



A new frog of the genus *Pristimantis* (Amphibia: Strabomantidae) from the high Andes of Southeastern Ecuador, discovered using morphological and molecular data

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Abstract

We describe a new small sized (SVL in females ≤ 25.7 mm; in males, ≤ 20.1 mm) *Pristimantis*, associated with bamboo-dominated forests in the Reserva Mazar, Andes of Southeastern Ecuador, at elevations between 2876–2989 m. This species is assigned to the *P. orestes* group, from whose members it differs by the absence of tubercles on heel and outer edge of tarsus, lacking a discoidal fold, presence of slightly expanded finger and toe pads, and bright yellow blotches on groin, axilla and anterior surfaces of thigh. The advertisement call of the new species consists of a series of short, indistinctly pulsed notes, and has a dominant frequency of 2.50–2.56 kHz. A molecular phylogeny based on a fragment of the mitochondrial gene 12S shows that the new species is sister to a clade formed by *Pristimantis simonbolivari* and an undescribed *Pristimantis*.

Key words: Cañar, new species, *Pristimantis orestes* group, Reserva Mazar

Resumen

Se describe una nueva especie de *Pristimantis* de tamaño pequeño (LRC en hembras ≤ 25.7 mm; en machos, ≤ 20.1 mm) asociada a bosques dominados por bambú en la Reserva Mazar, Andes del sureste de Ecuador, a elevaciones de 2876–2989 m. Asignamos esta especie al grupo *P. orestes*, de cuyos miembros difiere por la ausencia de tubérculos en el talón y porción externa del tarso, ausencia de pliegue discoidal, presencia de discos ligeramente expandidos en los dedos de las manos y los pies, y manchas amarillo brillante en la ingle, axila y superficie anterior del muslo. El canto de la nueva especie consiste de una serie de notas pulsadas, con una frecuencia dominante de 2.50–2.56 kHz. Una filogenia molecular inferida a partir de un fragmento del gen mitochondrial 12S muestra que la especie nueva es hermana a un clado formado por *Pristimantis simonbolivari* y una especie no descrita de *Pristimantis*.

Palabras claves: Cañar, Especie nueva, Grupo *Pristimantis orestes*, Reserva Mazar

Introduction

With 437 described species, no other frog genus in the world is as species-rich as *Pristimantis* (AmphibiaWeb 2011). Although this spectacularly diverse group of anurans is distributed throughout southern Central America and northern South America, its diversity elsewhere is not nearly as rich as in the Andes of Colombia, Ecuador, and Peru (Heinicke *et al.* 2007; Hedges *et al.* 2008).

The high diversity of *Pristimantis* in relation to closely related genera (e.g., *Oreobates*, *Phrynopus*) is not well-understood, but several factors, including high mutation rates, access to novel niches through its reproductive mode (i.e., direct development), phenotypic traits, the effect of mountain gradients, and susceptibility to barriers that do

not affect other amphibians (i.e., rivers; see Vences & Wake 2007), could be involved (see González-Voyer *et al.* 2011).

Also, the topographic and ecological complexity of the Andes may favor isolation among populations given a certain degree of niche conservatism (see Wiens 2004). This could be particularly true for frogs of the *Pristimantis orestes* species group (Lynch, 1997), as they are restricted to particular habitat types, mostly montane forests and paramos (Lynch 1979; Duellman & Lehr 2009; Wiens & Coloma 1992). Given that these two habitat types are both poorly surveyed and patchily distributed throughout the Andes (Valencia *et al.* 1999), it is to be expected that, isolated populations have the opportunity to radiate into new species.

This allopatric pattern of speciation may have occurred multiple times in the southern Ecuadorian Andes, where a frog species believed to be associated to the *Pristimantis orestes* group has recently been discovered. Just like all other 14 recognized members of this group —*Pristimantis atrabracus* (Duellman & Pramuk 1999), *P. chimu* (Lehr 2007), *P. cordovae* (Lehr & Duellman 2007), *P. corrugatus* (Duellman, Lehr, & Venegas 2006), *P. melanogaster* (Duellman & Pramuk 1999), *P. orestes* (Lynch 1979), *P. pataikos* (Duellman & Pramuk 1999), *P. pinguis* (Duellman & Pramuk 1999), *P. seorsus* (Lehr 2007), *P. simonbolivari* (Wiens & Coloma 1992), *P. simonsii* (Boulenger 1900), *P. stictoboubonus* (Duellman, Lehr, & Venegas 2006), *P. ventriguttatus* (Lehr & Kohler 2007), and *P. vidua* (Lynch 1979)— the new species is small with short snout, robust body, relative narrow head, and proportionally short limbs. Below, we describe this new taxon and present notes on its ecology, distribution, phylogenetic relationships, and vocalizations.

Methods

Terminology and morphological data. Generic and family names are according to the taxonomy proposed by Hedges *et al.* (2008). Specimens were sacrificed with 20% benzocaine, fixed in 10% formalin and stored in 70% ethanol. Diagnoses and descriptions generally follow Duellman & Lehr (2009). We examined comparative alcohol-preserved specimens from the herpetology collection at the Museo de Zoología of the Pontificia Universidad Católica del Ecuador (QCAZ) and Natural History Museum and Biodiversity Research Center of University of Kansas (KU). See Appendix I. All frogs were weighed live to the nearest 0.1 g using a Polder® electronic scale; when providing the standard deviation, we use the \pm symbol. Morphological measurements were taken with digital calipers to the nearest 0.1 mm, as described by Lehr & Coloma (2008), and are as follow: (1) snout–vent length (SVL); (2) tibia length; (3) foot length; (4) head length; (5) head width; (6) eye diameter; (7) interorbital distance; (8) upper eyelid width; (9) internarial distance; (10) eye–nostril distance. Sexual maturity was determined by the presence of vocal slits in males and by the presence of eggs or convoluted oviducts in females.

Molecular data. Amplification and Sequencing: We obtained DNA sequences of all available species in the *Pristimantis orcesi* group; as outgroups, we included two species from the *P. unistrigatus* group (i.e., *P. croceoguinis*, *P. ventrimarmoratus*). Genomic DNA was extracted from frozen tissue with the DNeasyTissue extraction kit (Qiagen Inc.) or a modified salt precipitation method (M. Fujita, unpubl.) based on the Puregene DNA purification kit (Gentra Systems). A fragment of the mitochondrial gene 12S was amplified using the primers t-Phe-frog (ATAGCRCTGAARAYGCTRAGATG) and Val-frog (TGTAAGCGARAGGCTTKGTTAAGCT) developed by Wiens *et al.* (2005), and primers 12.2L4E (GCTTAAAACCCYAARGGAYTTGACG) and 16H50 (TARACCA-TRATGCAAAGGTAC) developed by Heinicke *et al.* (2007). DNA amplification was achieved using the following protocol: 1 cycle of 2 min at 94°C, 30 sec at 50°C, 1 min at 72°C; 10 cycles of 30 sec at 94°C, 30 sec at 50°C, 1 min at 72°C; 29 cycles of 30 sec at 94°C, 30 sec at 58°C, 1 min at 72°C; 1 cycle of 5 min at 72°C (Wiens *et al.* 2005). Single PCR products were visualized in 7% agarose gel, and unincorporated primers and DNTPs were removed from PCR products with ExoSap (ExoSap-it, Affimetrix). Cycle sequencing was completed with the corresponding PCR primers and BigDye Terminator 3.1 chemistry (Applied Biosystems) using a standard cycle sequencing profile (96°C/3 min; 35 cycles of 96°C/10 s, 50°C/15 s, 60°C/3 min; and 72°C/7 min). Cycle sequencing reactions were conducted by a commercial company MacroGen Inc. Data from heavy and light stands were compared to generate a consensus sequence for each DNA fragment using Sequencer 4.8.

Aligning and Phylogenetic Analyses: Genbank sequences from *Phrynopis simonsii* (AY819406; Wiens *et al.* 2005), *Pristimantis melanogaster* (EF493826; Heinicke *et al.* 2007) and sequences obtained during this work (Appendix II) were aligned using MAFFT v.6 (Multiple alignment program for amino acid or nucleotide

sequences: <http://mafft.cbrc.jp/alignment/software/index.html>), with the Q-INS-i strategy. MacClade 4.07 (Maddison & Maddison 2005) was used to visualize the alignment (no modifications were necessary). Maximum likelihood (ML) trees were estimated using GARLI 0.951 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl 2006). GARLI uses a genetic algorithm that finds the tree topology, branch lengths, and model parameters that maximize lnL simultaneously (Zwickl 2006). Individual solutions were selected after 10,000 generations with no significant improvement in likelihood, with the significant topological improvement level set at 0.01; then, the final solution was selected when the total improvement in likelihood score was lower than 0.05, compared to the last solution obtained. Default values were used for other GARLI settings, as per recommendations of the developer (Zwickl 2006). Bootstrap support was assessed via 500 pseudoreplicates under the same settings used in tree search.

Vocalizations. Sound recordings were made with an Olympus LS-10 Linear PCM Field Recorder and a Sennheiser K6-ME 66 unidirectional microphone. Calls were analyzed in the Sound Analysis Software RAVEN (Charif et al. 2004). Call terminology follows Duellman and Trueb (1994). Dominant frequency, note duration, internote interval, maximum energy and repetition rate were calculated for each analyzed note.

Systematics

Pristimantis bambu Arteaga-Navarro & Guayasamin, new species

Holotype. QCAZ 46740, an adult male (Figs. 1–3) obtained by Alejandro F. Arteaga-Navarro on January 07, 2010, at La Libertad, Reserva Mazar (02.54804 S, 78.69741 W; 2976 m.a.s.l.), Cantón Azogues, Provincia Cañar, Ecuador; airline distance from Rivera is 6.6 km.

Paratypes. All 24 paratypes (Figs. 4A, B) were collected by Alejandro F. Arteaga-Navarro on January and March 2010 at the Reserva Mazar, southern Sangay National Park, east of Pallcayacu river, within the Cantón Azogues, Provincia Cañar, Ecuador. From these, 16 are adult males (QCAZ 46704, 46706–7, 46710–12, 46724–27, 46741–42, 47555, 47558, 47556–57); 6 are adult females (QCAZ 46705, 46708, 46739, 46743–44, 7559); and 2 are juvenile females (QCAZ 46713–14).

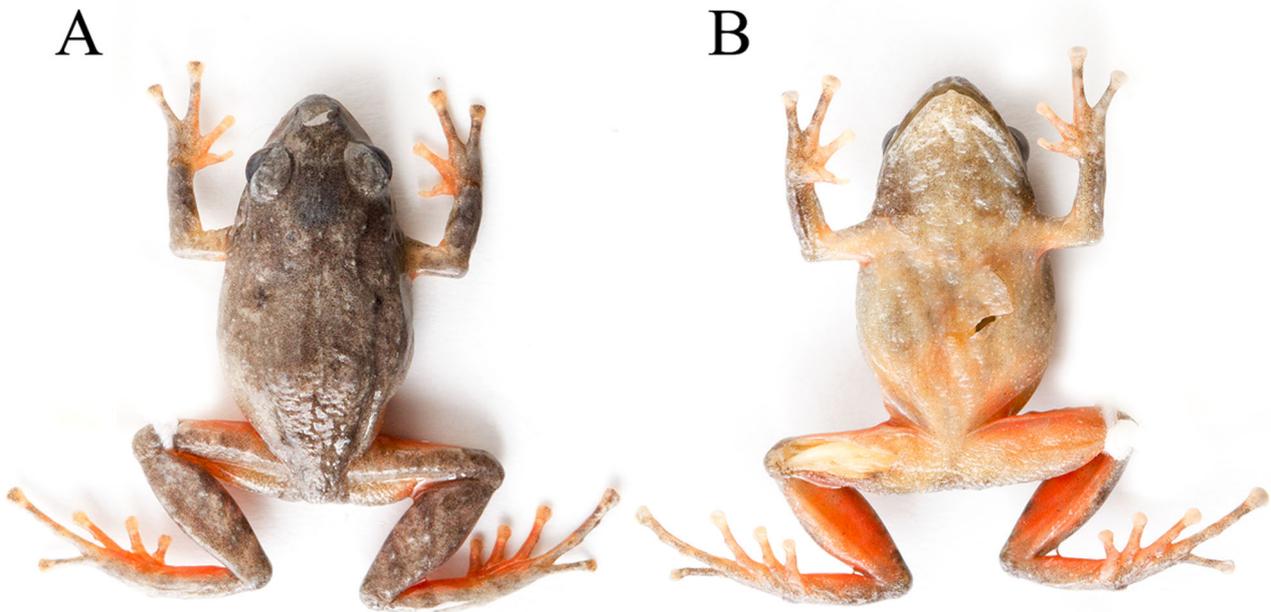


FIGURE 1. Adult male holotype of *Pristimantis bambu*, QCAZ 46740, SVL 20.0 mm.

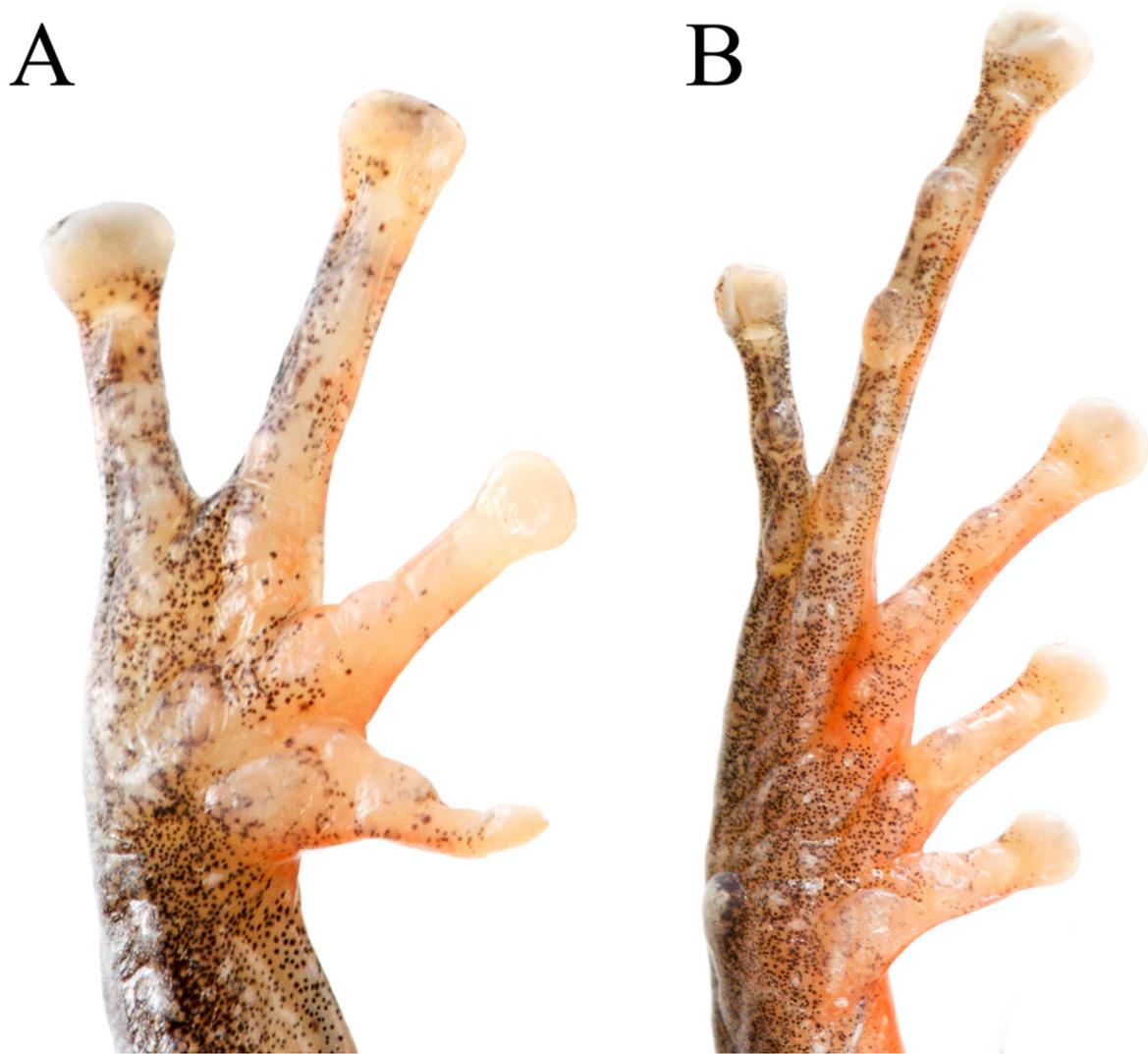


FIGURE 2. (A) Right hand of *Pristimantis bambu*; (B) Right foot of *Pristimantis bambu*. Holotype, adult male (QCAZ 46740).

Diagnosis. The new species is placed in the genus *Pristimantis*, as diagnosed by Hedges *et al.* (2008), because of the presence of the following traits: cranial crests absent, dentigerous process of the vomers present, and T-shaped terminal phalanges. *Pristimantis bambu* is diagnosed by having: (1) skin texture of dorsum shagreen with few widely interspersed low tubercles; middorsal fold barely noticeable and fragmented when present (Fig. 1A); dorsolateral folds ill-defined and incomplete, sometimes absent; venter areolate, lacking discoidal or thoracic folds (Fig. 1B); (2) tympanic membrane absent; tympanic annulus present, 40–71% of eye length in females and 42–65% in males, with upper rim obscured by supratympanic fold; (3) snout short, acuminate in dorsal view and rounded in profile; (4) upper eyelid lacking enlarged tubercles; when present, tubercles are low and almost undistinguishable from surrounding skin texture; upper eyelid width 49–83% of interorbital distance; cranial crests absent; (5) dentigerous process of vomers well developed, oblique in outline, positioned posterior to level of choanae and separated medially by distance less than width of odontophore, each process bearing 4–10 teeth; (6) males with a small subgular vocal sac; vocal slits present; nuptial pads absent; (7) first finger shorter than the second; discs on fingers expanded and rounded to slightly truncated (Fig. 2A), except for Finger I that is only barely expanded; (8) fingers bearing narrow lateral fringes; outer palmar tubercle divided at least distally, inner portion larger and elliptical and outer portion smaller and round; supernumerary tubercles round (Fig. 2A); (9) ulnar tubercles coalesced into fold; (10) no heel or tarsal tubercles; (11) toes bearing narrow fringes; webbing absent; Toe V slightly longer than Toe III; toe discs expanded, rounded to slightly truncated (Fig. 2B); (12) inner metatarsal tubercle elliptical, about 1.2–2 times the size of outer, rounded and enlarged metatarsal tubercle; supernumerary plantar

tubercles round and weakly developed (Fig. 2B); (13) in ethanol, dorsum pale brownish gray in males (blackish or slate gray in females), with minute dark spots on the scapular and sacral region (Fig. 3), and faint barring on the dorsal surfaces of limbs (absent in females); dark postocular stripe and faint supralabial bars present; flanks with some localized rusty tinge on the axilla, groin and hidden surfaces of hind limbs; pale cream spots enclosed by blackish pigment may be present on the groin and posterior surfaces of the thigh (bolder and invariably present in females); ventral surfaces uniformly grayish tan with very fine, dark, localized mottling on throat. In life (Fig. 3), dorsal surfaces of males tan, with or without dark symmetrical markings overall (uniformly blackish olive drab in females); dark transverse bars on the hind limbs poorly marked if present (absent in females); black postocular blotch present; in males, background color of throat, belly and ventrolateral surfaces khaki, with or without a yellowish tint on the throat that may shift to orange-red on the belly; rest of underparts, grayish brown to rosy brown; in females, all ventral surfaces uniform gray or deep purplish brown; a rusty tinge on the hidden surfaces of hind limbs is present in both males and females, almost always surrounding one or several amber to orange blotches in the axilla, groin and posterior surfaces of the thigh; these marks may be enclosed by black pigment; upper half of the iris pale goldenrod yellow; lower half, deep coppery brown; (14) SVL in females 24.6–26.4 (mean = 25.3 ± 0.6 , $n = 5$), in males 17.4–20.1 (mean = 19.2 ± 0.7 , $n = 13$); (15) body mass in living females 0.9–1.6 g, in males 0.5–0.8 g.



FIGURE 3. *Pristimantis bambu* in life. QCAZ 46740, SVL 20.0 mm, adult male, holotype. Photographs by: Luis A. Coloma.

Similar species. *Pristimantis bambu* differs from all other members of the *P. orestes* group (i.e., *P. atrabrachus*, *P. chimu*, *P. cordovae*, *P. corrugatus*, *P. melanogaster*, *P. orestes*, *P. pataikos*, *P. pinguis*, *P. seorsus*, *P. simonbolivari*, *P. simonsii*, *P. stictoboubonus*, *P. vetriguttatus*, *P. vidua*; sensu Hedges *et al.* 2008) by having slightly expanded, rounded to slightly truncate finger pads. *Pristimantis corrugatus* (Duellman & Lehr 2009) may as well have expanded discs, but these are elliptical and wider than those of *P. bambu*. The new species may be further distinguished from all other species in the group (Table 1), except from *P. chimu*, *P. pinguis*, *P. seorsus* and *P. simonsii* (Duellman & Lehr 2009), by having ulnar tubercles coalesced into a low fold. *Pristimantis chimu*, *P. seorsus* and *P. simonsii* differ from *P. bambu* by lacking vocal slits; *P. pinguis* differs by having a differentiated tympanic membrane. From those species in the group bearing small eyelid tubercles (*P. chimu*, *P. cordovae*, *P. orestes*, and *P. vetriguttatus*; Duellman & Lehr 2009; Lynch 1979), it differs by lacking a distinct discoidal fold. Finally, in life, *P.*

bambu is unique among the species assigned to the *P. orestes* group on the basis of the rusty pigment of the hidden surfaces of the hind limbs and the bold, black-bordered amber blotches on the groin and posterior surface of the thighs.

TABLE 1. Character states in species placed in the *Pristimantis orestes* group.

Species	Eyelid tubercles	Discs on outer fingers	Ulnar tubercles	Heel and tarsal tubercles	Vocal slits
<i>P. atrabrachus</i>	Absent	Barely wider than digit proximal to disc	Absent	Absent	Present
<i>P. bambu</i>	Several, barely visible; or 1-2 small	Slightly expanded, rounded to slightly truncate	Coalesced into fold	Absent	Present
<i>P. chimu</i>	Several, small	Narrow, rounded	Coalesced into short fold	Absent	Absent
<i>P. cordovae</i>	Several, small	Barely expanded, emarginate	Present	Small tubercle on heel; absent on tarsus	Present
<i>P. corrugatus</i>	One prominent, conical tubercle	Expanded, elliptical, half the width of digit proximal to disc	Small, elongate	Conical tubercle on heel; one low, diffuse tubercle on tarsus	Present
<i>P. melanogaster</i>	Absent	Rounded, barely wider than digit proximal to disc	Absent	Absent	Absent
<i>P. orestes</i>	Several, small, non-conical	Moderately dilated	Present, obscure	Small, non-conical	Present
<i>P. pataikos</i>	Absent	Rounded, barely wider than digit proximal to disc	Absent	Absent	Absent
<i>P. pinguis</i>	Absent	Rounded, barely wider than digit proximal to disc	Coalesced into short fold	Absent	Present
<i>P. seorsus</i>	Absent	Narrow, truncate	Present as tubercles or as a fold	Conical tubercle on the heel; those of tarsus forming ridges	Absent
<i>P. simonbolivari</i>	Absent	Barely expanded	Absent	Present only on heel	Present
<i>P. simonsii</i>	Absent	Rounded, barely wider than digit proximal to disc	Coalesced into low ridge	Absent	Absent
<i>P. stictoboubonus</i>	Absent	Narrow	Absent	Absent	Absent
<i>P. ventriguttatus</i>	Several, small	Broadly expanded, truncate	Present	Small, present on heel and tarsus	Present
<i>P. vidua</i>	Indistinct	Narrow	Absent	Present only on tarsus	Present

Description of the holotype. Adult male (QCAZ 46740; Figs. 1–3). Head slightly narrower than body, slightly longer than wide; upper eyelid bearing several, feebly visible tubercles; head width 35% of SVL; head length 36% of SVL; snout relatively short (snout to eye distance 17% of SVL), acuminate in dorsal view (Fig. 1A) and rounded in profile; tongue longer than wide, posterior half notched and not adherent to floor of mouth; eye diameter slightly larger than eye–nostril distance; nostrils not protuberant, directed anterolaterally; canthus rostralis weakly concave in profile; loreal region slightly concave; upper eyelid width 58% of interorbital distance; cranial crests absent; tympanic annulus distinct, round; tympanic membrane absent; two enlarged postrictal tubercles, situated posterovertrally to tympanic annulus; choanae round, not concealed by palatal shelf of maxillary; vomerine odontophores posteromedial to choanae, oblique in outline, about the same size of choana, separated medially by distance less than width of odontophore, each bearing 5 teeth; vocal slits and median, subgular vocal sac present; skin on dorsum shagreen with widely interspersed low tubercles; dorsolateral fold visible on either side but only on anterior half of the body; middorsal fold fragmented, barely noticeable (Fig. 1A); skin on venter areolate with no discoidal or thoracic folds (Fig. 1B); cloacal sheath absent; cloacal region bordered ventrally by small, closely packed

warts; ulnar tubercles coalesced into fold, continuous throughout forearm; outer palmar tubercle divided, inner portion larger and elliptical, outer portion smaller and round (Fig. 2A); subarticular tubercles round in section; supernumerary palmar tubercles low and rounded, much lower than subarticular tubercles; fingers bearing narrow lateral fringes; Finger I shorter than Finger II; disc of Finger I barely expanded; all other discs expanded, rounded to slightly truncate (Fig. 2A); ventral pads well defined by circumferential grooves; nuptial pads absent.

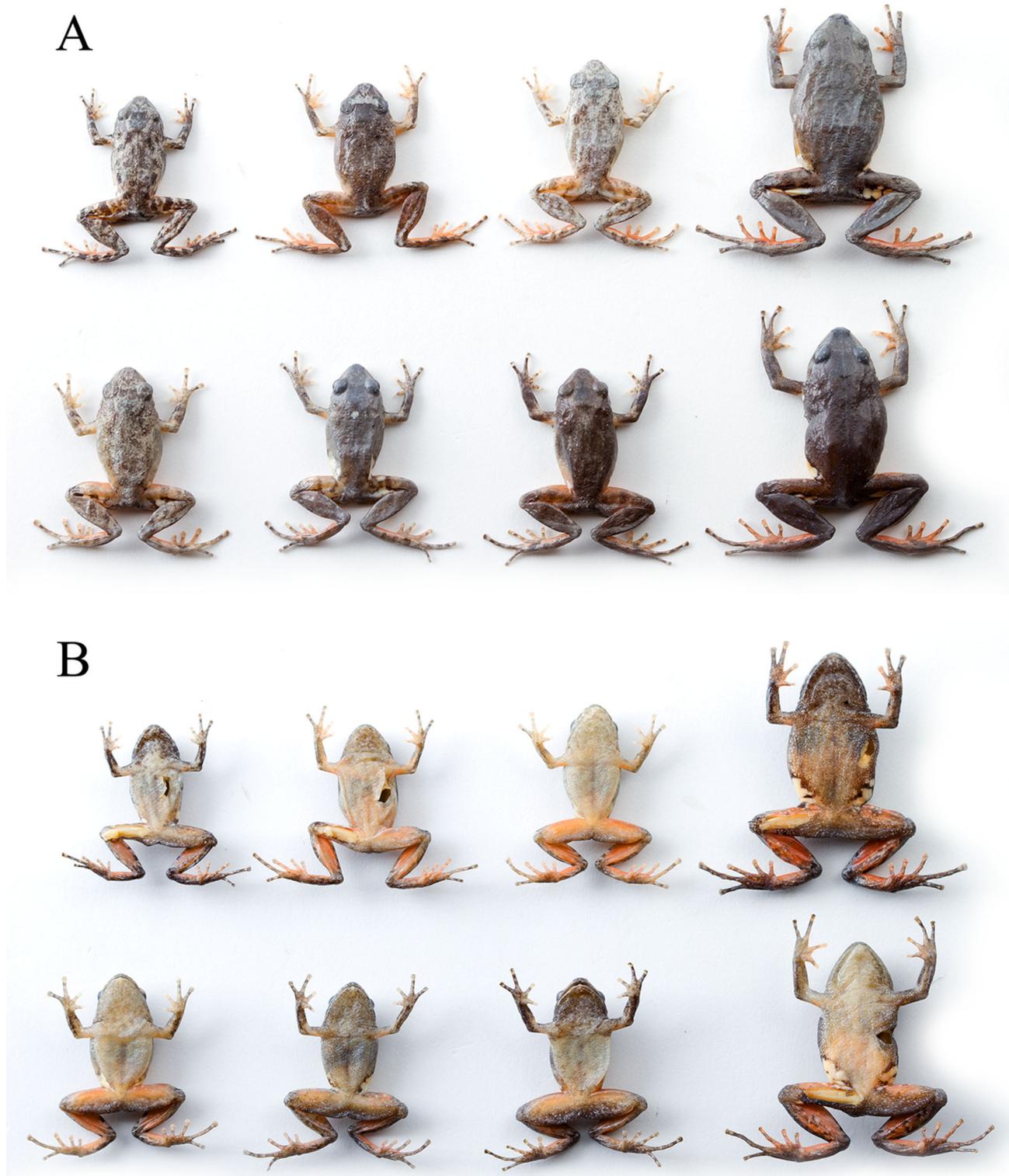


FIGURE 4. Color variation in eight paratypes of *Pristimantis bambu*. (A) dorsal view; (B) ventral view. Males are shown in columns 1–3, females in column 4.

Tibia length 47% of SVL; foot length 45% of SVL; inner metatarsal tubercle present, about the same size of the outer, rounded and enlarged tubercle; subarticular tubercles round in section; plantar supernumerary tubercles indistinct; toes bearing narrow lateral fringes; webbing absent; discs of Toe I barely expanded; all other toe discs expanded, rounded to slightly truncated; toes with ventral pads well defined by circumferential grooves; relative length of toes: I < II < III < V < IV; Toe V slightly longer than Toe III (Fig. 2B).

Measurements of holotype (in mm). SVL 20.04; tibia length 9.4; foot length 9.0; head length 4.5; head width 7.2; eye diameter 2.3; interorbital distance 2.6; upper eyelid width 1.5; internarial distance 2.0; eye-nostril distance 1.8.

Coloration of holotype in preservative. Dorsal surfaces pale brownish gray, with minute dark dots on the scapular and sacral region, distributed symmetrically on each half of the body, and with faint, dark transverse bars on the hind limbs (Fig. 1A); upper lip with dark brown bars below eyes; dark brown supratympanic stripe present; background color of ventral surfaces khaki with a localized rusty tinge on the hidden surfaces of the hind limbs, which is also present but much fainter on the lower belly, palms and axillary region (Fig. 1B).

Coloration of holotype in life (based on observations in the field and photographs; Figs. 3A, B). Dorsal surfaces light orangish brown, with faint, dark transverse bars on the hind limbs; flanks with same coloration as dorsum, with a dark brown postocular stripe accompanied by faint vertical supralabial stripes; background color of ventral surfaces khaki, finely mottled with dark pigment on the throat and strongly tinged with yellow on the throat and upper chest; palms, soles, ventral surfaces of hind limbs and groin intensely tinged with rusty to yellow, which becomes deep saffron on the belly as it fuses with the yellowish pigment of the throat; upper half of iris light goldenrod, lower half coppery brown.

Variation. Meristic variation is presented in Tables 2 and 3. Some paratypes have two eyelid tubercles (QCAZ 46705, 46714, 46712, 46739, 46711, 26724), and others have only one (QCAZ 46725, 46704, 41444, 41606, 41616, 41620, 41672, 41694, 41738; Figs. 4, 5B); several paratypes have no visible dorsolateral fold (QCAZ 46706, 46714, 46725, 46711, 46742, 46724) nor middorsal fold (QCAZ 46725, 46711, 46742, 46724). In ethanol, the dorsum of one paratype is densely covered throughout with symmetrically distributed dark marks (QCAZ 46742; Fig. 6A), another one has a pale gray middorsal spot (QCAZ 46714), and yet another one (QCAZ 46710) has a light drab frontal patch. The dorsal background color of three paratypes is darker than the holotype (QCAZ 47555, 47556, 47557; Fig. 6D) or completely dark grayish brown altogether (QCAZ 46707); three male paratypes (QCAZ 46712, 47555, 47557) have no localized rusty to light reddish brown pigment on the groin, axilla and hidden surfaces of the hind limbs. Most male paratypes (QCAZ 47555, 46726, 47556, 47558, 47557, 46711, 46712, 46714, 46725, 46713, 46724, 46727, 46706, 46742) have pale cream spots on the groin and posterior surface of the thighs. Throat mottling is much more extensive in two male paratypes (QCAZ 46706, 46707). In preservative, the dorsal color becomes grayer and the rusty and yellowish tinge of the ventral surfaces is extensively lost. Specimens in life have well-defined tubercles and folds on the dorsum and legs, but those skin marks are much fainter in preservative.

Vocalization. The advertisement call of QCAZ 47558 (an adult male recorded on Reserva Mazar on 11 March 2010 at 22:08 h, temperature not recorded) can be characterized as a series of short, indistinctly pulsed, non-modulated notes in amplitude. The dominant frequency ranges between 2.50 and 2.56 kHz (maximum energy is 122.54 ± 1.74 dB; $n = 20$). The note repetition rate is 54.42 notes per minute, and the note length ranges between 0.005 and 0.006 s (Fig. 5). Internote intervals range between 0.816 and 1.782 s (mean = 1.195 ± 0.310 s; $n = 20$). Out of six recordings, call duration ranged between 12 and 103 s, with significant variation among note repetition rate. Vocalizing males decreased note intensity when approached by an observer. This is the first call described for a species in the *P. orestes* group.

Etymology. This specific epithet *bambu* refers to the habitat preference of the new species, which is found in bamboo-dominated montane forests (*Chusquea* sp). The epithet is a noun in apposition.

Distribution and natural history. *Pristimantis bambu* is known only from La Libertad, Reserva Mazar, (S2.54804, W78.69741), at elevations between 2876 and 2989 m.a.s.l (Fig. 6). This site, a family-owned alpaca ranch, is located in the eastern mountain range of Ecuador, on the southern edge of Sangay National Park, Cañar province. The area is a patchwork of native evergreen montane forest (Valencia *et al.* 1999), shrub, secondary forest and pastures (FCT 2008). Average temperatures at La Libertad are 18°C and precipitation is 1200 mm per year. There are two distinct seasons: the dry season (Oct/Nov – Jan/Feb) and the wet season (FCT 2008). Individuals of *P. bambu* were active during night on leaf litter and vegetation (20–155 cm above ground) within regrowth mon-

tane forest where bamboo (*Chusquea* sp) is dominant. Individuals were seen active under overcast and cloudy nights, even under light drizzle, but not under heavy showers. During early morning, *P. bambu* was found active on the leaf litter, later during the day, individuals were found hidden in the leaf litter. Two individuals were hidden under piled up eucalyptus lumber and dead leaves far from the forest border, in pastures. *Pristimantis bambu* was found both near rivers and far from significant watercourses; but it was never found within primary forest. The species appears to be closely associated with secondary forests where the canopy cover is not complete, a habitat composed mainly of small trees, shrubs and bamboo. The species was also found on the forest border.

TABLE 2. Measurements (in mm) of adults of *Pristimantis bambu*. Range is followed by mean and standard deviation. SVL = snout–vent–length.

	Females (n = 5)	Males (n = 13)
SVL	24.6–26.4 (25.3 ± 0.6)	17.4–20.1 (19.2 ± 0.7)
Tibia length	11.9–12.4 (12.1 ± 0.2)	8.7–9.8 (9.3 ± 0.3)
Foot length	11.3–11.7 (11.5 ± 0.2)	7.5–9.1 (8.4 ± 0.4)
Head length	8.3–9.0 (8.6 ± 0.3)	6.1–7.2 (6.7 ± 0.3)
Head width	8.4–9.3 (8.7 ± 0.3)	6.2–7.1 (6.6 ± 0.3)
Interorbital distance	3.0–3.3 (3.1 ± 0.1)	2.2–2.6 (2.4 ± 0.1)
Upper eyelid width	1.6–2.1 (1.9 ± 0.2)	1.5–2.0 (1.7 ± 0.1)
Radioulina length	5.4–6.3 (5.8 ± 0.3)	4.2–4.7 (4.5 ± 0.1)
Eye to nostril distance	2.2–2.4 (2.3 ± 0.1)	1.5–1.9 (1.7 ± 0.1)
Snout to eye distance	4.0–4.3 (4.1 ± 0.1)	3.0–3.6 (3.3 ± 0.2)
Eye diameter	2.5–2.7 (2.6 ± 0.1)	2.1–2.4 (2.2 ± 0.1)
Tympanum diameter	1.0–1.5 (1.3 ± 0.2)	0.7–1.3 (1.1 ± 0.2)
Hand length	6.1–6.4 (6.2 ± 0.1)	4.3–5.2 (4.9 ± 0.2)
Finger I length	3.6–4.0 (3.7 ± 0.2)	2.6–3.1 (2.9 ± 0.2)

TABLE 3. Ranges of morphological proportions (in percentages) of adults of *Pristimantis bambu*. SVL = snout–vent–length.

	Females (n = 5)	Males (n = 13)
Tibia length/SVL	46.9–48.8	45.3–56.4
Foot length/SVL	43.7–46.7	40.9–45.9
Foot length/tibia length	93.2–95.8	77.0–96.1
Head width/SVL	32.9–37.0	23.8–36.2
Head length/SVL	33.0–35.9	32.1–37.4
Head length/head width	96.0–102.5	94.3–108.1
Eye-to-nostril distance/eye diameter	86.1–91.0	66.5–87.8
Upper eyelid width/IOD	49.3–69.8	59.2–82.8
Radioulina length/SVL	20.6–24.4	22.8–24.8
Hand length/radioulina length	98.9–118.4	101.5–115.0
Finger I length/hand length	55.2–66.0	52.9–65.7

During March 2010, a vocalizing male was heard. When searching it, a female *Pristimantis bambu* was found, presumably attracted by the male's vocalization. They were both bagged together and began amplexus 72 min afterwards. During the wet season, five males were heard vocalizing simultaneously along with another species (*Pristimantis pycnodermis*). A gravid female released 30 unfertilized eggs inside a plastic collecting bag. Sympatric species of *P. bambu* include, *P. pycnodermis*, *P. gagliardoi*, *P. riveti*, and two undescribed *Pristimantis* species.

During night, *P. bambu* remains still when approached by an observer; calling males may continue to vocalize even when approached, though some individuals may stop calling or foraging, even jumping away at times. At night, most individuals were found by standing 0.4–1.8 m from the vegetation and carefully scanning thin (2–10 mm in diameter) twigs, ferns, and leaves.

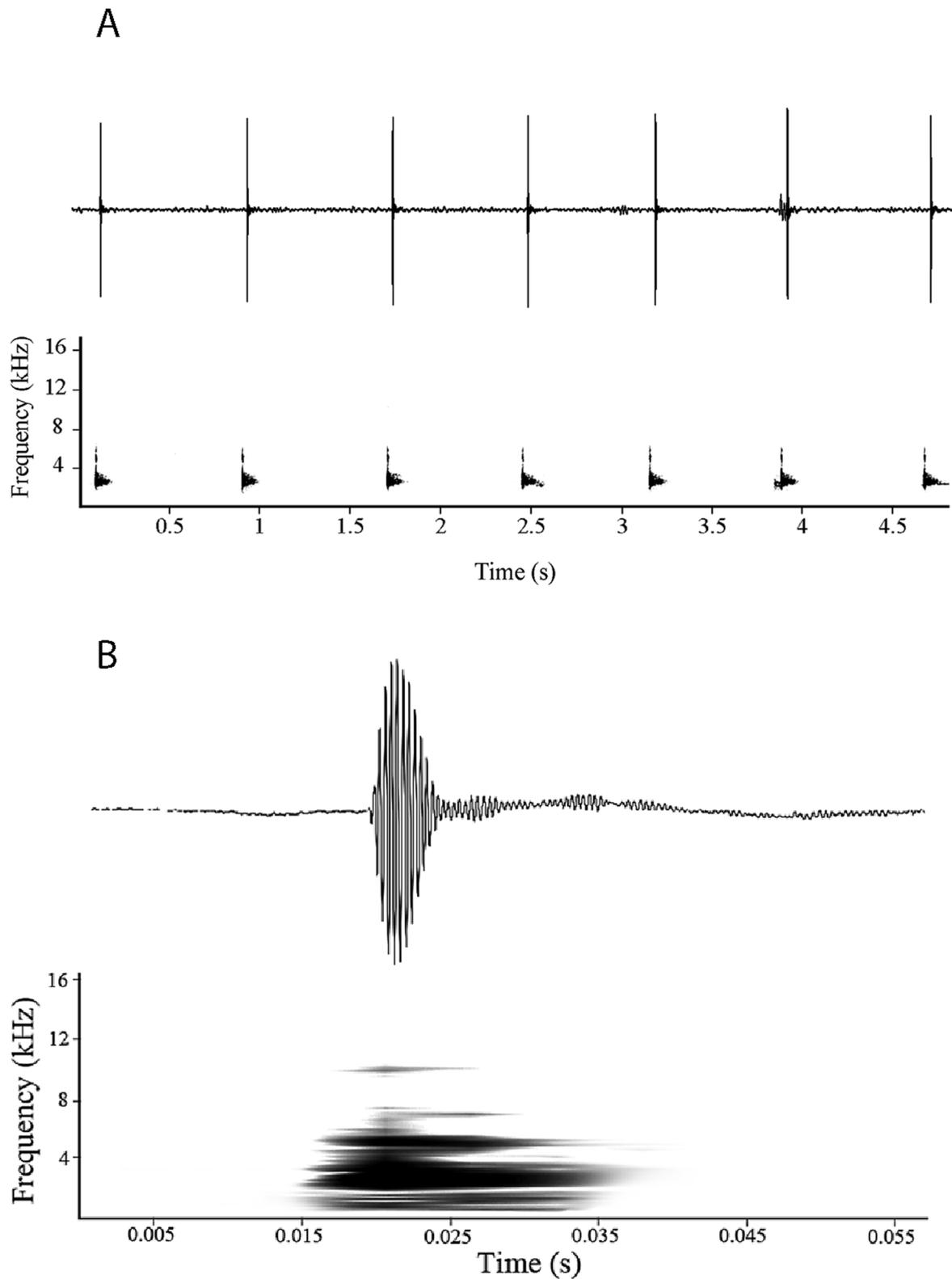


FIGURE 5. Oscillogram and sonogram of the advertisement call of *Pristimantis bambu* (QCAZ 47558).

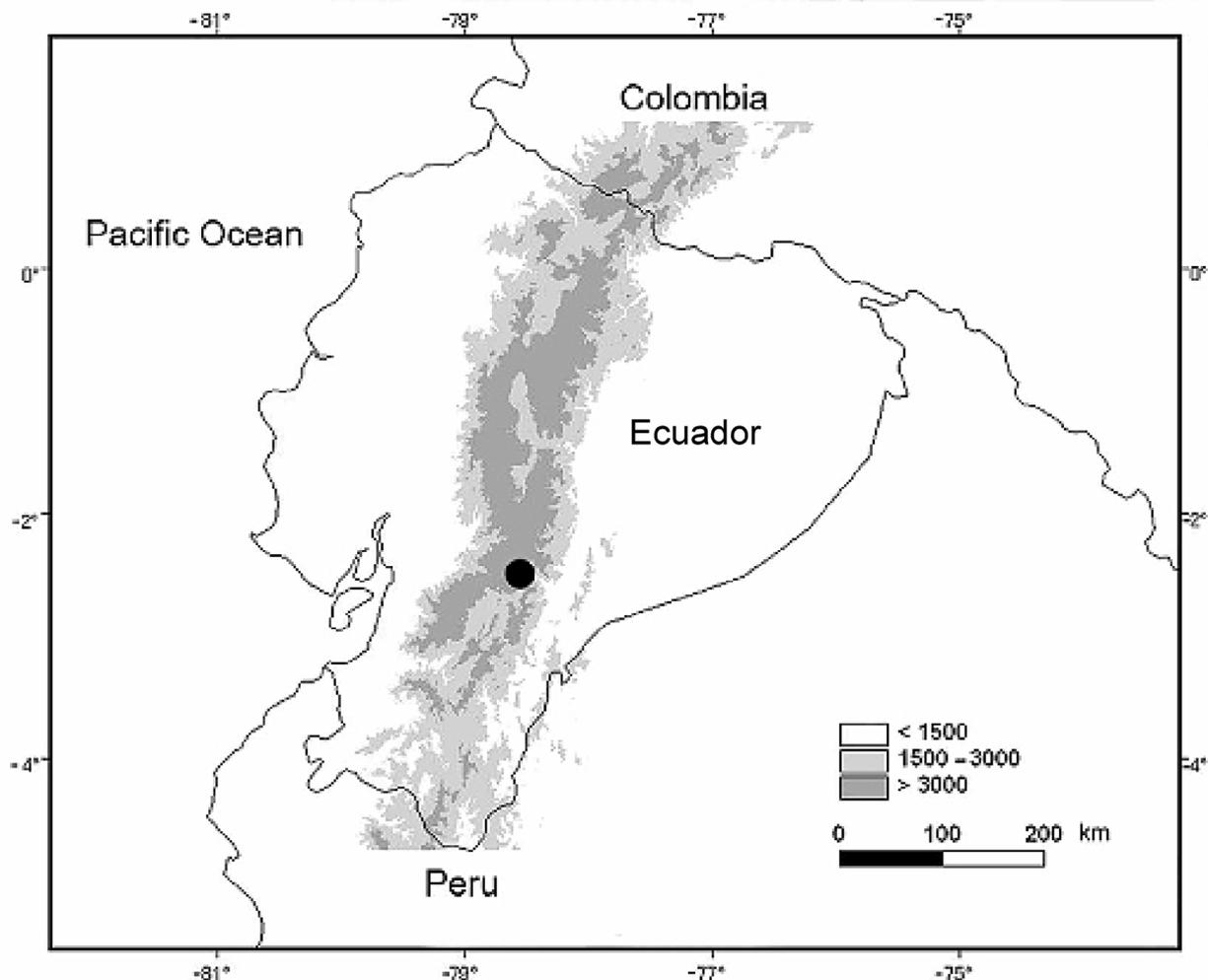


FIGURE 6. Distribution of *Pristimantis bambu* in Ecuador. The dot indicates the type locality, the only locality where the species is known to date.

Discussion. The most recent comprehensive phylogeny of strabomantids includes four species assigned to the *Pristimantis orestes* group; two of them, *P. orestes* and *P. simonbolivari*, cluster together, but the other species (*P. simonsii* and *P. melanogaster*) do not (Hedges *et al.* 2008). Two scenarios explain the observed pattern: (i) the molecular phylogeny is accurate and the shared morphologies of the species included in the *Pristimantis orestes* group represent convergent evolution to cope with similar habitats (Hedges *et al.* 2008), or (ii) the molecular phylogeny is not congruent with the evolutionary history of these amphibians, and the *Pristimantis orestes* group is monophyletic. Since only a molecular phylogeny is at hand at the moment, we favor the first scenario. Both morphology and molecules support the lineage distinctiveness of *P. bambu*. Given our taxon sampling, *P. bambu* is most closely related to *Pristimantis* sp. (Fig. 7; corrected genetic distance = 5.9–6.8%), *P. simonbolivari* (corrected genetic distance = 7.7–7.8%), and *P. orestes* (corrected genetic distance = 8.9%; Fig. 7).

Acknowledgements

John Lynch and José Manuel Padial provided valuable comments on an earlier version of this manuscript. The discovery of *Pristimantis bambu* was made possible by the support of Stuart White and Catherine Schloegel from Fundación Cordillera Tropical. We are highly grateful to Jesse Lewis, Seth Adams and all the enthusiastic people involved in the Round River Conservation Studies (RRCS) programs at Reserva Mazar during 2009 and 2010 for their assistance and companionship in the field. For access to collection specimens, we thank the Museo de Zoología of the Pontificia Universidad Católica del Ecuador and the Natural History Museum and Biodiversity Research Center of University of Kansas. Photographs of the holotype in life were provided by Luis A. Coloma.

Sequencing was funded by a grant from the Secretaria Nacional de Ciencia y Tecnología del Ecuador (SENACYT: PIC 08-470). Research permits were issued by the Ministerio de Ambiente del Ecuador (N° 008-09 IC-FAU-DNB/MA).

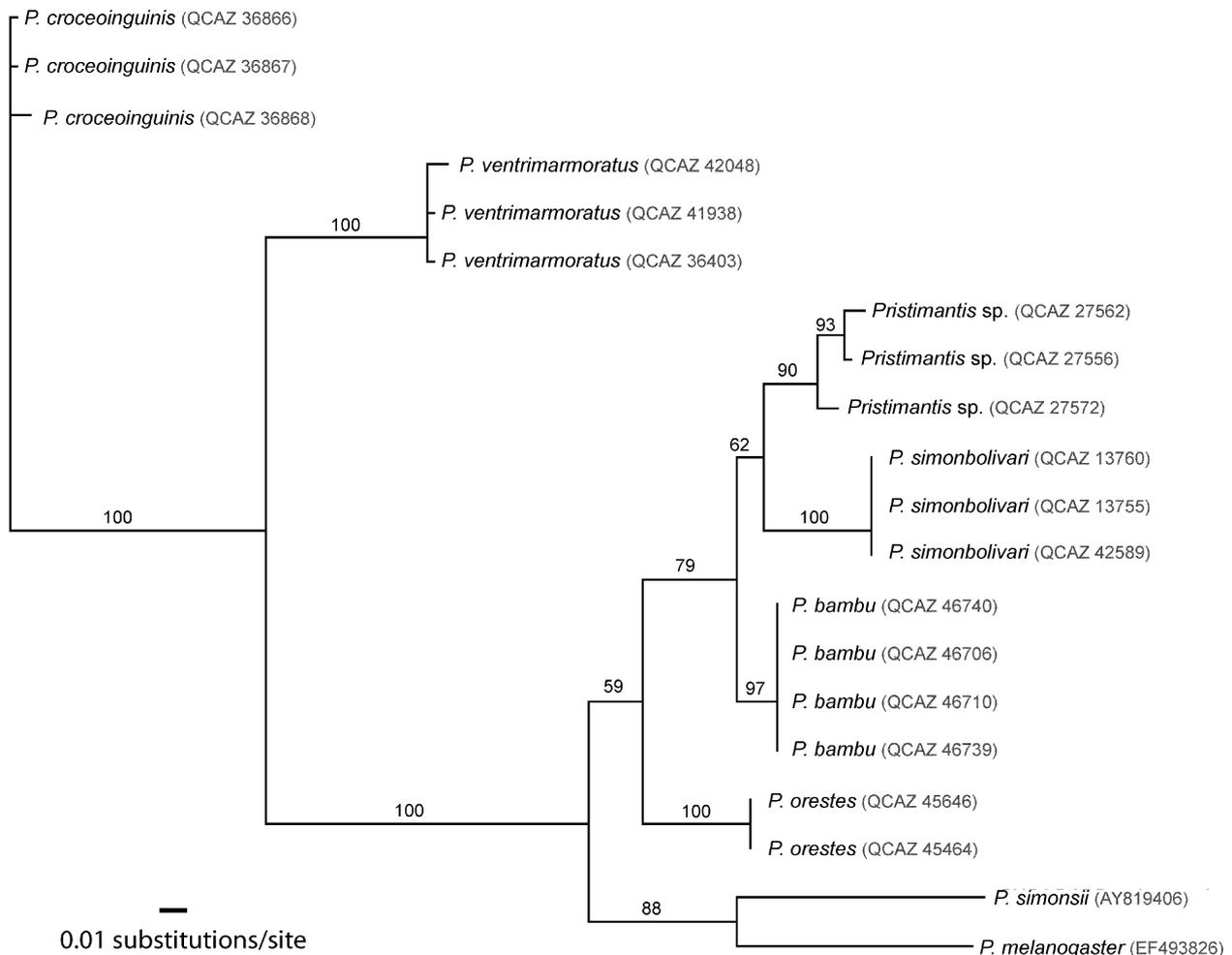


FIGURE 7. Maximum likelihood phylogeny of sampled species in the *Pristimantis orestes* group inferred using GARLI (lnL = -1384,3649; evolution model = GTR + G + I). *Pristimantis croceinguinis* and *P. ventrimarmoratus* are used as outgroups.

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APPENDIX I. Specimens examined.

- Pristimantis orestes*.— Ecuador: *Provincia Loja*: 10 km S Saraguro, KU 165553; Parque Nacional Podocarpus, Cajanuma, QCAZ 45646, 45464.
- Pristimantis simonbolivari*.— Ecuador: *Provincia Bolívar*: Bosque Protector Cashca Totoras, QCAZ 13763, 10816, 13760, 13755, 16816, 16821, 42589.
- Pristimantis vidua*.— Ecuador: *Provincia Zamora Chinchipe*: 15 km E Loja, KU 120085.
- Pristimantis* sp.— Ecuador: *Provincia Cañar*: Reserva Mazar, La Libertad, QCAZ 27556, 27562, 27572.
- Pristimantis ventrimarmoratus*.— Ecuador: *Provincia Morona Santiago*: General Leonidaz Plaza Gutiérrez (Limón), Río Napinaza, QCAZ 24048, 41938; *Provincia Napo*: Cascada de San Rafael, QCAZ 36403.

APPENDIX II. Species, museum numbers and corresponding GenBank accession numbers of the sequences analyzed in this study.

- Pristimantis bambu*: QCAZ 46740-Genbank JF906322, QCAZ 46706-Genbank JF906319, QCAZ 46710-Genbank JF906320, QCAZ 46739-Genbank JF906321. *Pristimantis croceoinguinis*: QCAZ 36866-Genbank JF906307, QCAZ 36867-Genbank JF906309, QCAZ 36868-Genbank JF906308. *Pristimantis orestes*: QCAZ 45646-Genbank JF906324, QCAZ 45464-Genbank JF906323. *Pristimantis simonbolivari*: QCAZ 13760-Genbank JF906317, QCAZ 13755-Genbank JF906316, QCAZ 42589-Genbank JF906318. *Pristimantis* sp.: QCAZ 27556-Genbank JF906313, QCAZ 27562-Genbank JF906314, QCAZ 27572-Genbank JF906315. *Pristimantis ventrimarmoratus*: QCAZ 42048-Genbank JF906312, QCAZ 41938-Genbank JF906311, QCAZ 36403-Genbank JF906310.