



## Revalidation of *Pristimantis brevicrus* (Anura, Craugastoridae) with taxonomic comments on a widespread Amazonian direct-developing frog

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Problems associated with delimiting species are particularly pronounced in taxa with high species-level diversity, as occurs in *Pristimantis* frogs. Herein, we resurrect *Pristimantis brevicrus*, nov. comb., from the synonymy of *P. altamazonicus*, a widespread species in the upper Amazon Basin, based on morphological, acoustic and genetic evidence. Both species are sympatric along the Upper Amazon Basin of Ecuador and northern Peru, up to ~1450 m. Phylogenetic analyses reveals that *P. altamazonicus* and *P. brevicrus* are sister taxa in a well-supported clade with *P. diadematus* and two unconfirmed candidate species. *Pristimantis altamazonicus* is distinguished from *P. brevicrus* by having a differentiated tympanic annulus, a smooth dorsum with scattered small tubercles towards the flanks, weakly areolate skin on the venter, red to bright orange groin with black mottling, on hidden surfaces of thighs (bluish-white to yellowish-white in *P. brevicrus*) of living specimens. The recognition of *P. brevicrus* and two unconfirmed candidate species suggest that the diversity of these frogs is inadequately understood, highlighting the need for more integrative taxonomic reviews of Amazonian amphibians.

**Key words:** Amazonia, Craugastoridae, Ecuador, frogs, integrative taxonomy, Peru, unconfirmed candidate species

### INTRODUCTION

The name *Pristimantis altamazonicus* (Barbour & Dunn, 1921) is applied to specimens distributed throughout the upper Amazon basin, and the species is characterised by partially or completely concealed tympana, bluish white, orange, black and cream or pink rose in hidden surfaces on limbs, and a tuberculated dorsum (Duellman, 1978a; Lynch, 1980; Rodríguez & Duellman, 1994; Duellman, 2005; Beirne & Whitworth, 2011). Such diagnostic characters were also applied to identify similar *Pristimantis* species characterised by dark marbled or barred groins and thighs (e.g. *P. diadematus*, *P. orcus*, *P. divnae*; Hedges & Schlüter, 1992; Lehr et al., 2009; Lehr & von May, 2009). *Eleutherodactylus brevicrus* was described by Andersson (1945) on the basis of a single specimen collected from Río Pastaza Watershed, 500 m, eastern Ecuador. In differentiating the latter species from *E. whymeri* (= *Pristimantis curtipes*; Boulenger, 1882), Andersson (1945) identified several diagnostic characters

like a concealed tympanum, a tuberculated dorsum, and the black and white cross bars on hidden areas of the thighs and tibiae. Lynch (1968) compared *E. brevicrus* to two new lowland species, *P. variabilis* (Lynch, 1968) and *P. croceinguinis* (Lynch, 1968) from northern Amazonia of Ecuador, commenting on the bright red colours on the flanks, groin, and posterior surfaces of the thighs in living specimens. However, Lynch (1974) later examined the holotype of *Pristimantis altamazonicus* and placed *E. brevicrus* as its junior synonym. Curiously, Lynch (1974) omitted that, according to the original description, the holotype of *P. altamazonicus* has a distinguishable tympanic annulus.

Problems associated with delimiting species are particularly pronounced in systems with high species-level diversity, as is the case for *Pristimantis* (Crawford & Smith, 2005; Elmer et al., 2007; Padial et al., 2009; Crawford et al., 2010; García et al., 2012). However, the increased use of genetic markers in combination with morphological and acoustic information has resulted in the discovery of

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a large number of cryptic *Pristimantis* species (Padial & De la Riva, 2009; Jansen et al., 2011). After reviewing the type series of *P. altamazonicus* and *E. brevicrus*, along with a large sample of specimens, we found evidence to support resurrecting the last as a valid taxon.

## METHODS

### Sampling

Field collections of specimens were made by the senior author at Limoncocha (Sucumbíos Province, March 2012 and May 2013), Estación Biológica Jatun Sacha (Napo Province, March and April 2012) and Tukupi (Morona Santiago Province, May 2012), Ecuador. Specimens were collected during night surveys (1800–0000 hours) at the end of the rainy season (February through May) using headlamps to find individuals on vegetation. We followed the technique of Visual Encounter Surveys (VES) provided by Crump & Scott (1994). Photographs, notes on colour variation and ecology were taken in the field for each specimen. Geo-references for each sampling point were obtained with the aid of a GPS Garmin® Montana 650.

Voucher specimens and tissue samples were prepared following ethical and technical protocols provided by McDiarmid (1994). Vouchers were euthanised with Lidocaine hydrochloride 2%, fixed in 10% buffered formalin and then later transferred to 70% ethanol. Liver and thigh muscle was preserved in 95% ethanol for DNA extraction prior to fixation. Voucher specimens and tissue samples were deposited at Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ).

### Terminology and morphological data

We applied the term Unconfirmed Candidate Species (UCS; Vieites et al., 2009) to distinguish deep genealogical lineages, largely unstudied and derived from geographically distant populations, for which general evidence suggest that they are distinct, undescribed species. The diagnosis was based on characters definitions and terminology used by Duellman & Lehr (2009). Bilateral characters were obtained from the right side of specimens, and, if this was not measurable, replaced by the corresponding left side. Sex was determined by direct inspection of gonads. A set of 14 measurements were taken (modified from Ortega-Andrade & Valencia, 2012) for each specimen with the aid of callipers (~0.1 mm precision). These morphometric characters were: (1) snout–vent length (SVL): distance from tip of snout to posterior margin of vent; (2) head width (HW): greatest width of head measured at level of jaw articulation; (3) head length (HL): distance from the tip of snout to posterior angle of jaw articulation; (4) eye diameter (ED): distance between anterior and posterior border of eye; (5) interorbital distance (IOD)=the shortest distance of the braincase between orbits; (6) eye–nostril distance (EN): distance from posterior margin of nostril to anterior margin of eye; (7) width of upper eyelid (EW): the perpendicular distance of the outer edge of the eyelid; (8) tympanic diameter (TD): distance between anterior and posterior outer borders of tympanic annulus; (9) femur length (FL): length of femur from vent to knee; (10) tibia length (TL): length of flexed leg from knee to heel; (11) foot

length (FoL): distance from heel to tip of toe IV, including in the measurement the length of tarsus and foot; (12) hand length (HaL): distance from proximal border of thenar tubercle to tip of Finger III; (13) disc diameter on Finger III (F3): width of the disc in Finger III; and (14) disc diameter on Toe IV (T4): width of the disc in Toe IV. We conducted multivariate analyses (principal component analysis, PCA) to assess the variation and ordination of specimens based on data from morphological measurements in males and females. To avoid size-dependent correlation effects, regression residuals on data were calculated using snout-vent length (SVL) as independent variable. All analyses and statistics were developed in PASW Statistics v18.0 (WinWrap Basic ©).

We examined the type specimens of *P. altamazonicus* (holotype MCZ 2028), high-definition photographs of the holotype of *E. brevicrus* (NRM 1920), *P. divnae* (holotype MUSM 19990), *P. eurydactylus* (paratype KU 218292), *P. orcus* (holotype MUSM 27435), *P. lindae* (holotype KU 162305), *P. ventrimarmoratus* (lectotypes BMNH.1947.2.15.73-76), and additional specimens referred as comparative material (Online Appendix S1), loaned from the following institutions: Bolivia: Museo de Historia Natural Noel Kempff Mercado, Santa Cruz (MHNNKM). Ecuador: Fundación Herpetológica Gustavo Orcés, Quito (FHGO); Museo Ecuatoriano de Ciencias Naturales, Quito (DHMECN); Museo de Zoología–Pontificia Universidad Católica del Ecuador, Quito (QCAZ). Peru: Centro de Ornitología y Biodiversidad, Lima (CORBIDI); Museo de Historia Natural Universidad Nacional de San Antonio Abad, Cusco (MHNC), Giuseppe Gagliardi's collection at Instituto de Investigaciones de la Amazonía Peruana, Iquitos (GGU-IIAP), Museo de Zoología de la Universidad Nacional de la Amazonía Peruana (MZUNAP). United States of America: American Museum of Natural History, New York, USA (AMNH); Museum of Comparative Zoology, Harvard University (MCZ); The University of Kansas Natural History Museum (KU), and National Museum of Natural History, Washington, D.C. (USNM).

### Genetic data

In order to determine the phylogenetic position of *P. altamazonicus*, we reconstruct a phylogenetic tree based on the protocols and molecular matrix for mitochondrial 16S rRNA, published by Ortega Andrade and Venegas (2014). In a second analyses, we assessed the molecular divergence between putative cryptic species and the closest morphological taxa by extraction of DNA from specimens identified as *P. altamazonicus* along its known distribution range in the upper Amazon basin (Online Appendix Table 1). Three mitochondrial genes (16S rRNA, 12S rRNA, and Cytochrome Oxidase sub-unit I) and exons of two nuclear genes (the Recombination Activating Gene 1 RAG-1 and the Tyrosinase Precursor Gene Tyr) were amplified. Polymerase chain reaction was carried out under locus-specific optimal annealing temperatures following protocols detailed by Pinto-Sánchez et al. (2012). PCR products were cleaned using the UltraClean PCR Clean-Up Kit (MO-BIO Laboratories, Inc., Carlsbad, CA, USA) or by Exo I/SAP digest and sequenced in both directions by Macrogen Co. Ltd. (South Korea). Sequences

were edited and aligned in Geneious v.5.4.7 (Biomatters, Auckland, New Zealand). Multiple sequence alignments were generated using MAFFT v.7.017 (Katoh et al., 2005) with default gap opening and extension costs and other settings configured in Geneious. Alignments were checked by eye, and leading and lagging ends were trimmed manually to remove regions with a high proportion of missing data at edges.

Because our combined data set comprised two ribosomal genes with secondary structure (12S and 16S), one protein-coding mitochondrial gene (COI), and two nuclear genes (RAG-1 and Tyr), a single nucleotide substitution model was unlikely to provide a good fit to the data (Brown & Lemmon, 2007). We used PartitionFinder (Lanfear et al., 2012) to select the optimal model of nucleotide substitution for each data partition, based on the Bayesian information criterion (BIC). The complete dataset is a combined mtDNA and nDNA matrix with eleven partitions (56 terminals, 3022 characters), one for each of the 12S and 16S genes and one by each codon position of the coding genes, COI+RAG-1+Tyr.

Phylogenetic analyses were conducted using Maximum Likelihood (ML) and Bayesian Methods (BM) on the aligned nucleotides. ML phylogenetic reconstruction was implemented in GARLI v.2.0 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl, 2006), through 20 independent searches, whereas the supports for the nodes were calculated by a search with 10000 bootstrap replications. Twenty searches were performed with stepwise-addition starting trees (streefname=stepwise), setting 5000000 generations as maximum to each run (stopgen=5000000), saving every 100 generations (saveevery=100), run termination threshold of 20000 generations without topology improvement (genthreshfortopoterm=20000), and a termination threshold value of 0.01 on the lnL increase required for any new topology along searches (significanttopochange=0.01); other parameters were left as default (Zwickl, 2006). A Majority-Rule Consensus 0.5 was applied on replications to generate a consensus tree with non-parametric bootstrap values in SumTrees (Sukumaran & Holder, 2010). Bayesian phylogenetic analyses were performed in MrBayes v.3.2.2 (Ronquist & Huelsenbeck, 2003) using two parallel runs of the Metropolis Coupled Monte Carlo Markov Chain (MCMCMC) algorithm for 20 million generations each, with three heated chains (0.1 heating parameter). Trees were sampled every 1000 generations. Convergence rate and stationarity of the Markov process were evaluated with average standard deviation of split frequencies between runs (values <0.01) and the Potential Scale Reduction Factor (values near 1.0 were considered adequate; Gelman & Rubin, 1992) visualised in the log file of MrBayes. The first 25% of generations were discarded as burn-in, after evaluating the stability and adequate "mixing" of sampled log-likelihood values assessed from the parameter estimates across generations (ESS>200) of both runs, visualised using Tracer v.1.6 (Rambaut & Drummond, 2013). Both, ML and Bayesian analyses, were performed in the CIPRES portal <<http://www.molecularevolution.org/index>>. Gene tree concordance was assessed by analysing each locus individually using Bayesian analysis. Genetic distances

between candidate species were calculated using the uncorrected p-distance in MEGA 6 (Tamura et al., 2013).

### Description of the mating call

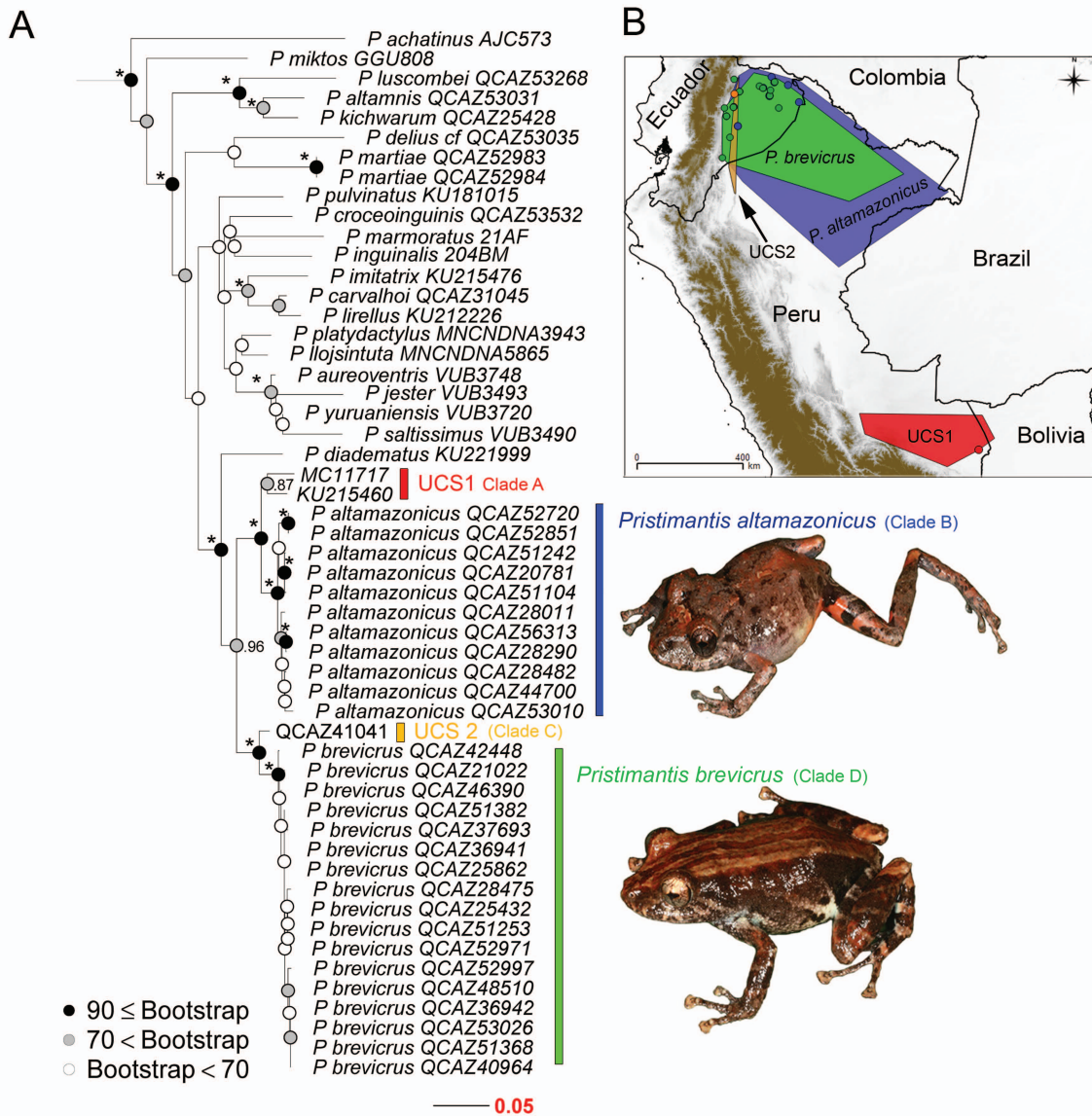
Mating call for *P. altamazonicus* was analysed from track #66 (photographed voucher, not collected), recorded from the National Park Yasuní, and published in the compact disc "Frogs of the Ecuadorian Amazon: a guide to their calls" produced by Read (2000). Additionally, we also analysed calls from *E. brevicrus* recorded from three individuals (QCAZ 52986, 52990 and 52992) using a Tascam DR-07mkII digital audio recorder, with built-in adjustable condenser microphones, and one specimen (Morley Read, Field Series SC51236) recorded using an Olympus LS14, with a Rode NTG1 Condenser Shotgun Microphone; all saved with a sample rate of 44.1 khz, in 16-bit/wav format. Mating calls from specimens QCAZ 52986 and 52990 were recorded while they called from a plastic bag. Specimen QCAZ 52992 was recorded in the field at a distance of ~2 m (21.9 °C / relative humidity ~97.6%). Specimen SC51236 was captured in amplexus, but separated in a plastic bag from where it was recorded calling at 1:02 am, on 2 July 2015. While call properties can be condition dependent (Baker & Richardson, 2006; Cunnington & Fahrig, 2010), calls emitted by frogs from plastic bags do not show marked distortions of acoustic characteristics (Bosch et al., 2000).

Five acoustic parameters were measured to describe the structure of each call (Cocroft & Ryan, 1995): (1) Call length: time from beginning to end of one call, measured from waveform in milliseconds; (2) dominant frequency: frequency containing the greatest amount of energy, measured along the entire call; (3) call rise time: time from beginning of the call to point of maximum amplitude; (4) interval between calls: time from ending of one call to the beginning of the next; and (5) call rate: number of calls/minute, from beginning of first call to beginning of last call. Measurement of fundamental frequency follows Hutter and Guayasamin (2012). The sonogram was produced and analysed using the program Raven Pro 1.5 (Charif et al., 2010). After a preliminary screening of the sound spectrogram, we applied a filter band-pass between 2200 and 3000 Hz to reduce noise and facilitate the measurement of the acoustic parameters. The dominant frequency was calculated with 512 samples in the spectrogram window. Recordings are deposited in the Sound Archive of the Museo de Zoología of Pontificia Universidad Católica del Ecuador (available at Amphibia Web Ecuador <<http://zoologia.puce.edu.ec/vertebrados/anfibios/>>).

## RESULTS

### Phylogenetic relationships

Genetic samples correspond to specimens identified as *P. altamazonicus sensu lato* from the upper Amazon basin in Ecuador and Peru (31 specimens; Online Appendix Table 1). The best topology (log likelihood -13,831.2) was obtained from an 11-partitioned matrix of combined mtDNA and nDNA, with an extended set of 3022 characters (Fig. 1). The most relevant results are: (i) The phylogeny recovered four divergent lineages resembling a complex of species



**Fig. 1.** Optimal maximum likelihood tree hypothesised from a combined mtDNA (12S+16S+COI by codon position) and nDNA (RAG-1 and Tyr by codon position) of an 11-partitioned matrix (log likelihood = -13,831.222; 3022 aligned sites); A) showing the phylogenetic relationships of *Pristimantis altamazonicus* (blue), *Pristimantis brevicrus* new combination (green), Unconfirmed Candidate Species 1 (UCS1, red) and Unconfirmed Candidate Species 2 (UCS2, orange). Outgroup (*Oreobates cruralis*, *O. saxatilis* and *Craugastor longirostris*) was removed from this figure. B) Distribution of the four clades recovered by the phylogeny along the upper Amazon Basin; areas correspond to convex hull polygons of localities recorded for each clade, whereas dots correspond to the genetic samples (see Online Appendix S1 for locations); shaded brownish areas correspond to an altitudinal range up from 2500 m a.s.l. in Andes. Supporting values of non-parametric bootstraps (colours on the nodes) and posterior probabilities (\*=1.0) are shown.

along the upper Amazon basin; and (ii) this complex is part of a well-supported clade, together with *P. diadematus* (Fig. 1, Online Appendix Fig. S1). The uncorrected distance between individuals of four clades range between 1.7 and 14.0% in mitochondrial genes, whereas sequences variation in nuclear genes varies between 0.5 and 1.6% (Online Appendix Table 2).

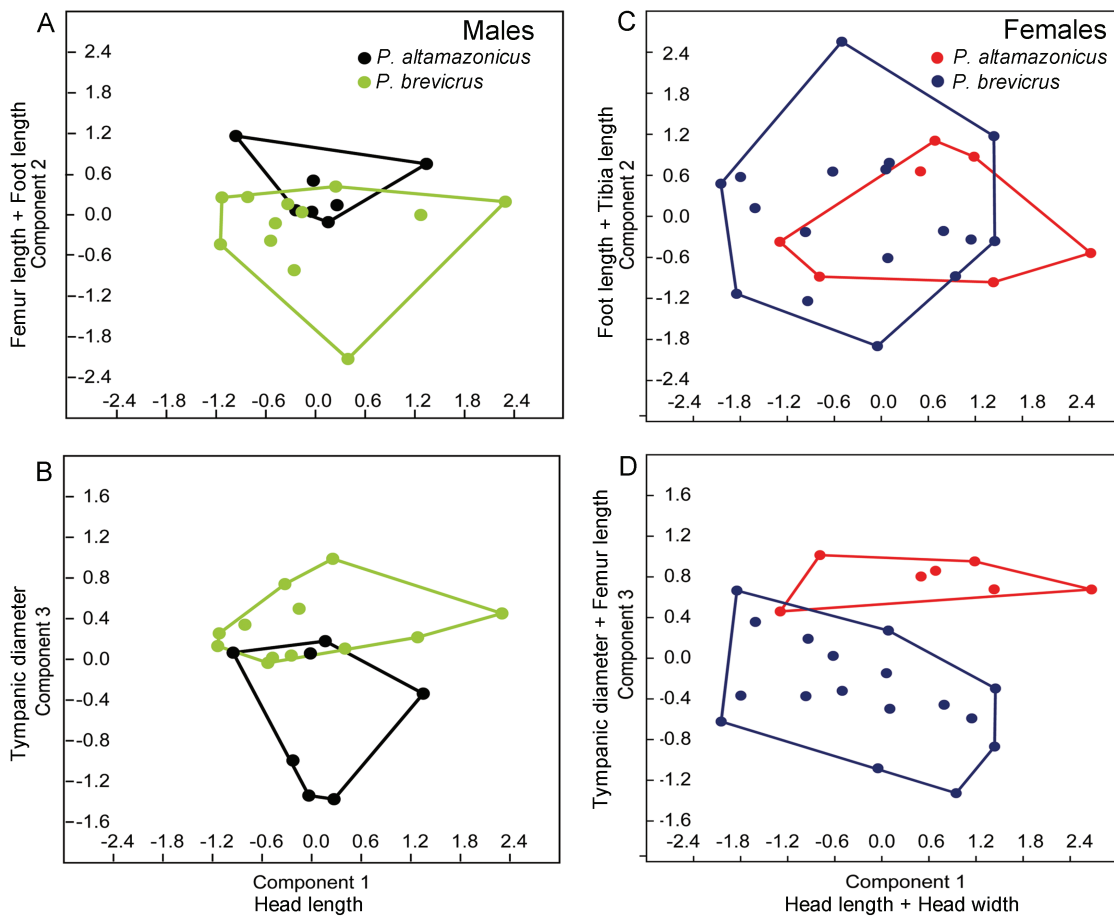
### Morphometrics

The first two principal components of morphometric variables barely separate specimens of *P. altamazonicus* from *E. brevicrus* (Fig. 2A, B). Head length and head width are highly related with the first axis, whereas limb

measurements (i.e. femur, foot, and tibia lengths) have high loadings in the second axis (Online Appendix Table 3). The ordination is improved when the presence/absence of tympanic annulus is considered (Fig. 2C, D), when the first three axes explain 73.1–73.4% of the observed variance (Online Appendix Table 3).

### Comparison of the holotypes and taxonomy

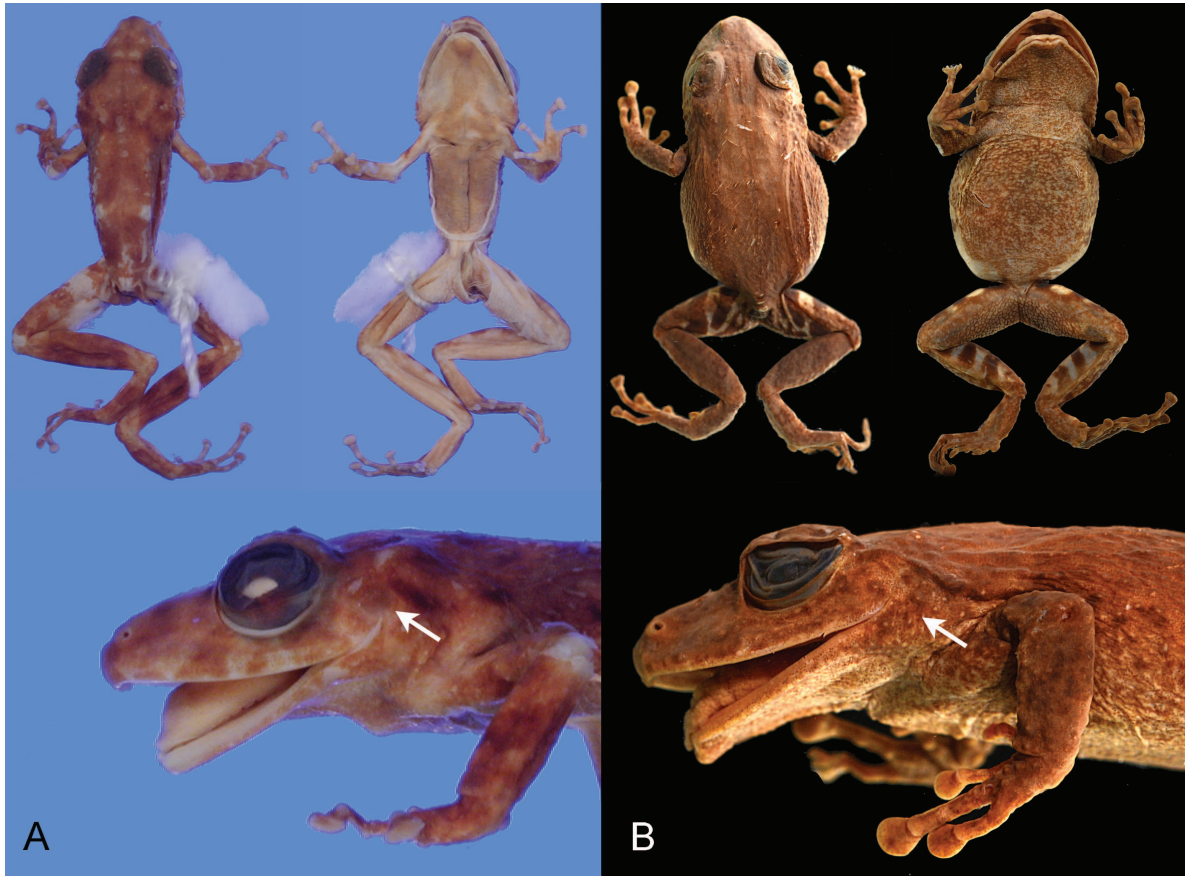
The holotypes of *P. altamazonicus* and *E. brevicrus* are in a good state of preservation (Fig. 3). The holotype of *P. altamazonicus* is a sub-adult female (SVL=20.61 mm), whereas the holotype of *E. brevicrus* is a gravid female (SVL= 33 mm). Differences in body size may be



**Fig. 2.** Ordination of specimens assessed by comparisons between Principal Components 1 vs. 2 (A, B), and 1 vs. 3 (C, D) on morphometric data for *Pristimantis altamazonicus* and *P. brevicrus*. Morphometric variables which most explained the variance of data are shown accordingly with each axis.

**Table 1.** Diagnostic characters and biogeography of the four clades resolved by a phylogeny of combined mtDNA and nDNA for *Pristimantis altamazonicus*, *Pristimantis brevicrus* and two Unconfirmed Candidate Species (UCS). The voucher specimens are detailed in Online Appendix S1.

Character/biogeography	<i>P. altamazonicus</i>	<i>P. brevicrus</i>	UCS 1	UCS 2
Skin texture	Smooth with scattered low tubercles	Shagreen commonly with warts or pustules on flanks	Tuberculated	Tuberculated
Tympanic annulus on skin	Present	Absent	Present	Absent
Colours on hidden surfaces of limbs and thighs in living specimens	Red to bright orange with black mottling	Bluish white to yellowish white colour with black mottling	Red to bright orange with black mottling	Bright yellow with black mottling
Distribution in Amazonia	Upper Amazon basin, lowlands in Ecuador, Peru and Colombia	Upper Amazon basin, lowlands in Ecuador, Peru and Colombia	Southern Amazon Basin, lowlands in Peru and Bolivia	Upper Amazon basin, piedmont on eastern slopes of Andes in Ecuador and Peru
Altitudinal range (mean±std.)	74–1462 m (372.3±241.8)	101–1102 m (459.4±279.4)	194–710 m (369.9±174.7)	486–1710 m (1154±460.7)
Latitudinal range	N0.29°–S6.52°	N0.11°–S4.27°	S11.41°–S13.19°	S0.03°–S4.04°



**Fig. 3.** Dorsal, ventral and lateral views of the holotypes of A) *Pristimantis altamazonicus* (MCZ 2028, sub-adult female, SVL=20.61mm, copyrighted by Museum of Comparative Zoology, Harvard University) and B) *Pristimantis brevicrus* (NRM 1920, gravid female, SVL=33.0 mm, photo by Sven Kullander, Swedish Museum of Natural History). Note the presence of tympanic annulus on the holotype of *Pristimantis altamazonicus*, and the absence of this character on the holotype of *P. brevicrus* (white arrows).

related to their age. However, we identified several differences in diagnostic characters between *E. brevicrus* and *P. altamazonicus* (condition of the latter, in parentheses): 1) the tympanic annulus is absent (*sensu* Duellman & Lehr, 2009) on skin (a differentiated tympanic annulus on skin), 2) warts or pustules on the flanks (dorsum smooth with scattered small tubercles towards the flanks), and 3) coarsely areolate skin texture on venter (skin of belly weakly areolate).

Herein, we resurrect the name *Pristimantis brevicrus* nov. comb., assigning it to the clade D in our phylogeny, whereas the name *Pristimantis altamazonicus sensu stricto* is assigned to clade B. This taxonomic resolution is based on the congruence of diagnostic characters and morphometric data assessed on the holotypes and revised material from both species (Table 1).

#### Systematics

Family CRAUGASTORIDAE (Hedges et al., 2008).

Genus *Pristimantis* (Jiménez de la Espada, 1870).

***Pristimantis altamazonicus* (Barbour & Dunn, 1921), Fig. 3.**

*Eleutherodactylus altamazonicus* Barbour and Dunn, 1921, Proc. Biol. Soc. Washington, 34: 161. Holotype: MCZ 2028, by original designation. Type

locality: "Upper Amazon and probably collected by the Thayer Expedition at Nauta", Departamento Loreto, Peru.

*Pristimantis altamazonicus* Heinicke, Duellman, and Hedges, 2007, Proc. Natl. Acad. Sci. USA, Suppl. Inform., 104: Table 2.

*Pristimantis (Pristimantis) altamazonicus* Hedges, Duellman, and Heinicke, 2008, Zootaxa, 1737: 128.

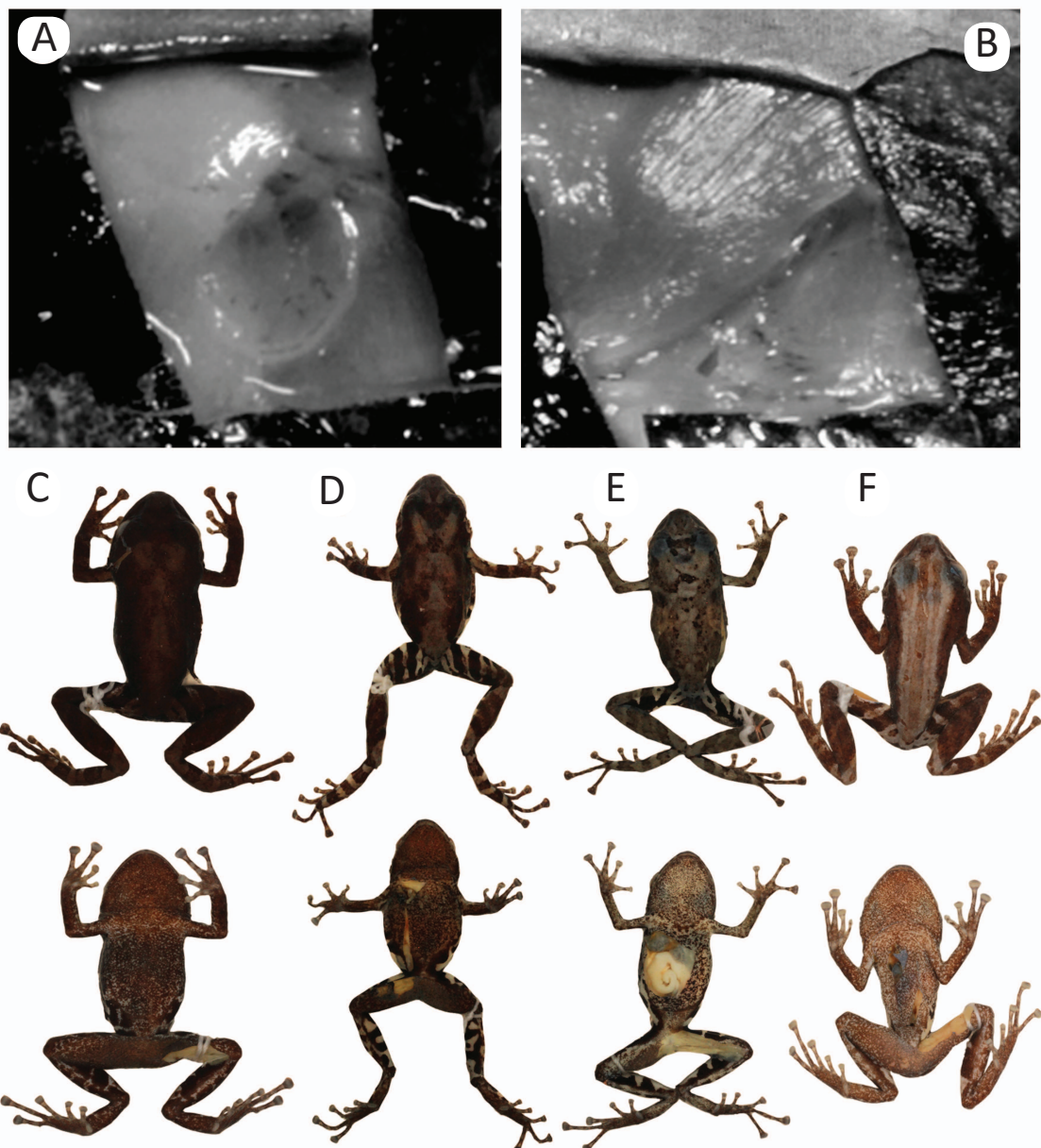
*Pristimantis (Pristimantis) altamazonicus* Duellman and Lehr, 2009. Nature und Tier Verlag: Münster, Germany: 138-139: Fig. 119.

**Characteristics.** *Pristimantis altamazonicus* is characterised by (1) skin of dorsum smooth, with scattered small tubercles in males; skin of belly weakly areolate; dorsolateral and discoidal folds absent; (2) tympanic annulus visible on skin; tympanic membrane not covered by muscle beneath skin; (3) snout moderately short, sub-acuminate in dorsal view and in profile; *canthus rostralis* weakly concave in dorsal view, rounded in cross-section; (4) upper eyelid about 70% of inter-orbital distance, bearing small tubercles; (5) dentigerous processes of vomer small, triangular, bearing 2–3 teeth; (6) males lack vocal slits and vocal sac; nuptial pads present; (7) fingers large and slender, first shorter than second; discs on outer fingers expanded, bluntly truncated, about 1.5x the width of digit proximal to pad; supernumerary tubercles large,

rounded; (8) fingers bearing narrow lateral fringes; (9) forearm bearing 2–3 ulnar tubercles, small and low; (10) heel lacking tubercles; outer border of tarsus smooth; inner border of tarsus bearing a small tarsal fold; (11) two metatarsal tubercles; inner elliptical, about 5x the outer tubercle; supernumerary plantar tubercles present, low and small; (12) toes with lateral fringes; webbing absent; discs equal in size or slightly smaller than those on fingers; Toe V longer than Toe III; (13) in life, dorsum tan to brown, with or without longitudinal cream stripes and/or dark blotches; groin, anterior and posterior surfaces of thighs are red to bright orange with black mottling; belly and ventral surfaces of the limbs are cream, light orange to light grey, heavy stippled brown or grey; iris coppery red

finely reticulated with black, and a black, narrow vertical streak through pupil. After preservation, all brown areas turn dark brown, anterior and posterior coloured surfaces on thighs turn into white mottled with black; venter cream with brown flecks; brown snout; (14) SVL in adult males  $18.4 \pm 0.9$  mm ( $n=7$ ; 17.1–19.8 mm); females  $29.2 \pm 0.6$  mm ( $n=7$ ; 28.4–30.1 mm).

**Diagnosis.** Comparison of *P. altamazonicus* with similar species from the Amazonian lowlands with black mottling or bars in groin and hidden surfaces of limbs are detailed in Table 2. This species is distinguished from *P. brevicrus* by having a differentiated tympanic annulus on skin (Fig. 4A), dorsum smooth with scattered small tubercles towards



**Fig. 4.** Examples of Tympanum Condition and chromatic variation in *Pristimantis brevicrus*. A) Tympanic annulus and membrane distinguished –not covered by muscle– beneath skin in *Pristimantis altamazonicus* (QCAZ 20781) and B) Tympanum covered by muscle beneath skin in *P. brevicrus* (QCAZ 25432). Extreme chromatic variation in dorsal and ventral views for specimens of *P. brevicrus*. C) a dark brown dorsal pattern, QCAZ 38768. D) W-shaped mark on scapular region and inverted chevrons, QCAZ 51382. E) brown dorsum with irregular blotches, QCAZ 52971. F) striped dorsal pattern, QCAZ 21022.

**Table 2.** Comparisons between *Pristimantis altamazonicus*, *Pristimantis brevicrus*, and relative species from the Amazonian lowlands. Characters were taken from the original species description and from the examined material (See Material and Methods). Presence of a character is indicated by “+”, its absence by “–”, “?” character unknown. Herein we report dorsal skin texture\* as a character assessed in preserved specimens; however see Guayasamin et al. (2015) for description of variation on skin texture observed in living *P. mutabilis* or *P. sobetes*.

Diagnostic character	<i>P. altamazonicus</i> This paper	<i>P. brevicrus</i> This paper	<i>P. diadematus</i> (Duellman & Lehr, 2009)	<i>P. divnae</i> (Lehr & von May, 2009)	<i>P. eurydactylus</i> (Hedges & Schlüter, 1992)	<i>P. imitatrix</i> (Duellman, 1978b)	<i>P. orcus</i> (Lehr et al., 2009)	<i>P. ventrimarmoratus</i> (Lynch & Duellman, 1980)
Snout-vent length (mm)	16.4–30.1	17.1–35.0	20.0–44.5	22.8–23.4, males	18.2–37.7	13.0–20.2	20.0–36.5	17.8–43.8
Dorsal skin texture*	Smooth with scattered low tubercles	Shagreen commonly with warts or pustules on flanks	Shagreen with tubercles	Shagreen with scattered minute tubercles	Tuberculate	Shagreen with scattered conical tubercles	Finely shagreen with scattered minute tubercles	Shagreen with tubercles
Ventral skin texture	Weakly areolate	Coarsely areolate	Areolate	Areolate	Weakly areolate	Coarsely areolate	Weakly areolate	Coarsely areolate
Tympanic annulus beneath skin	+	–	+	+	+	–	+	–
Vocal slits	–	–	+	–	–	+	–	–
Nuptial pads	+	–	+	–	+	–	+	–
Discoidal fold	–	+	+	+	–	Barely evident	+	–
Ulnar tubercles	+	+	+	–	+	+	+	+
Tubercles in heel	–	–	Small	Small	Small	–	Small	–
Tarsal fold	+	+	+	+	+	–	+	+
Relative size inner/outer metatarsal tubercles	5x	5x	10x	6x	3x	3x	3x	6x
Supernumerary plantar tubercles	+	Absent or barely visible	+	+	+	–	+	+
Toe webbing	–	–	–	+	+	–	+	–

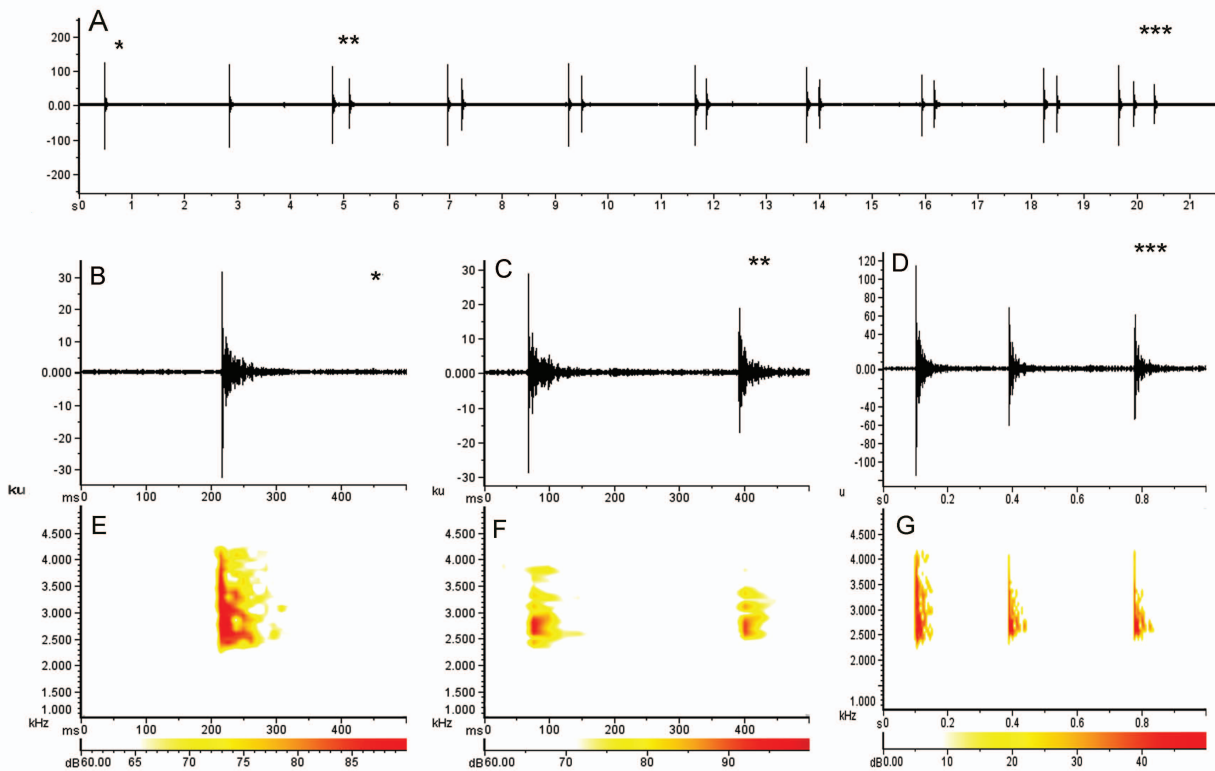


Table 2. Continued.

Diagnostic character	<i>P. altamazonicus</i> This paper	<i>P. brevicrus</i> This paper	<i>P. diadematus</i> (Duellman & Lehr, 2009)	<i>P. divinae</i> (Lehr & von May, 2009)	<i>P. eurydactylus</i> (Hedges & Schlüter, 1992)	<i>P. imitatrix</i> (Duellman, 1978b)	<i>P. orcus</i> (Lehr et al., 2009)	<i>P. ventrimarmoratus</i> (Lynch & Duellman, 1980)
Dorsal colouration in life	Tan to reddish brown, with or without longitudinal cream stripes	Tan to dusty dark brown, with longitudinal tan stripes in females	Tan to reddish brown	Brown with dark brown W-shaped mark on scapula and a dark brown chevron	After preservation, greyish tan with brown markings	Brown to reddish brown with dark markings	Greyish brown with irregularly shaped tan or green blotches	Brown above with diffuse gray flecks or vermiculations
W-shaped mark on scapular region	+	+	—	W-shaped scapular ridge	+	—	—	+
Groin and hidden surfaces of thighs	Red to bright orange with black mottling	Bluish white to yellowish white colour with black mottling	Bluish white, yellowish tan, pink or pale green with diagonal brown bars	Yellow with brown marks	Cream with diagonal brown bars	Mottled black and creamy white	White or whitish blue and black	Creamy white with brown spots or bars
Chest and belly colouration in life	Cream heavy stippled with grey	Black, dark brown to gray with bluish white flecks	Creamy white with brown mottling	Cream with dark brown blotches	Pale cream or occasionally with small spots or flecks	Pale bluish gray with dark brown flecks or reticulations	Dark brown and tan mottled	White, spotted or marbled with black
Iris colouration	Coppery red finely reticulated with black	Coopery red to silver, finely reticulated with brown, and a black, narrow vertical streak through pupil	Greenish bronze with a median horizontal red streak or reddish cooper	Golden with fine black reticulations and a vertical bar above and below the iris forming a cross	?	Bronze with a median horizontal red streak	Gold with a copper tint and fine black reticulations	Pale bronze with fine black reticulations



**Fig. 5.** Lateral and ventral views of living specimens of A–C) *Pristimantis brevicrus*; D) *P. altamazonicus*; E) Unconfirmed Candidate Species 1 (photos by Juan Carlos Chaparro); and F) Unconfirmed Candidate Species 2. Note the dorsal and ventral variation in color pattern for A, an adult male, QCAZ 53026; B, a sub-adult female, QCAZ 52971; and C, an adult female, FHGO 7203 of *P. brevicrus*. Furthermore, *P. altamazonicus* (D, QCAZ 53010) having a smooth dorsum with scattered tubercles and orange-red on groin and hidden surfaces of thighs, compared with the tuberculated dorsum and bluish-white or bluish-yellow groin and thighs in *P. brevicrus*. The unconfirmed Candidate Species 2 (F, FHGO 6544) is differentiated from the other three species by the bright yellow marks in hidden surfaces of limbs and groin, and variation in the tuberculation of dorsum.



**Fig. 6.** Mating call of *Pristimantis altamazonicus* (Read, 2000: record #66). Oscillograms (A–D) and their respective spectrograms (E–G) for singletons (B), doubletons (C) and triplets (D). Asterisks in B–D represent the location of each call type along the complete record in A. Color bars represents the intensity of each call expressed in decibels.

**Table 3.** Descriptive statistics for morphometric measurements (in mm) for males and females of *Pristimantis altamazonicus* and *P. brevicrus*. Mean±SD are given, with range in parentheses. The tympanic annulus is absent in the species (–).

Morphometrics	<i>Pristimantis altamazonicus</i>		<i>Pristimantis brevicrus</i>	
	Females (n=7)	Males (n=7)	Females (n=17)	Males (n=12)
Snout–vent length	29.2±0.6 (28.4–30.1)	18.4±0.9 (17.1–19.8)	31.3±2.6 (27.2–35)	19.9±2.2 (17–24.4)
Head width	12.8±1.2 (11.3–14.8)	8.1±0.8 (7–9.2)	13.1±1.6 (10.9–16.2)	8.4±1 (7.2–10.6)
Head length	11.3±0.7 (10.6–12.4)	6.6±0.4 (6.1–7.1)	11.7±1.2 (9.8–13.9)	7.2±0.7 (6–8.4)
Horizontal eye diameter	3.8±0.3 (3.4–4.1)	2.8±0.2 (2.6–3.1)	3.9±0.4 (3.2–4.9)	3.1±0.4 (2.6–3.9)
Interorbital distance	3.7±0.2 (3.4–3.9)	2.1±0.4 (1.4–2.5)	3.9±0.5 (3–4.7)	2.6±0.3 (2.1–3.2)
Eye–nostril distance	3.6±0.2 (3.5–4)	2.4±0.2 (2.2–2.7)	3.6±0.3 (3.2–4.1)	2.5±0.4 (1.9–3.1)
Width of upper eyelid	2.6±0.2 (2.3–2.9)	1.8±0.3 (1.4–2.1)	2.9±0.5 (2.3–4)	2.1±0.3 (1.6–2.6)
Tympanic diameter	1.3±0 (1.3–1.4)	0.9±0.1 (0.8–1)	–	–
Femur length	12.9±0.6 (11.8–13.8)	8.8±0.6 (8.1–9.8)	13.4±1.1 (11.4–15)	9.7±1.1 (8.5–12.9)
Tibia length	13.1±0.7 (12.2–13.8)	9.3±0.4 (8.9–9.9)	13.6±0.9 (12–15)	9.6±0.5 (8.8–10.5)
Foot length	18.4±0.8 (17.1–19.7)	12.3±0.7 (10.9–12.9)	19.5±1.3 (16.7–21.7)	13.2±1 (11.6–14.8)
Hand length	7.3±0.3 (6.9–7.6)	4.6±0.3 (4.4–5.2)	7.8±0.6 (6.7–8.8)	5.1±0.7 (4.1–6.2)
Disc diameter on finger III	1.6±0.2 (1.4–1.8)	1±0.1 (0.8–1.2)	1.8±0.2 (1.4–2.3)	1.1±0.3 (0.7–1.7)
Disc diameter on toe IV	1.6±0.1 (1.4–1.8)	1±0.2 (0.8–1.2)	1.7±0.2 (1.3–2.1)	1.1±0.2 (0.7–1.5)

the flanks, skin of belly weakly areolate and by the red to bright orange with black mottling in groin, anterior and posterior surfaces of thighs in living specimens (Fig. 5).

**Variation.** Measurements and proportions of reviewed specimens are detailed in Table 3. Male *P. altamazonicus* are smaller (mean SVL=18.4±0.9 mm; range 17.1–19.8 mm) than females (SVL=29.2±0.6 mm; range 28.4–30.1 mm). Furthermore, adult males and juveniles are darker and have a more tuberculate dorsum than females (differences are less evident in preserved specimens). In life, most specimens have a well-defined W-shaped mark on scapular region, but some females and juveniles present a stripped dorsal pattern (e.g., QCAZ 28011, 44700). In specimens with the latter pattern, a black canthal stripe is common.

**Colouration in life** (Fig. 5). The dorsum and flanks are brown with reddish and black stains; a well-defined W-shaped mark is present in scapular region. Interorbital bar, subocular stripes, scapular and sacral marks are present; sides of head are coloured as dorsum, canthal stripe is absent; groin, anterior and posterior surfaces of thighs are red to bright orange with black mottling and transversal bars; ventral surfaces of belly, throat, foot and hand are cream, pale red or light grey with, heavily stippled with grey. Iris coppery red to coppery brown, finely reticulated with black, and a black, narrow vertical streak through pupil. By day, tan or brownish surfaces turn into dark brown.

**Colouration in preservation.** Brownish areas in life turn to dark brown; forearms and hind limbs have well-defined dark brown transversal bars. Venter, throat, chest, ventral

surfaces of limbs, and palms, cream densely stippled with minute brown flecks (visible under magnification); red or orange areas in anterior and posterior surfaces on thighs turn to white mottled with black; brown snout.

**Natural history and distribution.** According to field notes and database records, specimens of *P. altamazonicus* were found active at night on leaves of low vegetation, up to 3 m above ground, in primary, secondary forest and clearings. This species is known from 104 localities along Amazonian evergreen lowlands of Colombia (Putumayo Department), Ecuador (Morona Santiago, Napo, Orellana, Pastaza and Sucumbíos Provinces), and northern Loreto Department in Peru, up to 1462 m altitude (Online Appendix S1). The occurrence area is calculated as about 336,865 km<sup>2</sup> in lowland evergreen forest in eastern Ecuador and northern Peru, but a wider distribution is probable in the upper Amazon basin (Fig. 1B).

**Mating Call.** Duellman (1978a) described the mating call like a soft “cluck”, repeated once or twice in succession. After analysing the mating call reported for *P. altamazonicus* from Parque Nacional Yasuní (Read, 2000), we found additional variation, which include repetitions in triads (Fig. 6). Comparisons of the acoustic parameters with the call of *Pristimantis brevicrus* are detailed in Table 4. In spite of several expeditions and time spend in trying to record additional specimens from *P. altamazonicus* in the Amazon basin, we only have a single call to analyse.

**Remarks.** Specimens referred by Duellman (1978a: 88–89), from Río Aguarico drainage in Amazonian Ecuador, correspond to *P. brevicrus*. These specimens lack

**Table 4.** Quantitative description of acoustic variables in the mating call of *Pristimantis altamazonicus* and *P. brevicrus* from Ecuador. Mean±Standard Deviation are given, with range in parentheses. Call type: A= Single, B= Double, C= Triple, D= Group of single calls\*, E= Trill

Species	<i>P. altamazonicus</i> (n=1)			<i>P. brevicrus</i> (n=4)		
	Type A (n=9)	Type B (n=16)	Type C (n=6)	Type A (n=392)	Type D (n=213)	Type E (n=24)
Call Type (Pulses)						
Call Length (Seconds)	0.057±0.004 (0.052–0.06)	0.066±0.01 (0.052–0.08)	0.06±0.011 (0.044–0.07)	0.013±0.005 (0.005–0.04)	0.055±0.009 (0.031–0.09)	0.053±0.005 (0.045–0.06)
Dominant Frequency (Hz)	2784.967±330.804 (2584–3359.2)	2740.075±175.733 (2584–3359.2)	2684.45±64.814 (2584–2756.2)	2624.293±47.303 (2437.5–2812.5)	2463.679±83.386 (2226.6–2953.1)	2511.861±26.091 (2437.5–2531.2)
Call Rise Time (Seconds)	0.005±0.002 (0.002–0.01)	0.01±0.014 (0.003–0.06)	0.009±0.007 (0.001–0.02)	0.004±0.002 (0.001–0.01)	0.023±0.009 (0.005–0.05)	0.021±0.008 (0.009–0.04)
Interval between calls (Seconds)	1.501±0.208 (1.186–1.91)	1.132±0.887 (0.149–2.3)	0.747±0.841 (0.173–2.29)	0.915±0.773 (0.357–10.29)	0.958±0.643 (0.295–9.31)	0.254±0.176 (0.104–0.87)
Call Rate (calls/minute)		48.889		52.939±17.792 (31.699–76.51)		

\* Group refers to call with a sequence of several single pulses separated by a large interval between them.

tympanum and show cream or bluish white colouration in groins with thighs cream or bluish white with black mottling. Based on our phylogenetic analysis, genetic distances (~3.1% in 16S), and differences in morphology (Figs 1 and 3, Tables 2–3), we consider herein specimens referred as *P. altamazonicus* from Madre de Dios in southern Peru (Duellman, 2005; Hedges et al., 2008; Duellman & Lehr, 2009; Pyron & Wiens, 2011) and from Bolivia (Padial et al., 2004) as Unconfirmed Candidate Species 1. Populations in southern Peru tend to have a more tuberculate dorsum than northern populations, but a visible tympanic annulus on skin and red groins and thighs with black mottling (Fig. 5). Furthermore, males (mean SVL=20.3–23.0 mm) and females (mean SVL=30.6 mm) seem to be larger in size than populations in the northern Amazon Basin (Duellman, 2005; Duellman & Lehr, 2009).

***Pristimantis brevicrus* (Andersson, 1945), new combination.**

Figs 1, 3–5.

*Eleutherodactylus brevicrus* Andersson, 1945, Ark. Zool., 37A (2): 40. Holotype: NRM 1920. Type locality: “Watershed, Rio Pastaza”, eastern Ecuador.

*Eleutherodactylus altamazonicus*. In part. Duellman, 1978. Misc. Pub. N° 65. The University of Kansas Museum of Natural History: Lawrence, Kansas. 8–89: Fig. 53.

*Eleutherodactylus altamazonicus*. In part. Lynch, 1980. American Museum Novitates 2696: 4.

*Eleutherodactylus altamazonicus*. In part. Rodríguez & Duellman, 1994. Special publication N° 22. The University of Kansas Museum of Natural History. Lawrence, Kansas: 55.

*Pristimantis (Pristimantis) altamazonicus*. In part.

Duellman & Lehr, 2009. Nature und Tier Verlag: Münster, Germany: 138–139: Fig. 119.

**Characteristics.** *Pristimantis brevicrus* is characterised by (1) skin of dorsum shagreen commonly with warts or pustules on flanks; dorsolateral folds absent; skin of belly coarsely areolate; discoidal fold barely evident; (2) tympanic annulus and membrane not visible on skin, covered by muscle beneath skin; (3) snout short, subacuminate in dorsal view, rounded in profile; lips not flared, *canthus rostralis* weakly concave in dorsal view, rounded in cross-section; (4) upper eyelid about 75% of inter-orbital distance, bearing small tubercles; (5) dentigerous processes of vomer small, triangular, bearing 2–4 teeth; (6) males lack vocal slits, vocal sac and nuptial excrescences; (7) fingers large and slender, first shorter than second; discs on outer fingers expanded, bluntly truncated, about 1.5x the width of digit proximal to pad; supernumerary tubercles large, rounded; (8) fingers bearing narrow lateral fringes; (9) forearm bearing 2–3 small ulnar tubercles; (10) heel lacking tubercles; outer border of tarsus smooth; inner border of tarsus bearing a small tarsal fold; (11) two metatarsal tubercles; inner elliptical, about 5x the outer tubercle; supernumerary plantar tubercles absent or barely visible; (12) toes with lateral fringes; webbing absent; discs equal in size or slightly smaller than those on fingers; Toe V longer than Toe III; (13) in life, dorsum tan to dusty dark brown with or without a W-shaped mark on scapular region; females frequently have longitudinal tan stripes; groin, anterior and posterior surfaces of thighs are whitish blue to yellowish blue with black mottling; belly and ventral surfaces of the limbs bluish white with dark brown or black flecks; throat, feet and hands brown; iris coopery red to silver, finely reticulated with brown, and a black,



**Fig. 7.** Dorsal, ventral and lateral views of an adult male collected of *Pristimantis brevicrus* at Estación Biológica Jatun Sacha, S 1.066°, W 77.617°, 396 m altitude, Napo Province, Ecuador (QCAZ 53026). Note the presence of tubercles on upper eyelid (black) and the absence of an exposed tympanum (white) indicated by the arrows.

narrow vertical streak through pupil. After preservation, all brown areas in life become dark brown, anterior and posterior surfaces of thighs white mottled with black; venter cream with brown flecks in males, black with creamy white flecks in females; brown snout; (14) mean SVL in adult males  $19.9 \pm 2.2$  mm (17–24.4 mm); females with  $31.3 \pm 2.6$  mm (27.2–35 mm).

**Diagnosis.** Comparison of *P. brevicrus* with similar species from the Amazonian lowlands with black mottling or bars in groin and hidden surfaces of limbs are detailed in Table 2. This species is distinguished from *P. altamazonicus* in lacking a visible tympanic annulus on skin (Figs 3–4, 7), having a coarsely areolate skin texture on venter, shagreen dorsum commonly with warts or pustules toward the flanks and by the bluish white to yellowish white colour with black mottling in groin and hidden surfaces of thighs (red to bright orange with black mottling and transversal bars in *P. altamazonicus*).

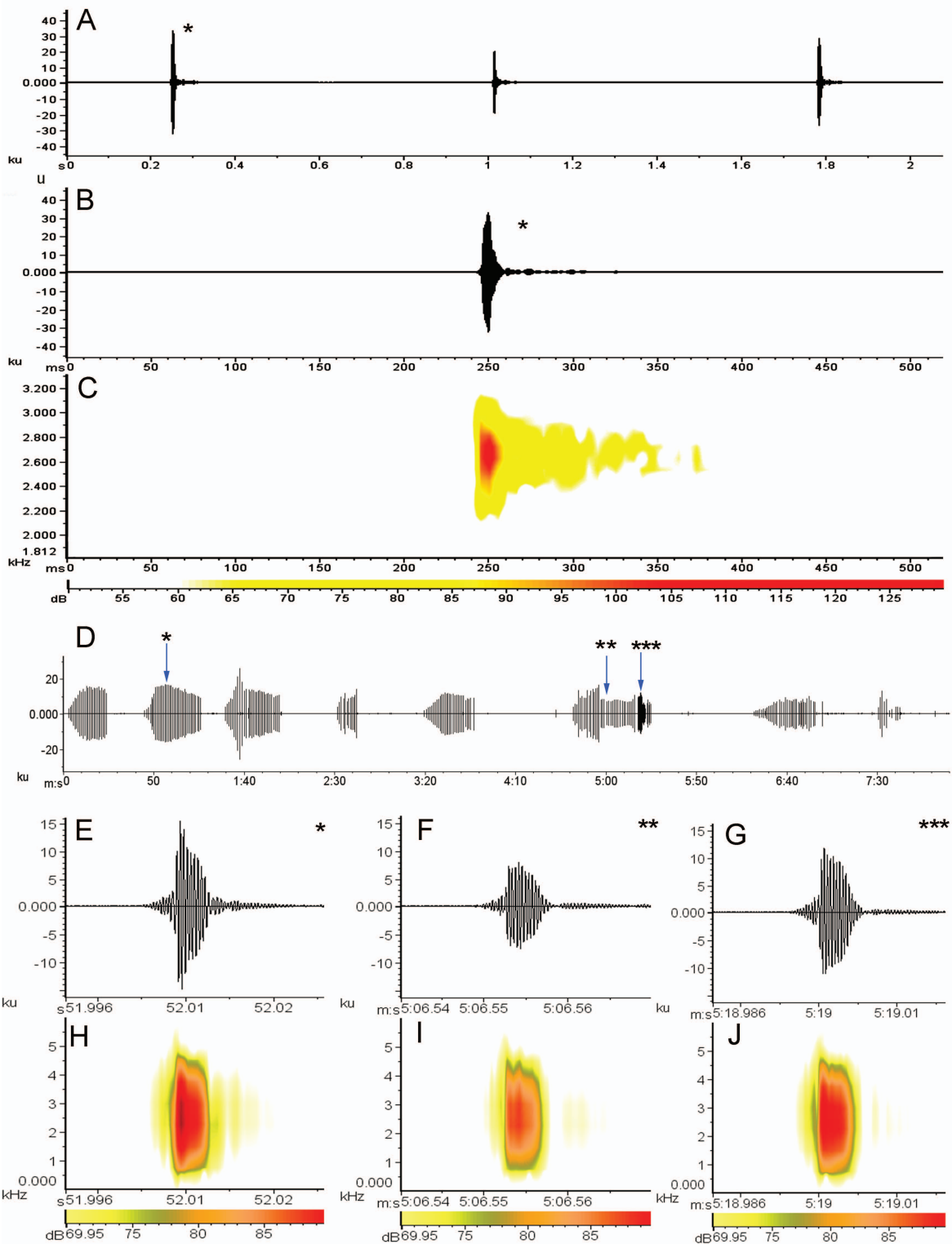
**Variation.** Measurements and proportions of reviewed specimens are detailed in Table 3. Males are smaller (mean SVL =  $19.9 \pm 2.2$  mm; 17.0–24.4 mm) than females (SVL =  $31.3 \pm 2.6$  mm; 27.2–35.0 mm), and commonly have a W-shaped mark on scapular region (e. g. QCAZ 52685, 56312) and a brighter greyish brown dorsum (Fig. 4). Females and juveniles commonly present cream to brown longitudinal stripes on the dorsum on a dark brown background (~60% of specimens reviewed).

**Colouration in life** (Fig. 5). At night, dorsum and flanks are reddish brown, with or without creamy brown or tan longitudinal stripes (stripes common to females and juveniles). In the day, dorsal colouration is darker than at night. In males, the dorsum is brown to dark grey, with a pale interorbital bar and a W-shaped mark in the scapular region. Canthal and postorbital stripes are absent, but lips are barred. The flanks are dark brown or grey with diagonal dark brown marks in the inguinal region. The dorsal surfaces of thighs are dark brown, but some individuals have tan to reddish brown heels. The groin, anterior and posterior surfaces of the thighs are mottled or barred with black stripes and bluish white or pale yellow blotches. The ventral surfaces are gray brown, dark brown or black with minute bluish white flecks. The iris is coppery red to silver, finely reticulated with brown, and with a black, narrow vertical streak through the pupil. By day, all brightly brown surfaces turn into dark brown or black.

**Colouration in preservation.** Dorsum grey brown with an interorbital bar and a W-shaped mark in scapular region, and with faint, dark transverse bars on the hind limbs; upper lip with dark brown bars below eyes; canthal, supratympanic, and postorbital stripes absent; the groin, anterior and posterior surfaces of the thighs are mottled or barred with black stripes and whitish cream blotches. The ventral surfaces are cream with minute brown flecks. Dark colouration is variable in preserved specimens, which may be related to the amount of time spent in preservative (Fig. 4).

**Natural history and distribution.** According to field notes and database records, specimens of *P. brevicrus* were found active at night on leaves of low vegetation, up to 3.5 m above ground. Based on specimen data from museum collections, this species has been collected throughout the year, with about 68% of specimens between January and June. This species is known from 74 localities along Amazonian evergreen lowlands of Ecuador in Morona Santiago, Napo, Orellana, Pastaza and Sucumbios Provinces, and eight localities from Amazonas and Loreto Departments in northern Peru, up to 1100 m altitude (Online Appendix S1). The occurrence area is calculated in about 206,739 km<sup>2</sup> on lowland evergreen forest in eastern Ecuador and northern Peru, but a wider distribution is probable for southern Colombia in the upper Amazon basin (Fig. 1B).

**Mating Call.** We analysed 629 calls from four males recorded at nights of May 17<sup>th</sup> (QCAZ 52986, 52990), 18<sup>th</sup> (QCAZ 52992) in 2012, at Limoncocha Biological Reserve (S0.40688°, W76.62063°), and the specimen SC51236 recorded calling at 0102 hours, on 2 July 2015 in Kurintza (S1.50423°, W77.51427°). Specimens QCAZ 52986 and QCAZ 52990 were captured in amplexus perching on a leaf and a stem of shrubs, about 1.4 to 2.1 m above ground. Specimen QCAZ 52992 was recorded calling from a shrub leaf, 3.0 m above ground. Specimen SC51236 was captured in amplexus, perching on a leaf of a shrub, about 2.4 m above ground on the night of 2 July 2015.



**Fig. 8.** Variation in the mating call of *Pristimantis brevicrus* for specimen QCAZ 52992 (A–C) and SC51236 (D–J). Oscillograms of each pulse and their respective spectrograms are detailed for singletons (B), groups of pulses (E) and a trill (F–G). Asterisks represent the location of each call type along the complete records in A and D. Colour bars represents the intensity of each call expressed in decibels.

Among the repertoire of these specimens, we identified three types of calls: 1) sequences of single clicks; 2) groups of pulses; and 3) a trill (Fig. 8). A detailed variation in call length, dominant frequency, call rise time, interval between calls and call rate of *P. brevicrus* and comparisons with calls from *P. altamazonicus* are described in Table 4.

**Remarks.** This species was misidentified as *P. altamazonicus* for populations in the northern Amazon Basin of Ecuador and Peru (Duellman, 1978a; Lynch, 1980; Rodríguez & Duellman, 1994; Duellman & Lehr, 2009), with bluish white and black mottling on hidden surfaces of thighs and minute brown flecks on the belly.

## DISCUSSION

We recognised *P. brevicrus* (Andersson, 1945) as a valid species, separated herein from the synonymy of *P. altamazonicus* (Lynch, 1974), based on morphological and genetic evidence. Furthermore, two Unconfirmed Candidate Species are also recognised as part of the complex (Figs 1 and 4, Table 1). However, additional specimens from these populations are necessary to resolve the taxonomic status of both entities.

We used a criterion of congruence between morphological diagnostic characters and the phylogenetic hypothesis (Table 1, Fig. 1) to distinguish *P. brevicrus* from *P. altamazonicus*. Variation in the presence/absence of the tympanic annulus, skin texture on dorsum and belly, colouration in the groin and belly in living specimens, were useful characteristics to identify and separate both species. Lacking tympanum (condition D *sensu* Duellman & Lehr, 2009), as occurs in *P. brevicrus*, is considered common (about 25% of *Pristimantis* species). The call of *P. altamazonicus*, a species with an exposed tympanic annulus and membrane, differs from *P. brevicrus* in the note repetition rate, dominant frequency and calling rate. Differences in mating call might suggest that reproductive barriers between sympatric populations of both species may be maintained via female mate choice (Guerra & Ron, 2008). Detailed physiological and ethological studies are necessary to understand the evolutionary implications of the tympanum condition in these populations, a structure that may influence mating behaviour, sexual selection, spatial orientation and territoriality (Robertson, 1986; Duellman & Trueb, 1994; Hetherington & Lindquist, 1999).

We have used the skin texture, presence/absence of dermal tubercles and colouration of groin and hidden surfaces of limbs to distinguish among living specimens of the *P. altamazonicus* complex. Recently, Guayasamin et al. (2015) described a notable variation in the depth of skin texture and tubercles in *P. mutabilis* which is related to stress, humidity, and background. Furthermore, Ortega-Andrade et al. (2015) described striking variation in body colouration of species from the *P. acuminatus* complex, which was related with the time of day that the specimens were prepared. In both cases, these authors suggest that skin and colour plasticity are associated with camouflage. Our observations on living specimens of the *P. altamazonicus* complex do not provide additional information on variation in colouration and skin texture.

The latitudinal replacement for monophyletic sister groups such as *P. altamazonicus* and the Unconfirmed Candidate Species 1 suggest an allopatric divergence between northern and southern Amazonia (Fig. 1B). In contrast, *P. brevicrus* (101–1102 m elevation, mostly in lowland forest in Amazonia) is a sister species to Unconfirmed Candidate Species 2 (486–1710 m, mostly in foothill forests along eastern Andes), suggesting divergence along an altitudinal gradient. However, *P. brevicrus* and *P. altamazonicus* can be found in sympatry along the lowland tropical forest in the northern Amazon basin. Similar biogeographic patterns

have been reported for congeneric species (Elmer et al., 2007; Padial & De la Riva, 2009) and other frogs (Funk et al., 2011; Ron et al., 2011; Caminer & Ron, 2014).

The *P. altamazonicus* complex is phylogenetically close to the larger *P. diadematus*, a species inhabitant of the mid to lowlands in the upper Amazon basin. However, neither genetic nor morphological evidence allowed an unambiguous assignment of these species to any of the multiple phenetic groups within *Pristimantis* (Lynch & Duellman, 1997; Hedges et al., 2008). The recognition of *P. brevicrus* as a valid species and two additional Unconfirmed Candidate Species highlight that the diversity of these frogs is far from being adequately understood.

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