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A NEW ANDEAN SPECIES OF THE *HYPSIBOAS PULCHELLUS* GROUP: ADULTS, CALLS, AND PHYLOGENETIC RELATIONSHIPS

EDGAR LEHR[1,5], JULIAN FAIVOVICH[2], AND KARL-HEINZ JUNGFER[3,4]

[1]*Department of Biology, Illinois Wesleyan University, PO Box 2900, Bloomington, IL 61701, USA*
[2]*División Herpetología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" CONICET, Angel Gallardo 470, 1405 Buenos Aires, Argentina*
[3]*Institute of Integrated Sciences, Department of Biology, University of Koblenz-Landau, Universitätsstr. 1, 56070 Koblenz, Germany*

ABSTRACT: We describe a new species of the *Hypsiboas pulchellus* Group from the eastern Andes of central Peru (Region Pasco). Calls of both *H. melanopleura* and the new species are described. The new species is more similar to *H. melanopleura* and *H. palaestes* but differs in morphological characters and in coloration pattern. The new species and *H. melanopleura* are included in a molecular phylogenetic analysis of the *H. pulchellus* Group that shows them to be sister species and forming a second, independent, Andean clade within the group. New collecting sites for *H. melanopleura* are provided with the first record in the Region of Junin and the distribution of *H. melanopleura*, *H. palaestes*, and the new species is illustrated in a map.

RESUMEN: Se describe una nueva especie del grupo de *Hypsiboas pulchellus* del Departamento de Pasco, en los Andes orientales de Perú. Las vocalizaciones de *H. melanopleura* y las de la nueva especie son descriptos y comparados. La nueva especie es similar a *H. melanpleura* y *H. palaestes* pero se diferencia por caracteres morfológicos y patrón de coloración de adultos. La nueva especie y *H. melanopleura* son incluidas en un análisis filogenético de secuencias de ADN, cuyo resultado muestra que estas especies son taxones hermanos y que conforman un segundo clado andino independiente dentro del grupo de *H. pulchellus*. Por primera vez se reportan localidades de *H. melanopleura* en el Departamento de Junín y se presenta un mapa con la distribución de esta especie, *H. palaestes*, y la nueva especie.

Key words: Advertisement call; *Hypsiboas aguilari*; *Hypsiboas melanopleura*; *Hypsiboas pulchellus* Group; New species; Phylogeny

THE GENUS *Hypsiboas* Wagler, 1830 contains 80 species (Frost, 2009), most of which are included in seven species groups. One of them is the *Hypsiboas pulchellus* Group that currently contains 34 species, which are distributed in Andean Peru, Bolivia, and Argentina at elevations of 500–3416 m, as well as in the lowlands and mountain regions of Argentina, Paraguay, Brazil, and Uruguay (updated from Frost, 2009). Besides molecular synapomorphies, the only morphological synapomorphy known for members of the *H. pulchellus* Group is the muscle depressor mandibulae that lacks the slip that arises in the dorsal fascia at the level of the m. dorsalis scapulae (Faivovich et al., 2005).

Three species (*Hypsiboas balzani*, *H. melanopleura*, and *H. palaestes*) of the *Hypsiboas pulchellus* Group are known to occur in Peru, whereas *H. andinus* from northern Bolivia is likely to occur in Peru (Frost, 2009), but has not been recorded. Field work in the eastern Andes of central Peru in 2003 led to the rediscovery of *Hypsiboas melanopleura* at its type locality (Lehr and von May, 2004), whereas a new species of *Hypsiboas* was found at nearby localities but at higher elevations. The new species differs from *H. melanopleura* in morphology and coloration. Some live specimens of both species were transported to a lab to study vocalization. Herein we describe the new species, including its advertisement call, and that of *H. melanopleura*. Furthermore we include both species in a phylogenetic analysis of the *H. pulchellus* Group, because *H. melanopleura* was not available to previous analyses (Antunes et al., 2008; Faivovich et al., 2004, 2005).

MATERIALS AND METHODS

Taxonomy of hylids follows Faivovich et al. (2005). The format of this description follows that of Antunes et al. (2008). Specimens were preserved in 96% ethanol, and stored in 70% ethanol. Sex was determined by observation of

[4] PRESENT ADDRESS: Panoramastr. 14, 74405 Gaildorf, Germany
[5] CORRESPONDENCE: e-mail, elehr@iwu.edu

presence or absence of secondary sexual characters (prepollical spine, vocal slits). Measurements of adults were made with digital calipers and rounded to the nearest 0.1 mm and are as follow: SVL (snout–vent length); TL (tibia length); FL (foot length, distance from proximal margin of inner metatarsal tubercle to tip of fourth toe); DF3 (width of disc on third finger); HL (head length, from angle of jaw to tip of snout); HW (head width, greatest width, measured between centers of tympana); ED (eye diameter); TY (tympanum diameter, horizontal); IOD (interorbital distance); EW (eyelid width); IND (internarial distance); and E–N (eye–nostril distance, straight-line distance between anterior corner of orbit and posterior margin of external naris). Relative lengths of digits were determined by adpressing adjacent digits to one another. Descriptions of color in life are based on field notes and photographs taken by the senior author. Webbing formula was determined by the method proposed by Savage and Heyer (1967), as modified by Myers and Duellman (1982). Drawings were made by the senior author using a stereomicroscope with drawing tube attachment. One specimen of *Hypsiboas melanopleura* (MTD 46505) and the new species (MTD 45204) were partly dissected to assess the condition of the muscle depressor mandibulae.

Natural history observations were recorded by E. Lehr and C. Aguilar. Comparative data were taken from original species descriptions, and the specimens examined (Appendix I). Museum acronyms are: KU = Natural History Museum, University of Kansas, Lawrence, Kansas, USA; MUSM = Museo de Historia Natural Universidad Nacional Mayor de San Marcos, Lima, Peru; MTD = Museum für Tierkunde Dresden, Dresden, Germany. Advertisement calls of the new species and *H. melanopleura* were recorded in captivity. Live specimens were placed in an aquarium of 70 × 40 cm, which was 50 cm high. Water level was about 6 cm, and consisted of some stones and twigs. As an imitation of rain, water was sprinkled by means of a pump four times at intervals of two hours during the day. Calls were recorded with a Sony WM D6C cassette recorder and a Sennheiser Me-66 directional microphone. Calls were analyzed with Cool Edit 2000 software digitizing the calls at 22,500 Hz and using FFT 256 for spectral analyses.

Phylogenetic Analysis

Relationships of the new species and *Hypsiboas melanopleura* were studied using the same taxonomic sampling and sequences recently employed by Antunes et al. (2008) with the addition of five specimens (paratypes) from two localities of the new species and five from two localities of *H. melanopleura*. Sequences of the added terminals for the mitochondrial genes 12S, the intervening tRNA valine, 16S (approx. 2420 base-pairs [bp]), and cytochrome b (385 bp), and the nuclear genes rhodopsin (316 bp), RAG-1 (428 bp, available only for the new species), and seven in absentia homolog 1(397 bp, available only for *H. melanopleura*) were generated using the primers and lab protocols of Faivovich et al. (2004, 2005). Sequences are deposited in Genbank with the numbers HM444756–HM444789.

The phylogenetic analysis was done using the program POY 4 (Varón et al., 2008). The rationale for using parsimony as an optimality criterion was advanced by Farris (1983) and recently discussed, among others, by Goloboff (2003) and Goloboff and Pol (2005). The preference for the treatment of sequence data as dynamic homologies simultaneously with tree search, as opposed to static homology hypotheses (multiple alignments) independent of tree search, has been justified by Wheeler (1996, 2002) and De Laet (2005).

We constructed 200 Wagner trees using random addition sequences, followed by a of round of tree bisection and reconnection (TBR) branch swapping keeping only one tree per replicate, and submitting the best topologies to a final round of TBR keeping all trees. Bremer supports (Bremer, 1988), were calculated with POY; parsimony jacknife values (Farris et al., 1996) were estimated using TNT (Goloboff, 2003; Goloboff et al., 2008), on the basis of the implied alignment generated by POY. Tree edition was done with Winclada (Nixon, 1999).

Systematics

Hypsiboas **aguilari** *sp. nov.*
(Figs. 1, 2)

Holotype.—MUSM 18656 (Figs. 1, 2), an adult male from San Alberto (10° 32′ 53.4″ S,

FIG. 1.—Dorsolateral (A) and dorsal (B) views of male *Hypsiboas aguilari* (MUSM 18656, holotype, SVL 41.7 mm), dorsolateral view (C) of male *H. aguilari* (MTD 45662, paratype, SVL 43.3 mm), and dorsolateral view (D) of *H. aguilari* (MTD 45663, Gosner Stage 45, SVL 20.9 mm). Photos by E. Lehr.

075° 22′ 16.6″ W; Fig. 3), 2200 m elevation, Provincia de Pasco, Departamento de Pasco, Peru, collected on 12 February 2003 by E. Lehr and C. Aguilar.

Paratypes.—24 (seven females: MUSM 19957, 23557–58, 24534, MTD 46302, 46494, 46500, 13 males: MUSM 18654–55, 19804–05, 19956, MTD 45202–04, 45662, 46303–04, 46338, 46499, two juveniles: MUSM 24533, MTD 46495, Gosner Stage 45: MTD 45663, Gosner Stage 46: MUSM 24807, MTD 45202–04, MUSM 18654–55 collected at the type locality with the holotype by E. Lehr and C. Aguilar; MUSM 24533–34 from San Alberto (10° 34′ 38.5″ S, 075° 23′ 32.2″ W), 1850 m elevation, on 25 June 2005 by M. Lundberg; MUSM 19956 at km 4.4 near road from Llamaquizu to radio antenna, Oxapampa, 10° 37′ 31.6″ S, 075° 21′ 13.9″ W, 2080 m elevation, on 3 July 2004 by M. Lundberg; MTD 46338 at km 4.4 near road from Llamaquizu to radio antenna, 10° 37′ 31.6″ S, 075° 21′ 13.9″ W, 2080 m elevation, on 3 July 2004 by E. Lehr and M. Lundberg; MTD 46304 at km 2.65 Llamaquizu to radio antenna, 10° 37′ 17.8″ S, 075° 21′ 53.8″ W, 2000 m elevation, on 10 July 2004 by M. Lundberg; MUSM 24806 from Maria Teresa (km 19 near road from Oxapampa to Yaupi), 10° 42′ 05.6″ S, 075° 27′ 22.2″ W, 1470 m elevation, on 29 May 2005 by M. Lundberg; MUSM 19957, MTD 46302–03 at km 24 near road from Oxapampa to Yaupi, 10° 43′ 39.0″ S, 075° 27′ 57.6″ W, 1530 m elevation, on 31 January 2003 by M. Lundberg; MUSM 23557–58 (collected on 2 October 2004), MTD 46494–95 (collected on 6 November 2004) from Tingo, approximately 10° 37′ 03.3″ S, 075° 32′ 58.9″ W, 1900 m elevation, by M. Lundberg; MTD 46499–500 from Palmazu, approximately 10° 29′ 45.6″ S, 075° 28′ 38.9″ W, 1800 m elevation, on 11 December 2004 by M. Lundberg; all from Provincia de Pasco, Departamento de Pasco, Peru. MTD 45662–63, MUSM 19804–05 from Pampa Hermosa ("house next to river"), approximately 10° 59′ 44.0″ S, 075° 26′ 04.9″ W, 1225 m elevation, Provincia de Tarma, Departamento de Junín, Peru, on 30 August 2003 by E. Lehr, C. Aguilar, and R. von May.

Diagnosis.—A medium-sized (SVL 33.7–43.8 in adult males [n = 14], 33.7–50.0 in adult females [n = 7]) member of the *Hypsiboas pulchellus* Group, as revealed by the muscle depressor mandibulae that lacks the slip that arises in the dorsal fascia at the level of the m. dorsalis scapulae. *Hypsiboas aguilari* is defined by the following combina-

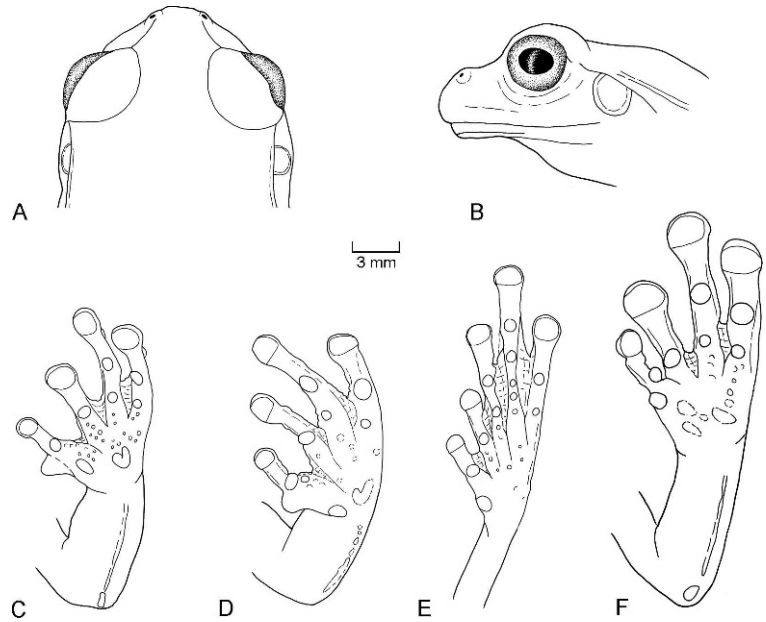

FIG. 2.—Dorsal (A) and lateral (B) views of head and ventral views of hand (C) and foot (E) of male *Hypsiboas aguilari* (MTD 46303, paratype), ventral view of hand (D) of male *H. melanopleura* (MTD 45680), and ventral view of hand (F) of female *H. aguilari* (MTD 46494, paratype). Drawings by E. Lehr.

tion of characters: slender body, head wider than body; snout rounded in dorsal view, slightly truncate in lateral view; large prominent eyes; upper half of palpebral membrane translucent, lower half with brown reticulations; small tympanum (tympanum diameter about half the size of eye diameter); narrow, slightly elevated dorsolateral fold; limbs long and slender; arms in males hypertrophied; enlarged prepollex with projecting bony spine in males; nuptial pad absent; no inner tarsal fold, tarsal tubercles absent; heel tubercles and calcars absent; cloacal sheath short; in life, head and dorsum pale beige with pale olive blotches; throat pale yellowish green, chest and belly white, extremities gray; thighs without any color pattern; iris beige or pale salmon with black reticulations.

Comparisons.—*Hypsiboas balzani*, *H. cordobae*, *H. cymbalum*, *H. ericae*, *H. guentheri*, *H. joaquini*, *H. marginatus*, *H. marianitae*, *H. prasinus*, and *H. pulchellus* have or may have a predominant green dorsal coloration (Caramaschi and Cruz, 2000; Duellman et al., 1997; Garcia et al., 2001, 2003; Lutz, 1973), not observed in *H. aguilari*. The new species differs from *Hypsiboas alboniger*, *H. andinus*, *H. balzani*, *H. bischoffi*, *H. caingua*, *H. cordobae*, *H. cymbalum*, *H. guentheri*, *H. marianitae*, *H. prasinus*, *H. pulchellus*, *H. riojanus*, and *H. secedens*, by the absence of pattern on posterior surface of thighs (most of these with vertical bars, a few with other designs; Duellman et al., 1997; Langone, 1997; Lutz, 1973). *Hypsiboas freicanecae* has a metallic triangle on dorsum of snout, and a calcar (Carnaval and Peixoto, 2004) like *H. secedens* (Lutz, 1963) both character states absent in *H. aguilari*. From *H. beckeri*, *H. buriti*, *H. cipoensis*, *H. goianus*, *H. guentheri*, *H. latistriatus*, *H. leptolineatus*, *H. phaeopleura*, *H. polytaenius*, and *H. stenocephalus*, *H. aguilari* differs by the absence of longitudinal stripes on the dorsum and by its more robust body. *Hypsiboas ericae*, *H. caipora*, *H. curupi*, *H. joaquini*, *H. marginatus*, *H. poaju*, *H. semiguttatus*, and *H. stellae* differ for having brown to dark brown anterior and posterior surfaces of thighs, furthermore these species tend to have large longitudinal blotches on dorsum (Antunes et al., 2008; Garcia and Haddad, 2008; Garcia et al., 2007, 2008; Kwet, 2008). The new species also differs from *H. marginatus* for lacking a black stripe

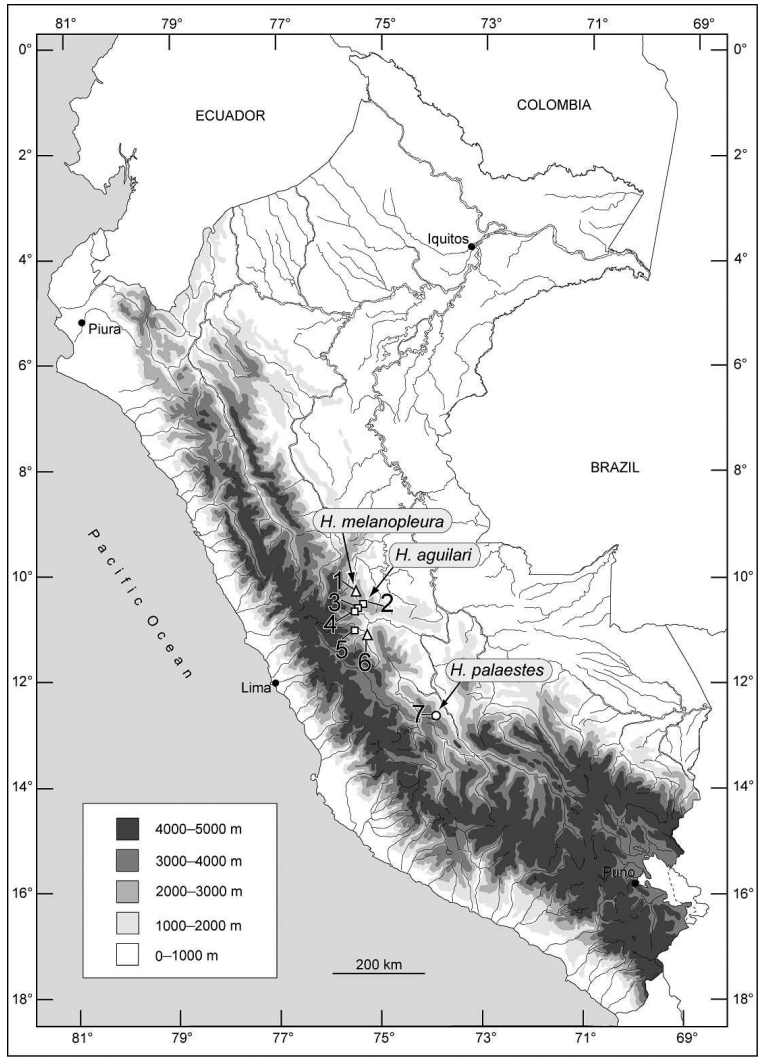

FIG. 3.—Map illustrating distributions of *Hypsiboas aguilari*, *H. melanopleura*, and *H. palaestes* in Peru. Arrows indicate type localities. 1 = Huancabamba (Pasco), 2 = San Alberto (Pasco), 3 = Llamaquizu (Pasco), 4 = Maria Teresa (Pasco), 5 = Pampa Hermosa (Junín), 6 = San Ramon (Junín), 7 = south edge of Tutumbaro (Ayacucho). See text for details on distribution of *H. aguilari* and *H. melanopleura*.

that extends from the nostril back to the sacral region (Garcia et al., 2001).

Hypsiboas aguilari is more similar to *H. melanopleura* and *H. palaestes*. *Hypsiboas aguilari* and *H. melanopleura* occur sympatrically but have not been found syntopically. *Hypsiboas aguilari* and *H. palaestes* have dorsolateral folds, which are absent in *H. melanopleura*. Furthermore, *H. aguilari* and *H. melanopleura* differ in coloration (characters of *H. melanopleura* in parenthesis): pale labial stripe is narrow (broad) without clear borders to surrounding coloration (well-defined with distinct borders), dorsum with transverse bars and irregularly shaped blotches (dorsum with small spots), flanks in both species bear white spots, but flanks in *H. aguilari* are grayish brown and slightly darker than dorsum (dark gray to black and much darker than dorsum), day coloration in some individuals pale whitish to pale bluish green (yellow), iris beige to pale salmon with black

reticulation (reddish-copper with black reticulation). The prepollex in *H. aguilari* (Fig. 2C) is pointed vs. blunt in *H. melanopleura* (Fig. 2D). Flanks have white spots (Fig. 1C) in *H. aguilari*, whereas the flanks are uniform tan in *H. palaestes*.

Description of holotype.—Adult male; SVL 41.7; body slender; head wider than body, nearly as wide as long, HW/HL = 1.06, widest below tympanum; snout rounded in dorsal view (Fig. 2A), slightly truncate in lateral profile (Fig. 2B); eye–nostril distance shorter than diameter of eye, E–N/ED = 0.84; canthus rostralis nearly straight in dorsal view, rounded in section; loreal region flat; lips thin, not flared; internarial region flat; nostrils slightly protuberant, directed dorsolaterally. Interorbital area flat, IOD/ED = 1.09, IOD/HW = 0.33; eyes large and protuberant, ED/HL = 0.32, ED/HW = 0.30; upper eyelid width, 3.6. Upper half of palpebral membrane translucent, lower half with brown reticulations. Supratympanic fold distinct, obscuring upper part of tympanic annulus, connecting to a narrow and slightly elevated dorsolateral fold, which is more prominent anteriorly (between posterior corner of upper eyelid to about middle of body) and becomes discontinous (fragmented) in its posterior run; tympanum small, distinct, directed dorsolaterally, separated from eye by a distance about half the size of tympanum diameter; tympanum diameter about half the size of eye diameter TY/ED = 0.49, and slightly larger than DF3. Arm short and hypertrophied, lacking an axillary membrane; ulnar tubercles coalescing to distinct, narrow ulnar fold extending from below base of hand to elbow; fingers moderately long, bearing moderately large, round discs, relative length of fingers I < II < IV < III (Fig. 2C); fingers bearing lateral fringes; subarticular tubercles distinct, not bifid, ovoid in ventral view, conical in profile, distal subarticular tubercles more prominent than proximal ones, most prominent on Finger IV; supernumerary tubercles small and numerous; carpal tubercles barely noticeable; inner metacarpal tubercle elongate, flat; outer metacarpal tubercle bifid, flat, large; nuptial pad absent; prepollex enlarged and pointed (Fig. 2C) and modified as a bony spine that is evident under the skin; nuptial pad absent; webbing basal between fingers one and two; webbing formula II 2–3 III 3–3 IV. Hind limbs long and slender; TL/SVL = 0.51; heels overlapping when hind limbs flexed perpendicular to axis of body; tarsal fold absent; calcars and heel tubercles absent; toes moderately long, bearing round discs, slightly smaller than those on fingers; relative lengths of toes I < II < III < V < IV (Fig. 2E); toes bearing lateral fringes; subarticular tubercles moderately large, round in ventral view, slightly conical in profile; supernumerary tubercles absent; outer metatarsal tubercle absent; inner metatarsal tubercle distinct, ovoid in dorsal view, conical in profile; webbing formula I 2–1½ II 1–3 III 2–2 IV 2–1 V; thickened layer of tissues at midline of webbing between Toes IV and V. Skin on all dorsal surfaces finely shagreen; skin on flanks finely shagreen with scattered, low warts; skin in axilla, groin, and on anterior surface of thighs smooth; skin on throat, chest, belly, and ventral surfaces of thighs coarsely areolate, skin on tibia and concealed surface of tarsus smooth. Cloacal opening directed posteriorly at upper level of thighs; cloacal sheath distinct; cloacal tubercles present, scattered, extending to midlevel of thighs. Tongue ovoid, barely free behind, posterior margin slightly notched; dentigerous processes of vomers prominent, in two nearly straight, transverse series, each bearing seven teeth, medially narrowly separated; choanae large, elongate; vocal slits moderately long, extending from midlateral base of tongue, almost reaching to angle of jaws; vocal sac single, median, subgular.

Measurements (in mm) of holotype.—SVL 41.7, TL 21.3, FL 16.7, DF3 2.0, HL 14.1, HW 14.9, ED 4.5, TY 2.2, IOD = 4.9, EW = 3.6, IND = 2.6, E–N = 3.8.

Coloration of holotype in preservative.—Dorsum tan with minute brown spots and four grayish-brown transversally oriented stripes from cloaca to midlength of dorsum, irregularly shaped grayish-brown blotches behind posterior margin of head and scapular region; grayish-brown, broadly U-shaped interorbital bar; dorsolateral fold pale gray; arms tan with minute brown spots and grayish-brown transversal bars; legs colored as arms with bars more distinct on tibia; anterior and posterior surfaces of thighs uniform tan; heel dorsolat-

TABLE 1.—Ranges (in mm) followed by mean and standard deviation and proportions of adult type specimens of *Hypsiboas aguilari* and adult specimens of *H. melanopleura* (see Appendix I).

	H. aguilari		*H. melanopleura*	
Characters	Males (n = 14)	Females (n = 7)	Males (n = 6)	Females (n = 7)
SVL	33.7–43.8 (40.4 ± 2.9)	33.7–50.0 (44.4 ± 5.4)	33.9–42.0 (39.8 ± 3.0)	43.1–45.8 (44.3 ± 1.2)
TL	17.2–22.6 (20.7 ± 1.4)	18.5–26.3 (24.2 ± 2.7)	19.3–22.8 (21.5 ± 1.3)	22.9–24.6 (23.5 ± 0.6)
FL	13.3–18.3 (16.2 ± 1.3)	14.1–22.6 (19.5 ± 2.6)	15.4–18.9 (17.8 ± 1.3)	18.4–20.9 (20.1 ± 0.8)
DF3	1.7–2.4 (2.0 ± 0.2)	1.9–2.7 (2.3 ± 0.3)	1.7–2.1 (1.9 ± 0.2)	2.0–2.4 (2.2 ± 0.1)
HL	11.7–15.3 (14.2 ± 0.9)	12.3–18.1 (15.6 ± 1.8)	12.1–14.5 (13.4 ± 0.9)	14.1–16.2 (14.9 ± 0.7)
HW	12.5–15.8 (14.5 ± 0.9)	12.3–17.9 (15.8 ± 1.8)	12.4–15.1 (14.3 ± 1.0)	14.6–16.7 (15.4 ± 0.7)
ED	3.7–4.8 (4.4 ± 0.3)	3.8–5.4 (4.6 ± 0.7)	3.6–4.5 (4.3 ± 0.4)	4.5–5.5 (4.9 ± 0.4)
TY	1.7–2.5 (2.1 ± 0.2)	1.7–3.0 (2.5 ± 0.4)	1.7–2.6 (2.2 ± 0.4)	2.5–2.9 (2.7 ± 0.2)
IOD	4.4–5.3 (5.0 ± 0.4)	4.0–5.6 (4.9 ± 0.5)	4.5–5.4 (5.0 ± 0.4)	4.6–5.3 (4.9 ± 0.2)
EW	2.2–4.3 (3.7 ± 0.5)	3.3–4.9 (4.0 ± 0.6)	2.9–3.7 (3.5 ± 0.4)	3.7–4.5 (4.1 ± 0.3)
IND	2.6–3.1 (2.8 ± 0.2)	2.6–3.2 (3.0 ± 0.2)	2.4–2.9 (2.7 ± 0.2)	2.7–3.1 (2.9 ± 0.1)
E–N	3.0–4.3 (3.9 ± 0.3)	3.7–4.7 (4.3 ± 0.4)	3.3–4.1 (3.8 ± 0.3)	3.5–4.3 (4.0 ± 0.3)
TL/SVL	0.47–0.56	0.52–0.59	0.51–0.57	0.51–0.57
FL/SVL	0.36–0.45	0.42–0.45	0.43–0.45	0.43–0.48
HL/SVL	0.33–0.39	0.33–0.36	0.30–0.36	0.33–0.35
HW/SVL	0.34–0.38	0.33–0.36	0.35–0.37	0.34–0.36
HW/HL	0.94–1.08	0.99–1.05	1.01–1.19	1.02–1.06
E–N/ED	0.81–0.96	0.87–1.00	0.84–0.93	0.72–0.91
EW/IOD	0.64–0.88	0.62–0.88	0.64–0.75	0.74–0.94
TY/ED	0.43–0.55	0.45–0.62	0.43–0.59	0.49–0.62
DF3/TY	0.84–1.24	0.90–1.12	0.73–1.00	0.77–0.92

erally with a white stripe; webbing pale grayish brown with tan longitudinal stripe in mid of webbing between Toes IV and V; white supracloacal stripe; narrow, pale cream dorsolateral stripe on each side of body; upper half of flanks grayish brown, lower half tan with minute brown spots and larger pale cream spots; sides of head grayish brown with narrow, pale cream labial stripe; ventral surfaces of hands and feet pale gray, all other ventral surfaces pale cream with minute brown spots on throat and chest; ulnar fold pale cream; iris gold with black reticulation, upper half of palpebral membrane translucent, lower half with brown reticulations.

Coloration of holotype in life.—Dorsally pale beige with pale olive blotches; throat pale yellowish green, chest and belly white, extremities gray; iris beige with black reticulations.

Variation.—Specimens differ remarkably with respect to dorsal pattern consisting of blotches in different numbers, sizes, and shapes. The posterior half of the dorsum usually consists of 3–5 transversal bars, whereas the anterior half of the dorsum contains irregularly shaped blotches forming a left-adjusted E (MUSM 19804), H (MUSM 19957), an interorbital Y (MUSM 19805), or a left-adjusted C (MUSM 24533). Two specimens (MUSM 18654, 18655) have the entire dorsum with numerous oval blotches and spots. Coloration in life for one male (MTD 45204) was noted as follows: head and dorsum pale olive with dark olive blotches; extremities pale beige with dark olive bars; sides of head dark brown; iris pale salmon with black reticulation; white spots on flanks; anterior and posterior surfaces of thighs gray; throat yellowish green with black spots, chest and belly white; extremities gray. Females are larger than males and lack hypertrophied arms, vocal slits, and a prepollex. See Table 1 for ranges and proportions of the type series.

Distribution and ecology.—*Hypsiboas aguilari* is known from several localities in the central Peruvian Regions of Pasco and Junín at 1225–2080-m elevation (Fig. 3). The species is commonly found in open areas of secondary forests, and in agriculturally used habitats such as pasture lands or irrigation canals. Specimens at San Alberto were found at night in lower vegetation next to water or in the water. Males were calling around stagnant water. Tadpoles of *H. aguilari* were found on 12 February and between 10 and 17 July in

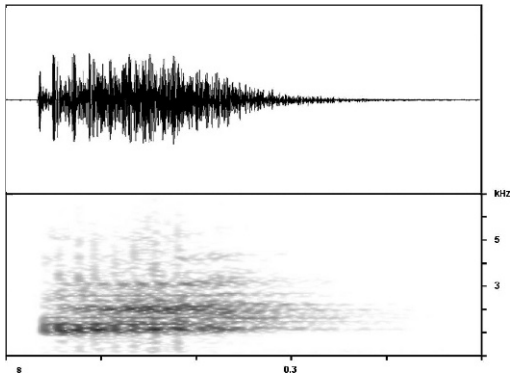

FIG. 4.—Sound spectrogram and corresponding waveform (above) of an advertisement call of *Hypsiboas aguilari* obtained from the type locality and kept in captivity by K.-H. Jungfer. Recording obtained in captivity on 25 February 2005 at 21.0 °C air temperature.

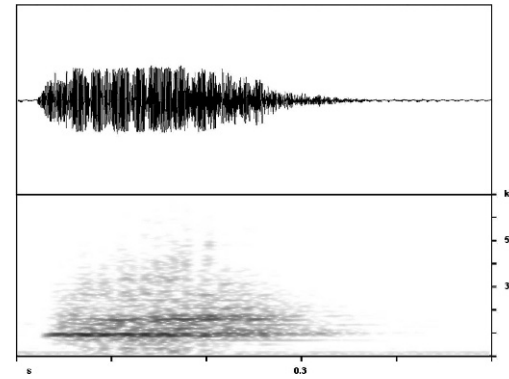

FIG. 5.—Sound spectrogram and corresponding waveform (above) of an advertisement call of *Hypsiboas melanopleura* obtained from the type locality and kept in captivity by K.-H. Jungfer. Recording obtained on 7 March 2005 at 21.4 °C air temperature.

swampy, slow-flowing creek on pasture together with adults of *H. aguilari*. At Llamaquizu (Pasco), tadpoles of *H. aguilari* and *Scinax oreites* were found syntopically on 10 and 17 July in a small pond of approximately 60 cm of diameter with a depth of 40 cm. At the bottom of the pond were small rocks, which the tadpoles used to hide. The pond is connected to a small rivulet. Tadpoles of *H. aguilari* and *H. melanopleura* will be described separately.

Etymology.—The new species is dedicated to our friend and colleague César A. Aguilar Puntriano (MUSM) for his contributions to Peruvian herpetology.

Bioacoustics

The advertisement call of *Hypsiboas aguilari* from the type locality recorded at 21.0 °C in captivity consisted of one note repeated sporadically. A note (Fig. 4) had a duration of 0.357 s. The dominant frequency was at 1080 Hz. Most energy is concentrated in harmonics at 1245, 1590, and 1934 Hz. Pulse rate was about 55 pulses/s. Five calls selected randomly had note lengths of 0.315–0.440 ($\bar{X}$ = 0.375), dominant frequencies of 944–1086 ($\bar{X}$ = 1055) Hz. Pulses, between 50/s and 60/s, were not always obvious. Note repetition rates varied considerably. Six recorded subsequent calls had a repetition rate of 5.6/min at intervals of 4.9–22.7 s.

The advertisement call of *Hypsiboas melanopleura* from the type locality (Huancabamba, Departamento Pasco, Peru) was recorded in captivity at 21.4 °C. It consisted of one note that was repeated sporadically. One note (Fig. 5) had a duration of 0.343 s. The dominant frequency was at 928 Hz. There was a harmonic at 1537 Hz. Five calls selected randomly had poorly pulsed note lengths of 0.328–0.444 ($\bar{X}$ = 0.360) s, dominant frequencies of 795–928 ($\bar{X}$ = 858) Hz and one harmonic each at 1219–1616 Hz ($\bar{X}$ = 1457). Note repetition rates differed greatly. Six subsequent calls had intervals of 20.1–53.2 s ($\bar{X}$ = 1.9 calls/min).

Natural history observations in captivity

Males were usually calling from twigs slightly above the water or from shallow water. From time to time a male would approach another male and they would start grappling, usually in the water. After about one minute, one of the males would quickly swim away. Males inspected after such grappling bouts always had scratches on the flanks, head, and dorsum caused by the prepollical spines that project through its skin cover. The same behavior was exhibited by *Hypsiboas melanopleura* males. Bouts were always short and we never observed any severe injuries in either species. Scars of scratches resulting from fights are also known in many other species of the *Hypsiboas pulchellus* Group (Duellman et al., 1997; Faivovich et al., 2005; K. H. Jungfer, personal observation).

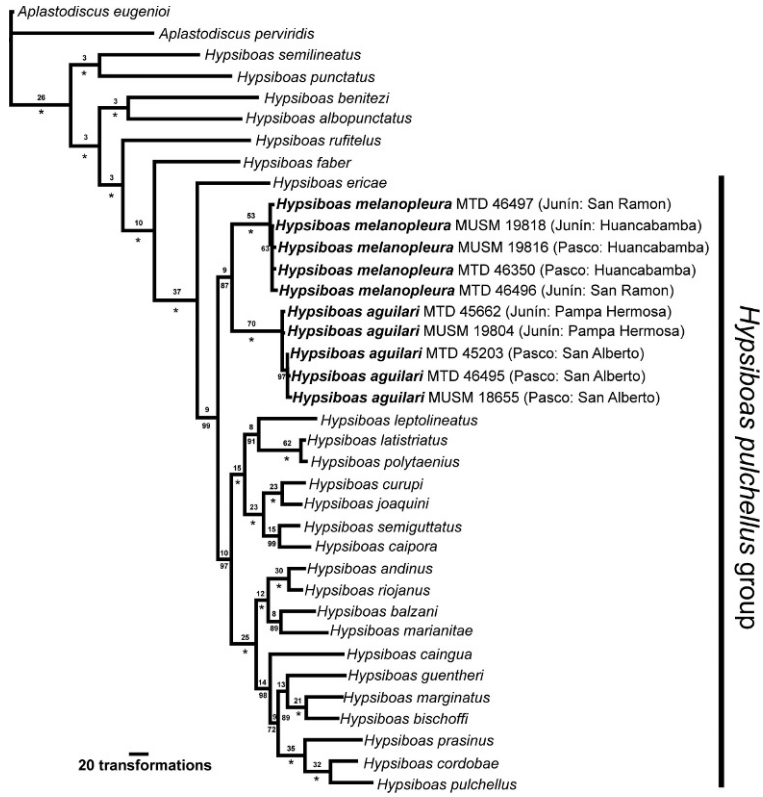

FIG. 6.—Strict consensus of the four equally most parsimonious trees resulting from a phylogenetic analysis of the *Hypsiboas pulchellus* Group plus several outgroups. Note the position of *H. aguilari* and *H. melanopleura*. Numbers above nodes are Bremer supports, numbers below, parsimony jacknife values. The asterisk denotes 100% jacknife support. Numbers next to locality names in parenthesis of *H. aguilari* and *H. melanopleura* refer to localities shown in Fig. 3.

A captive pair laid about 440 eggs in several small clutches below the water's surface. Eggs were attached to substrate, and were black with a white vegetal pole and a diameter of about 3 mm. Juveniles were about 21 mm in SVL after metamorphosis and had small red spots on green ground color dorsally that turned creamy white toward the dorsolateral margins. Laterally they were brown with a greenish or bluish hue and bluish white ventrally.

Phylogenetic analysis

The analysis resulted in four most parsimonious trees of length 4715. The best length was hit 34 times doing Random addition sequences plus TBR branch swapping. Most of the conflict among the alternative resolutions is due to the relationships among the different exemplars of both *H. aguilari* and *H. melanopleura*. These two are sister species, forming the sister clade of most species of the *H. pulchellus* Group with the single exception of *H. ericae*, the sister species of all species of the group (Fig. 6). Otherwise, the relationships implied by the strict consensus tree for the *H. pulchellus* Group are identical to those obtained by Faivovich et al. (2004, 2005). The *H. polytaenius* clade is recovered as monophyletic, unlike the recent analysis of Antunes et al. (2008) where it was not monophyletic.

DISCUSSION

Taxonomy

Taxonomy of members of the *Hypsiboas pulchella* is known to be complicated because of morphological similarity and intraspecific variation with respect to coloration (Duellman

et al., 1997; Lehr and von May, 2004; Lutz, 1973). Additional genetic, bioacoustic, or developmental characters are useful for species delimination and, thus, helpful in avoiding synonyms. For 92 years the type series of *H. melanopleura* comprising one male and four females were the only existing specimens until Lehr and von May (2004) described an additional eight males obtained at the type locality. These authors recognized discrepancies regarding the existence of nuptial pads in male *H. melanopleura* (Lehr and von May, 2004). Duellman et al. (1997) revised the type series of *H. melanopleura* including the single male and described "nuptial spines absent; nuptial excrescence brown on inner side of base of thumb in breeding male," whereas Lehr and von May (2004) did not observe any nuptial pad structure in their males (Lehr and von May, 2004). Because nuptial pads could be present only during breeding season, Lehr and May (2004) recommended assessing this character when sexually active specimens become available. On the basis of observations made on males of *H. melanopleura* obtained during the breeding season and from those observed in captivity (K. H. Jungfer, personal observation), nuptial pads are absent in *H. melanopleura*, as in all other species of the *H. pulchellus* Group, and in general most species of *Hypsiboas* (see Faivovich et al., 2006 for cases where they do occur).

Herein we report for the first time dorsolateral folds for two species of the *Hypsiboas pulchellus* Group. This fold is slightly elevated, more prominent anteriorly between the posterior corner of upper eyelid to about middle of body length, and becomes discontinuous (fragmented) more posteriorly. The coloration of the fold is slightly paler compared to the surrounding skin. In preserved specimens the dorsolateral folds are less distinct. Photos taken of several live specimens of *H. aguilari* (Fig. 1A–C) and of *H. palaestes* (Duellman et al., 1997; Fig. 3) clearly show this fold. Obviously this diagnostic character was overseen in the past. A review of preserved specimens and photos showing live specimens of the *pulchellus* Group would be necessary to assess the distribution of dorsolateral folds in the group.

Bioacoustics

The nearly indistinguishable calls in *Hypsiboas aguilari* and *H. melanopleura* were not expected and are unusual for species having a sympatric but not syntopic distribution, as the elevational distribution of *H. melanopleura* lies within the range of *H. aguilari* (1780 m vs. 1225–2080 m in *H. aguilari*). Currently, *H. melanopleura* is known from the type locality that is about 25 km (air line) away from the closest known locality of *H. aguilari* at an elevation of 2080 m, and from San Ramon (Departmento de Junín).

We are somewhat hesitant to believe that similarities in the calls of both species resulted from artifacts, because both were recorded under the same conditions (i.e., in the same aquarium at almost the same temperatures [21.0 °C vs. 21.4 °C]). However, we expect that as additional recordings from these species under natural conditions become available, we will be able to better appreciate the degree of differentiation of their advertisement calls.

The advertisement calls of *Hypsiboas aguilari* and *H. melanopleura* (Figs. 4, 5) seem to differ significantly from that of *H. palaestes* (Duellman et al., 1997:29, Fig. 17). Available recordings were taken at different air temperatures (14 °C for *H. palaestes* and 21.0–21.4 °C for *H. aguilari* and *H. melanopleura*, respectively). Because of this reason, call differences with *H. palaestes* have not been included in the comparisons accompanying the diagnosis of *H. aguilari*, and the following comparison should be considered tentative. Calls of *H. aguilari* and *H. melanopleura* consist of one note repeated sporadically, while in *H. palaestes* it is a series of soft, low, trill-like notes. In the recordings of *H. palaestes* reported by Duellman et al. (1997), only a single harmonic is evident at 4.43–4.45 KHz, while in those of *H. aguilari* and *H. melanopleura* the dominant frequency is much lower (795–1080 Hz), and at most five harmonics are evident (maximum frequency: 1934 Hz). Thus, there are considerable differences in the structures of the calls of *H. aguilari/melanopleura* and *H. palaestes*. Temperature is known to have a negative correlation with note length and pulse rate (i.e., both of them decrease with rising temperatures, and dominant frequency rises

with higher temperatures [e.g., Blair, 1958; Schneider, 1977]). Single notes, however, are still longer in *H. aguilari/melanopleura* than in *H. palaestes*, although they were taken at higher temperatures. Likewise, dominant frequencies are still lower in *H. aguilari/melanopleura* than in *H. palaestes*. The opposite should be expected in both cases.

Phylogenetic relationships

Faivovich et al. (2004) included as exemplars of the Andean species of the *H. pulchellus* Group *H. andinus*, *H. balzani*, *H. marianitae*, and *H. riojanus*. These formed a monophyletic group in their best hypotheses, implying a single origin for the Andean species of the group. In the absence of other evidence, the authors suggested that the Andean species that then were not available for the analysis (*H. alboniger*, *H. melanopleura*, and *H. palaestes*) were also part of this single Andean clade. The results obtained here do not support this suggestion; instead, they indicate the existence of not one but two independent clades of Andean species in the *H. pulchellus* Group. The first of these clades is composed of *H. aguilari* and *H. melanopleura*. On the basis of its remarkable similarity with the former, we tentatively associate *H. palaestes*, not included with this clade. The three species from this clade are distributed in the Central Andes of Peru. The other clade is composed of *H. andinus*, *H. balzani*, *H. marianitae*, and *H. riojanus*; *H. callipleura* is tentatively associated with this clade on the basis of its similarity with *H. balzani* (it has been considered a junior synonym until recently, see Köhler, 2000). Tentatively we also associate *H. alboniger*, from the highlands of southern Bolivia, with this clade. All species from this clade are distributed in the Central Andes and adjacent mountains in southern Peru, Bolivia and Argentina, extending southwards up to La Rioja Province.

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Appendix I

Specimens Examined

Hypsiboas andinus: ARGENTINA: Catamarca: 3.4 km NE La Merced, 950 m: KU 160372–406. *Hypsiboas balzani*: PERU: Cuzco: 4 km WSW Santa Isabel, 1700 m: KU 139212; 4 km SW Santa Isabel, Rio Cosñipata, 1700 m: KU 163315–18. *Hypsiboas palaestes*: PERU: Ayacucho: Tumbaro, Rio Piene, 1840 m: KU 16306–14, 19844–53, MTD 46160 (all paratypes). *Hypsiboas melanopleura*: PERU: Junín: San Ramon: MTD 46496–98, 46501–07; Pasco: Huancabamba, 1780 m: MTD 45678, 45680–81, Huancabamba: BMNH 1947.2.13.56 (lectotype), 1947.2.13.54 (type).