



Three new species of leaf litter frogs from the upper Amazon forests: cryptic diversity within *Pristimantis* “*ockendeni*” (Anura: Strabomantidae) in Ecuador

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Abstract

We describe three new species of *Pristimantis* leaf litter frogs from the upper Amazon basin of Ecuador: *Pristimantis achuar*, *Pristimantis altamnis*, and *Pristimantis kichwarum*. Each of these new species was previously considered to be part of a single species, formerly known as *Eleutherodactylus ockendeni* (Boulenger) morph B. However, analysis of DNA sequences together with examination of the morphology of more than 600 specimens from Ecuador reveals that multiple species were confused under the same name. The morphological differences among the three new species are subtle but consistent: presence or absence of a black canthal stripe and/or supratympanic ridge, snout-vent length, and ventral colouration. The three species are generally allopatric and geographically restricted, although at some localities two of the species occur sympatrically.

Resumen

Describimos tres especies nuevas de ranas del sotobosque de la cuenca Amazónica Ecuatoriana: *Pristimantis achuar*, *Pristimantis altamnis* y *Pristimantis kichwarum*. Estas especies fueron consideradas antes como una sola especie bajo el nombre *Eleutherodactylus ockendeni* (Boulenger) morfo B. Sin embargo, el análisis de secuencias de ADN y la examinación de la morfología de más que 600 especímenes Ecuatorianos indican que en realidad existen tres especies. Sus diferencias morfológicas son sutiles pero constantes: presencia o ausencia de una raya cantal negra y/o de un pliegue supratimpánico y su coloración ventral. Las tres especies tienen distribuciones alopátricas y distintas aunque en algunas localidades se puede encontrar dos de las tres especies juntas.

Key words Anura; new species; terrestrial frog; South America; *Pristimantis achuar*; *Pristimantis altamnis*; *Pristimantis kichwarum*

Introduction

The upper Amazon region, particularly of Ecuador, is distinguished for having a great number of species of *Pristimantis* frogs (Lynch 1980; Flores & Vigle 1994; Zimmerman & Simberloff 1996; Coloma 2007). Though the upper Amazon rainforest of Ecuador is considered one of the most intensively studied areas in South America (Heyer 1988; Flores & Vigle 1994), the region is nonetheless vast, often scientifically unexplored, and biologically and topographically complex: unidentified specimens, new species, and range extensions of *Pristimantis* frogs are frequently discovered (recent examples include Cisneros-Heredia 2005, 2006; Elmer 2004; Guayasamín et al. 2006; KRE unpubl. data, G. Vigle unpubl. data).

The name *Pristimantis ockendeni* (previously, *Eleutherodactylus ockendeni*) has been generally applied to a leaf-litter frog found in primary and secondary forests throughout the upper Amazon basin from southern Peru to Colombia (Boulenger 1912; Lynch 1974, 1980; Pearman 1997; Doan & Arizabal Arriaga 2002; Padial et al. 2004; Elmer et al. 2007a,b). In some areas, such as the upper Napo and upper Arajuno river drainages, it is one of the most common *Pristimantis*, though this abundance is not equal across its Ecuadorean range (Pearman 1997; KRE unpubl. data). Recent genetic analyses of *P. "ockendeni"* collected from throughout eastern Ecuador demonstrated minimally three highly distinct genetic lineages, sufficiently divergent to suggest they are different species (Elmer et al. 2007b). We conducted an *a posteriori* examination of more than 600 specimens (from Elmer et al. 2007a,b and additional museum specimens) and found previously unrecognized subtle but highly consistent morphological differences among the three genetic lineages. We here describe them as new species, while also clarifying some taxonomic issues surrounding *P. ockendeni* and species synonymized with it.

Materials and Methods

Specimens were fixed in 10% formalin and stored in 70% alcohol. Diagnoses and descriptions generally follow Lynch (1974) and Duellman & Pramuk (1999). We examined alcohol-preserved specimens from the herpetology collection (as of Feb. 2007) at the Museo de Zoología of the Pontificia Universidad Católica del Ecuador (QCAZ). Colour photographs of a syntype of *Hylodes ockendeni* (British Museum of Natural History, BMNH 1947.2.16.89) (provided by J.M. Padial) and the holotype of *Syrrhophus calcaratus* in the Naturhistoriska Riksmuseet, Stockholm, NRM 1921 (provided by E. Åhlander) were examined.

Measurements in Diagnosis and Description refer to all or a subset of the paratype series including the holotype. Measurements in Table 2 are of paratypes and a subset of examined QCAZ adult specimens. Paratype specimens were selected on the criteria of being from a single locality with relatively large sample sizes, collected by KRE, and many having mtDNA sequence publicly available. Morphological measurements were taken with digital callipers for all or a subset of: snout-vent length (SVL), tibia length, foot length, head width, head length, interorbital distance (IOD), eye to nostril distance (END), eye diameter, tympanum diameter, femur length, forelimb length, and internarial distance. Sex and maturity were determined by the presence of ovarian eggs or convoluted oviducts in females, the presence of vocal slits and/or testes in males, or by the collection of pairs in amplexus. The numbered list of characters in the Diagnosis follows Duellman & Pramuk (1999). We used a Wild Heerbrugg M3B dissecting scope for fine detail observations. Colour patterns in life were taken from KRE's field notes. Details of DNA sequencing can be found in Elmer et al. (2007b). DNA sequences were compiled in MacClade v4 (Maddison and Maddison 2003), redundant DNA sequences were determined in Collapse v1.2 (Posada 2004) and genetic distances were calculated in PAUP* beta v4 (Swofford 2003).

Heinicke et al. (2007) and Hedges et al. (2008) use the genus *Pristimantis* for a large clade of South American species. We feel that this causes regrettable instability to the taxonomy of eleutherodactyline frogs. Following the preference of the first author, we describe these new species under the genus *Pristimantis*. Similarly, the placement of this genus in the family Strabomantidae is followed. However, the second author (DCC) prefers different taxonomic concepts for both taxa that require fewer changes in names; these concepts will be elaborated elsewhere.

Species descriptions

Pristimantis achuar sp. nov.

Figures 1–4, 6.

Holotype. QCAZ 25463 (field number SC11438) an adult female collected at Kapawi Jungle Lodge, Pastaza province, Ecuador (S 02° 32.32', W 76° 51.50', altitude 239 m) on 24 June 2003 by KRE and I.G. Tapia.

Paratypes. (25 specimens: 10 males, 10 females, 5 juveniles) QCAZ 25454, 25456–63, 25490–92, 25523–30, 25532, 25534, 25538, 25545, 25547, and tissues were collected at Kapawi Jungle Lodge (type locality) from 24–27 June 2003 by KRE and I.G. Tapia.

Referred specimen. QCAZ 25273 collected from Auca 14 Rd near Dayuma, Orellana province, Ecuador (S 00°41.85', W 76°43.79', altitude 255 m) on 20 March 2003 by KRE, P. Menéndez-Guerrero, and S. Padilla.

Etymology

This species is named for the Achuar indigenous nation of the upper Amazon basin, who are ardent protectors of their biodiversity and generously granted permission to KRE to sample frogs from their land at Kapawi Lodge. The epithet is a noun in apposition.

Diagnosis

(1) skin of dorsum smooth to finely shagreened with W-shaped occipital-scapular ridges; skin of venter areolate; discoidal folds absent; dorsolateral folds absent; (2) tympanum distinct, tympanic annulus barely or slightly discernible, approximately one-third eye width; (3) snout subacuminate (weakly pointed) in dorsal view, rounded in lateral profile, canthus rostralis from anterior corner of eye to nostril straight in profile, concave in dorsal view, sharply angled in cross-section; (4) upper eyelid bearing one or two low tubercles in some individuals; (5) odontophores weakly raised, oval, oblique; (6) males with moderately sized vocal slits, nuptial pads absent; (7) first finger shorter than second; all fingers bearing pads with circumferential grooves; discs slightly truncate, almost rounded, discs on fingers IV and III largest; (8) fingers fringed with blunt edges; (9) ulnar tubercles absent; (10) heel bearing two small subconical tubercles; inner tarsus tubercles absent; outer edge of tarsus bearing low tubercles in some specimens; (11) inner metatarsal tubercle elliptical, 4–5 times conical outer metatarsal tubercle; plantar supernumerary tubercles on finger I and II; (12) toes weakly fringed, webbing absent; toe V much longer than III; discs approximately equal size and shape as finger discs; (13) dorsum medium to dark brown, W-shaped scapular ridge, outer lines of W black, black dots at base in all; postsacral bar dark brown or black; posterior surface of thighs uniform brown; venter cream; (14) adults small size, males 14.3–16.6 mm, females 19.6–23.5 mm SVL (paratype series).

Description

Head slightly wider than body, approximately as long as wide; head width of females 36–39% SVL (\bar{x} = 37%, N = 8), of males 34–39% SVL (\bar{x} = 37% N = 9); snout subacuminate (weakly pointed) in dorsal view, in lateral profile rounded; upper jaw slightly overhanging lower jaw at anterior tip; snout of moderate length, eye-nostril distance of females 88–107% of eye width (\bar{x} = 99%, N = 8), of males 68–103% (\bar{x} = 87%, N = 9); canthus rostralis from anterior corner of eye to nostril straight in profile, canthus rostralis concave in dorsal view, sharply angled in cross-section; loreal region slightly concave, lips not flared; nostrils slightly protuberant, directed laterally with slight dorsal inclination; interorbital space flat; cranial crests absent, interorbital distance in females 52–62% of head width (\bar{x} = 58%, N = 8), in males 52–60% (\bar{x} = 56%, N = 9); temporal region vertical, sloping slightly laterally towards jaw; supratympanic ridge absent, postrictal tubercle present, in some specimens line of tubercles posterior to tympanum; tympanum visible, tympanic membrane distinct in most individuals, tympanic annulus barely or slightly discernible, in females tympanum width 34–44% of eye width (\bar{x} = 40%, N = 9), in males tympanum width 30–50% eye width (\bar{x} = 37%, N = 9), size and shape

not sexually dimorphic; distance from tympanum to eye less than diameter of tympanum; choanae medium-sized, round, well within borders of jaws; vomerine odontophores weakly raised, oval, and oblique (less raised in females), slightly smaller than and posterior to choanae, separated medially by approximately two times width of odontophore, females more teeth than males (female median 4 left/5 right, male median 1 left/1 right), teeth of males somewhat covered by mucosal tissue; tongue longer than wide, slight posterior notch, attached at front of mouth, posteriorly and laterally not adherent to floor of mouth; in males lateral vocal slits moderate sized, in some male specimens slightly distended subgular vocal sac visible. Nuptial pads absent.

Skin of dorsum smooth to finely shagreened; skin of venter areolate, skin of flanks shagreened; no discoidal fold (that can be discerned from preservation artefact); pair of W-shaped prominent ridges from posterior eyelid to scapular region, two scapular tubercles at posterior end of W; one or two low tubercles on mid-eyelids in some; two low tubercles on dorsum equidistant between posterior end of sacral vertebrae and vent, dark coloured bar in some (postsacral bar); tubercles on elbow absent or low, forearm smooth; palmar tubercle bifid, creased into thirds in some, wider than elongate thenar tubercle; palm bearing elongate tubercles smaller than subarticular tubercles; subarticular tubercles elliptical, supernumerary tubercles on finger I and II, distal subarticular tubercles flattened and rounded; proximal subarticular tubercles subconical; fingers bluntly fringed; all fingers bearing slightly truncate discs on pads, all pads wider than long, pads in decreasing order of size IV=III-II-I (barely wider than digit); decreasing finger length III-IV-II-I.

Heel bearing one or two small subconical tubercles; inner edge of tarsus lack tubercles, outer edge of tarsus low tubercles in some specimens; tarsal fold weak; inner metatarsal tubercle elliptical, 4–5 times longer than smaller and conical outer metatarsal tubercle; subarticular tubercles subconical; toes weakly fringed, lacking webbing; toe IV much longer than V; toe discs wider than long, approximately equal size and shape to finger discs, in order of descending size, IV=V-III-II-I; hindlimbs of moderate length, shank of female 53–60% SVL ($\bar{x} = 57\%$, $N = 8$), of males 58–63% SVL ($\bar{x} = 61\%$, $N = 9$).

Colour in preservation

Central dorsum medium to dark brown, paler on flanks, diagonal brown stripes on flanks, thin white line extending from tip of snout to vent in one specimen; sacral chevron present in some, dark anal triangle present; scapular W-shaped ridge black in most, base of W with two black dots on each side separate or longitudinally joined; background colour of head and scapular region the same colour as dorsum in most specimens, with more grey or darker brown in some specimens, grey interorbital bar in one specimen; prominent dark labial bar at anterior margin of eye; less prominent labial bar at posterior margin of eye in some; eyelids dark grey; dark band at mid-forearm, upper arm lacking markings, elbow with dark spot; brown bands on thigh, shank, and tarsus; inner and posterior surfaces of thighs uniform brown; postsacral dorsal bar dark brown or black; discs of fingers and toes approximately same colour as remainder of digits; venter cream, small melanophores concentrated around jaw and flanks in some specimens; underside of thighs brown, paler line along mid-ventral region of thigh and shank due to absence of melanophores.

One specimen (QCAZ 25273) from Auca 14 Rd, Orellana province (approximately 200 km N of Kapawi Lodge) differs from the paratype series by having a darkened supratympanic ridge. Phylogenetic analysis of mtDNA cytochrome *b* and 16S has confirmed that this specimen is *P. achuar* (Elmer et al. 2007b, see discussion below).

Colour in life

Dorsum pale, medium or dark brown, grey brown in some individuals, lighter coloured tinged either grey (e.g. QCAZ 25454) or reddish laterally (e.g. QCAZ 25547); some individuals with scattered cream flecks on dorsum (e.g. QCAZ 25456), some individuals with dark brown or light brown reticulate pattern or stripes on dorsum, some individuals with thin white snout-vent line (e.g. QCAZ 25526); outer lines of W almost invariably black, sometimes accompanied by a reddish line alongside the black (e.g. QCAZ 25454, 25456, 25524,

25528); yellow-orange patch at base of W in a few (e.g. QCAZ 25457, 25458, 25463, 25532); often lighter or darker patch from snout to top of head between eyes (e.g. QCAZ 25458, 25461, 25463, 25490, 25492, 25529) or from top of head between eyes to W (e.g. QCAZ 25491) some individuals with tan interorbital bar at top of head (e.g. 25526); venter cream, light speckling on throat and venter in some individuals, throat yellow in some males (e.g. QCAZ 25457, 25458, 25460, 25490, 25523, 25524, 25528, 25529, 25530, 25547). Colour of iris not noted.

Description of holotype

Measurements (in mm): 21.4 SVL, 12.4 tibia length, 16.1 foot length, 8.3 head width, 7.9 head length, 4.6 IOD, 2.9 END, 2.8 eye width, 1.2 tympanum. Colouration in preservation: light brown dorsum, outer arms of W thin black, two points at base of W are separate, tan coloured from the top of the head (between orbitals) to nose, lacking stripes and chevrons, dark bands on forearms, dark bar eye from anterior corner of eye to lip; very little speckling on throat. Colouration in life: “light brown dorsum; black W; orange mark at base of W; lighter from inter-orbital to nose; vague or absent stripes on body, some on legs”.

Molecular diversity

Cytochrome *b* DNA sequences examined for five paratype specimens (QCAZ 25457-25459, 25526, 25527) and the holotype are identical (one haplotype). rDNA 16S sequences for four paratypes (QCAZ 25457, 25462, 25492, 25527) are also identical (one haplotype). GenBank accession numbers (cytochrome *b*/16S): QCAZ 25457 EU130668/ EU130618, QCAZ 25458 EU130669/, QCAZ 25459 EU130670/, QCAZ 25462 /EU130619, QCAZ 25492 /EU130620, QCAZ 25526 EU130671/, QCAZ 25527 EU130672/ EU130621.

Natural History

Individuals were found in primary forest and presumably can also be found in secondary forest. By night they were often perched on the leaves of low vegetation from 0.2 to 1.3 m elevation. By day they were found in the leaf litter.

Distribution

Pristimantis achuar is found in the lowlands of the Pastaza and Napo rivers in southern Ecuador (see Fig. 5). The species is probably more widely distributed into northern Peru.

***Pristimantis altamnis* sp. nov.**

Figures 2–4.

Holotype. QCAZ 25311 (field number SC 11271), an adult female collected at Cando community, upper Napo river, parroquia Talag, Napo province, Ecuador (S 01° 05.71', W 77° 55.47', altitude 680 m) on 3 May 2003 by KRE and T. Sugahara.

Paratypes. (12 specimens: 8 males, 2 females, 2 juveniles) QCAZ 25302–304, 25306–310, 25313, 25324–326, and tissue samples were collected in the area of Cando community on 3 May 2003 by KRE and T. Sugahara.

Etymology

Diagnosis

(1) Skin on dorsum smooth to finely shagreened with W-shaped occipital-scapular ridges, that on venter areolate; discoidal folds absent; dorsolateral fold absent; (2) tympanum distinct, tympanic annulus present, its width approximately one-third eye width; supratympanic ridge not or barely pigmented; (3) snout subacuminate (weakly pointed) in dorsal view

Heel bearing one or two small subconical tubercles; inner and outer edge of tarsus lack tubercles; tarsal fold weak; inner metatarsal tubercle raised, oval, 4–5 times longer than smaller and conical outer metatarsal tubercle; subarticular tubercles subconical, most distal subarticular tubercles flatter and smaller; toes weakly fringed, lacking webbing; toe discs wider than long, approximately equal size and shape to finger discs, in order of descending size, IV–V=III–II–I; hindlimbs of moderate length, shank of female 55–58% SVL (\bar{x} = 57%, N = 3), of males 53–63% SVL (\bar{x} = 60%, N = 8).

Colour in preservation

Central dorsum dark to medium brown, paler on flanks, diagonal brown stripes on flanks; sacral chevrons mostly absent, dark anal triangle present; ridge of scapular W-marking black in most, base of W with two black dots on each side separate or longitudinally joined; background colour of head and scapular region brown or grey, similar to that of dorsum; prominent dark labial bar at anterior margin of eye; less prominent labial bar at posterior margin of eye in some; grey interorbital bar in some specimens; upper eyelids grey; dark band at mid-forearm, upper arm lacking markings, elbow with dark spot in some; brown bands on thigh, shank, and tarsus; a few with postsacral dorsal bar black, in most a pair of postsacral dark tubercles; discs of fingers and toes paler than remainder of digits; venter moderately dense distribution of melanophores giving a slightly grey appearance, cream background (under microscope small deposits of guanine pigment visible on ventral aroli warts), melanophores more concentrated around chin and pectoral region, producing a fine reticulum under chin; underside of thighs brown, lighter line along mid-ventral region of thigh and shank due to absence of melanophores.

Colour in life

Dorsum medium to dark brown, lighter laterally, some individuals with stripes; scapular W mark with black points at base in all, black W in most, sometimes also outlined in reddish brown; some individuals with light brown patch from snout or top of head, extending to W; venter cream with mottled speckling, throat with yellow flush in some males.

Some specimens from northern Ecuador (610 masl, locality 1 in Fig. 5) (QCAZ 25819–38) had slighter paler dorsal and ventral colouration than most *P. altamnis*. MtDNA sequence data of cytochrome *b* and 16S confirms their identity to the remainder of the specimens at that locality, and thus part of the *P. altamnis* clade (Elmer et al. 2007b). Colour of iris not noted.

Description of holotype

Measurements (in mm): 27.6 SVL, 15.2 tibia length, 19.4 foot length, 10.8 head width, 9.8 head length, 5.4 IOD, 4.3 END, 3.5 eye width, 1.3 tympanum width. Colour in preservative: medium brown dorsum, lighter stripes laterally, dark bars on thighs and shanks, thin black line of W, most thick around base tips; venter cream, some light speckling under throat. Colour in life: “medium brown dorsum, grey-brown laterally; black W”.

Molecular diversity

MtDNA cytochrome *b* sequences examined for eight paratype specimens (QCAZ 25306–25310, 25324–25326) are identical (one haplotype). MtDNA 16S sequences for three paratypes (QCAZ 25306, 25309, 25313) are also identical (one haplotype). MtDNA GenBank accession numbers (cytochrome *b*/ 16S): QCAZ 25308 EU130652/ , QCAZ 25309 EU130653/ EU130607, QCAZ 25310 EU130654/, QCAZ 25313 / EU130608, QCAZ 25324 EU130647/ EU130605, QCAZ 25325 EU130648/, QCAZ 25326 EU130649/.

Natural history

Individuals were found in primary and secondary forest. By night they are often perched on the leaves of low vegetation. By day they were found in leaf litter.

Distribution

Pristimantis altamnis has been found throughout the humid upland tropical forests of eastern Ecuador, at elevations of approximately 400–1000 masl (see Fig. 5). To the east it is replaced by *P. kichwarum* or *P. achuar*. Its range limits to the north and south are not known. In the upper Napo River area (Jatun Sacha Biological Station), *P. altamnis* has been found sympatrically with *P. kichwarum*.

Pristimantis kichwarum sp. nov.

Figures 2–4, 6.

Holotype. QCAZ 18128 (field number SC7783), an adult male collected at Jatun Sacha Biological Station, parroquia Ahuano, Napo province, Ecuador (S 01°04.45', W 77°36.93', altitude 472 m), on 14 November 2001 by KRE and F. Ayala-Varela. MtDNA cytochrome *b* Genbank accession number EF581033.

Paratypes. (45 specimens: 29 males, 13 females, 3 juveniles) QCAZ 18023, 18027, 18030, 18033, 18039, 18041, 18045, 18053, 18069, 18071, 18077, 18085, 18089, 18092, 18096, 18098, 18110, 18112, 18117, 18126, 18132, 18137, 18139, 18153, 18165, 25287, 25288, 25290, 25291, 25295, 25552, 25554, 25555, 25558, 25561, 25564, 25573, 25575, 25579, 25580, 25751, 25757, 25759, 25773, and 25782 were collected between 4 November 2001 and 14 April 2003 from Jatun Sacha Biological Station and adjacent areas (Librarius Study Center and Inner Vision Lodge [currently known as Arajuno Jungle Lodge]) (Appendix 1).

Referred. Specimens collected adjacent to Jatun Sacha Biological Station (at Inner Vision Lodge) by KRE and T. Sugahara, QCAZ 25752 (male) 13 April 2003 and QCAZ 25783 (male) 17 April 2003.

Etymology

This species is named for the indigenous Kichwa communities in the Oriente of Ecuador. They are recognized in this small collective way as a token of appreciation for the engagement of individuals and communities in this research, and for sharing their biodiversity and knowledge. The patronym is a Latin feminine plural genitive of the nation name.

Diagnosis

(1) Skin of dorsum smooth to finely shagreened with W-shaped occipital-scapular ridges, that of venter areolate; no obvious discoidal folds; dorsolateral fold absent; (2) tympanum and tympanic membrane distinct, tympanic annulus barely or slightly discernible, tympanum width approximately one-third eye width; supratympanic ridge dark; (3) snout subacuminate (weakly pointed) in dorsal view, rounded in lateral profile, canthus rostralis from anterior corner of eye to nostril slightly convex in lateral profile, concave in dorsal view, sharply angled in cross-section; (4) upper eyelid with or without two low tubercles; cranial crests absent; (5) odontophores moderately raised, oval, oblique; (6) males with moderately sized vocal slits, nuptial pads absent; (7) first finger shorter than second; all fingers bearing pads with circumferential grooves; discs slightly truncate, almost rounded, those on IV and III largest; (8) fingers slightly fringed; (9) ulnar tubercles very low or absent; (10) heel bearing small subconical tubercles; tarsal tubercles absent, tarsal fold weak; (11) inner metatarsal tubercle raised, elliptical, lateral margin more convex than medial margin, 4–5 times longer than conical outer metatarsal tubercle; few supernumerary plantar tubercles; (12) toes weakly fringed, lacking webbing; toe V much longer than III; discs approximately of equal size and shape as finger discs; (13) dorsum dark to light brown; W-shaped scapular ridge black in some, black dots at the lower points of the W in all; canthal stripe black; postsacral bar slightly or completely black; posterior surface of thighs brown; venter cream with some dark flecks; (14) adults small, males 17.5–21.9 mm ($N = 30$), females 24.4–27.0 mm ($N = 13$) SVL.

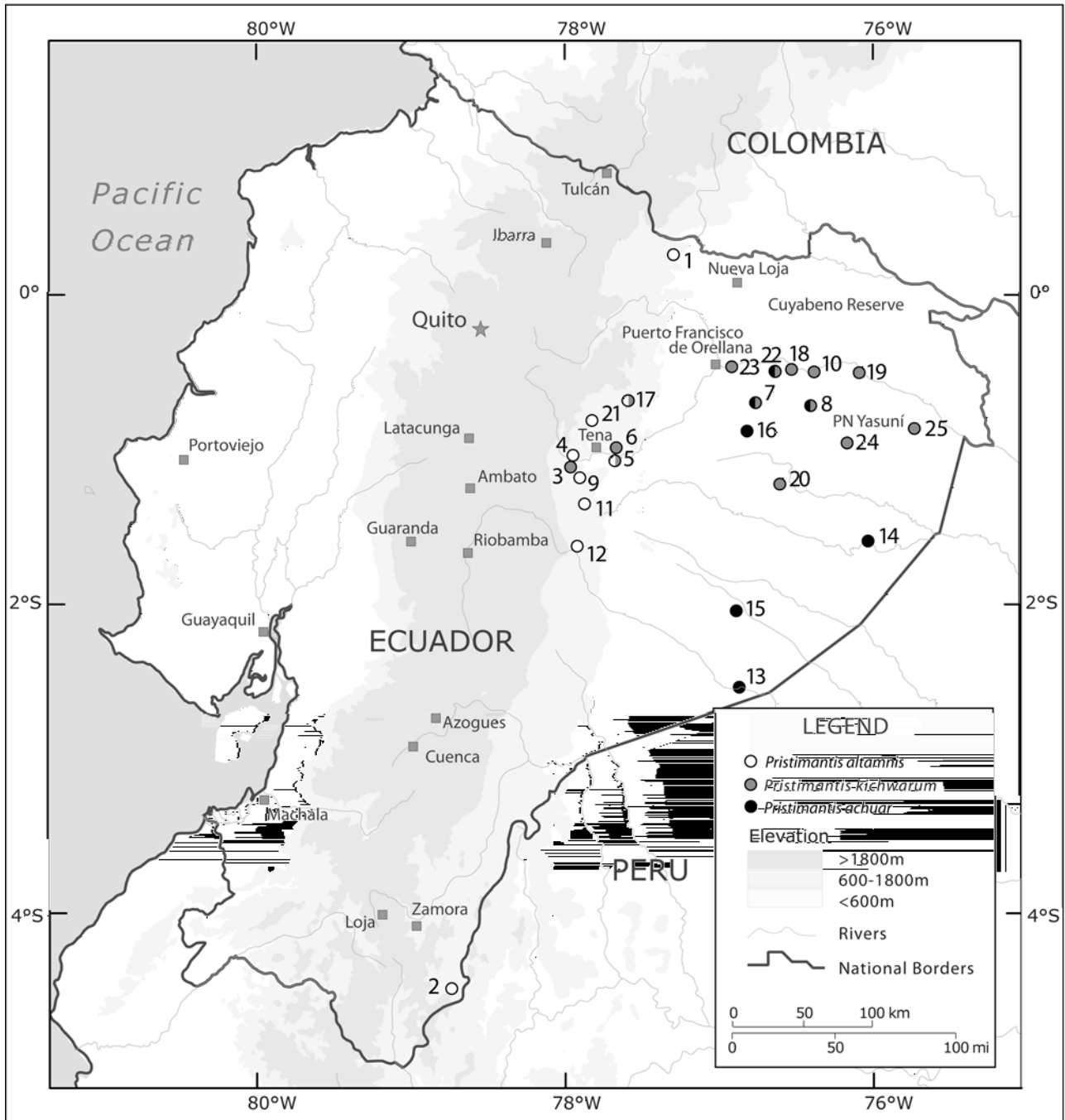


FIGURE 5. Map of localities for reviewed specimens of *P. altamnis* (white dot), *P. kichwarum* (grey dot), and *P. achuar* (black dot). The general area of the Cuyabeno Reserve and Yasuní National Park are noted, as mentioned in the text for some collections. For simplicity, some nearby localities share a dot. (1) Chonta Yacu, Lumbaqui; (2) Centro Shuar Yawi; (3) Serena; (4) Talag or Cando; (5) Jatun Sacha Biological Station and Arajuno Jungle Lodge; (6) Puca Chicta, Misahuallí; (7) Auca 14 Rd near Dayuma; (8) Parque Nacional Yasuní: Estación Científica, Onkonegare, and pozo Capiron; (9) Llanganates Mountains, Canton Arosamena Tola; (10) La Selva Lodge, Pañacocha; (11) Y of road Tena to Puyo, Canton Sta Clara; (12) Fundación Hola Vida, El Porvenir; (13) Kapawi Lodge; (14) Lorocachi; (15) Petrolera Garza 1, Montalvo; (16) Bloque Shiripuno, Parque Nacional Yasuní; (17) Rio Huataracu, via Hollín-Loreto; (18) Indillama; (19) Yuturi; (20) Bataburo Lodge, Rio Tiguño; (21) Santa Rita; (22) Parque Nacional Yasuní, Carretera Pompeya Sur-Iro Km 21, 38 or 41; (23) Comuna El Descanso; (24) Parque Nacional Yasuní Road to MAXUS km 107 and AMO 1; (25) Parque Nacional Yasuní Bloque 31.

Description

Head slightly wider than body, approximately as long as wide; head width of females 36–39% SVL (\bar{x} = 37%, N = 5), of males 33–39% SVL (\bar{x} = 36%, N = 9); snout subacuminate (weakly pointed) in dorsal view, in lateral view rounded; upper jaw overhanging lower jaw at anterior tip; snout of moderate length, eye-nostril distance of females 90–98% of eye width (\bar{x} = 94%, N = 5), of males 78–104% (\bar{x} = 93%, N = 9); canthus rostralis from anterior corner of eye to nostril slightly convex in lateral profile, canthus rostralis concave in dorsal view, sharply angled in cross-section; loreal region slightly concave, lips not flared; nostrils slightly protuberant, directed laterally with slight dorsal inclination; interorbital space flat; cranial crests absent, interorbital distance in females 49–58% of head width (\bar{x} = 53%, N = 5), in males 52–61% (\bar{x} = 57%, N = 9); two low tubercles on mid eyelids in some specimens; temporal region vertical, sloping slightly laterally towards jaw; supratympanic ridge, darkened, not obscuring tympanum, postrictal tubercle present; tympanum visible, tympanic membrane distinct, tympanic annulus barely or slightly discernable, in females tympanum width 27–44% of eye width (\bar{x} = 34%, N = 5), in males tympanum width 29–43% of eye width (\bar{x} = 33%, N = 9), size and shape not sexually dimorphic; distance from eye to tympanum less than diameter of tympanum; choanae medium-sized, round, moderately elliptical, well within borders of jaws; odontophores moderately raised and oblique (less raised in females), slightly larger than and posterior to choanae, separated medially by 2–3 times width of odontophore, females with more teeth than males (median 5 left/5 right in females, median 2 left/2 right in males), teeth of males somewhat covered by mucosal tissue in some specimens; tongue longer than wide, with slight posterior notch, attached at front of mouth, posteriorly and laterally not adherent to floor of mouth; vocal slits lateral, moderate sized; in some male specimens slightly distended subgular vocal sac visible. Nuptial pads absent.

Skin of dorsum smooth to finely shagreened; skin of venter areolate, skin of flanks shagreened; no apparent discoidal folds (that can be distinguished from preservation artefact); pair of W-shaped prominent ridges from posterior eyelid to scapular region, two posterior scapular tubercles located at base of W; postsacral bar with low tubercles; small (generally low) tubercles on elbow, smooth forearm, ulnar tubercles low or absent; palmar tubercle bifid, crease at base of U in most specimens, larger than elongate thenar tubercle; elongate palmar tubercles smaller than subarticular tubercles; subarticular tubercles elliptical, raised and flat in lateral view, supernumerary tubercles on finger I and II; distal subarticular tubercles flattened and rounded; proximal subarticular tubercles subconical; fingers with weak lateral fringes; all fingers bearing discs on pads, all pads wider than long, slightly truncate, equally distinct on fingers IV-II, pads in decreasing order of size IV=III-II-I (barely wider than digit); decreasing finger length III-IV-II-I.

Heel bearing one or two small subconical tubercles; inner and outer edge of tarsus lacking tubercles; tarsal fold weak; inner metatarsal tubercle raised, elliptical and lateral margin more convex than medial margin, 4–5 times longer than smaller conical outer metatarsal tubercle; subarticular tubercles subconical, most distal subarticular tubercles flatter and smaller; toes weakly fringed, lacking webbing; toe discs wider than long, slightly truncate, approximately equal size to finger discs, in order of descending size, IV-V=III-II-I; hindlimbs of moderate length, shank of female 53–58% SVL (\bar{x} = 55%, N = 5), of males 54–60% SVL (\bar{x} = 58%, N = 9).

Colour in preservation

Central dorsum dark to light brown, paler on flanks, diagonal brown stripes on flanks, thin white mid-dorsal line extending from tip of snout to vent in some (e.g. QCAZ 18030, 18110, 18126, 25554, 25558); sacral chevron present in some, dark anal triangle present; scapular W-shaped ridge, outer lines of W black, inner lines of W black in some, and/or with white along ridge (in individuals with thin white snout-to-vent line), base of W with two black dots on each side separate or longitudinally joined; background colour of head and scapular region brown or grey, approximately same colour as dorsum; canthal stripe black; prominent dark labial bar at anterior margin of eye; less prominent labial bar at posterior margin of eye in some; supratym-

panic ridge with thin black line; eyelids grey; dark band at mid-forearm, upper arm lacking markings, elbow with dark spot in some; brown bars on thigh, shank, and tarsus; anterior and posterior surfaces of thighs uniform brown; postsacral dorsal bar partially or fully black; discs of fingers and toes paler than remainder of digits; venter cream (under microscope small deposits of guanine pigment visible on ventral areolar warts) with small dark spotting, more concentrated around jaw and flank; ventral surface of thighs brown, mid-ventral region of thigh and shank with absence of melanophores producing a lighter line.

Colour in life

Dorsum light to medium brown, lighter laterally, occasionally with postero-lateral stripes and/or reticulate pattern, with or without darker mid-dorsal chevron(s); some specimens with reddish tinge lateral to W and around upper arms (e.g. QCAZ 18085, 18165), some specimens with random grey dorsal flecks (e.g. QCAZ 18033, 18039, 18069, 18077, 18085, 18132, 18137, 18165), some specimens with thin white snout-vent line (e.g. QCAZ 18030, 18110, 18126, 25554, 25558); points at base of scapular W black, W-shaped ridge most commonly black (except, e.g. QCAZ 18071, 18092); some specimens with lighter brown patch extending from nose to top of head (between eyes) (e.g. QCAZ 18030, 18077, 18085, 18139) or to W (e.g. QCAZ 18117), or extending from top of head (between eyes) to W (e.g. QCAZ 18069); venter cream, lightly specked especially around throat, throat tinged with yellow in some males (e.g. QCAZ 25552, 25555, 25558, 25564). Iris is bronze with a darker lateral stripe, lighter bronze above and darker below median.

Description of the holotype

Measurements (in mm): SVL 20.2, tibia length 12.3, foot length 15.7, head width 7.6, head length 7.9, IOD 3.9, END 2.9, eye width 2.6, tympanum width 0.9. Colour in preservative: medium brown with reticulate patterning, scapular W marking white with very thin black outline, base points of W black, thin white snout-vent line, finger and toe pads lighter than digits. Right foot of the holotype with unusually small toe discs. Colour in life: “mottled medium dark brown dorsum, black and light (paler) outlines of scapular W, thin white snout-vent line”.

Molecular diversity

MtDNA cytochrome *b* sequences examined for 44 paratype specimens and the holotype resulted in five haplotypes with a maximum uncorrected *p*-distance between them of 0.63%. MtDNA 16S sequences for six paratypes resulted in three haplotypes with a maximum uncorrected *p*-distance of 0.39%. See Appendix 1 for cytochrome *b* and/or 16S GenBank accession numbers.

Natural history

Individuals were found in primary and secondary forest. By night they were found perched on the leaves or stems of low vegetation from 0.2 to 1.5 m elevation. By day they were found in the leaf litter. The following specimens were collected in amplexus: QCAZ 25287 (paratype female) with 25288 (paratype male), 25751 (paratype female) with 25752 (referred male), and 25782 (paratype female) with 25783 (referred male).

Distribution

Pristimantis kichwarum has been found throughout the humid tropical forests of eastern lowlands of Ecuador in the Napo, Orellana, and Sucumbíos provinces, at elevations of approximately 500 masl and below (see Fig. 5). The extent of its distribution to the east and north is not known. The species is apparently replaced by *P. achuar* to the south in the Pastaza basin. In the upper regions of the Napo River (Jatun Sacha Biological Station), *P. kichwarum* has been found sympatrically with *P. altamnis*.

TABLE 1. Morphological characters used to distinguish various upper Amazonian or east Andean *Eleutherodactylus* (+ indicates presence, - indicates absence, ? indicates not available or inconclusive).

	W on scapular region	SVL (mm)	Canthal stripe	Eyelid tubercles	Tympanum	Thigh colour, posterior surface
<i>P. achuar</i>	present as thin ridge, usually black	♀ 19.6-23.5; ♂ 14.3-16.6	-	+	visible	uniform brown
<i>P. altamnis</i>	present as thin ridge, usually black	♀ 18.8-28.3; ♂ 15.1-22.3	-	+	visible	uniform brown
<i>P. kichwarum</i>	present as thin ridge, usually black	♀ 19.3-30.2; ♂ 15.2-22.4	+	+	visible	uniform brown
<i>P. acuminatus</i>	absent	♀ 26-34; ♂ 17-23[9]	+ [3]	-	concealed[3]	pale green
<i>P. carvalhoi</i>	as a marking, present or absent[9]	♀ 17.4-21.9; ♂ 12.8-18.2[2]	-[9]	+ [9 photo]	concealed[1] or barely evident [9]	?
<i>P. croceinguinis</i>	present as low thick ridge	♀ 24.9; ♂ 17.2-21.6[8]	-	+	concealed[6]	grey
<i>P. frater</i>	?	♀ 23.7-25.9; ♂ 15.6-18.8;[1]	?	?	visible[8]	uniform brown[8]
<i>P. incomptus</i>	present as marking [1]	♀ 27.5-45.4; ♂ 21.7-27.9[1]	-[8]	-[1]	visible[1]	brown[1]
<i>P. lanthanites</i>	absent, but with chevron markings and scapular points	♀ 18-23; ♂ 13-17[4]	usually[3]	+	visible	brown with small yellow-tan flecks[3]
<i>P. librarius</i>	no, but some pale chevron markings	♀ 16-19; ♂ 13-17[4]	-	two low tubercles	partially concealed[8]	reddish in life
<i>P. martiae</i>	no, but other markings present[4]	♂ 15.3-18.7[7]	-[4]	low flat warts[4]	concealed[1]	brown
<i>P. paululus</i>	some scapular markings, but not a ridge	♀ 23; ♂ 19[3]	+ [3]	-	visible[3]	greyish-tan[3]
<i>P. pecki</i>	absent; brown interorbital triangle[7]	♀ 24.6-31.3; ♂ 19.6-22.5[8]	-[7]	+ nonpungent[7]	prominent [7]	uniform brown[7]
<i>P. pseudoacuminatus</i>	absent	♀ 21.9-26.8; ♂ 15.7-22.0[6]	-, + in some[3]	-[3]	visible[3]	uniform pale green to grey-green[3]
<i>P. quaquaversus</i>	absent	♀ 24.6-31.3; ♂ 19.6-22.5[4]	-	+	concealed[3]	purplish with white flecks[3]
<i>P. variabilis</i>	absent	♀ 22.0-27.0; ♂ 19[3]	+ [6]	?	visible	brown[6]

..... continued on the next page

TABLE 1 (continued).

	Dorsum colour in life	Venter colour	Dorsal skin	Canthus shape, cross-section	Snout, dor- sal view	Elevation (masl)
<i>P. achuar</i>	medium brown	cream	smooth to finely shagreened	sharp	subacuminate	255-240
<i>P. altamnis</i>	medium to dark brown	cream with brown flecks forming retic- ulate	smooth to finely shagreened	sharp	subacuminate	400-1000
<i>P. kichwarum</i>	light to dark brown	cream, some brown flecks	smooth to finely shagreened	sharp	subacuminate	240-515
<i>P. acuminatus</i>	green	white	smooth	sharp	pointed (acuminate)	300-1000[2]
<i>P. carvalhoi</i>	dull brown[9]	cream with small brown flecks; groin yellow[9]	finely tuber- culate[9]	rounded[9 photo]	moderately long and pointed[9]	lowland[5]
<i>P. croceinguinis</i>	yellow-brown to brown[6], grey	grey to black (usu- ally brown) flecked with cream[6]; orange mark in groin	pustular	moderately sharp	subacuminate	200-400m[5]; also higher altitude[KRE]
<i>P. frater</i>	?	?	?	rounded[8]	subacumi- nate[8]	1000-1600[5]
<i>P. incomptus</i>	tan with darker brown mark- ings[1]	brown, grey, dirty cream with brown flecks[1]	bearing low flat warts[1]	rounded[1]	subacuminate to round[1]	1370-1910[5]
<i>P. lanthanites</i>	olive-brown	cream-white with grey flecks[3]; brown with yellow mottling; throat grey/ brown with strong medial yellow/white stripe	dorso-lateral dermal folds	moderately sharp	long and sub- acuminate	200-1630 [5]
<i>P. librarius</i>	golden tan	stippled with brown	smooth	moderately sharp	almost pointed (acuminate)	220-560[5]
<i>P. martiae</i>	brown	pale brown with brown suffusion[4]	finely shagreened with low warts[4]	moderately sharp	subacuminate to rounded with pointed tip[4]	100-1300[5]
<i>P. paululus</i>	dull green[3]	green with white spots[4]	smooth[4]	slightly rounded	rounded[3]	300-600[5]
<i>P. pecki</i>	brown, darker laterally[7]	cream[7]	smooth[7]	round[7]	subacumi- nate[7]	1550-1830[7]
<i>P. pseudoacuminatus</i>	green[3]	white[3]	smooth[3]	gently rounded[3]	narrow and rounded[3]	330-570[2]
<i>P. quaquaversus</i>	tan, reddish brown	bronze-white[3]	smooth	slightly rounded	subacuminate	200-1900[5]
<i>P. variabilis</i>	grey, brown, or dark brown[6]	cream to white with black flecks, black- edged yellow-white area in groin[6]	shagreened[6]	well-defined[6]	acuminate[6]	100-600[5]

[1] Lynch and Duellman 1980; [2] Lynch 1980; [3] Duellman 1978; [4] Lynch 1974; [5] IUCN et al.; [6] Lynch 1968; [7] Duellman & Lynch 1988; [8] Flores & Vigle 1994; [9] Rodriguez & Duellman 1994; [no citation] KRE/DCC review of QCAZ specimens.

Discussion

Distinguishing Species:

Morphology

The three new species could be most easily confused with the sympatric congeners *Pristimantis martiae*, *P. croceoinguinis*, *P. librarius*, or *P. variabilis* (see Table 1 for notes on the characters of these and other upper Amazonian species). *Pristimantis achuar*, *P. altamnis*, and *P. kichwarum* can be distinguished from these other species most reliably by the presence of a thin W-shaped epidermal ridge (not just black markings) along the scapular region, usually fully or mostly black: no other species in the area has this ridge. *Pristimantis martiae* has a shorter snout than *P. achuar*, *P. altamnis*, or *P. kichwarum* and a pale brown venter. *Pristimantis croceoinguinis* has a softer canthus, eyes placed more closely together (i.e., smaller IOD), a taller head in profile, grey dorsal and ventral colouration, and a distinctive orange spot in the groin. *Pristimantis librarius* prefers secondary forest and disturbed habitats such as grazing areas and new successional growth, has a light brown ventral colouration, and an orangish or reddish colouration on the anterior, posterior, and ventral surfaces of the thighs in life. The three new species described here are easily distinguished from *P. variabilis* by its long snout, large pale brown melanophores on the venter, a more rounded canthus, more robust digits, and the presence of a yellowish (in life, white in preservation) patch in the groin.

A more difficult task is to distinguish *P. achuar*, *P. altamnis*, and *P. kichwarum* from each other. Other authors have stated that *P. ockendeni* lacks a canthal stripe (e.g., Duellman 1978; Lynch 1980; Flores & Vigle 1994; Duellman & Pramuk 1999). In reviewing Peruvian and Ecuadorean *E. ockendeni*, Lynch (1974) does not mention either presence or absence of a canthal stripe. We have found the canthal stripe to be an important distinguishing character that corresponds to the mitochondrial lineages (Figure 7). *Pristimantis kichwarum*, which has been found so far only at elevations below 510 m, has a black canthal stripe, a dark supratympanic ridge, and a cream venter with melanophores producing a pattern of brown flecking. *Pristimantis altamnis* is generally found in the uplands (400 to 1000 masl) but is also sympatric with *P. kichwarum* at a few localities (e.g., Jatun Sacha Biological Station). *Pristimantis altamnis* can be distinguished from *P. kichwarum* by lacking a black canthal stripe and having a darker ventral colouration (due to a heavier pattern of flecks). In the specimens we reviewed, no *P. altamnis* specimen had a thin white snout-vent line as was found occasionally in *P. kichwarum* and *P. achuar*. An additional subtle but consistent difference between the new species is the shape of the head in profile: in *P. kichwarum* the area of the snout from eye to nostril is slightly convex in profile and the snout somewhat rounded, while in *P. altamnis* and *P. achuar* the profile from nose to eye is straight and the snout blunter. *Pristimantis kichwarum* and *P. altamnis* are the same body size (Table 2). *Pristimantis achuar* lacks a canthal stripe and supratympanic ridge and can be distinguished from *P. altamnis* by a much paler venter (near absence of melanophores) and a smaller body size (Table 2). Additionally, *P. achuar* has a lowland geographic distribution (to date less than 350 masl). Figures 2–4 and Table 1 illustrate the morphological differences between the three new species.

In addition to the specimens ascribed to new species (see Specimens examined), we reviewed specimens from Reserva de Producción Faunística Cuyabeno in the north of Ecuador. We identified two morphs in that reserve: one lacks a canthal stripe and has a darker venter (like *P. altamnis*) (QCAZ 27826, 27831, 27833, 27938, 27985, 28019, 28021, 28026, 28030, 28032, 28048, 28049, 28058, 28140, 28183, 28195); the other morph has a black canthal stripe and a paler venter (like *P. kichwarum*) (QCAZ 28041, 27873, 28087, 25576, 25577). MtDNA sequences have shown QCAZ 25576 and 25577 to be sister taxa to *P. kichwarum* (approximately 6% *p*-distance at cytochrome *b*) and resolve into the same clade as *P. kichwarum* relative to *P. altamnis* and *P. achuar* (Figure 7 and Elmer et al. 2007b). The specimens of these two morphs were collected from three different localities in the reserve, separated by 10–80 km. In general, the specimens lacking a canthal stripe (like *P. altamnis*) are from the western portion of the reserve while those from the more eastern portion have a canthal stripe (like *P. kichwarum*). We have declined to assign either of these morphs to any of the three

new species because sample sizes are too small and there are many juveniles in the sample. However, it is most probable, based on morphology and genetics, that the specimens with a black canthal stripe are *P. kichwarum*.

TABLE 2. Mean and standard deviation (in mm) of *P. achuar*, *P. altamnis*, and *P. kichwarum* morphological measurements by sex.

	<i>P. achuar</i>			<i>P. altamnis</i>			<i>P. kichwarum</i>		
	Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev	N
Females									
Males–									
SVL	21.8	1.3	8	25.5	3.1	15	25.3	2.9	32
Femur Length	11.0	0.7	8	12.6	1.5	15	12.2	1.3	28
SVL		0.05		0.58			0.65		
Forelimb Length	10.3	0.2	8	12.4	1.5	15	11.7	1.3	26
Femur Length		0.880		0.96		83			8
Head Width	8.0	0.4	8	9.7	1.3	15	9.3	1.1	28
Forelimb Length	16.1	0.7	8	18.9	2.1	15	18.1	1.9	24
Foot Length	16.1	0.7	8	18.9	2.1	15	18.1	1.9	24
Head Width	0.90	0.		0.63	0.8		0.53		
D									
0 De-878(ng)- h	124-73011.2(1)3937596(1)1.24.			(.)932					
Nos297.1tsrl-48501.9(2)-0.4(80)-3330.8(0.)20.6(2)-2920.2(8)-4291.330.						063	1549		

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Molecular differentiation

The observed mtDNA sequence divergence between *P. achuar*, *P. altamnis*, and *P. kichwarum* is high. Cytochrome *b* uncorrected *p*-distance between the new species' paratypes is 17.1% between *P. achuar* and *P. altamnis*, 14.9–16.1% between *P. kichwarum* and *P. achuar*, and 11.8–12.7% between *P. kichwarum* and *P. altamnis*. 16S difference is approximately half that of cytochrome *b*: between *P. achuar* and *P. altamnis* is 8.9%; between *P. achuar* and *P. kichwarum* 8.5–8.9%; between *P. altamnis* and *P. kichwarum* 5.3–5.6%.

With larger geographic sampling than the present study, DNA sequence data compiled from Elmer et al. (2007b) show that maximum intraspecific uncorrected *p*-distance for cytochrome *b* is low: 4.6% within *P. achuar* (their “Southeastern Clade”), 4.9% within *P. altamnis* (“Upland Clade”), and 6.4% within *P. kichwarum* (“Lowland Clade”). However, cytochrome *b* *p*-distances between clades are high: 16.4–17.8% between *P. achuar* and *P. altamnis*, 14.9–16.1% between *P. achuar* and *P. kichwarum*, and 12.0–13.3% between *P. altamnis* and *P. kichwarum*. At 16S, maximum distance within species is 1.9% within *P. achuar*, 1.7% within *P. altamnis*, and 3.4% within *P. kichwarum*. *P*-distances between species range from 8.7–9.4% between *P. achuar* and *P. altamnis*, 8.5–9.2% between *P. achuar* and *P. kichwarum*, and 4.5–5.8% between *P. altamnis* and *P. kichwarum*.

The sister taxon relationship of *P. altamnis* to *P. kichwarum* relative to *P. achuar* is also reflected in a phylogeny of the three species that includes samples from across Ecuador (Figure 7, which is a simplified version of Elmer et al. 2007b Figure 2). We designate *P. kichwarum* as two subclades: the more diverse Napo clade that includes the type locality, and the Cuyabeno clade that is found in the north of Ecuador, which is drawn separately to reflect its higher intraspecific divergence relative to the rest of the clade (discussed above).

There has been extensive discussion about what level of genetic diversity may represent new candidate species of amphibians (see Moritz and Cicero 2004, Vences et al. 2005, and references therein). In these three new species, there is no overlap between intra- and inter-specific *p*-distances: a maximum threshold of 7% divergence at cytochrome *b* and 4% divergence at 16S is sufficient to demarcate intraspecific diversity, while interspecific diversity exceeds 11% for cytochrome *b* and 4% for 16S. Therefore, the mtDNA genetic diversity within and between these neotropical *Pristimantis* are comparable to levels as also found in some other terrestrial tropical frogs at similar geographic scales (see Vences et al. 2004, Vences et al. 2005, Fouquet et al. 2007).

Taxonomy

The new species described here—*P. achuar*, *P. altamnis*, and *P. kichwarum*—are not *ockendeni*, though they have been known by this specific epithet in Ecuador since 1974. Genetic data evidenced here and in Elmer et al. (2007b) clearly distinguishes these three species, and these data enabled detection of subtle but consistent morphological differences among the species.

The three species are also quite different from type specimens of possible nominal species to which they might have been assigned. Having reviewed the original description of *Hylodes ockendeni* (Boulenger 1912 p. 667) and photographs of a syntype (BM 1947.2.16.89, adult female, SVL = 31.42), we are confident that the three species we describe here are not *P. ockendeni*. First, one of the most distinguishing features of *P. altamnis*, *P. kichwarum*, and *P. achuar* relative to other frogs in the area is the presence of an occipital W-shaped ridge. However, this key feature is not mentioned in the description of *Hylodes ockendeni* Boulenger 1912 (based on three specimens) and is absent in the syntype we reviewed. Second, the size of the holotype and syntype specimens (34 and 31 mm SVL, respectively), is larger than the species we describe here. Third, photographs demonstrate notable morphological differences between *P. ockendeni* and the species described here, for example: the snout is shorter, the canthus rostralis less sharp in cross-section, the finger discs larger, and the venter more pale in *P. ockendeni*. Fourth, and perhaps most obviously, Boulenger’s specimens come from La Union, Carabaya, a locality in extreme southeastern Peru approximately 1500 km from our Ecuadorean localities. Given the limited geographical ranges of most Andean and Amazonian amphibians (Lynch & Duellman 1997; Loughheed et al. 1999; Wynn & Heyer 2001; Graham et al. 2004), it is very unlikely that any of the new species would be the same species as one from southern Peru.

Given that we have described three new species, it is important to rule out the possibility that these might be referable to species synonymized under *P. ockendeni*. Two nominal taxa are relevant: *Syrrhophus calcaratus* Andersson 1945, and *Hylodes hylaeformis* Melin 1941.

Syrrhophus calcaratus was described from the Rio Cosanga, 800 m altitude in Napo province, Ecuador (Andersson 1945). Without examining specimens, Lynch (1968) transferred it to the genus *Eleutherodactylus* as part of a set of nomenclatural actions designed to revise the genus *Syrrhophus*. Because the name *calcaratus* was occupied by *Eleutherodactylus calcaratus* Boulenger 1908, Lynch (1968) provided the replacement name *Eleutherodactylus anderssoni*. Later, Lynch (1974) synonymized *E. anderssoni* with *E. ockendeni* based on similar morphology between the two species.

After reviewing holotype photographs (NRM 1921), the original species description (Andersson 1945 p. 27–28), and the description in Lynch (1974), we are confident that none of the three species described here is *E. anderssoni*. First, though there is a W-shaped occipital marking in the *E. anderssoni* holotype, it is clearly not an elevated ridge nor is the notable character of a W-shaped ridge mentioned in the species description (Andersson 1945). In fact, the first mention of the occipital W-shaped ridge is by Lynch (1974), who associated this mark with specimens described as morph B of *ockendeni*, while morph A (like the types of *Hylodes ockendeni* and *Syrrhophus calcaratus*) lacks the ridge. Second, the implication of Andersson's placement of the new species he described into the genus *Syrrhophus* was that it lacked vomerine teeth, a feature then considered to define *Syrrhophus*. Lynch (1974) noted that the vomerine odontophores were concealed in the holotype of *S. calcaratus*. The species we describe here have obviously exposed vomerine odontophores, as well as vomerine teeth. Third, there are notable morphological differences between the holotype of *S. calcaratus* and the new species (Figure 8); for example, the tip of the snout is more pointed in *S. calcaratus*, the snout is shorter, and the canthus rostralis less defined than the species we describe here; the limbs are shorter and plumper, and the head is narrower in *S. calcaratus*; ventral colouration is paler than *P. kichwarum* or *P. altamnis*.

In 1941 Melin described *Hylodes hylaeformis* from Roque in the Department of San Martín in northern Peru. Bokermann (1958) proposed *E. melini* as a replacement name because *E. hylaeformis* was preoccupied. Lynch (1980) synonymized *Hylodes hylaeformis* (and by implication *E. melini*) under *E. ockendeni*. However, he did not examine any material of the species, and simply noted that "in the absence of differences, *E. melini* (and *H. hylaeformis*) is added to the synonymy of *E. ockendeni*" (page 12).

Lynch (1980) examined more than 350 specimens of what he called *E. ockendeni* from Colombia, southern Ecuador, and northern Peru (including San Martín), many part of the Harvey Bassler collections at the American Museum of Natural History. Clearly his concept of *E. ockendeni* was a broader one than that supported by the evidence presented in this paper. Like Lynch, we also did not examine any material of *E. melini*. Given the geographic pattern of genetic divergence observed among the three species described herein, and the geographic distance between our new species and the type locality of *E. melini* in the Department of San Martín, it is unlikely that any of the species described here are referable to *E. melini*. Although we have no direct evidence, it is also unlikely that *E. melini* is conspecific with the type material of *E. ockendeni*.

Lynch (1980) noted that samples of *E. ockendeni* collected from southern Amazonian Ecuador are notably smaller than those from northern Ecuador. We found that although *P. altamnis* and *P. kichwarum* are almost identical in size, *P. achuar* are significantly smaller than *P. altamnis* or *P. kichwarum* (t-test, $P < 0.001$) (Table 3). Based on geographic location (Cuisime in Morona-Santiago province is approximately 100 km WSW from Kapawi [Figure 5 locality 13]) and their snout-vent length (23.4 mm females, 17.3 mm males), we expect that Malkin's *ockendeni* from Cuisime (Lynch 1980) are actually *P. achuar* (though it is unclear from the text whether the Malkin specimens are morph A, morph B, or a mixture).

In recent years herpetologists in Ecuador have considered the species *P. ockendeni* to be typified as only morph B, i.e., having a W-shaped black occipital ridge. For example, the entire collection of *P. ockendeni* (excluding some misidentifications) in Ecuador's largest museum collection, QCAZ, have the W-shaped ridge; morph A was thought not to exist in Ecuador (pers. comm. L.A. Coloma). When this change in concept of "*ockendeni*" in Ecuador—from morph A to exclusively morph B—occurred is unclear but to our eye the photograph of *E. ockendeni* in Duellman (1978) is not the same frog that has come to be known as *P. ocken-*

deni, so this shift presumably happened in the last 20 years. *Pristimantis achuar*, *P. altamnis*, and *P. kichwarum* surely taxonomically replace “*P. ockendeni* morph B” in Ecuador and their description clarifies some taxonomic uncertainty about leaflietter *Pristimantis* in Ecuador and the dissonance about *P. ockendeni* morphs. We have not examined all available material of *P. "ockendeni"* so the status of some records are unclear and confusion remains about the species-level status and definition of *P. "ockendeni"* morph A in Ecuador, Peru, and elsewhere.

TABLE 3. Snout-vent lengths of female and male “*E. ockendeni*” as previously published (Lynch 1980) and compared to current measurements for *P. altamnis*, *P. kichwarum* and *P. achuar* across Ecuador.

	females	males
“ <i>E. ockendeni</i> ” from northern Ecuador ^a	27.2 ± 0.5 (n = 37)	19.4 ± 0.5 (n = 18)
<i>P. altamnis</i> ^b	25.5 ± 3.1 (n = 15)	18.5 ± 1.5 (n = 60)
<i>P. kichwarum</i> ^b	25.3 ± 2.9 (n = 32)	18.8 ± 1.6 (n = 74)
“ <i>E. ockendeni</i> ” from southern Ecuador (Malkin) ^a	23.4 ± 0.3 (n = 18)	17.3 ± 1.0 (n = 4)
<i>P. achuar</i> ^b	21.8 ± 1.4 (n = 8)	15.5 ± 0.7 (n = 10)

^adata from Lynch (1980) Table 1; ^bdata from this study

We can summarize our taxonomic decisions as follows: *Pristimantis ockendeni* is not conspecific with any of the new species, nor is it likely to be found in Ecuador, given its type locality. “*Eleutherodactylus anderssoni*” is a species distinct from any of the species described herein; it is also distinct from *P. ockendeni*. It is poorly characterized, however, and its distribution unknown except for the type locality. Although we have no direct evidence, by extrapolation from what we know about the new species described herein, it is likely that *E. melini* from northern Peru is also distinct from *P. ockendeni*, as well as from the Ecuadorean species we describe here.

Given Lynch's (1974) description of two morphs and the common difficulty of distinguishing *Pristimantis* species, we encourage researchers to (1) provide more details about the morphological characters (canthal stripe, etc.) used to assign specimens to species that are similar to *P. "ockendeni"*, (2) photograph a variety of morphs in life, and (3) collect vouchered tissue samples for deposition in institutional collections. Clearly, in the case of *P. ockendeni* in Ecuador, a “species” that was presumed to be well-known, a sample of toe clips without voucher specimens would have been worthless.

Pristimantis in Ecuador are speciose and often difficult to distinguish and therefore are not as well-studied as more colourful or sonorous frogs. However, of the *Pristimantis*, the species referred to *P. ockendeni* are relatively common in many Ecuadorean forests and has been studied previously (e.g., articles mentioned above, as well as: Lynch & Duellman 1980; Izquierdo et al. 2000; Cisneros-Heredia 2003; Coloma 2005; Cisneros-Heredia 2006; Elmer et al. 2006). Given our revised taxonomy, the single locality studies of “*E. ockendeni*” in Elmer et al. (2006) and (2007a) are *P. kichwarum*. The Upland, Lowland, and Southeastern “*E. ockendeni*” clades of Elmer et al. (2007b) are *P. altamnis*, *P. kichwarum*, and *P. achuar* respectively. The species identified as “*E. ockendeni*” in studies from elsewhere in the upper Amazon are unlikely the same species as the three described here (e.g., Heyer & Berven 1973; Toft & Duellman 1979; Toft 1980; Aichinger 1987; Duellman & Salas 1991; Rodríguez & Duellman 1994; Duellman & Mendelson 1995; Duellman & Thomas 1996; Zimmerman & Simberloff 1996; Duellman & Pramuk 1999; Acosta-Galvis 2000; Doan & Arizábal Arriaga 2002; Padial et al. 2004; Heinicke et al. 2007; Lehr et al. 2007). Duellman (2005) reported one specimen of *P. ockendeni* from Cusco Amazónico. His description is very general and matches all of the leaflietter *Pristimantis* described herein from Ecuador. The photo included (plate 83) is of a specimen from northern Peru, Loreto. Geographically, this frog would most likely be *P. achuar*.

Conclusion

The morphological conservatism among these three new species—*P. achuar*, *P. altamnis*, and *P. kichwarum*—is striking. In fact, without the genetic analyses to demonstrate high divergence among clades it is unlikely they would have been recognized as distinct species rather than geographic variants or polymorphic populations. However, with detailed *a posteriori* revision of specimens we were able to identify minor but reliable morphological differences by which the three new species can be discerned based on morphology alone, even in absence of geographical or genetic information. Other recent genetic studies have demonstrated that many neotropical amphibian taxa are composed of highly divergent lineages and cryptic species (e.g., Chek et al. 2001; Crawford 2003; Camargo et al. 2006; Ron et al. 2006; Crawford et al. 2007; Fouquet et al. 2007). Thus, the diversity of amphibians in the upper Amazon of Ecuador is certainly much greater than current estimates and our incomplete catalogues.

Specimens examined

Pristimantis achuar [10 specimens]

ECUADOR: *Orellana*: Bloque Shiripuno, QCAZ 9555, 9572; Yasuní carretera Pomeya Sur-Iro km 38, QCAZ 8101, 8112; Yasuní pozo Capiron 2, QCAZ 17331. *Pastaza*: Kapawi Lodge, QCAZ 11189; Lorocachi, QCAZ 10006; Loc. Petrolera Garza 1, NE of Montalvo, QCAZ 1213, 1217, 1221.

Pristimantis altamnis [146 specimens]

ECUADOR: *Napo*: Jatun Sacha Biological Station, QCAZ 2887, 18116, 18127, 18130, 18147, 18148, 18150-152, 18154, 18167, 25439, 25440-442, 25445-447, 25553, 25556, 25557, 25559, 25560, 25562, 25563, 25574, 25578; Llanganates Mtns, Canton Arosamena Tola, QCAZ 25327, 25328; Talag, QCAZ 4516; Sta Rita, QCAZ 7024. *Pastaza*: Y of road Tena to Puyo, Canton Sta Clara, QCAZ 25334, 25337, 25338, 25342-345, 25348-353, 25356-359, 25361, 25362, 25364, 25366, 25372, 25374, 25377, 25378, 25381, 25383; Fundación Hola Vida, El Porvenir, QCAZ 25164, 25165, 25601, 25654, 25655, 25658-664, 25692-700, 25742. *Orellana*: Rio Huataraco, via Hollín-Loreto, QCAZ 7084. *Sucumbíos*: community Chonta Yacu, via Lumbaquí-Tulcan, QCAZ 25786, 25796, 25804-25813, 25819-25838. *Zamora-Chinchi*: Centro Shuar Yawi, QCAZ 31031.

Pristimantis kichwarum [388 specimens]

ECUADOR: *Napo*: Jatun Sacha Biological Station and adjacent Arajuno Jungle Lodge, QCAZ 2888, 9182-9214, 18021, 18022, 18024-026, 18028, 18029, 18031, 18032, 18034-18038, 18040, 18042-044, 18046-052, 18054-068, 18070, 18072-076, 18078-084, 18086-088, 18090, 18091, 18093-095, 18097, 18099-109, 18111, 18113-115, 18118-125, 18129, 18131, 18133-136, 18138, 18140-146, 18149, 18155, 18164, 18166, 25292, 25293, 25299, 25440-442, 25446, 25553, 25555-557, 25559, 25560, 25562, 25563, 25574, 25578, 25754-756, 25760-765, 25767, 25770, 25772, 25775, 25776, 25778-781; Puca Chicta, east of Misahuallí, QCAZ 18156, 18157; Serena, south side Rio Napo, QCAZ 25839-847, 25854-860, 25863, 26864, 25866-25870, 25873. *Orellana*: Parque Nacional Yasuní, Onkonogare, QCAZ 5103, 5208, 6916, 6955, 6958; Estación Científica Yasuní, QCAZ 7928-931, 7933, 7934, 7964, 8449, 8450, 11659, 13100, 16638, 16640, 16641, 16647, 16655, 16761, 19208, 19221, 19330, 19332, 20846, 20847, 20849-853, 23843, 24305, 25154; Parque Nacional Yasuní, AMO 1, km 96-98, QCAZ 5330, 5349, 5359, 5369, 5382, 5431, 6556; Parque Nacional Yasuní, road to MAXUS km 107, QCAZ 6915, 6919-921, 6923, 6924, 6927-929, 6942-954, 6956, 6959-963, 6966; Parque Nacional Yasuní, Pomeya Sur-Iro Road km 38-41, QCAZ 8086, 8091, 8099, 8100, 8102, 8105, 8114, 8119, 8128, 9336, 9350, 17332, 17334, 17349, 17355, 17356, 17359, 17363, 17368, 17370, 20139; Parque Nacional Yasuní, pozo Capiron 2, QCAZ 9347, 17333; Parque Nacional Yasuní, Bloque Shiripuno, QCAZ 9550, 9552; Parque Nacional Yasuní, Bloque 31, QCAZ 11872-878; Parque Nacional Yasuní (general), QCAZ 5078, 5090, 5092, 5093, 5095, 5104, 5137, 5142, 5152, 5156, 5163, 5164, 5203, 5205, 5328,

5329, 5342, 5343, 5346, 5456, 5457, 5465, 5466, 5468, 5469, 6694-696, 6913, 8174, 8938, 9006, 9338, 9349, 9689, 10150, 10576-578, 10580, 11867, 12073, 12074, 12076, 12077, 13302, 14890-899, 15083, 17366, 20484, 22265, 22460, 24292; Rio Huataraco, via Hollín-Loreto, QCAZ 7085, 7087-090; Yuturi, QCAZ, 10417; Auca 14 Road south of Dayuma, QCAZ 25277, 25599; Comuna El Descanso, QCAZ 12547; Indil-lama, 15 km east Pompeya, QCAZ 4652-655, 5016, 11440. *Pastaza*: Bataburo Lodge, Rio Tiguño, QCAZ 20037, 20067. *Sucumbíos*: La Selva Lodge, Pañacocha, QCAZ 4375, 8514-516, 25429.

Pristimantis sp. (“*Pristimantis ockendeni*” not re-assigned) [26 specimens]

ECUADOR: *Sucumbíos*: Zábalo, QCAZ 27826, 27831, 27833, 27938, 27985, 28019, 28021, 28026, 28030, 28032, 28048, 28049, 28058, 28140; Puerto Bolivar, QCAZ 28183, 28195; Rey de los Andes (Rio Cuyabeno at road), QCAZ 27873, 28087; PUCE station, Lag. Cuyabeno, QCAZ 25576, 25577. *Orellana*: Parque Nacional Yasuní, QCAZ 8101, 8112, 17331, 17343, 17575, 19208.

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1



6



P. kichwarum



P. achuar



P. altamnis

4

FIGURE 1. Photograph of *P. achuar* holotype (QCAZ 25463) in life.

FIGURE 4. Lateral view of specimens of *P. kichwarum* (QCAZ 18033), *P. achuar* (QCAZ 25463), and *P. altamnis* (QCAZ 25311). Note the presence of a canthal stripe and darkened supratympanic ridge in *P. kichwarum* and their absence in *P. achuar* and *P. altamnis*.

FIGURE 6. *Eleutherodactylus kichwarum* holotype (QCAZ 18128) in life.

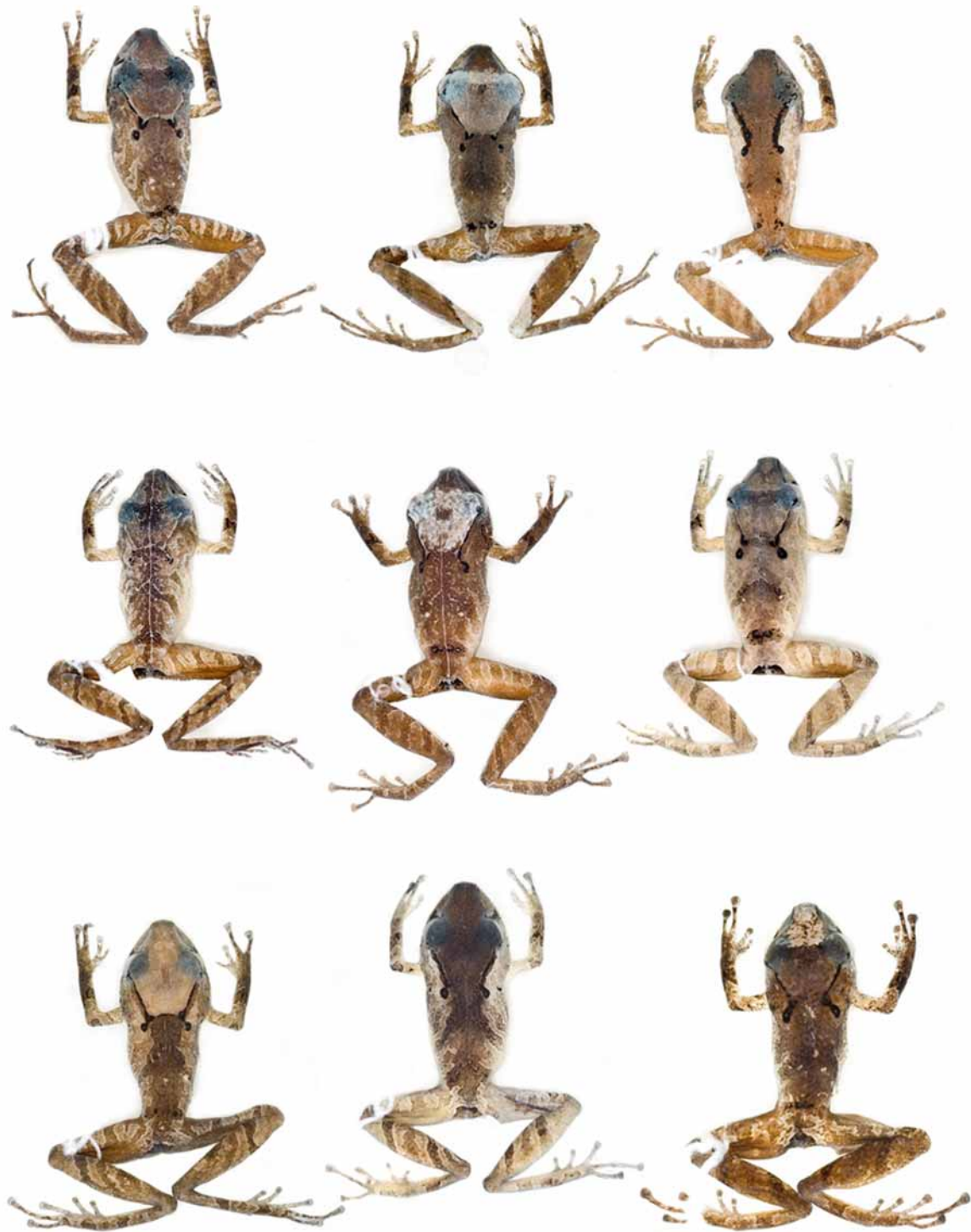


FIGURE 2. Dorsal view of three paratypes of *P. altamnis* (top row; QCAZ 25313, 25302, 25304), *P. kichwarum* (middle row; QCAZ 25287, 27762, 25782), and *P. achuar* (bottom row; QCAZ 25538, 25490, 25491). Note the presence of a canthal stripe in *P. kichwarum* and its absence in *P. altamnis* and *P. achuar*.

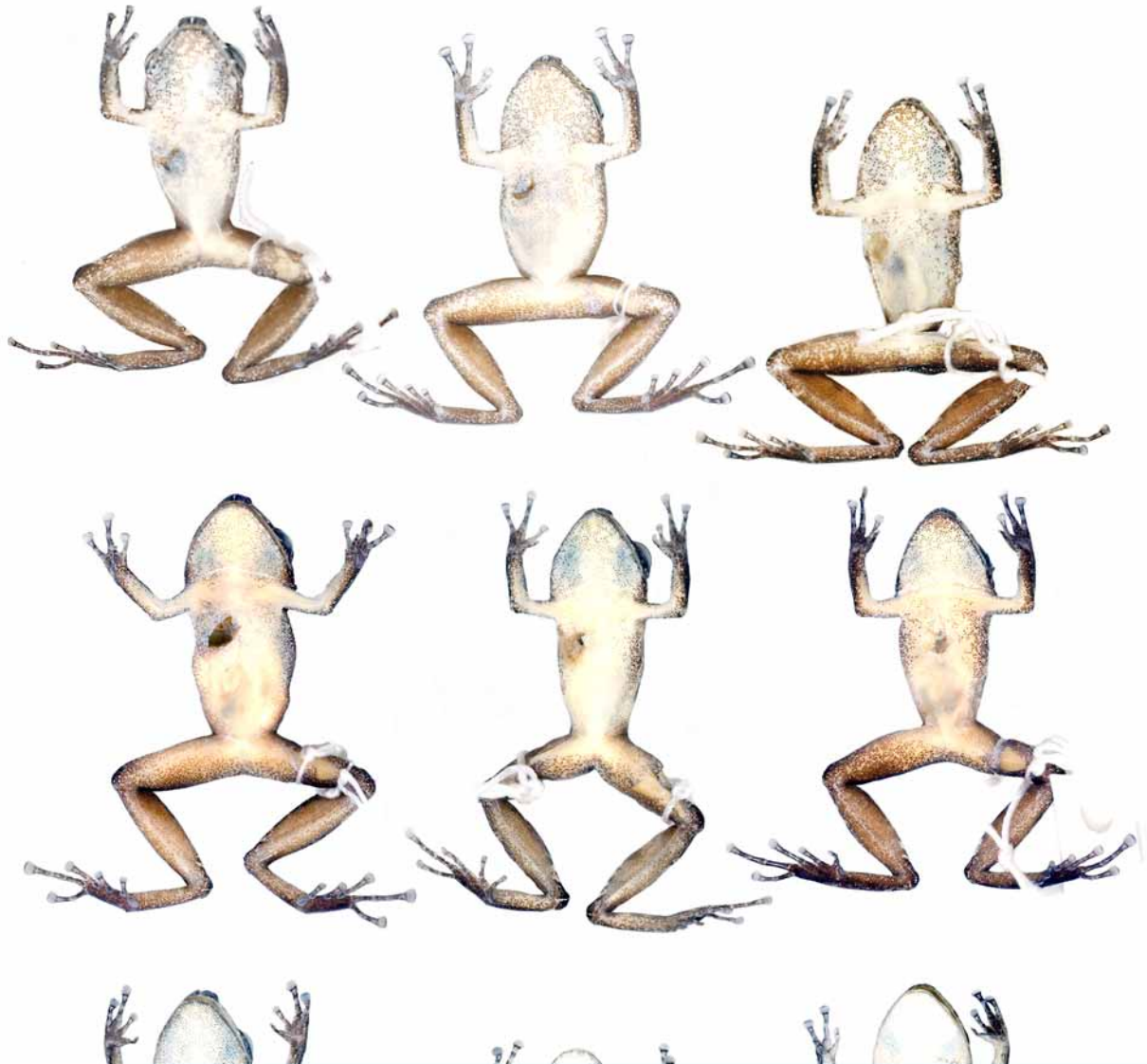


FIGURE 3. Ventral view of three paratypes of *P. altamnis* (top row; QCAZ 25445, 25442, 25447), *P. kichwarum* (middle row; QCAZ 25287, 25558, 25759), and *P. achuar* (bottom row; QCAZ 25461, 25530, 25534). Note the difference in ventral colouration between the three species, with *P. altamnis* having the most dense flecking of melanophores and *P. achuar* having the palest venter.



FIGURE 7. Bayesian phylogenetic tree of *P. achuar*, *P. altamnis*, and *P. kichwarum* based on mtDNA cytochrome *b* and 16S. Posterior probabilities are labelled at nodes. Two subclades are noted within *P. kichwarum*. The size of terminal triangles reflect the amount of diversity when samples from across the range are included (from Elmer et al. 2007b).



FIGURE 8. Dorsal view of two specimens of *P. altamnis* (left and right; QCAZ 25313 and 25304) and the holotype of *S. calcaratus* (centre; NRM 1921). Note the lack of a darkened W-shaped occipital ridge, the pointed snout, different colour patterning, and shorter, plumper hindlimbs of *S. calcaratus* compared to *P. altamnis*.

Appendix 1. Paratypes of *Pristimantis kichwarum* with museum catalogue number, field number, locality, date of collection, collectors, and Genbank accession numbers for mtDNA cytochrome *b* and 16S [with acceptance]. EBJS = Jatun Sacha Biological Station, parroquia Ahuano, Canton Tena, Provincia Napo, Ecuador; FAV = F. Ayala-Varela; TS = T. Sugahara. Samples used for measurement and colour diagnosis in the species description are marked with an asterisk.

Museum QCAZ #	Field Number	Local-ity	Alti-tude (m)	Latitude	Longitude	Sex	Date of Collec-tion (dd-mm-yyyy)	Field Collectors	Genbank accession no. (cyt b/ 16S)
18023	SC7625	EBJS	461	S01°04.057	W77°37.008	male	04-11-2001	KRE, FAV	/EU130581
18027	SC7639	EBJS	448	S01°04.070	W77°36.980	female	04-11-2001	KRE, FAV	EF581014/
18030	SC7654	EBJS	456	S01°04.214	W77°36.989	male	05-11-2001	KRE, FAV	EF581015/
18033	SC7657	EBJS	452	S01°04.204	W77°36.949	female	05-11-2001	KRE, FAV	EF581016/
18039	SC7663	EBJS	449	S01°04.122	W77°37.159	male	05-11-2001	KRE, FAV	EF581017/
18041	SC7667	EBJS	456	S01°04.209	W77°37.003	male	05-11-2001	KRE, FAV	EF581018/
18045	SC7673	EBJS	451	S01°04.092	W77°37.142	male	05-11-2001	KRE, FAV	EF581019/
18053	SC7686	EBJS	463	S01°04.062	W77°37.091	female	06-11-2001	KRE, FAV	EF581020/
18069	SC7706	EBJS	420	S01°04.110	W77°36.887	male	08-11-2001	KRE, FAV	EF581021/ EU130582
18071	SC7708	EBJS	439	S01°04.195	W77°36.863	male	08-11-2001	KRE, FAV	EF581022/
18077	SC7717	EBJS	440	S01°04.229	W77°36.840	male	09-11-2001	KRE, FAV	EF581023/
18085	SC7725	EBJS	414	S01°04.286	W77°36.834	male	09-11-2001	KRE, FAV	EF581024/
18089	SC7741	EBJS	423	S01°04.420	W77°36.698	male	11-11-2001	KRE, FAV	EF581025/
18092	SC7744	EBJS	418	S01°04.339	W77°36.787	male	11-11-2001	KRE, FAV	EF581026/
18096	SC7748	EBJS	416	S01°04.425	W77°36.685	male	11-11-2001	KRE, FAV	EF581027/
18098	SC7750	EBJS	419	S01°04.326	W77°36.814	male	11-11-2001	KRE, FAV	EF581028/
18110	SC7762	EBJS	423	S01°04.054	W77°37.008	female	12-11-2001	KRE, FAV	EF581029/
18112	SC7766	EBJS	415	S01°04.254	W77°36.896	male	13-11-2001	KRE, FAV	EF581030/
18117*	SC7771	EBJS	n/a	n/a	n/a	female	13-11-2001	KRE, FAV	EF581031/
18126	SC7781	EBJS	419	S01°04.460	W77°36.795	female	14-11-2001	KRE, FAV	EF581032/
18132*	SC7790	EBJS	419	S01°04.520	W77°36.650	female	16-11-2001	KRE, FAV	EF581034/ EU130583
18137	SC7795	EBJS	371	S01°04.463	W77°36.678	male	16-11-2001	KRE, FAV	EF581035/
18139	SC7798	EBJS	424	S01°04.788	W77°36.787	male	19-11-2001	KRE, FAV	EF581036/
18153*	SC7822	EBJS	432	S01°04.705	W77°36.595	female	30-11-2001	KRE, FAV	EF581037
18165	SC7595	EBJS	399	S01°03.895	W77°37.029	male	02-11-2001	KRE, FAV	EF581013/
25287	SC11099	EBJS (Inner Vision Lodge)	408	S01°06.113	W77°35.582	female	09-04-2003	KRE, TS	EF581048/
25288*	SC11100	EBJS (Inner Vision Lodge)	408	S01°06.113	W77°35.582	male	09-04-2003	KRE, TS	EF581049/
25290*	SC11102	EBJS (Inner Vision Lodge)	433	S01°06.091	W77°35.648	male	09-04-2003	KRE, TS	EF581051/
25291*	SC11103	EBJS (Inner Vision Lodge)	396	S01°06.116	W77°35.594	male	09-04-2003	KRE, TS	EF581050/
25295	SC11107	EBJS (Inner Vision Lodge)	396	S01°06.110	W77°35.590	juve- nile	09-04-2003	KRE, TS	EF581052/ EU130595
25552*	SC11010	EBJS (Librar- ius Study Cen- ter)	383	S01°03.619	W77°36.923	male	20-02-2003	KRE	EF581039/
25554*	SC11007	EBJS (Librar- ius Study Cen- ter)	390	S01°03.700	W77°36.962	female	20-02-2003	KRE	EF581040/

25555*	SC11001	EBJS (Librarius Study Center)	390	S01°03.754	W77°36.882	male	20-02-2003	KRE	EF581038/
25558	SC11009	EBJS (Librarius Study Center)	384	S01°03.627	W77°36.937	male	20-02-2003	KRE	EF581041/
25561	SC11006	EBJS (Librarius Study Center)	392	S01°03.661	W77°36.927	juvenile	20-02-2003	KRE	EF581042/
25564	SC11013	EBJS (Librarius Study Center)	404	S01°03.839	W77°36.879	male	21-02-2003	KRE	EF581043/
25573	SC11022	EBJS (Librarius Study Center)	400	S01°03.841	W77°36.866	male	22-02-2003	KRE	EF581044/
25575*	SC11024	EBJS (Librarius Study Center)	405	S01°03.825	W77°36.861	female	22-02-2003	KRE	EF581045 /
25579	SC11027	EBJS (Librarius Study Center)	390	S01°03.740	W77°36.879	juvenile	13-03-2003	KRE	EF581046/ EU130592
25580*	SC11029	EBJS (property of Don Gabriel)	404	S01°04.102	W77°36.694	male	16-03-2003	KRE	EF581047 /
25751*	SC11125	EBJS (Inner Vision Lodge)	398	S01°06.110	W77°35.850	female	13-04-2003	KRE, TS	EF581055 /
25757*	SC11122	EBJS (Inner Vision Lodge)	419	S01°06.049	W77°35.067	male	13-04-2003	KRE, TS	EF581054 /
25759	SC11118	EBJS (Inner Vision Lodge)	398	S01°06.110	W77°35.856	male	13-04-2003	KRE, TS	EF581053/ EU130596
25773	SC11145	EBJS (Inner Vision Lodge)	413	S01°05.720	W77°35.901	male	16-04-2003	KRE, TS	EF581060 /
25782	SC11154	EBJS (Inner Vision Lodge)	416	S01°06.225	W77°35.662	female	17-04-2003	KRE, TS	EF581062 /