ascending frequency modulation. Average values for lowest, highest and peak frequencies of notes in continuous calls were 5550.2 ± 102.1 Hz, 6728.0 ± 260.2 Hz, and 6197.5 ± 26.3 Hz, respectively. Notes of continuous calls tend to have lower peak frequencies than those of regular trills but frequency bandwidth is highly similar between call types. Differences in peak frequency could be attributed to a slight difference in average body size between individuals recorded (mean SVL between individuals emitting regular trills is 15.3 mm, and between individuals emitting notes continuously is 15.5 mm), although the small number of recorded specimens prevents reliable statistical evaluations.

Advertisement calls recorded at RFAD (Manaus, Amazonas) and SJB (Roraima) generally matched the same note arrangements (Fig. 3). Calls formed by regular trills were recorded on both locations. Descriptive parameters relating to the entire trills broadly overlapped between sampling locations, whereas most note parameters of *A. sumtuosus* calls recorded in SJB had values out of the range measured in the remaining locations (Table 1). Continuous, irregularly arranged calls were only recorded at RFAD. Variation in call traits of continuous calls largely overlapped with values measured from calls recorded at *A. sumtuosus* type locality at REBio Trombetas. However, peak frequencies of notes were generally higher among males recorded at RFAD (Table 2).

The small number of individuals recorded in each location, differences in air temperature ranges at the time of recording procedures and the lack of information on body size of all individuals recorded at RFAD and SJB hinder the application of tests to evaluate the statistical significance of differences in continuous call traits between sampling sites. However, observed call arrangement is conservative among sampling locations.

TABLE 1. Temporal and spectral characterization of advertisement calls of *Allobates sumtuosus* emitted in regular trills. Recordings proceeded from three locations in Brazilian Amazonia: Reserva Biológica do Rio Trombetas (REBio Trombetas), Reserva Florestal Adolpho Ducke (RFAD), and São João da Baliza (SJB). Temperature (°C) refers to air temperature at the time of recording and SVL to snout-to-vent length of vouchered males. NT, TD, and TSI refer to characteristics of trills. ND, NSI, NPF, NLF, and NHF refer to characteristics of notes and intervals within trills. A more comprehensive description of call trait characteristics and recording locations is given in the text.

	REBio Trombetas	RFAD	SJB
N° of recorded males	4	2	3
Calls/Trills analyzed	15	8	8
Notes analyzed	75	40	40
Temperature (°C)	23.5–24.1	27.3	25.9
SVL (mm)	14.5–16.0	14.8	15.3
NT (Mode)	32	19	30
NT (Range)	23–35	15–20	28–32
TD (s)	5.034 ± 0.528 (3.949–5.878)	2.560 ± 0.351 (1.950–2.930)	4.624 ± 0.271 (4.320–5.140)
TSI (s)	22.958 ± 7.928 (4.509–32.647)	34.280 ± 32.365 (10.910–101.640)	21.886 ± 3.877 (15.800–25.420)
ND (s)	0.049 ± 0.004 (0.043–0.051)	0.048 ± 0.001 (0.048–0.049)	0.071 ± 0.005 (0.065–0.075)
NSI (s)	0.111 ± 0.006 (0.105–0.119)	0.096 ± 0.008 (0.091–0.103)	0.079 ± 0.006 (0.074–0.086)
NPF (Hz)	6325.4 ± 126.3 (6172.3–6480.1)	6157.9 ± 250.4 (5980.8–6335.0)	5857.0 ± 218.9 (5609.4–6024.9)
NLF (Hz)	5638.1 ± 129.5 (5486.4–5793.6)	5582.7 ± 319.3 (5356.9–5808.4)	4994.6 ± 140.1 (4879.4–5150.6)
NHF(Hz)	6800.9 ± 120.2 (6680.9–6940.7)	7009.5 ± 228.7 (6847.8–7171.2)	6298.5 ± 205.8 (6065.8–6456.8)

TABLE 2. Temporal and spectral characterization of advertisement calls of *Allobates sumtuosus* emitted continuously (with no evident note arrangement), and values for corresponding acoustic traits in *Allobates spumaponens*, as reported in the species description (Kok & Ernst 2007). *Allobates sumtuosus* recordings proceeded from two locations in Brazilian Amazonia: Reserva Biológica do Rio Trombetas (REBio Trombetas), and Reserva Florestal Adolpho Ducke (RFAD). Temperature (°C) refers to air temperature range at the time of recordings and SVL to snout-to-vent length of vouchered males. ND, NSI, NPF, NLF, and NHF refer to characteristics of notes. A more comprehensive description of variables and recording locations is given in the text.

	REBio Trombetas	RFAD	*Allobates spumaponens*
N° of recorded males	4	5	2
Notes analyzed	80	100	10
Temperature (°C)	23.5–24.1	24.5–25.2	24.0–25.0
SVL (mm)	15.2–15.8	14.7–14.8	15.7
ND (s)	0.046 ± 0.005 (0.041–0.052)	0.049 ± 0.001 (0.048–0.051)	0.066 ± 0.003 (0.06–0.07)
NSI (s)	0.312 ± 0.077 (0.224–0.401)	0.421 ± 0.064 (0.341–0.476)	
NPF (Hz)	6197.5 ± 26.3 (6160.6–6223.1)	6362.2 ± 114.3 (6279.1–6549.3)	~ 6500.0
NLF (Hz)	5550.2 ± 102.1 (5403.8–5626.4)	5567.4 ± 213.3 (5444.7–5947.4)	5339.2 ± 83.9 (5186.4–5472.8)
NHF(Hz)	6728.0 ± 260.2 (6350.0–6919.2)	6843.9 ± 82.5 (6768.3–6961.9)	6802.8 ± 62.4 (6713.8–6920.6)

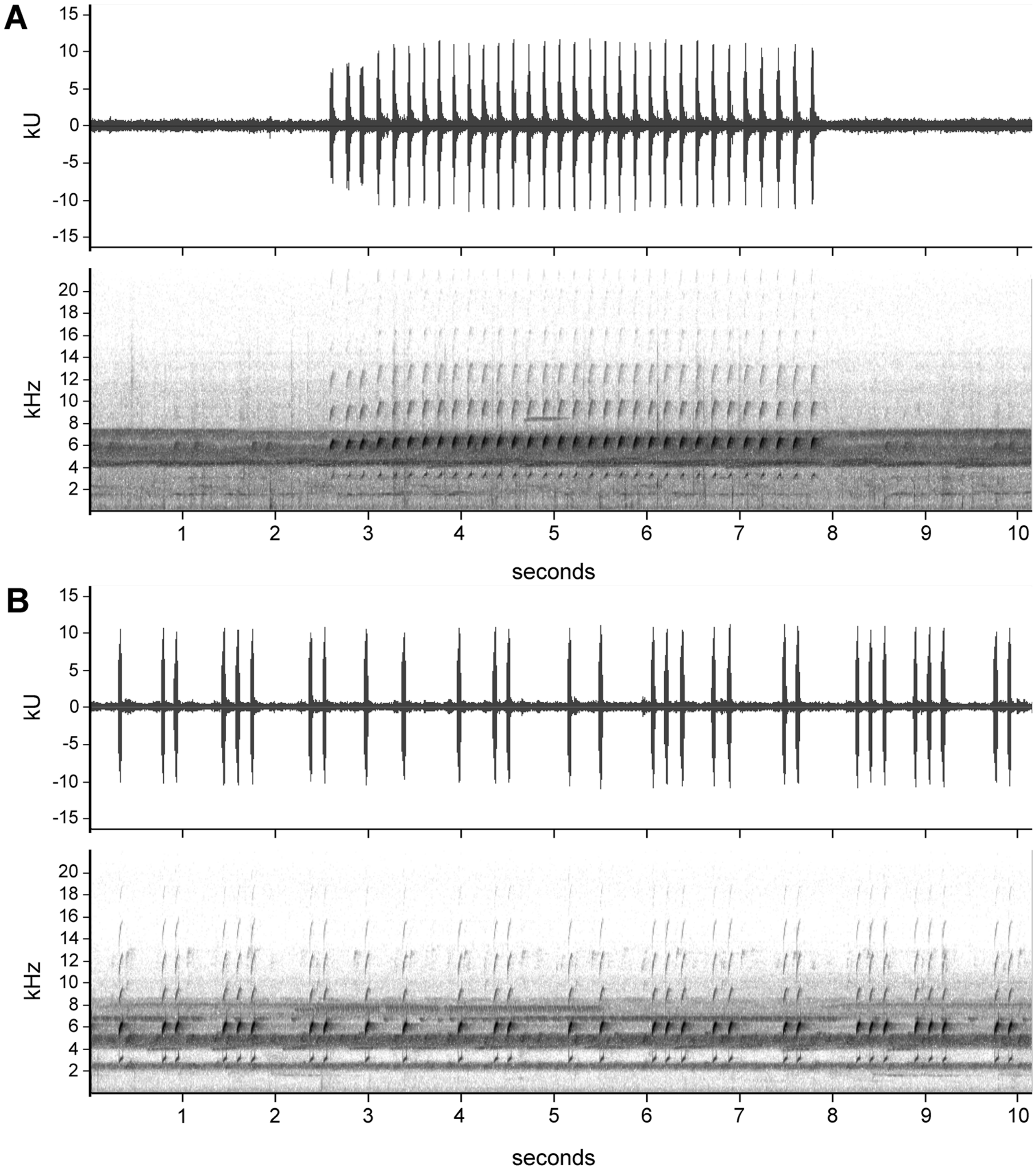

FIGURE 2. Waveforms (upper graphs) and spectrograms (lower graphs) of the advertisement calls of *Allobates sumtuosus* recorded at the species type locality in Reserva Biológica do Rio Trombetas, State of Pará, Brazil. Male *A. sumtuosus* emit two forms of advertisement calls: (A) Calls emitted as regular trills of notes, between long silent intervals. (B) Irregular calls, with no evident pattern of note arrangement, emitted continuously between short, highly variable silent intervals.

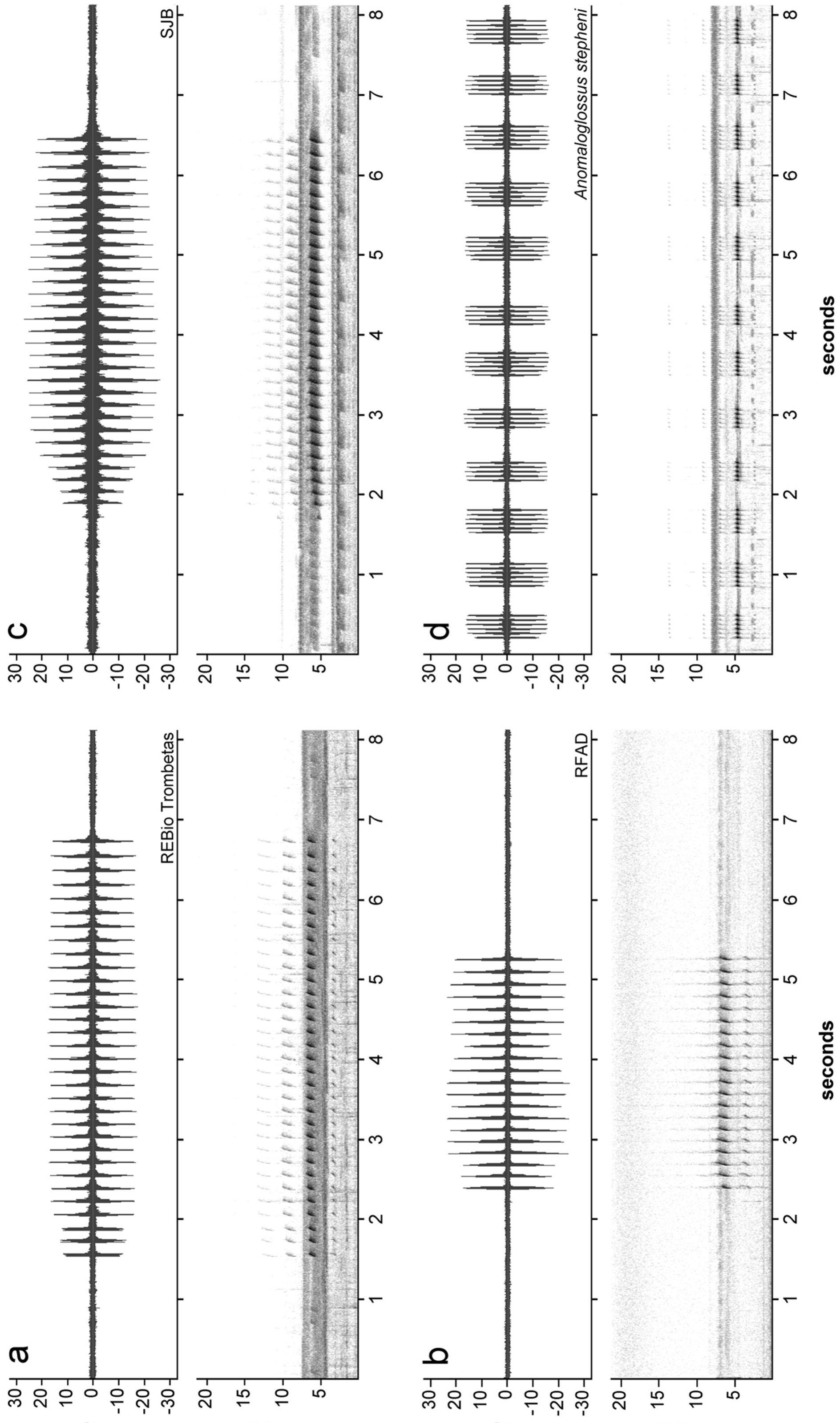

FIGURE 3. Advertisement calls of *Allobates sumtuosus* recorded in three locations in Brazilian Amazonia: (A) At the species type locality at Reserva Biológica do Rio Trombetas (REBio Trombetas), State of Pará; (B) at Reserva Florestal Adolpho Ducke (RFAD), in Manaus, State of Amazonas; and (C) in São João da Baliza (SJB), State of Roraima. Air temperatures at the time of recording were 23.5, 27.3 and 25.9°C, respectively. (D) Calls of *Anomaloglossus stepheni*, recorded in REBio Trombetas, at 27.0°C. The species is the only cryptically colored aromobatid frog known to be syntopic to *A. sumtuosus* along its type locality. However, calls of the two species are easily distinguished by the arrangement and duration of notes and intervening silent intervals.

Comparisons with calls of *A. spumaponens* are also limited by the small number of males for which calls have been sampled in detail and for lack of available information on mean values and amplitude of variation of some call parameters (Table 2). Sonograms provided in the description of *A. spumaponens* depicts a call arrangement similar to the continuous calls of *A. sumtuosus* described above (see top graphs in Fig. 7 of Kok & Ernst 2007). Spectral properties of individual notes generally overlap between calls of *A. spumaponens* and calls of *A. sumtuosus* (Table 2). Considering only continuous calls, note duration is longer in *A. spumaponens*, ranging between 0.06 and 0.07 s. However, *A. sumtuosus* recorded in SJB while producing calls in regular trills also produced notes with duration equivalent to that of *A. spumaponens* (Table 1).

Morphological reappraisal of *Allobates sumtuosus*. Body measurements of eight male voucher specimens collected at REBio Trombetas are provided in Table 3. Body robust, head as wide as long (average HL/HW = 0.98), head length corresponding to 0.32 times the snout-to-vent length (Fig. 4). Diameter of eye always larger than distance from anterior corner of the eye to nostril (average EN/EL = 0.77). Nares located posterolaterally to tip of snout. Nostrils directed anterolaterally, visible in anterior, ventral and lateral views, but not in dorsal view. Distance between nostrils less than half of head width (average IN/HW = 0.42). Tympanum round, diameter shorter than maximum diameter of eye (average TYM/EL = 0.58). Ventral margins of tympanum inconspicuous to the naked eye, not extending beyond the dark brown band along the lateral surface of body. Vocal sac single, round when inflated. In preserved individuals, when retracted, vocal sac evident as loose skin on the gular region (*i.e.* not restricted to lateral vocal slits). Gular and pectoral regions unpigmented. When present, small number of melanophores restricted to tip and sides of chin.

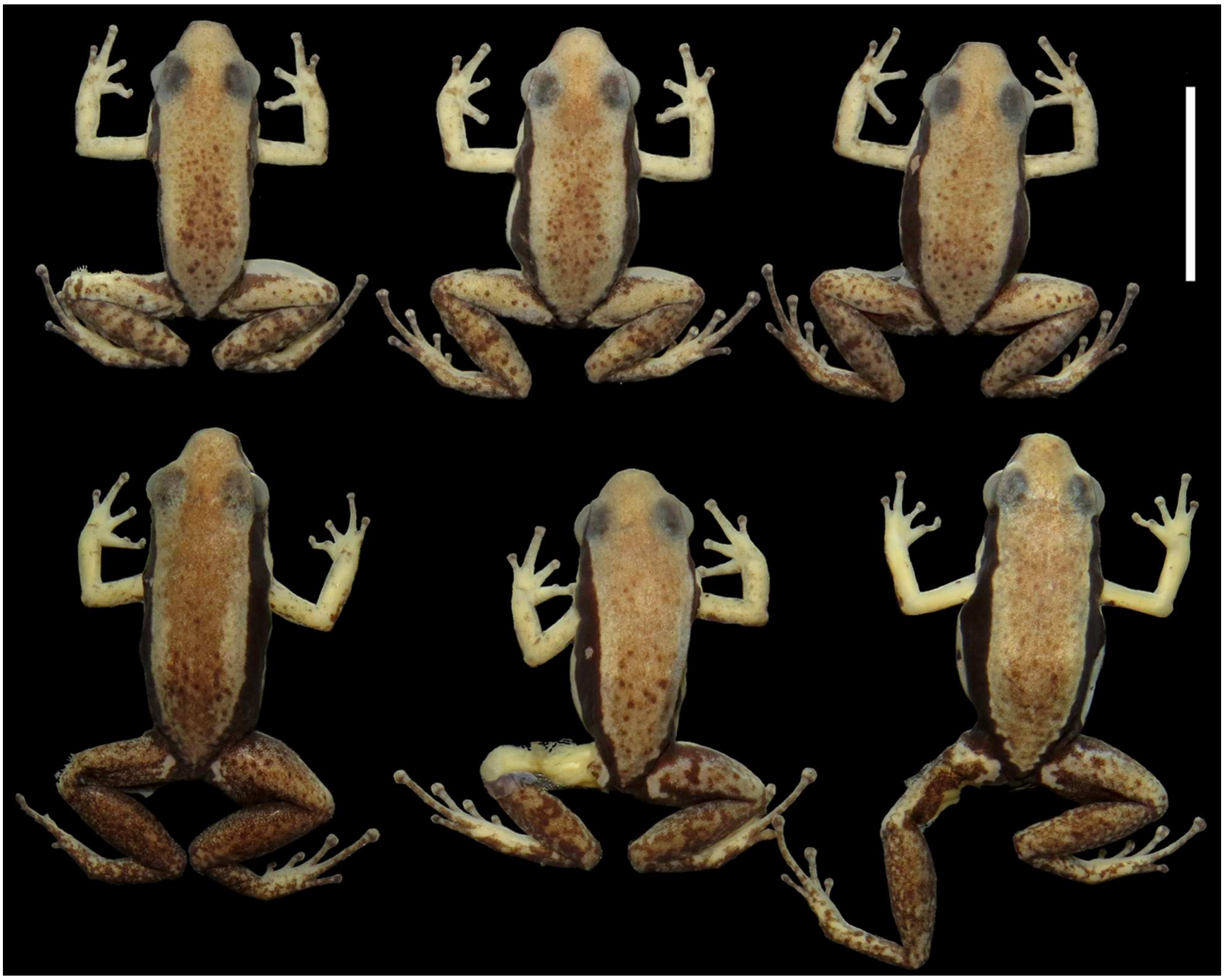

FIGURE 4. Variation in dorsal color patterns among preserved *Allobates sumtuosus* specimens. Upper line: typical male specimens proceeding from Reserva Biológica do Rio Trombetas (INPA-H 31952, 31958, 31960). Lower line: male (INPA-H 31950, center) and female (INPA-H 31948–31949, left and right, respectively) specimens proceeding from Reserva Florestal Adolpho Ducke, in Manaus. Note variation in conspicuousness and width of pale dorsolateral stripe, evident only as a weakly pigmented stretch along the lateral edges of dorsum. Scale bar corresponds to 10.0 mm.

TABLE 3. Measurements (in mm) of eight *Allobates sumtuosus* male specimens collected at the species type locality in Reserva Biológica do Rio Trombetas, State of Pará, Brazil, in February 2011, and measurements of six male *Allobates spumaponens* from the species type locality in Guyana, as provided in the original species description by Kok & Ernst (2007). Values represent mean ± one standard deviation (range of variation). Third column: available measurements of *A. sumtuosus* holotype (USNM303591), provided in the original species description (Morales 2002).

	A. sumtuosus	*A. spumaponens*	*A. sumtuosus*
	Males (*n* = 8)	Males (*n* = 6)	Holotype (USNM303591)
SVL	15.4 ± 0.3 (15.0–15.8)	15.0 ± 1.0 (13.2–16.0)	15.6
HL	5.0 ± 0.1 (4.8–5.2)	4.8 ± 0.2 (4.3–5.0)	
HW	5.3 ± 0.3 (4.9–5.7)	4.8 ± 0.3 (4.3–5.4)	5.6
SL	2.5 ± 0.1 (2.5–2.7)	2.5 ± 0.2 (2.3–2.8)	
EN	1.5 ± 0.1 (1.4–1.7)	1.4 ± 0.1 (1.2–1.5)	
IN	2.2 ± 0.1 (2.1–2.4)	1.9 ± 0.1 (1.7–2.1)	2.0
EL	2.0 ± 0.1 (1.9–2.1)	2.3 ± 0.2 (2.0–2.5)	2.2
IO	1.8 ± 0.1 (1.7–2.0)	2.0 ± 0.1 (1.8–2.2)	1.9
TYM	1.2 ± 0.2 (1.0–1.5)	0.7 ± 0.1 (0.6–0.9)	
FAL	3.5 ± 0.1 (3.3–3.7)	3.4 ± 0.2 (3.0–3.8)	
UAL	3.8 ± 0.2 (3.6–4.2)		
HAND1	2.9 ± 0.2 (2.7–3.2)		
HAND2	2.5 ± 0.1 (2.4–2.7)		
HAND3	3.5 ± 0.1 (3.3–3.6)	3.4 ± 0.2 (3.0–3.6)	
HAND4	2.2 ± 0.2 (2.0–2.6)		
WFD	0.5 ± 0.04 (0.5–0.6)	0.4 ± 0.05 (0.4–0.5)	
WPF	0.4 ± 0.05 (0.4–0.5)		
DPT	0.6 ± 0.1 (0.5–0.7)		
DTT	0.5 ± 0.1 (0.4–0.6)		
LL	7.3 ± 0.2 (7.0–7.7)		6.9
TL	7.6 ± 0.3 (7.2–7.9)	7.3 ± 0.4 (6.6–7.8)	7.5
FL	6.4 ± 0.2 (6.2–6.8)	6.1 ± 0.4 (5.3–6.6)	
WTD	0.6 ± 0.05 (0.6–0.7)	0.5 ± 0.05 (0.5–0.6)	

Length of forearm equivalent to 0.23 times the snout-to-vent length. Palmar tubercle round to drop-shaped (Fig. 5A). Thenar tubercle conspicuous, elliptical, shorter than palmar tubercle in maximum diameter (DTT/DPT = 0.76). Metacarpal ridge and carpal pad absent. Outgrowth or callosities on thumbs and black arm glands absent in male specimens. Relative lengths of fingers: IV < II < I < III (Fig. 5A). Finger IV lacks distal subarticular tubercle, when appressed, length never reaches the distal subarticular tubercle of Finger III. Length of Finger II approximately 14% smaller than Finger I. Relative width of distal phalange of Finger III generally over 0.8 times the maximum width of the respective finger disc (difference between widths of disc and distal phalange never larger than 0.1mm). Discs of all fingers weakly expanded.

Tibia length equivalent to 0.49 times the snout-to-vent length, in average. A tarsal keel present, tuberclelike, and strongly curved at proximal end, extending from metatarsal tubercle (Fig. 5B). Metatarsal fold absent. Tarsal fringe absent. Relative lengths of toes: I < II < V < III < IV. Basal webbing present only between Toes III and IV (Fig. 5B). Fringes appear only partially between Toes III and IV, as an extension of basal webbing. Fringes absent on remaining toes. Discs of all toes weekly expanded.

Surface of dorsum weakly granular, granules with tips indicated by aggregations of brown melanophores. Granules more evident on dorsal surface of urostyle region and shanks. A pale dorsolateral stripe with diffuse inner edge present, variable in width and conspicuousness among preserved specimens (Fig. 4). Skin smooth along lateral and ventral body surfaces. Dermal flap above cloaca absent.

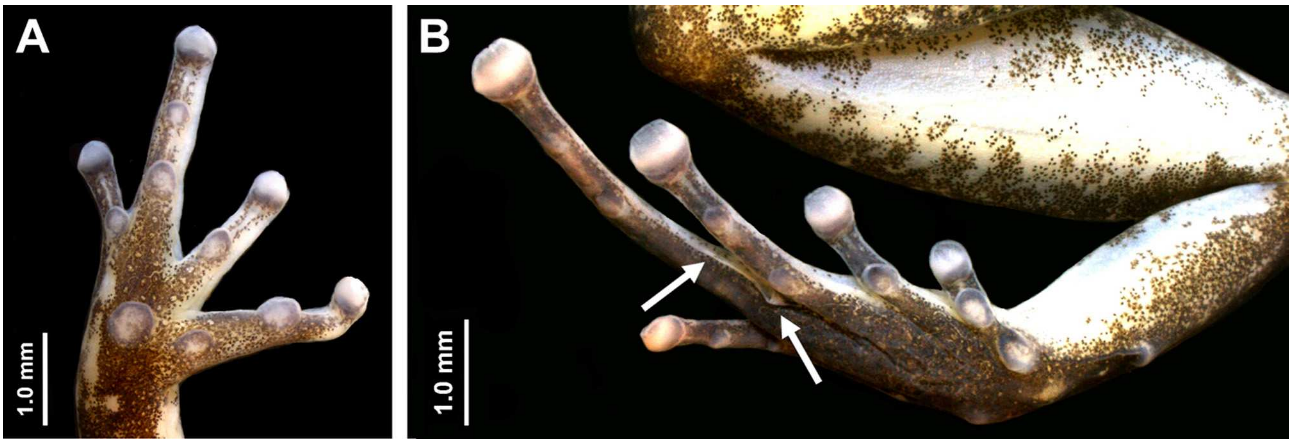

FIGURE 5. (A) Hand and (B) foot of a male specimen of *Allobates sumtuosus* (INPA-H 31960) collected at the species type locality in Reserva Biológica do Rio Trombetas, State of Pará, Brazil. Lower arrow in (A) indicates position of basal webbing between Toes III and IV. Upper arrow indicates the extension of basal webbing that could be interpreted as a dermal fringe. However, it is restricted to the proximal internal margin of Toes III and IV, not extending along the length of these toes, or between Toes II and III.

Genetic distances and evolutionary relationships. Evolutionary analysis based on a fragment of the 16S rDNA mitochondrial gene suggests that *Allobates sumtuosus* constitutes a well-supported clade, monophyletic in relation to *Allobates* species known to occur in Brazil and along the Guiana Shield, except *A. spumaponens* (Fig. 6). Specimens phenotypically similar to *A. sumtuosus* collected at RFAD, SJB and BR 174 road form the sister clade to the clade constituted by typical *A. sumtuosus* and *A. spumaponens*. Average uncorrected pairwise genetic distance between typical *A. sumtuosus* and *A. spumaponens* are around 1%, and around 3% between typical and putative *A. sumtuosus* from the remaining localities. No changes in magnitude of genetic distances were observed when correcting distances by applying a standard K2P evolutionary model (Table 4). Average uncorrected pairwise genetic distances between typical *A. sumtuosus* and the remaining *Allobates* ranged from 7 to 14% (7–19% considering K2P genetic distances).

Basal clades were generally weakly supported, and their arrangement should not be considered to reflect the phylogenetic relationships between distantly related taxa (Fig. 6). However, samples attributed to *A. gasconi* collected in different river basins in Brazil (rivers Juruá and Ituxi) represent two clearly distinct evolutionary lineages, samples from Juruá having been collected closer to *A. gasconi*'s type locality at Jainu, on the left bank of the Juruá River. Samples attributed to *A. conspicuus* collected in Porto Walter, State of Acre, Brazil (~450 km northwest of the species type locality in Manu National Park, Peru), are closely related to typical *A. subfolionidificans*. Genetic divergence between the latter species pair does not exceed 3% (Table 4). These species are distinguished based on color traits of preserved specimens (dorsolateral and ventrolateral stripes are absent in *A. subfolionidificans*), but data are lacking about calls, tadpoles and reproductive behavior of *A. conspicuus* (Morales 2002; Lima *et al.* 2007).

Geographic distribution and variation. Based on the examination of live specimens from type locality, analysis of advertisement calls and 16S rDNA genealogical relationships, *Allobates sumtuosus* is distributed across forested areas in the Amazonian low and highlands east of the Branco River (Fig. 7). Several *A. spumaponens* records were provided in a geographically extensive herpetofaunal survey across the northern State of Pará, in Brazil (Ávila-Pires *et al.* 2010—Fig. 7). A thorough examination of voucher specimens and field data is necessary to confirm species identification, but we tentatively consider these records as *A. sumtuosus*, and suggest the southeastern reaches of FLOTA Paru (00°56'38" N, 53°14'10" W), a conservation unit in the municipality of Almeirim, State of Pará, as the eastern limit of the species distribution. The species is not known to occur south of the Negro and Amazon rivers (see Simões *et al.* 2013 for additional localities sampled for *Allobates* species). If we consider *A. spumaponens* a junior synonym of *A. sumtuosus*, its northern distribution might encompass Guyana and reach northern Suriname.

Along its geographic range, *A. sumtuosus* is fairly conservative in relation to color of live specimens, with a uniformly copper to tan-brown dorsum (with a variable number of darker brown dots), predominantly bluish-gray dorsal surfaces of thighs and shanks (with a variable number of small, irregular brown marks) and iridescent white mottling on ventrolateral surface of body (Fig. 8). The most conspicuous case of color variation relate to the shades of yellow on ventral surfaces of body. In most male individuals, shades of yellow appear on inguinal and axillary regions. Sexual dimorphism in color of throat, chest and abdomen occurs in individuals collected at RFAD, near Manaus (ventral surfaces are unpigmented in males, and predominantly yellow in females, from chin to posterior abdomen—Fig. 8) and at *A. spumaponens* type locality in Suriname (belly yellow in females, hyaline-white in males—Kok & Ernst 2007). Color of live and preserved female specimens remains unknown for *A. sumtuosus* in REBio Trombetas, but the presence or absence of sexual dimorphism in color of ventral surfaces seem to be conservative within cryptically colored *Allobates* species (Caldwell *et al*. 2002; Caldwell & Lima 2003; Grant *et al*. 2006; Lima *et al.* 2007).

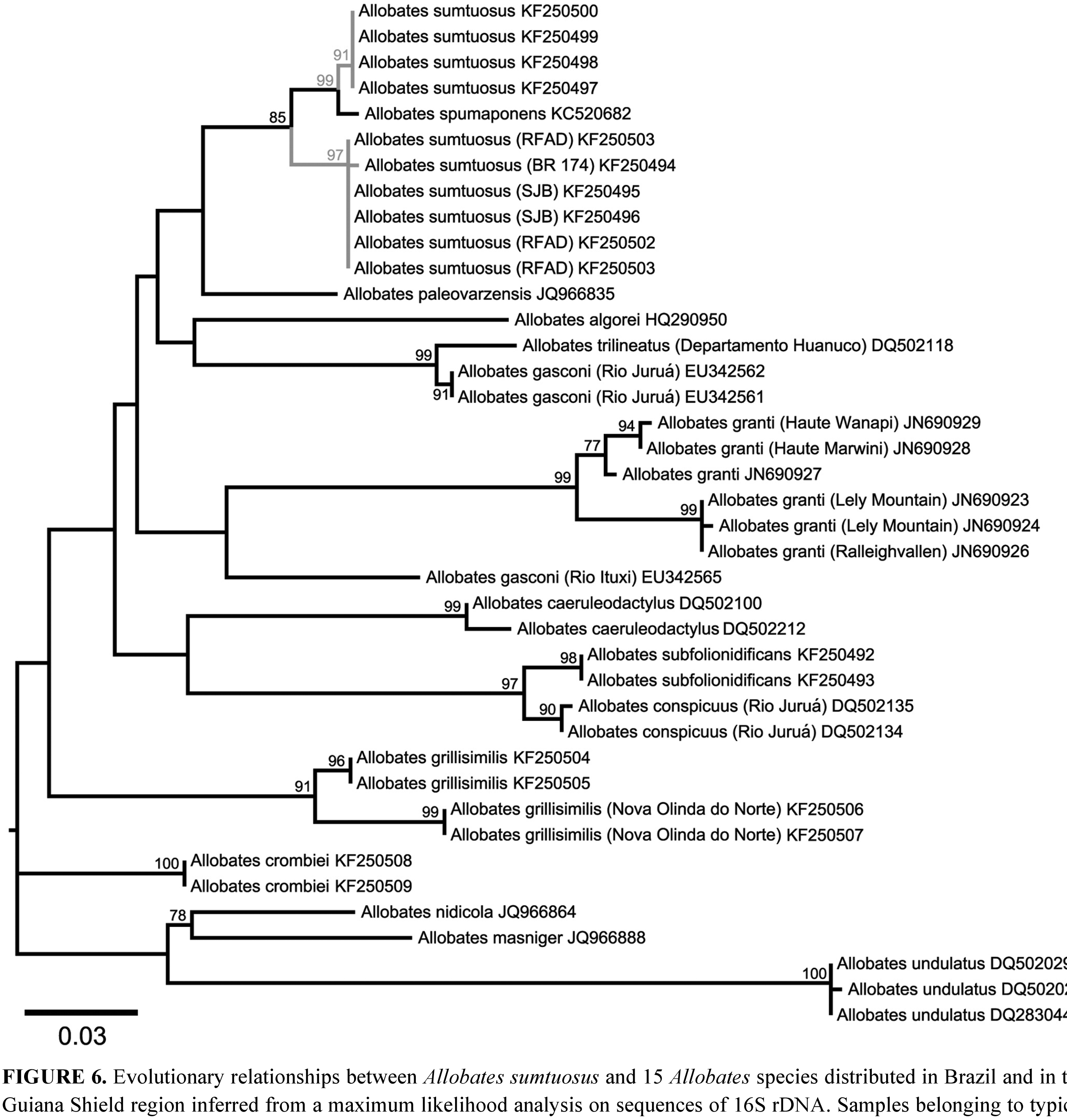

FIGURE 6. Evolutionary relationships between *Allobates sumtuosus* and 15 *Allobates* species distributed in Brazil and in the Guiana Shield region inferred from a maximum likelihood analysis on sequences of 16S rDNA. Samples belonging to typical and putative *A. sumtuosus* are highlighted in gray. Clade labels indicate support values from 5000 bootstrap replicates (only values above 70 are shown). Except when noted within parenthesis, sequences proceeded from samples collected at the referred type locality of each species. Due to differences in the length of sequences obtained from GenBank, positions with less than 95% site coverage (> 5% of sequences missing data for that position) were eliminated. A total of 346 positions were used in the final dataset.

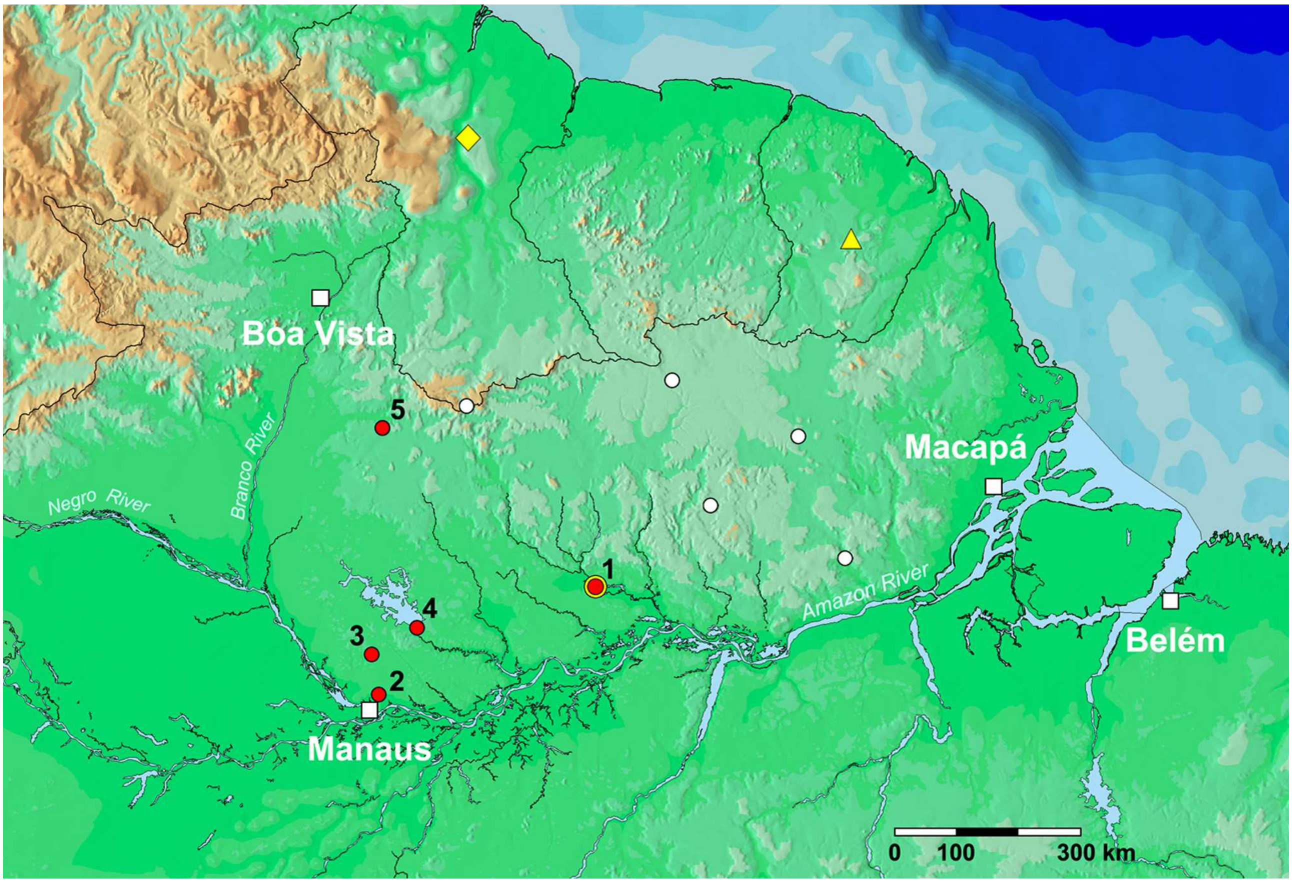

FIGURE 7. Geographic distribution of *Allobates sumtuosus* records in Brazilian Amazonia (red dots), and type localities of two morphologically similar species, *Allobates spumaponens* (yellow diamond) and *Allobates granti* (yellow triangle) in Guyana and French Guiana, respectively. *Allobates sumtuosus* records correspond to: 1—Reserva Biológica do Rio Trombetas, State of Pará; 2—Reserva Florestal Adolpho Ducke, in Manaus, Amazonas; 3—Fazenda Dimona, part of the Biological Dynamics of Forest Fragmentation Project (BDFFP), north of Manaus; 4—Vila de Balbina, near the city of Presidente Figueiredo; 5—São João da Baliza, in southeast Roraima. White dots indicate records of specimens identified as *Allobates spumaponens* by Ávila-Pires *et al*. (2010). State capitals in Brazil (white squares) are used as geographic references.

Discussion

As with many cryptically colored congeneric species, *Allobates sumtuosus* has not been diagnosed in the field for over a decade since its description. The species remained undetected in surveys across areas of well-documented amphibian fauna in central Amazonia, such as Reserva Florestal Adolpho Ducke (Lima *et al*. 2006, Menin *et al.* 2008) and the Biological Dinamics of Forest Fragments Project (BDFFP—Deichmann *et al*. 2010), as well as in large-scale amphibian assessments conducted within the species putative distribution (Ávila-Pires *et al.* 2010). Despite the small number of specialists in dendrobatid frogs residing in the region, and the yet shy investment in intensive field and collection surveys, we conclude that the short species description, based on a small set of external morphological characters, prevented herpetologists from identifying this apparently widespread species.

Some general morphological and color traits have proved useful for distinguishing among groups of similar *Allobates* species (*e.g.* SVL range, color of throat in males, hourglass or diamond-shape patterns on dorsum, etc.). However, continuous traits subject to large intraspecific variation, or to changes over large periods of preservation, can be a source of confusion during diagnosis of this large morphologically conserved group. For example, the presence of a pale dorsolateral stripe, coded as a diagnostic character of *A. sumtuosus* (Morales 2002), is not evident in live specimens, and is more or less conspicuous among preserved individuals. The presence of fringes on fingers and toes can also be a subjective statement. Sole and toe surfaces of *A. sumtuosus* holotype (Fig. 9) are similar to those of the specimens described herein. If fringes are present on toes, they seem to be short projections of basal webbing between Toes III and IV, not extending between Toes III and II (as pointed in Morales 2002). We argue that the adoption of a morphological or color trait as a diagnostic character among cryptically colored *Allobates* should depend on blatant and consistent interspecific differences. Moreover, the integration of acoustic and molecular data to morphological information seems to be crucial if a more reliable taxonomy of *Allobates* is intended.

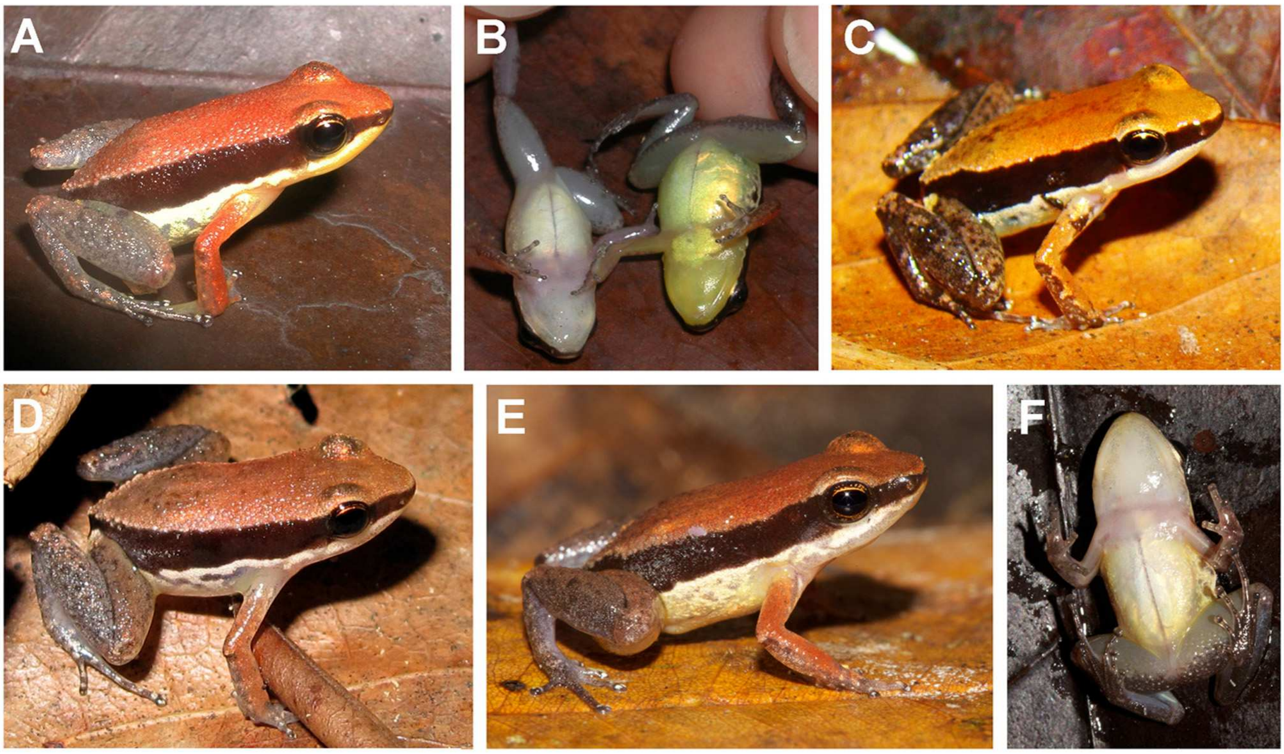

FIGURE 8. Color variation in live specimens of *Allobates sumtuosus*. (A) Reserva Florestal Adolpho Ducke (RFAD), Manaus, Amazonas. (B) Ventral view of male (left) and female (right) specimens from RFAD. (C) Fazenda Dimona, Manaus, Amazonas, about 60 km north of RFAD. (D) Vila de Balbina, Amazonas. (E) São João da Baliza, Roraima. (F) Ventral view of the same individual. Photographs A–B: Albertina P. Lima; C–D Pedro I. Simões; E–F: Santiago Castroviejo-Fisher.

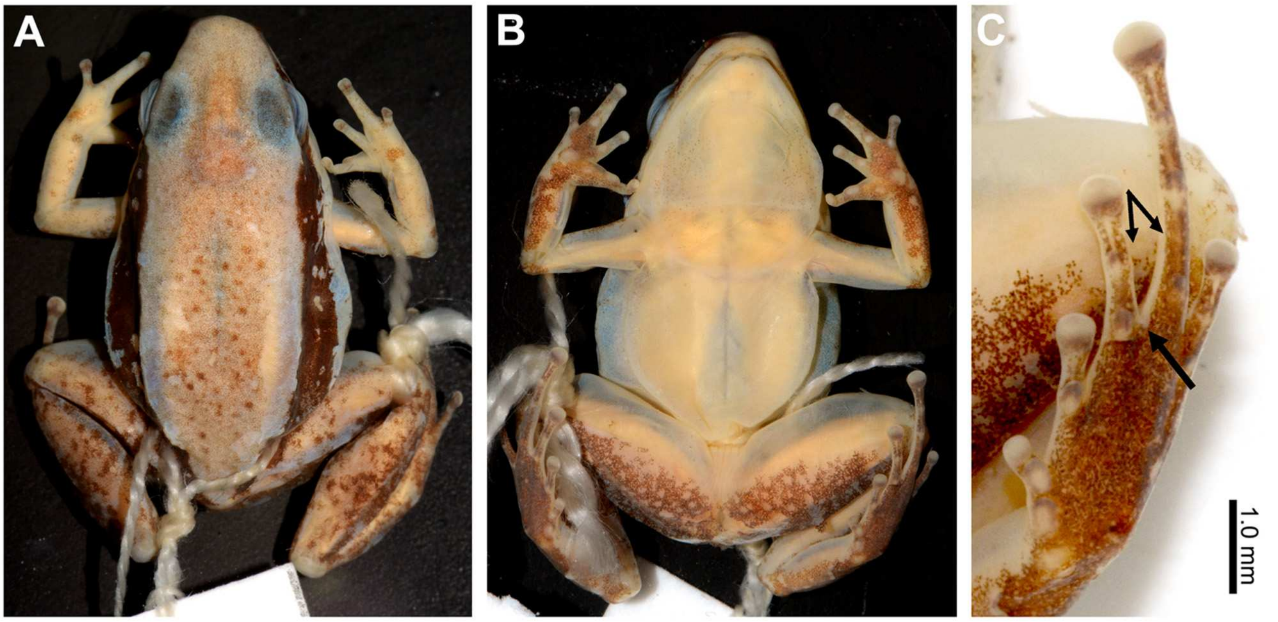

FIGURE 9. Dorsal view (A), ventral view (B), and sole (C) of *Allobates sumtuosus* holotype (USNM 303591), collected in 1979 by R.I. Crombie and L.C. Branch at REBio Trombetas. Photos are courtesy of Pedro L. V. Peloso. Lower arrow indicates basal webbing between Toes III and IV. Upper arrows indicate the absence of dermal fringes along Toes III and IV.

Although presenting an appreciable level of geographic variation in continuous traits, advertisement calls seem to be quite conservative in relation to arrangement of notes or pulses within cryptically colored *Allobates* species (Kaefer & Lima 2012; Kaefer *et al.* 2012). Alternatively, call arrangements are generally differentiated and diagnosable between species of the genus. As noted in *A. sumtuosus*, there are differences in note arrangement that support the existence of two forms of advertisement calls. Attributing different classifications to each call type (e.g. territorial calls, mating calls, etc.) is inadequate until comprehensive observations on the behavioral context of their emissions are available. However, the existence of multiple call arrangements could be used as a diagnostic character itself. To date, the ability to produce more than one arrangement has been reported for a few species of *Allobates*. These include species with distinct long-range advertisement calls and short-range courtship calls (*A. caeruleodactylus, A. femoralis*, *A. hodli*) and species with alternative long-range advertisement calls which could not be associated with any specific behavioral context (*A. brunneus*, *A. flaviventris*, *A. marchesianus*, *A. myersi*) (Caldwell *et al*. 2002; Lima *et al.* 2002; Lima *et al.* 2009; Simões *et al.* 2010; Simões & Lima 2011; Montanarin *et al.* 2011; Melo-Sampaio *et al.* 2013). This observation reinforces the need of a large number of recordings in order to correctly characterize each species' vocal repertoire, or to remotely identify a call sample based on a reference collection.

Despite the reduced sample sizes, an integrative appraisal considering morphological, acoustic and genetic data supports the hypothesis that *A. spumaponens* is a junior synonym of *A. sumtuosus*. The former species has been described from the Upper Demerara-Berbice region in Guyana, approximately 750 km northwest of REBio Trombetas, based on seven specimens (Kok & Ernst 2007). Species comparisons were largely based on external morphology. Understandably, more thorough comparisons were presented between *A. spumaponens* and *A. granti*, a species widespread in French Guiana, which shares many similar morphological traits with *A. spumaponens* (Kok *et al.* 2006). However, when combined with a few morphological traits (relative length of fingers and presence of a dorsolateral stripe), the advertisement calls of *A. granti* (formed by the regular emission of note pairs or double pulses) are sufficient to distinguish both species (Kok & Ernst 2007).

On the other hand, *A. sumtuosus* was distinguished from *A. spumaponens* by the presence of fringes between Toes II and III, and between Toes III and IV, and also by swelling of Finger III on males (both character states being absent in *A. spumaponens*). As pointed above, by examining recently collected adult specimens of *A. sumtuosus*, we have no reason to support evidence on the presence of fringes on toes, except basally between Toes III and IV. We also find the definition of Finger III swelling by Morales (2002) difficult to evaluate, as it apparently categorizes several states that might vary continuously (from wide to narrow swellings of the internal lateral surface of Finger III). We argue that swelling of Finger III would be better applied as a diagnostic character when analyzed as a sexually dimorphic trait and then presented quantitatively as the relative width of phalanges in relation to width of the finger disc in both males and females. By comparing the morphology of Finger III among male *A. sumtuosus* specimens examined herein and that provided in illustrations along the original species descriptions (Fig. 20 of Morales 2002; Fig. 3 of Kok & Ernst 2007), we found no appreciable differences in relation to Finger III swelling, the width of finger discs always being slightly larger than that of their respective distal and medial phalanges. Moreover, additional body measurements of male adults greatly overlap between the two species (Table 3), as well as morphological traits of their tadpoles (Simões & Lima 2012).

Advertisement calls proved useful in distinguishing *A. spumaponens* from *A. granti*, but a comparison between call traits of typical *A. spumaponens* and *A. sumtuosus* does not indicate differences in call arrangement. Acoustic signals of *A. spumaponens* were described based on advertisement calls of the holotype and one unvouchered individual, of which only calls of the former were analyzed in detail (Table 2). Notes are emitted continuously, and silent intervals between them are irregular, thus matching the second call type described herein for *A. sumtuosus*. Values of spectral and temporal traits of notes fall within (or very close to) the range observed among calls of typical *A. sumtuosus*. The only exception is note duration, which ranged from about 0.04 to 0.05 s in *A. sumtuosus* calls recorded at REBio Trombetas. However, similar values for note duration were observed for *A. sumtuosus* calls at SJB. Temporal traits of anuran advertisement calls, such as the duration of notes and silent intervals, are usually more variable in range than spectral traits (Gerhardt 1991; Tárano 2001; Kaefer *et al*. 2012), and are influenced by environmental and metabolic factors (Prestwich 1994; Gerhardt & Huber 2002). Thus, a larger extent of variation is not unexpected in intraspecific comparisons, especially when considering small samples.

We also agree with the notion that the choice of sites for tadpole deposition by *A. spumaponens* could be circumstantial, occurring as an alternative reproductive strategy in the absence of rain puddles during the drier months of the year (Kok & Ernst 2007). Although the rearing of tadpoles in foam nests of leptodactilid frogs represents a strikingly novel and interesting facet of the complex reproductive behavior of Dendrobatoidea, the lack of information regarding its frequency among individuals identified as *A. spumaponens* should hinder its adoption as a fixed (and potentially diagnostic) behavioral trait. Although fairly conservative within amphibian species, some degree of plasticity has been described for behavioral traits related to reproductive modes of many dendrobatoids (Poelman and Dicke 2007; Brown *et al.* 2011; Schulte *et al.* 2013).

Based on a short fragment of the 16S rRNA mithocondrial gene, genetic distances between samples of *A. sumtuosus* and *A. spumaponens* from their respective type localities were only 1%, below thresholds commonly suggested for the recognition of different anuran species in genetic barcode studies (see Vieites *et al*. 2009). After *A. spumaponens*, the species more closely related to typical *A. sumtuosus* based on 16S rDNA genetic distances was *A. paleovarzensis*. However *A. paleovarzensis* is distributed south of the Negro and Amazon rivers, and can be easily distinguished from *A. sumtuosus* by its larger size (SVL of males 18.3–22.4 mm), color of throat (grayish-violet, highly pigmented in males) and advertisement calls (emitted as short groups of notes, 0.7–3.0 s long, within a 4.1–4.7 kHz frequency bandwidth) (Lima *et al*. 2010).

The evidence presented and discussed above does not exclude the possibility of genetic introgression from *A. sumtuosus* into *A. spumaponens*, as such evolutionary processes have been revealed among a few Amazonian dendrobatoid species (Brown & Twomey 2009; Simões *et al.* 2012). At least one color trait, the color of dorsal surface of thighs, should also be investigated in specimens proceeding from intermediate localities between northern Brazilian Amazon and Guyana, in order to clarify its potential application as an effective diagnostic character or to test the existence of clinal variation.

We argue that the possibility of genetic introgression and differences observed in a single color trait is not sufficient to support the recognition of *A. spumaponens* as a distinct species, and hereby consider it a junior synonym of *A. sumtuosus*. We reaffirm Reserva Biológica do Rio Trombetas, in State of Pará, Brazil, as the species type locality, while noting that three paratypes of *A. sumtuosus* proceeding from Loreto, Peru (MUSM 17718–20), should be re-examined for confirmation of species identity.

Finally, we call attention to the importance of preserving areas that contain specific type localities. The unequivocal location of typical specimens and the reassessment of diagnostic traits based on behavior and color of live specimens were only possible because the type locality of *A. sumtuosus* is contained within a well-protected conservation unit. As demonstrated by this study, we advocate the use of material from type localities to be regarded as a foundation of taxonomic practices. Such framework allows researchers to discover new species, revise their taxonomic status, and provide distribution data applicable to the conservation of biodiversity.

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References

Ávila-Pires, T.C., Hoogmoed, M.S. & Rocha, W.A. (2010) Notes on the Vertebrates of northern Pará, Brazil: a forgotten part of the Guianan Region, I. Herpetofauna. *Boletim do Museu Paraense Emílio Goeldi, Ciências Naturais*, 5, 13–112. http://dx.doi.org/10.5123/s1981-81142008000200001

Barrio-Amorós, C.L. & Santos, J.C. (2009) Description of a new *Allobates* (Anura, Dendrobatidae) from the eastern Andean piedmont, Venezuela. *Phyllomedusa*, 8, 89–104.

Brown, J.L. & Twomey, E. (2009) Complicated histories: three new species of poison frogs of the genus *Ameerega* (Anura: Dendrobatidae) from north-central Peru. *Zootaxa*, 2049, 1–38.

Brown, J.L., Twomey, E., Amézquita, A., Barbosa de Souza, M., Caldwell, J.P., Lötters, S., von May, R., Melo-Sampaio, P.R., Mejía-Vargas, D., Perez-Peña, P., Pepper, M., Poelman, E.H., Sanchez-Rodriguez, M. & Summers, K. (2011) A taxonomic revision of the Neotropical poison frog genus *Ranitomeya* (Amphibia: Dendrobatidae). *Zootaxa*, 3083, 1–120.

Caldwell, J.P. & Lima, A.P. (2003) A new Amazonian species of *Colostethus* (Anura: Dendrobatidae) with a nidicolous tadpole. *Herpetologica*, 59, 219–234. http://dx.doi.org/10.1655/0018-0831(2003)059[0219:anasoc]2.0.co;2

Caldwell, J.P., Lima, A.P. & Keller, C. (2002) Redescription of *Colostethus marchesianus* (Melin, 1941) from its type locality. *Copeia*, 2002, 157–165. http://dx.doi.org/10.1643/0045-8511(2002)002[0157:rocmmf]2.0.co;2

Charif, R.A., Clark, C.W. & Fristrup, K.M. (2004) *Raven 1.2 User's Manual*. Cornell Laboratory of Ornithology, Ithaca, NY, 191 pp.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772. http://dx.doi.org/10.1038/nmeth.2109

Deichmann, J.L., Williamson, G.B., Lima, A.P. & Allmon, W.D. (2010) A note on amphibian decline in a central Amazonian lowland forest. *Biodiversity Conservation*, 193, 619–3627. http://dx.doi.org/10.1007/s10531-010-9920-z

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11–15.

Fouquet, A., Noonan, B.P., Rodrigues, M.T., Pech, N., Gilles, A. & Gemmell, N.J. (2012) Multiple quaternary refugia in the eastern Guiana shield revealed by comparative phylogeography of 12 frog species. *Systematic Biology*, 61, 461–489.

Fouquet, A., Pineau, K., Rodrigues, M., Mailles, J., Schneider, J.-B., Ernst, R. & Dewynter, M. (2013) Endemic or exotic: The phylogenetic position of the Martinique Volcano Frog *Allobates chalcopis* (Anura: Dendrobatidae) sheds light on its origin and challenges current conservation strategies. *Systematics and Biodiversity*, 11, 87–101. http://dx.doi.org/10.1080/14772000.2013.764944

Frost, D.R. (2013) Amphibian Species of the World: an Online Reference. Version 5.6 (9 January 2013). Electronic Database. American Museum of Natural History, New York, USA. Available from: http://research.amnh.org/herpetology/amphibia/index.html (accessed 2 December 2013)

Gerhardt, H.C. (1991) Female mate choice in treefrogs: static and dynamic acoustic criteria. *Animal Behaviour*, 42, 615–635. http://dx.doi.org/10.1016/s0003-3472(05)80245-3

Gerhardt, H.C. & Huber, F. (2002) *Acoustic Communication in Insects and Anurans. Common Problems and Diverse Solutions.* University of Chicago Press, USA, 542 pp.

Grant, T. & Rodríguez, L.O. (2001) Two new species of frogs of the genus *Colostethus* (Dendrobatidae) from Peru and a redescription of *C. trilineatus* (Boulenger, 1883). *American Museum Novitates*, 3355, 1–24. http://dx.doi.org/10.1206/0003-0082(2001)355<0001:tnsofo>2.0.co;2

Grant, T., Frost, D.R., Caldwell, J.P., Gagliardo, R., Haddad, C.F.B., Kok, P.J.R., Means, B.D., Noonan, B.P., Schargel, W. & Wheeler, W.C. (2006) Phylogenetic systematics of dart-poison frogs and their relatives (Anura: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History*, 299, 1–262. http://dx.doi.org/10.1206/0003-0090(2006)299[1:psodfa]2.0.co;2

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.

Kaefer, I.L. & Lima, A.P. (2012) Sexual signals of the Amazonian frog *Allobates paleovarzensis*: geographic variation and stereotypy of acoustic traits. *Behaviour*, 149, 15–33. http://dx.doi.org/10.1163/156853912x623757

Kaefer, I.L., Tsuji-Nishikido, B.M. & Lima, A.P. (2012) Beyond the river: underlying determinants of population acoustic signal variability in Amazonian direct-developing *Allobates* (Anura: Dendrobatoidea). *Acta Ethologica*, 15, 187–194. http://dx.doi.org/10.1007/s10211-012-0126-0

Kaefer, I.L., Tsuji-Nishikido, B.M., Mota, E.P., Farias, I.P. & Lima, A.P. (2013) The early stages of speciation in Amazonian forest frogs: Phenotypic conservatism despite strong genetic structure. *Evolutionary Biology*, 40, 228–245. http://dx.doi.org/10.1007/s11692-012-9205-4

Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.
http://dx.doi.org/10.1007/bf01731581

Kok, P.J.R., MacCulloch, R.D., Gaucher, P., Poelman, E.H., Bourne, G.R., Lathrop, A. & Lenglet, G.L. (2006) A new species of *Colostethus* (Anura, Dendrobatidae) from French Guiana with a redescription of *Colostethus beebei* (Noble, 1923) from its type locality. *Phyllomedusa* 5, 43–66.

Kok, P.J.R. & Ernst, R. (2007) A new species of *Allobates* (Anura: Aromobatidae: Allobatinae) exhibiting a novel reproductive behaviour. *Zootaxa*, 1555, 21–38.

Lima, A.P. & Caldwell, J.P. (2001) A new Amazonian species of *Colostethus* with sky blue digits. *Herpetologica*, 57, 133–138.

Lima, A.P., Caldwell, J.P. & Biavati, G.M. (2002) Territorial and reproductive behavior of an Amazonian dendrobatid frog, *Colostethus caeruleodactylus*. *Copeia*, 2002 (1), 44–51.
http://dx.doi.org/10.1643/0045-8511(2002)002[0044:tarboa]2.0.co;2

Lima, A.P., Magnusson, W.E., Menin, M., Erdtmann, L.K., Rodrigues, D.J., Keller, C. & Hödl, W. (2006) *Guide to the frogs to Reserva Adolpho Ducke, Central Amazonia*. Manaus, Editora Attema, 168 pp.

Lima, A.P., Sanchez, D.E.A. & Souza, J.R.D. (2007) A new Amazonian species of frog genus *Colostethus* (Dendrobatidae) that lays its eggs on undersides of leaves. *Copeia*, 2007, 114–122.
http://dx.doi.org/10.1643/0045-8511(2007)7[114:anasot]2.0.co;2

Lima, A.P., Caldwell, J.P. & Strussmann, C. (2009) Redescription of *Allobates brunneus* (Cope) 1887 (Anura: Aromobatidae: Allobatinae), with a description of the tadpole, call, and reproductive behavior. *Zootaxa*, 1988, 1–16.

Lima, A.P., Caldwell, J.P., Biavati, G. & Montanarin, A. (2010) A new species of *Allobates* (Anura: Aromobatidae) from Paleovárzea Forest in Amazonas, Brazil. *Zootaxa*, 2337, 1–17.

Melo-Sampaio, P.R., Souza, M.B. & Peloso, P.L.V. (2013) A new, riparian, species of *Allobates* Zimmermann and Zimmermann, 1988 (Anura: Aromobatidae) from southwestern Amazonia. *Zootaxa*, 3716 (3), 336–348.
http://dx.doi.org/10.11646/zootaxa.3716.3.2

Menin, M., Waldez, F. & Lima, A.P. (2008) Temporal variation in the abundance and number of species of frogs in 10,000 ha of a forest in Central Amazonia, Brazil. *South American Journal of Herpetology*, 3, 68–81.
http://dx.doi.org/10.2994/1808-9798(2008)3[68:tvitaa]2.0.co;2

Montanarin, A., Kaefer, I.L. & Lima, A.P. (2011) Courtship and mating behaviour of the brilliant-thighed frog *Allobates femoralis* from Central Amazonia: implications for the study of a species complex. *Ethology Ecology & Evolution*, 23, 141–150.
http://dx.doi.org/10.1080/03949370.2011.554884

Morales, V.C. (2000, "2002") Sistemática y biogeografía del grupo *trilineatus* (Amphibia, Anura, Dendrobatidae, *Colostethus*), con descripcíon de once espécies nuevas. *Publicaciones de La Asociacíon de Amigos de Doñana*, 13, 1–59.

Palumbi, S.R. (1996) Nucleic acids II: the polymerase chain reaction. *In*: Hillis, D.M., Moritz, C. & Mable, B.K. (Eds.), *Molecular Systematics*. Sinauer & Associates Inc., Sunderland, Massachusetts, pp. 205–247.

Peloso, P.L.V. (2010) A safe place for amphibians? A cautionary tale on the taxonomy and conservation of frogs, caecilians and salamanders in the Brazilian Amazonia. *Zoologia*, 27, 667–673.
http://dx.doi.org/10.1590/s1984-46702010000500001

Poelman, E.H. & Dicke, M. (2007) Offering offspring as food to cannibals: oviposition strategies of Amazonian poison frogs (*Dendrobates ventrimaculatus*). *Evolutionary Ecology*, 21, 215–227.
http://dx.doi.org/10.1007/s10682-006-9000-8

Prestwich, K.N. (1994) The energetics of acoustic signaling in anurans and insects. *American Zoologist*, 34, 625–643.
http://dx.doi.org/10.1093/icb/34.6.625

Salas, A. (2004) *Allobates sumtuosus*. IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. Available from: www.iucnredlist.org (accessed 31 May 2013)

Santos, J.C. & Cannatella, D.C. (2011) Phenotypic integration emerges from aposematism and scale in poison frogs. *Proceedings of the National Academy of Sciences USA,* 108, 6175–6180.
http://dx.doi.org/10.1073/pnas.1010952108

Santos, J.C., Coloma, L.A., Summers, K., Caldwell, J.P., Ree, R. & Cannatella, D.C. (2009) Amazonian amphibian diversity is primarily derived from Late Miocene Andean lineages. *PLOS Biology*, 7, 1–14.
http://dx.doi.org/10.1371/journal.pbio.1000056

Schulte, L.M. & Lötters, S. (2013) The power of the seasons: rainfall triggers parental care in poison frogs. *Evolutionary Ecology*, 27, 711–723.
http://dx.doi.org/10.1007/s10682-013-9637-z

Simões, P.I. & Lima, A.P. (2011) The complex advertisement calls of *Allobates myersi* (Pyburn, 1981) (Anura: Aromobatidae) from São Gabriel da Cachoeira, Brazil. *Zootaxa*, 2988, 66–68.

Simões, P.I. & Lima, A.P. (2012) The tadpole of *Allobates sumtuosus* (Morales, '2000' 2002) (Anura: Aromobatidae) from its type locality at Reserva Biológica do Rio Trombetas, Pará, Brazil. *Zootaxa,* 3499, 86–88.

Simões, P.I., Lima, A.P. & Farias, I.P. (2010) The description of a cryptic species related to the pan-Amazonian frog *Allobates femoralis* (Boulenger 1883) (Anura: Aromobatidae). *Zootaxa*, 2406, 1–28.

Simões, P.I., Lima, A.P. & Farias, I.P. (2012) Restricted natural hybridization between two species of litter frogs on a threatened landscape in southwestern Brazilian Amazonia. *Conservation Genetics*, 13, 1145–1159. http://dx.doi.org/10.1007/s10592-012-0362-x

Simões, P.I., Sturaro, M.J., Peloso, P.L.V. & Lima, A.P. (2013) A new diminutive species of *Allobates* Zimmermann and Zimmermann, 1988 (Anura, Aromobatidae) from the northwestern Rio Madeira/Rio Tapajós interfluve, Amazonas, Brazil. *Zootaxa*, 3609 (3), 251–273. http://dx.doi.org/10.11646/zootaxa.3609.3.1

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution,* 28, 2731–2739. http://dx.doi.org/10.1093/molbev/msr121

Tárano, Z. (2001) Variation in Male Advertisement Calls in the Neotropical Frog *Physalaemus enesefae*. *Copeia*, 2001 (4), 1064–1072. http://dx.doi.org/10.1643/0045-8511(2001)001[1064:vimaci]2.0.co;2

Verdade, V.K., Valdujo, P.H., Carnaval, A.C., Schiesari, L.,Toledo, L.F., Mott, T., Andrade, G.V., Eterovick, P.C., Menin, M., Pimenta, B.V.S., Nogueira, C., Lisboa, C.S., de Paula, C.D. & Silvano, D.L. (2012) A leap further: the Brazilian Amphibian Conservation Action Plan. *Alytes,* 29, 27–42.

Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F. & Vences, M. (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *PNAS*, 106 (20), 8267–8272. http://dx.doi.org/10.1073/pnas.0810821106

APPENDIX I. Origin and accession numbers of 16S rDNA sequences of *Allobates* species occurring in Brazil and across the Guiana Shield region obtained in this study or available in GenBank.

Species	Locality	Coordinates	16S	Reference
Allobates sumtuosus	REBio Trombetas, Pará, Brazil	01°22’12” S, 56°51’08” W	KF250500	This study
Allobates sumtuosus	REBio Trombetas, Pará, Brazil	01°22’12” S, 56°51’08” W	KF250499	This study
Allobates sumtuosus	REBio Trombetas, Pará, Brazil	01°22’12” S, 56°51’08” W	KF250498	This study
Allobates sumtuosus	REBio Trombetas, Pará, Brazil	01°22’12” S, 56°51’08” W	KF250497	This study
Allobates sumtuosus	Reserva Ducke, Manaus, Amazonas, Brazil	02°55’–03°01’S, 59°53’–59°59’W	KF250503	This study
Allobates sumtuosus	Reserva Ducke, Manaus, Amazonas, Brazil	02°55’–03°01’S, 59°53’–59°59’W	KF250502	This study
Allobates sumtuosus	Reserva Ducke, Manaus, Amazonas, Brazil	02°55’–03°01’S, 59°53’–59°59’W	KF250501	This study
Allobates sumtuosus	São João da Baliza, Roraima, Brazil	00°57’10” N, 59°55’43” W	KF250495	This study
Allobates sumtuosus	São João da Baliza, Roraima, Brazil	00°57’10” N, 59°55’43” W	KF250496	This study
Allobates sumtuosus	Sítio Tamaga, BR 174, km 80, Amazonas, Brazil	02°13’23” S, 60°03’55” W	KF250494	This study
Allobates algorei	Road to Rio Negro, Tachira, Venezuela		HQ290950	Santos & Cannatella 2011
Allobates caeruleodactylus	Castanho, Amazonas, Brazil	03°37’10” S, 59°86’78” W	DQ502100	Grant *et al.* 2006
Allobates caeruleodactylus	Castanho, Amazonas, Brazil	03°37’10” S, 59°86’78” W	DQ502212	Grant *et al.* 2006
Allobates conspicuus	Porto Walter, Rio Juruá, Acre, Brazil	08°15’31” S, 72°46’37” W	DQ502135	Grant *et al.* 2006
Allobates conspicuus	Porto Walter, Rio Juruá, Acre, Brazil	08°15’31” S, 72°46’37” W	DQ502134	Grant *et al.* 2006
Allobates crombiei	Cachoeira do Espelho, Altamira, Pará, Brazil	03°39’00” S, 52°22’33” W	KF250508	This study

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APPENDIX 1. (Continued)

Species	Locality	Coordinates	16S	Reference
Allobates crombiei	Cachoeira do Espelho, Altamira, Pará, Brazil	03°39'00" S, 52°22'33" W	KF250509	This study
Allobates gasconi	Porto Walter, Rio Juruá, Acre, Brazil	08°15'31" S, 72°46'37" W	EU342561	Santos *et al*. 2009
Allobates gasconi	Porto Walter, Rio Juruá, Acre, Brazil	08°15'31" S, 72°46'37" W	EU342562	Santos *et al*. 2009
Allobates gasconi	Madereira Scheffer, Rio Ituxi, Amazonas, Brazil		EU342565	Santos *et al*. 2009
Allobates granti	Lely Mountain, Suriname		JN690923	Fouquet *et al.* 2012
Allobates granti	Lely Mountain, Suriname		JN690924	Fouquet *et al.* 2012
Allobates granti	Saul, French Guiana		JN690927	Fouquet *et al.* 2012
Allobates granti	Haut Marwini, French Guiana		JN690928	Fouquet *et al.* 2012
Allobates granti	Haute Wanapi, French Guiana		JN690929	Fouquet *et al.* 2012
Allobates grillisimilis	Nova Olinda do Norte, Amazonas, Brazil	05°52'05" S, 61°17'13" W	KF250506	This study
Allobates grillisimilis	Nova Olinda do Norte, Amazonas, Brazil	05°52'05" S, 61°17'13" W	KF250507	This study
Allobates grillisimilis	Borba, Amazonas, Brazil	04°26'03" S, 59°37'25" W	KF250504	This study
Allobates grillisimilis	Borba, Amazonas, Brazil	04°26'03" S, 59°37'25" W	KF250505	This study
Allobates masniger	Parque Nacional da Amazônia, Pará, Brazil	4°32'51" S, 56°18'13" W	JQ966888	Kaefer *et al.* 2013
Allobates nidicola	Autazes Road Km 12, Amazonas, Brazil	03°28'02" S, 59°49'09" W	JQ966864	Kaefer *et al.* 2013
Allobates paleovarzensis	Careiro, Amazonas, Brazil	03°22'26" S, 59°52'06" W	JQ966835	Kaefer *et al.* 2013
Allobates spumaponens	Mabura Hill Forest Reserve, Guyana		KC520682	Fouquet *et al.* 2013
Allobates subfolionidificans	Rio Branco, Acre, Brazil	09°57' S, 67°57' W	KF250492	This study
Allobates subfolionidificans	Rio Branco, Acre, Brazil	09°57' S, 67°57' W	KF250493	This study
Allobates trilineatus	Panguana, Rio Llullapichis, Huanuco, Peru		DQ502118	Grant *et al.* 2006
Allobates undulatus	Cerro Yutaje, Amazonas, Venezuela	05°46' N, 66°08' W	DQ502028	Grant *et al.* 2006
Allobates undulatus	Cerro Yutaje, Amazonas, Venezuela	05°46' N, 66°08' W	DQ502029	Grant *et al.* 2006
Allobates undulatus	Cerro Yutaje, Amazonas, Venezuela	05°46' N, 66°08' W	DQ283044	Grant *et al.* 2006