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A new species of *Hyalinobatrachium* (Anura: Centrolenidae) from the Amazonian slopes of the central Andes, with comments on the diversity of the genus in the area

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Abstract

We describe a new species of *Hyalinobatrachium* from the Amazonian slopes of the Andes in Peru and Bolivia on the basis of morphological, bioacoustic and genetic characteristics. *Hyalinobatrachium carlesvilai* sp. nov. can be distinguished from other species of *Hyalinobatrachium* by the combination of the following characters: (1) truncate snout in dorsal and lateral view; (2) white pericardium; (3) enameled dorsal, tarsal and cloacal folds; (4) hand webbing formula **III** $2^{-} – 1^{+}$ **IV**; (5) iris cream; (6) advertisement call consisting of a single, frequency-modulated note with a pulsed section followed by a tonal section. The new species had been previously identified as *Hyalinobatrachium munozorum* and *H. bergeri*. The advertisement call of the new species was previously assigned to *H. bergeri*. Here we describe the previously unknown call of *Hyalinobatrachium bergeri*. Additionally, we study the taxonomic status of *H. lemur* and *H. pellucidum* and place the former as synonym of the later. We extend the distribution of *H. pellucidum* to Departamento Cusco in southern Peru.

Key words: Bioacoustics; Centrolenid frogs; Cryptic species; Glassfrogs; *Hyalinobatrachium bergeri*; *Hyalinobatrachium lemur*; *Hyalinobatrachium munozorum*; *Hyalinobatrachium pellucidum*; mitochondrial DNA; Synonymy; Taxonomy

Introduction

Centrolenid frogs, also known as glassfrogs, constitute a monophyletic group (reviewed by Guayasamin *et al*. 2008a) with roughly 150 recognized species arranged in 12 monophyletic genera (Guayasamin *et al*. 2009) distributed throughout the Neotropical wet forests from southern Mexico to southern Bolivia. A recent taxonomic effort on this group of batrachians during the last years has led to an increase of its alpha-diversity; however, several taxonomic problems are pending resolution (e.g. Kok & Castroviejo-Fisher 2007; Castroviejo-Fisher *et al*. 2008).

In this work, we describe a new species of the genus *Hyalinobatrachium* that has been previously misidentified with *H. bergeri* (Cannatella 1980) and *H. munozorum* (Lynch & Duellman 1973). We use morphological character states, morphometrics, bioacoustics and genetics to support the new species. Moreover, we compare advertisement calls previously assigned to *H. bergeri* with new recordings of this species and the one described herein, and conclude that some previous identifications were erroneous. Accordingly, we describe for the first time the advertisement call of *H. bergeri*. Additionally, we compare

material of *H. lemur* Duellman & Schulte (1993) and *H. pellucidum* (Lynch & Duellman 1973) (including all type specimens) and conclude that the former is a junior synonym of the later.

We work under the theoretical framework that defines a species as a temporal segment of a populational or metapopulational lineage evolving separately from other lineages (Simpson 1961, modified by Wiley 1978; Frost & Kluge 1994; Mayden 1997, 2002; de Queiroz 1998, 2005a, b, c, 2007), and use an integrative taxonomic approach that exploits multiple lines of evidence to delimit species boundaries (Dayrat 2005; Padial *et al.* 2009).

Material and methods

Nomenclature and terminology. We followed Guayasamin *et al.* (2009) classification. Institution acronyms are those of Frost (2009) with the addition of Colección de Vertebrados del Centro de Biodiversidad y Genética (CBG), Cochabamba, Bolivia. When possible, field numbers for type specimens of the new species are indicated with the initials of the collectors Ignacio De la Riva and José M. Padial.

Morphology. Specimens examined other than those used for the description of the new species are listed in Appendix I. Voucher specimens were preserved in 70% ethanol and fixed in formalin (4–10%). For the description of morphological and color characteristics we followed Lynch & Duellman (1973), Flores (1985), Señaris & Ayarzagüena (2005), Cisneros-Heredia & McDiarmid (2007), and Kok & Castroviejo-Fisher (2008). Diagnostic characters were arranged according to Cisneros-Heredia & McDiarmid (2007). Terminology for webbing formula follows Savage & Heyer (1967) as modified by Guayasamin *et al*. (2006).

With a digital caliper to the nearest 0.1 mm, we took the following measurements: snout-vent length (SVL); head length (from rictus to tip of snout, HL); head width (at the level of rictus, HW); shortest interorbital distance (IOD); eye diameter (horizontal, EL); upper eyelid width (EW); distance from anterior margin of eye to tip of snout (ES); width of the disc of the third finger (FIII); thigh length (distance from the middle of the cloacal slit to the proximal part of the femur-tibia articulation, TL); shank length (from the femur-tibia articulation to the tibia-heel proximal articulation, SL); foot length (FL). Throughout this paper, the observed range for each measurement is followed by mean ± standard deviation.

Color characteristics were noted from living individuals, field photographs, descriptions, and photographs in the literature (Lynch & Duellman 1973; Duellman & Schulte 1993; Señaris & Ayarzagüena 2005).

Bioacoustics. We recorded frog vocalizations in the field. Sound recording equipment included a Sony WM D6C tape recorder and a Sennheiser Me 80 directional microphone. Recordings were processed on an Apple Macintosh computer. The sounds were digitized and edited at a sampling frequency of 44.1 KHz and 16 bit resolution with a Delta 66 digitizing board and Peak 3.2 software. We also analyzed the recordings of the advertisement calls of purported *H. bergeri* included in Márquez *et al*. (1996) and De la Riva (2002). All calls were edited using Audacity 1.2.6 for MacOS X (Mazzoni & Dannenberg 1999). The software Praat 4.5.02 for MacOS X (Boersma & Weenink 2006) was used to obtain numerical information and to generate audiospectrograms and oscillograms. Frequency information was obtained through Fast Fourier Transformations (FFT) (width, 1024 points). Air temperature was measured immediately after sound recording. We measured the following acoustic variables: call duration, dominant frequency, lower and upper frequency (minimum and maximum frequency at the fundamental call), and call rate. For acoustic variables, we follow the terminology of Márquez *et al*. (1995).

Genetics. We used two criteria to delimit species boundaries using DNA data: reciprocal monophyly and genetic distances. The first criterion is based on the assumption that coalescent patterns in gene genealogies are related to historical processes that originate separate lineages (e. g. Avise 2000; Knowles & Carstens 2007). The second assumes that genetic variation within a species tends to be relatively small because of constant gene flow, whereas variation among species increases with time; however, following Padial *et al*. (2009), we do not use thresholds to delimit species boundaries.

Specimens used in the genetic analyses and their GenBank accession numbers are listed in Appendix II. Genomic DNA was extracted from 35 specimens belonging to eight species using a standard phenol-

chloroform extraction protocol (Sambrook *et al.* 1989). A fragment of approximately 850 bp of the mitochondrial ribosomal gene 16S was amplified and sequenced using previously described primers (16SC-5' and 16Sbr-3') and PCR conditions (Hillis *et al.* 1996). Sequences from heavy and light strands were compared to generate a consensus sequence for each specimen using Sequencher (Gene Codes Corporation 2000). Additionally, we used eight DNA sequences available at GenBank. The 16S fragment was aligned with the software Mafft (Katoh *et al.* 2005) under the L-INS-i strategy and default parameters. We used the program MODELTEST 3.7 (Posada & Crandall 1998) to select the model of sequence evolution that best fits the data. The model and the parameter estimates were chosen by Akaike's information criterion (Akaike 1974). The chosen model was GTR + I + G (General Time Reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites). For Bayesian phylogenetic analyses (Rannala & Yang 1996) we used MrBayes version 3.2.1 (Huelsenbeck & Ronquist 2001). The majority rule consensus tree was produced from two independent runs, each with one cold (the head chain) and three heated Monte Carlo Markov chains (MCMC) (Yang & Rannala 1997), run for five million generations (Metropolis-coupled MCMC). Trees were sampled every 1000 generations. Burn-in was evaluated by examination of the standard deviation of split frequencies and the likelihood -lnL.

We used PAUP* 4.0b10 (Swofford 2002) to calculate uncorrected pair-wise distances (p). The same program was used to construct Maximum Parsimony (MP) under heuristic searches (1000 stepwise random additions with TBR branch swaping) and neighbor joining (NJ) phylogenetic trees (using uncorrected p–distances). Based on the phylogeny of Guayasamin *et al.* (2008a), we used *Centrolene gorzulae* (Ayarzagüena 1992) to root the phylogenetic tree. The support of the internal nodes in the trees was assessed using 1000 nonparametric bootstrap pseudoreplicates for the MP and NJ trees, and Bayesian posterior probabilities (BPP) for the MrBayes analyses.

Hyalinobatrachium carlesvilai **new species**
(Figs. 1A–C, 2A–C, 3A, 7A–B)

Holotype. MHNCP 5339 (IDL 4545), adult male from a point between Santa Rosa and San Juan del Oro (14º12'49.1" S, 69º08'09.5" W; 1135 m), Provincia Sandia, Departamento Puno, Peru, collected by I. De la Riva, S. Castroviejo-Fisher, J.C. Chaparro, J. Bosch and J. M. Padial on 12 February 2006.

Paratopotype. MNCN 43689 (IDLR 4546), adult male, same data as holotype.

Paratypes. MNCN 43690–2 (IDLR 4557–9), adult males; MHNCP 5344 (IDLR 4560), adult female from a point three kilometers from the type locality (14º12'49.1" S, 69º08'09.5" W; 1135 m), Provincia Sandia, Departamento Puno, Peru, collected by I. De la Riva, S. Castroviejo-Fisher, J.C. Chaparro, J. Bosch and J.M. Padial on 12 February 2006; MNCN 44213 (IDLR 4759), adult male from a point placed fifteen kilometers from Quincemil towards Puerto Maldonado (13°12'03.6'' S, 70°40'28.9'' W; 572 m), Provincia Quispicanchis, Departamento Cusco, Peru, collected by I. De la Riva, S. Castroviejo-Fisher, J.C. Chaparro and J.M. Padial on 02 February 2007; MHNCP 6688, adult male from Cueva de los Guácharos (09°19'33.4" S, 76º01'45.7" W; 674m), Tingo Maria, Provincia Leoncio Prado, Departamento de Huánuco, Peru, collected by J.C. Chaparro, J.A. Ochoa and R. Gutiérrez on 02 November 2007; MHNCP 5434, adult male, first "*chacra*" (crop clearing) after the Puesto de Vigilancia 3 de Mayo (09º25'10.5" S, 75º58'15.0" W; 723 m), Parque Nacional Tingo María, Distrito Mariano Damazo Veraun, Provincia Leoncio Prado, Departamento Huánuco, Peru, collected by J.C. Chaparro on 15 September 2006; MNCN 42797 (JMP 920), adult male from Paractito-los Guácharos (17º03' S, 65º28' W; 500 m), Provincia Chapare, Departamento Cochabamba, Bolivia, collected by J.M. Padial on 01 March 2004; CBG 1139,1140, adult males from Río Leche (17º16' S, 64º45' W; 500 m), Provincia Carrasco, Departamento de Cochabamba, Bolivia, collected by R. Aguayo and R. Rivas on 24 October 2004; ZFMK 75238, adult male from 7 km on road south of Paractito (17°04' S, 65°29' W; 500 m), Provincia Chapare, Departamento Cochabamba, Bolivia, collected by J. Köhler on 03 February 1998.
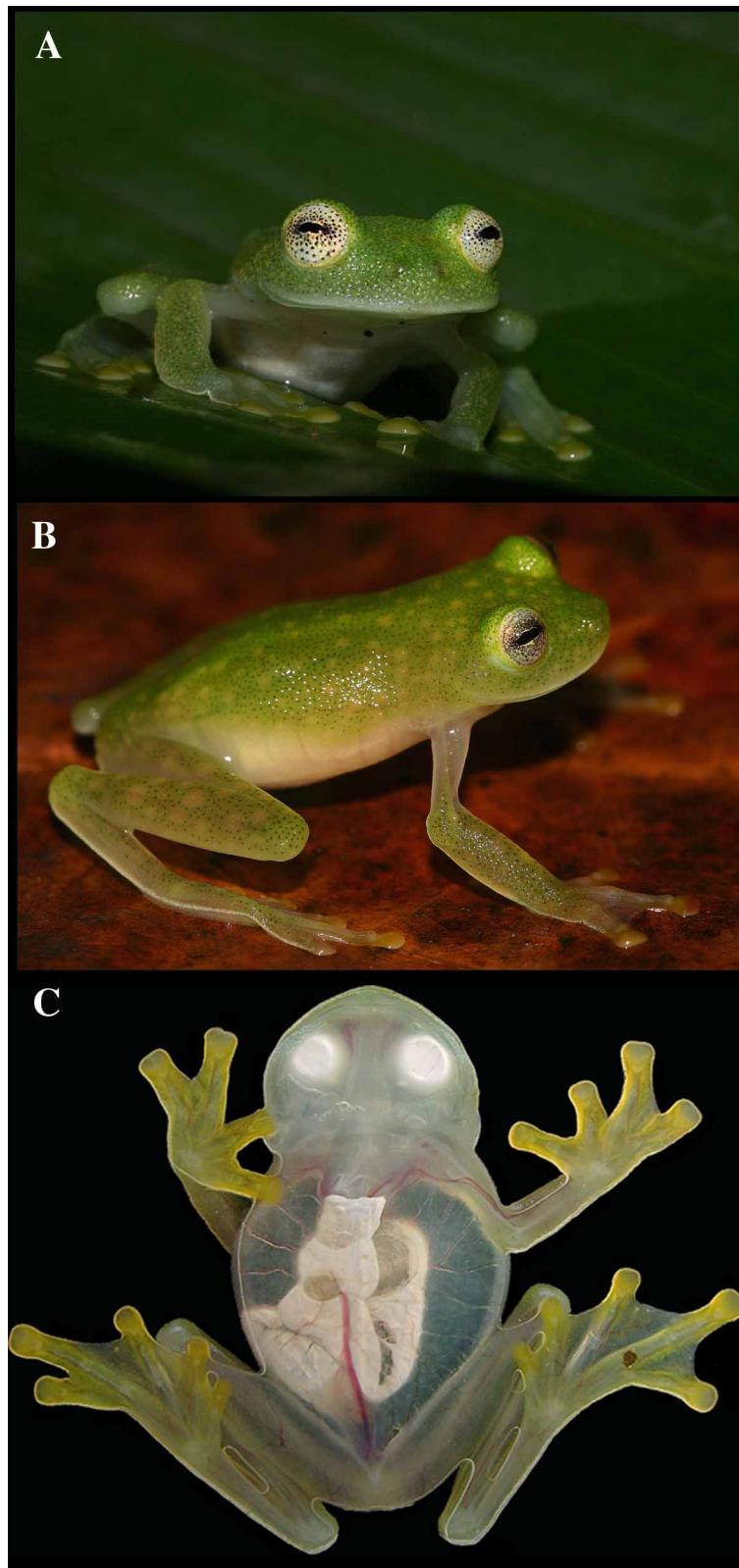
FIGURE 1. *Hyalinobatrachium carlesvilai* **sp. nov. (A)** paratype MNCN 44213 (photo JMP); **(B)** paratype MHNCP 5434 (photo JCC); **(C)** CET (adult male, specimen not yet catalogued, photo J. Ayarzagüena).
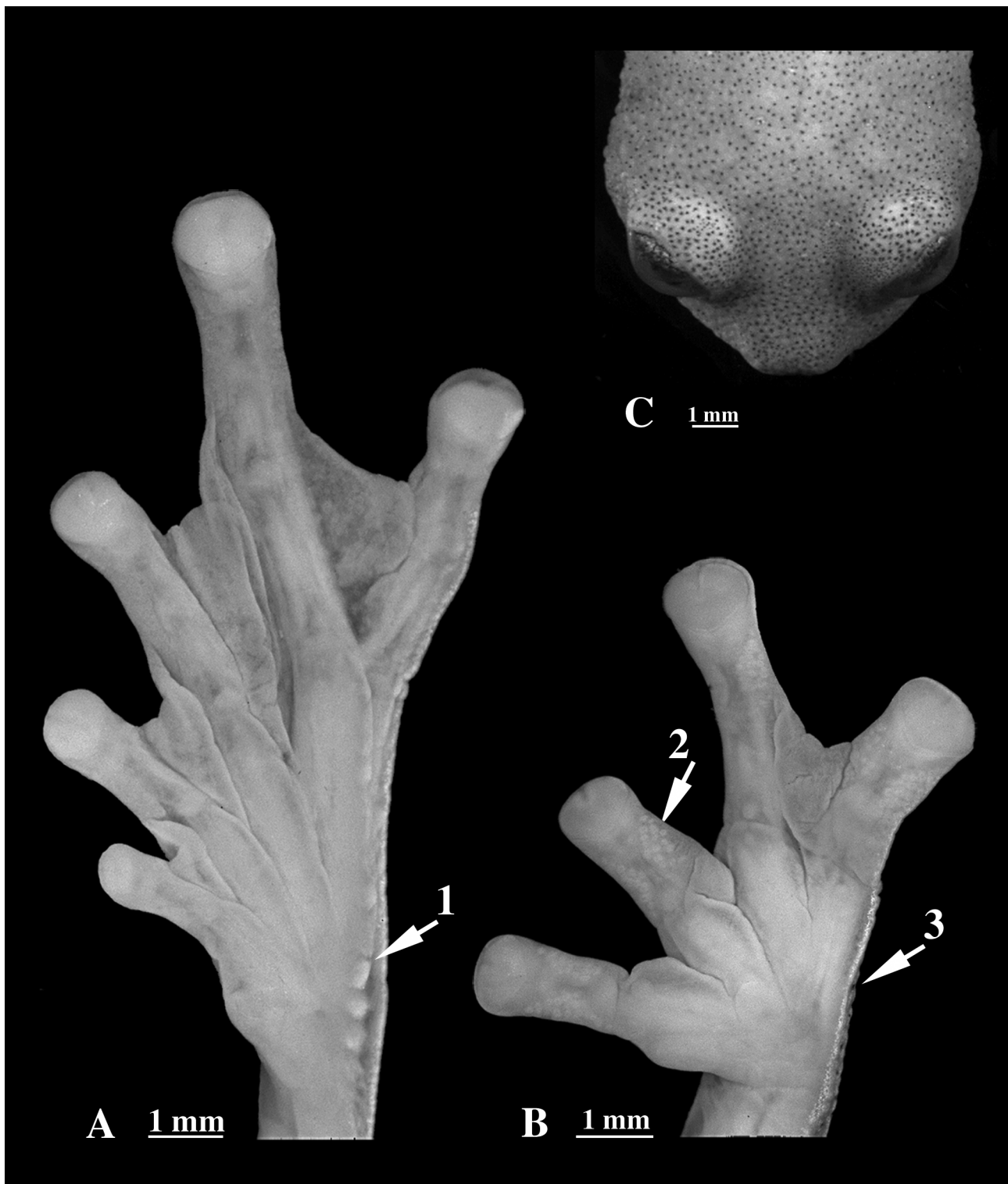
FIGURE 2. Details of the foot **(A)**, hand **(B)**, and head **(C)** of *Hyalinobatrachium carlesvilai* **sp. nov.** paratype MNCN 43691 (photos SCF). 1 = enameled tarsal fold; 2 = nuptial glands; 3 = enameled tarsal fold.

Diagnosis. The new species is placed in the genus *Hyalinobatrachium* because of the following characters: (1) humeral spine absent (Savage 1967); (2) digestive tract and bulbous liver covered by white peritonea (Savage 1967); (3) completely transparent ventral parietal peritoneum (Savage 1967); (4) white bones in life (Savage 1967); (5) dorsal coloration in preservative white or cream (Savage 1967); (6) males lack conspicuous dorsal spinules during breeding season; (7) when present, nuptial pad small and restricted to

the inner edge of Finger **I** in males (Type V of Cisneros-Heredia & McDiarmid 2007); (8) dentigerous process of the vomer and vomerine teeth absent (Ruiz-Carranza & Lynch 1991; Savage 1967); (9) males usually vocalize from the underside of leaves, and females deposit one layer of eggs on the underside of leaves (Ruiz-Carranza & Lynch 1998).
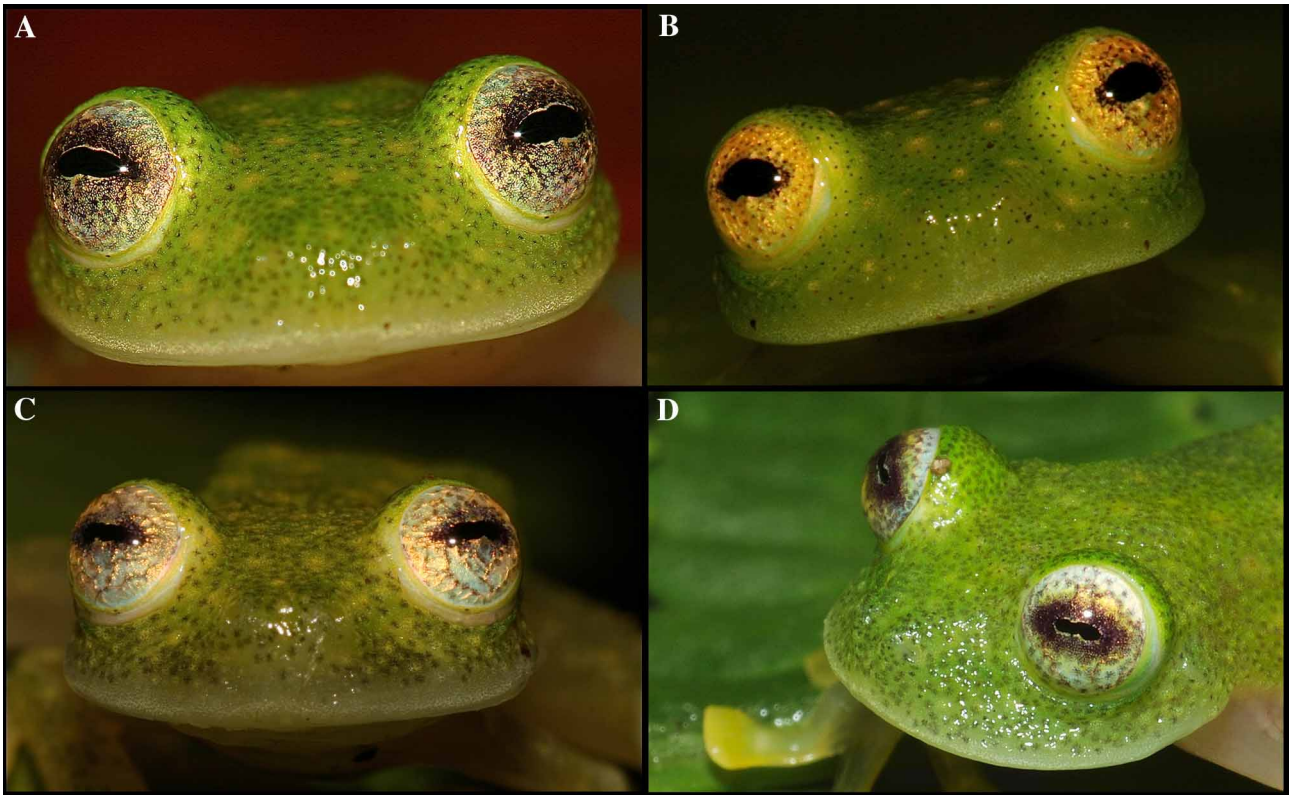

FIGURE 3. Irises of *Hyalinobatrachium carlesvilai* **sp. nov.** paratype MNCN 44213 **(A)** (photo JMP); *H. pellucidum* MHNCP 4880, adult male **(B)** (photo JMP); *H. bergeri* MHNCP 5713, gravid female **(C)** (photo JMP) and **(D)** (photo IDLR).

The following combination of characters distinguishes *Hyalinobatrachium carlesvilai* **sp. nov.** from other species of the genus: (1) dentigerous process on vomer absent; (2) snout truncate in dorsal and lateral view; (3) tympanum absent, tympanic annulus indistinct; (4) dorsal skin finely shagreened in life and preservative; (5) ventral skin granular, cloacal ornamentation consisting of small enameled tubercles and folds, enlarged paired round tubercles below vent absent; (6) parietal peritoneum transparent, pericardial, hepatic and visceral peritonea white, all other peritonea transparent; (7) liver bulbous; (8) humeral spine in adult males absent; (9) finger webbing **III** 2^- – 1^+ **IV**, absent between Fingers **I** and **II** and basal between Fingers **II** and **III**; (10) toe webbing **I** $1 - 2^-$ **II** $1 - 2^-$ **III** $1 - 1^{1/2}$ **IV** $1^{1/2} - 1$ **V**; (11) fringe on postaxial edge of Finger **IV** present and enameled, metacarpal fold present and enameled, ulnar fold present and enameled; fringe on postaxial edge of Toe V present and enameled, metatarsal fold present and enameled, tarsal fold present and enameled; (12) nuptial excrescence formed by a group of glands on Finger **I** (Type V), extending to the lateral fringes and membranes of the other fingers; glands slightly visible in the webbing between toes **IV** and **V**; prepollex not enlarged; prepollical spine not projecting (spine not exposed); (13) when adpressed, Finger **I** longer than **II**; (14) diameter of eye about 2X width of disc on Finger **III**; (15) coloration in life: dorsal surface lime green with small yellow spots and minute melanophores, bones white; (16) coloration in preservative: dorsum cream with dark melanophores; (17) iris coloration in life: cream with black flecks and an incomplete pale yellow ring around pupil; (18) distribution of melanophores on digits constant, only present on the proximal edge of Fingers **I** and toes **IV**–**V**; in life, hands and feet light green, tips of fingers and toes orange; (19) males call from the under side of leaves; advertisement call consisting of a single note, the first third being pulsed

and the second third tonal; call duration 0.102–0.152 s, dominant frequency 4617.33–4915.36 Hz; (20) clutches deposited on vegetation overhanging streams on the under side of leaves, clutch size 27–32 eggs ($n =$ 2); clutches observed were guarded by males; (21) SVL in males 20.6–23.9 mm ($n = 7$); in females 23.2 mm ($n = 1$). Combat behavior and tadpoles are unknown for the new species.
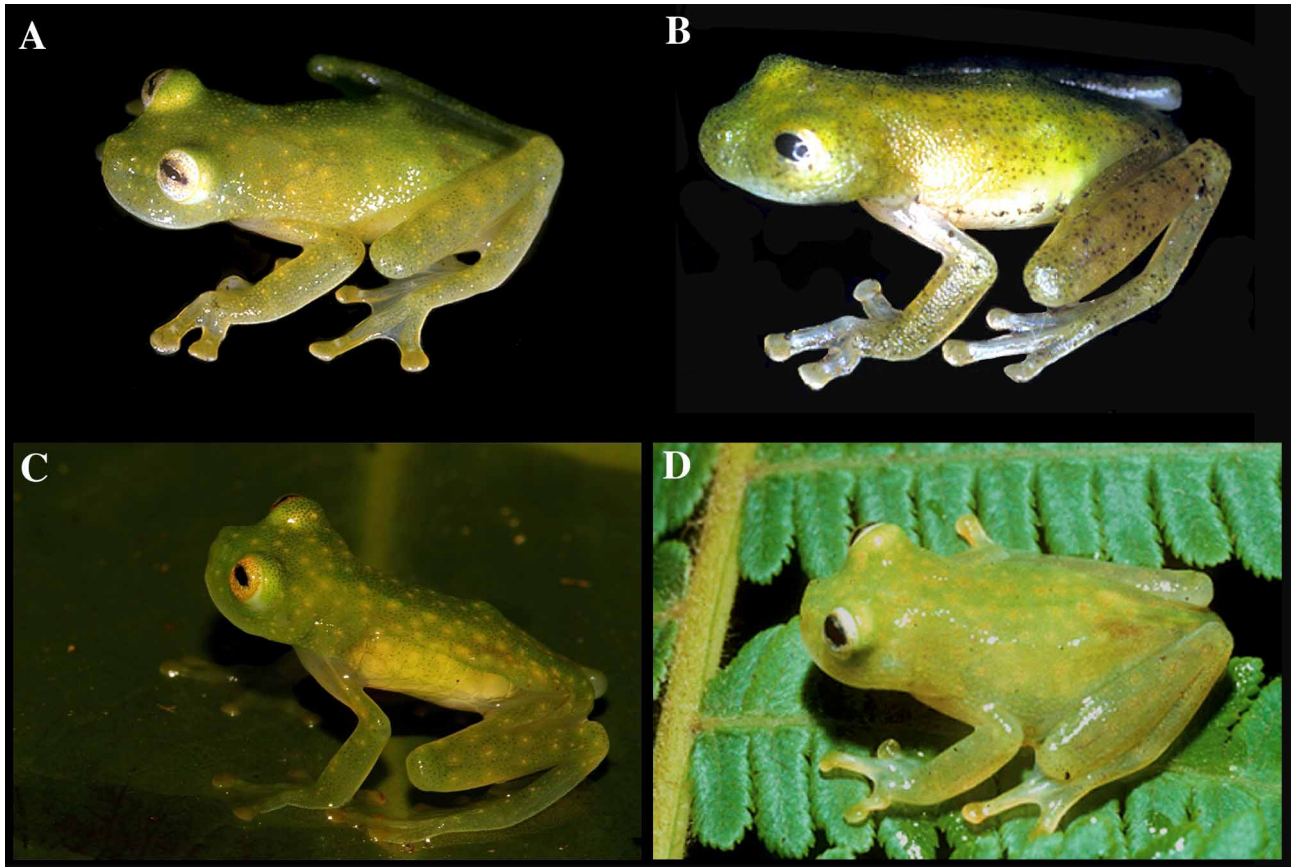

FIGURE 4. *Hyalinobatrachium pellucidum*, holotype (**A**) (photo Natural History Museum, University of Kansas); *Hyalinobatrachium munozorum*, holotype (**B**) (photo Natural History Museum, University of Kansas); *Hyalinobatrachium pellucidum*, adult male, MHNCP 4880 (**C**) (photo JMP); *Hyalinobatrachium pellucidum,* holotype of *H. lemur* (**D**) (photo Natural History Museum, University of Kansas).

Comparisons. All described species of *Hyalinobatrachium* known to occur in the Amazonian slopes of the Andes are endemic to this area. Because the new species described here inhabits this ecoregion, we constrict our comparisons to those *Hyalinobatrachium* species occurring in Amazonian slopes of the Andes. *Hyalinobatrachium carlesvilai* **sp. nov.** can be distinguished from *H. bergeri* (SE Peru and Bolivia), by (character of the former in parentheses) webbing formula on hand **III** $3 – 2^+$ **IV** (**III** $2^- – 1^+$ **IV**; Fig. 2), pericardium at least partially transparent (completely white), and presence of a dark grey ring around the pupil (absent; Fig. 3). *Hyalinobatrachium esmeralda* Ruiz-Carranza & Lynch (1998), from the eastern Cordillera of Colombia, has a hand webbing formula **III** $2^+ – 2^+$ **IV** (**III** $2^- – 1^+$ **IV**), transparent pericardium [but see Ruiz-Carranza & Lynch (1998; 4C)] (white pericardium), *canthus rostralis* not defined (defined), and round snout in dorsal and lateral view (truncate; Fig. 2). *Hyalinobatrachium lemur* (Fig. 4D), from Departamento San Martín in northern Peru (but see below), has a transparent pericardium (white), and has weak or absent humeral and tarsal folds (enameled ulnar and tarsal folds). *Hyalinobatrachium munozorum* (Fig. 4B), from the eastern Amazonian lowlands of Colombia, Ecuador and Peru (but see below for Peruvian localities), has a round snout in dorsal and lateral view (truncate), and lacks humeral and tarsal dermal folds (enameled ulnar and tarsal folds). *Hyalinobatrachium pellucidum* (Figs. 4A, C), from the Amazonian versant of the Andes in Ecuador (but see below for geographical extension), has a round snout in dorsal and lateral view (truncate),

golden iris (creamy white; Fig. 3), webbing formula on hand **III** 2^+ – 2 **IV** (**III** 2^- – 1^+ **IV**), and a transparent pericardium (white). *Hyalinobatrachium ruedai* Ruiz-Carranza & Lynch (1998), from the Amazonian slopes of the Andes in Colombia and Ecuador, has medium sized melanophores (absent), and an intense golden iris with a pupillary ring (cream and pupillar ring absent). We summarize in Table 1 characteristics that distinguish these species of *Hyalinobatrachium*.

TABLE 1. Character states in species of the *Hyalinobatrachium fleischmanni* Group from the Amazonian lowlands and slopes of the Andes, plus the similar species *H. fleischmanni* and *H. tatayoi*. Males snout-vent length (SVL) in mm; snout shape in profile.

Taxa	SVL	Snout	Hand webbing	Pericardium	Iris
H. bergeri	20.3–22.4	Truncate	III $3 - 2^+$ IV	Partially transparent	Dark grey ring around pupil
H. carlesvilai	20.6–23.6	Truncate	III $2^- - 1^+$ IV	White	Creamy with dark flecks
H. esmeralda	21.2–22.4	Round	III $2^+ - 2^+$ IV	White/Transparent	Golden with brownish flecks
H. fleischmanni	19.0–28.0	Subacuminated	III $2^{1/}_2 - 2$ IV	White	Yellow with dark flecks
H. munozorum	18.8–20.5	Round	III $2^- - 1^+$ IV	White/Transparent	Pale gold
H. pellucidum	20.4–21.4	Truncate	III $2 - 2$ IV	Transparent	Yellow with dark flecks
H. ruedai	20.2–22.6	Truncate	III $2 - 1^{3/}_4$ IV	White	Golden with dark flecks
H. tatayoi	21.5–22.4	Semiround	III $2^- - 1^{1/}_2$ IV	White	Yellow with dark flecks

Description of the holotype. Adult male of small size, SVL 22.4 mm; head slightly wider than body, HW 36% of SVL; head slightly wider than long (HW/HL = 1.2); snout truncate in dorsal view and profile; ES/EL = 0.75 and ES/IOD = 1.0; loreal region slightly concave; nostrils slightly prominent, round; internarial region depressed; *canthus rostralis* defined; eyes small, directed antero-laterally; EL 47% of HL; EW/IOD = 0.8; tympanic annulus indistinct, tympanic membrane absent, supratympanic fold absent; dentigerous processes on vomers absent; choanae small, circular, widely separated; tongue elongate, ovoid, not attached to mouth posteriorly for about one sixth of its length; vocal slits extending from the sides of the base of tongue to the level of the mandibular joints. Forearms slim; diameter of forearms about one and a half times the diameter of upper arms; enameled ulnar fold remarkably evident; humeral spine absent; relative length of fingers: **II** < **I** < **IV** < **III**; finger discs wide, truncated and larger than those of toes; FIII 40% of EL; webbing absent between fingers **I**–**II** and basal between **II**–**III**, webbing formula on hand **III** $2^- - 1^+$ **IV**; subarticular tubercles round and small; supernumerary tubercles slightly appreciable; palmar tubercle round and small, thenar tubercle small and elongated; nuptial excrescences Type V, glands present on the lateral fringes of fingers and the sides of membrane between fingers **III** and **IV**; hind limbs slender; SL 55% of SVL; enameled tarsal fold remarkably evident; discs of toes round, truncate in profile; inner metatarsal tubercle small and ovoid; outer metatarsal tubercle absent; supernumerary tubercles slightly appreciable; webbing formula of feet **I** $1 - 2^-$ **II** $1 - 2^-$ **III** $1 - 1^{1/2}$ **IV** $1^{1/2} - 1$ **V**. In preservative, dorsal skin scarcely covered with enameled granules, area around tympanum almost granular; skin on belly and thighs granular, other ventral surfaces smooth; cloacal opening directed posteriorly at upper level of thighs, concealed by a dermal fold and flanked by evident and enameled irregular folds and warts.

Coloration in life. Dorsal surfaces light green dusted with minute black melanophores and with dull yellow spots. Enameled tarsal and dorsal folds. Cloacal ornamentation consisting of enameled warts and folds. Dorsal surface of tip of fingers orange. Dull creamy iris dotted with dark flecks. Parietal peritoneum transparent, pericardium, hepatic, and visceral peritonea white, peritonea covering the gall bladder and other internal organs (urinary bladder, gonads, kidneys) not mentioned before transparent.

Coloration in preservative. General appearance cream. Dorsal surfaces dotted by a coat of minute dark melanophores, which leave uncovered cream spots. Dorsum with a fine layer of guanophores only appreciable

under magnification. Enameled tarsal, ulnar and anal folds. Iris white. Other surfaces cream. Peritonea as stated above.

Measurements. Holotype morphometrics are as follow: SVL = 22.4; HL = 6.8; HW = 8.1; IOD = 2.4; EL = 3.2; EW = 1.9; ES = 2.4; FIII = 1.3; TL = 12.3; SL = 12.2; FL = 10.8. Measurements of the complete paratype series but ZFMK 75238, which was not measured, are in Table 2.

TABLE 2. Measurements in mm of the type series (excluding paratype ZFMK 75238) of *Hyalinobatrachium carlesvilai* **sp. nov.**

	MNCN 44213	MHNCP 5339	MNCN 43689	MNCN 43690	MNCN 43691	MNCN 43692	MNCN 42797	MHNCP 5344	CBG 1139	CBG 1140
SVL	23.9	23.1	23.6	22.4	22.7	20.6	23.5	23.2	21.92	23.9
HL	6.5	7.1	6.9	6.8	7.2	6.2	6.7	6.6	6.9	7.2
HW	8.5	8.2	8.3	8.1	8.4	8.1	8.7	8.5	8.4	8.7
IOD	2.3	2.3	2.7	2.4	2.6	2.5	2.7	2.7	2.3	2.5
EL	2.2	3.5	3.5	3.2	3.1	3.1	3.5	3.5	2.5	2.6
EW	2.1	2.3	2.2	1.9	1.9	1.7	2.0	1.8	2.0	2.1
ES	2.4	2.3	2.2	2.4	2.4	1.7	2.8	2.2	1.7	1.8
FIII	2.4	1.1	1.4	1.3	1.2	1.2	1.1	1.4	1.1	1.4
TL	12.5	10.5	12.1	12.3	11.9	10.4	11.7	12.4	12.4	12.8
SL	13.4	11.4	12.3	12.2	12.6	11.0	12.4	12.7	12.6	13.4
FL	10.6	10.0	10.8	10.8	11.0	9.8	10.5	10.3	9.9	10.6

Variation. No significant variation was appreciated through the type series.

Distribution and ecology. *Hyalinobatrachium carlesvilai* **sp. nov.** is known from the Amazonian forest of the Andean slopes in Peru and Bolivia (Fig. 5). In Peru it is known from Tingo María and the valleys of Marcapata and upper Tambopata, Departments of Huánuco, Cusco, and Puno respectively, and in Bolivia it has been found in the Provinces of Chapare and Carrasco (Departamento Cochabamba) and the Amboró National Park (Provincia Ichilo, Departamento Santa Cruz), covering an airline distance of approximately 1000 km (Fig. 6). We have found specimens between 300 and 1200 m. We found specimens on leaves (1–3 m above water) along streams and rivers between dusk and midnight. All males collected were calling from the underside of leaves (Fig. 7A). The only female collected (paratype MHNCP 5344; Fig. 7B) was on the same leaf as the paratype MNCN 43690, which was attending an egg clutch (Fig. 7C). The holotype MHNCP 5339 was found guarding a clutch of 27 eggs in advanced state of development (Fig. 7D), both the specimen and the clutch were in the same leaf. The egg clutch was collected and preserved in ethanol 70% (MNCN/DNA 8999). We did not visit egg clutches during the day. *Hyalinobatrachium carlesvilai* **sp. nov.** occurs in sympatry with *H. bergeri*, both occupy approximately the same altitudinal range and have the same reproductive strategy. Despite this apparent overlap in their niches, both species are abundant, suggesting little effect of potential competition. In neighboring areas occupied by *H. carlesvilai* **sp. nov.** in SE Peru, we collected other anurans, namely *Atelopus* sp., *Teratohyla* aff. *amelie, Amereega simulans, Hypsiboas balzani, Hypsiboas boans, Pristimantis fenestratus,* and *P.* cf. *martiae*.

Advertisement call. We recorded and analyzed a total of 23 notes from two males (MHNCP 5339 and MNCN 4557); air temperature was 21°C. The advertisement call consists of a single high-pitched note that was clearly audible at long distances, the first third being pulsed and the second third tonal (Fig. 8). It lasts 0.102–0.152 seconds ($\bar{X} = 0.134 \pm 0.013$) with a call repetition rate of 4.6 calls/minute. The dominant frequency is 4617.33–4915.36 Hz ($\bar{X} = 4837.95 \pm 85.79$) and the call rises in frequency from 3606.40–4112.80 Hz ($\bar{X} = 4019.6 \pm 137.85$) at the beginning of the call to 50693.30–5519.40 Hz ($\bar{X} = 5225.33 \pm 140.99$) at the end. The first third of all recorded calls starts with a group of short pulses (2–4) that

shows a fast rise in frequency. The following two thirds of the call consist of a tonal section at slightly increasing frequency. The maximum amplitude of the call is reached at the beginning of the tonal section.

We realized that the call here described is basically identical to that described by Márquez *et al*. (1996) and later published by De la Riva (2002) for Bolivian purported *H. bergeri*. Accordingly, we analyzed those recordings and compare them to our new recordings of *H. bergeri*. We summarize the results in Table 3 and Figure 9. Our analysis shows that the call assigned to *H. bergeri* by Márquez *et al*. (1996) and De la Riva (2002) actually corresponds to the new species herein described. Both calls overlap in all the parameters studied and show the same structure. On the other hand, the calls emitted by *H. bergeri* (twenty eight calls corresponding to the vouchers MHNCP 5394 and MHNCP 5408, plus a non-collected specimen and recorded at 20–25°c) are remarkably different. The call of *H. bergeri* is completely tonal lacking the characteristic pulsed start of that of *H. carlesvilai* **sp. nov.** It is emitted at a constant frequency between 3775.20–4337.90 Hz ($\bar{X}$ = 4084.68 ± 165.28) and 4788.10–5013.10 Hz ($\bar{X}$ = 4876.36 ± 60.12) with a dominant frequency of 4489.60–4659.91 Hz ($\bar{X}$ = 4599.08 ± 69.94).

TABLE 3. Characteristics of the advertisement call of *Hyalinobatrachium bergeri* and *H. carlesvilai* **sp. nov.** *Hyalinobatrachium carlesvilai** refers to the call previously assigned to *H. bergeri* by Márquez *et al*. (1996) and De la Riva (2002) and that we identified as *H. carlesvilai*. Time is given seconds and frequency in Hertz.

	H. bergeri (n = 28)	*H. carlesvilai** (n = 8)	*H. carlesvilai* (n = 23)
Structure	Completely tonal at constant frequency	First third pulsed and then tonal, increasing frequency	First third pulsed and then tonal, increasing frequency
Maximum amplitude	Beginning of the call	Beginning of the tonal section	Beginning of the tonal section
Notes per call	1	1	1
Duration	0.129–0.198 (0.154 ± 0.019)	0.134–0.163 (0.146 ± 0.009)	0.102–0.152 (0.134 ± 0.013)
Dominant frequency	4489.6–4659.91 (4599.08 ± 69.94)	4489.60–4574.75 (4574.75 ± 45.52)	4617.33–4915.36 (4837.95 ± 85.79)
Lower frequency	3775.20–4337.90 (4084.68 ± 165.28)	3381.30–3775.20 (3648.60 ± 174.74)	3606.40–4112.80 (4019.06 ± 137.85)
Upper frequency	4788.00–5013.10 (4876.36 ± 60.12)	4788.00–4956.80 (4886.46 ± 58.23)	5069.30–5519.40 (5225.33 ± 140.99)
Other frequencies	9000; 13500; 18000	9000	–

Phylogenetic relationships. Our genetic analyses (Figs. 9–10) support the hypothesis of *Hyalinobatrachium carlesvilae* **sp. nov.** as an independently evolving lineage. Our results indicate that the sequences of the mtDNA gene analyzed are reciprocally monophyletic to all the other sequences analyzed, with little variation between and within populations (genetic distances 0–1 %) but clearly divergent from its sister clade *H. fleischmanni* + *H. tatayoi* (genetic distances 4.8–6.2 %). The topologies of the MP and NJ trees are congruent with our Bayesian tree (Figs. 9–10). Additionally, Guayasamin *et al.* (2008a) provided genetic analyses of six loci that fully support recognition of *H. carlesvilai* **sp. nov.** (referred as *Hyalinobatrachium* aff. *munozorum* therein) as an independent evolving lineage that has been isolated from other recognized species of *Hyalinobatrachium* for a large time scale.

Etymology. The name is a patronym for Carles Vilà and is a noun in the genitive case. We take pleasure to dedicate this species to our dear friend in recognition of his scientific work on animal conservation, evolutionary biology, and domestication and for his continuous support on centrolenid research and companionship both in the field and the lab.

FIGURE 5. Habitat of *Hyalinobatrachium carlesvilai* **sp. nov.** in Peru. Quincemil, Cusco (left and bottom right); between Santa Rosa and San Juan del Oro, Puno (top right).

Discussion

Species of the genus *Hyalinobatrachium* show subtle morphological differences, which make species identification, comparison, and description a difficult task. This situation is evidenced by the confusing taxonomic status of several species (see Kok & Castroviejo-Fisher 2008; Castroviejo-Fisher *et al.* 2008) and has led to the recent recognition of several synonyms (Cisneros-Heredia & McDiarmid 2007; Guayasamin *et al.* 2008b).

Hyalinobatrachium species from the Amazonian slopes of the Andes and neighboring lowland forests show some taxonomic problems. The description of *Hyalinobatrachium lemur* falls within the variability exhibited by *H. pellucidum*, to the point that Cisneros-Heredia & McDiarmid (2007) suggested that they could be conspecifics. We studied the type series of both species and additional specimens deposited at KU (see Appendix I for details). Additionally, we studied the morphology and genetics of one specimen from southern Peru (MHNCP 4880) and the sequence and photograph of one Ecuadorian specimen (Appendix II). We compared the two diagnostic characters proposed by Duellman & Schulte (1993: 29) to distinguish *Hyalinobatrachium lemur* from *H. pellucidum* (characters of the latter in parentheses) and demonstrate how our new observations justify the consideration of *H. lemur* as a junior synonym of *H. pellucidum*. (1) Dorsal skin shagreened (smooth); we observed that both holotypes have a shagreened dorsal skin, although is more marked in the holotype of *H. pellucidum*. (2) Two free phalanges in the web between Fingers **III**–**IV** (one free phalange); both specimens have the same hand webbing formula **III** 2 – 2 **IV**. Although not discussed by Duellman & Schulte (1993) the holotype of *H. pellucidum* has marked and enameled ulnar, tarsal and cloacal folds, while that of *H. lemur* only shows weak and not enameled folds. We interpret these differences as products of preservation artifacts (the muscles of dried out specimens might contract leaving more marked folds) and intraspecific variation. Two additional specimens coming from the same locality, and nearby the type locality of *H. lemur* (~ 45 Km straight line), show intermediate states regarding ulnar, tarsal and cloacal folds. The specimen KU 217297 has enameled but weak ulnar fold, weak but not enameled tarsal fold, and an enameled and weak cloacal fold; on the other hand, KU 217295 has enameled and marked ulnar fold, enameled but weak tarsal fold, and enameled and marked cloacal fold (the specimen KU 217296, although coming from the same locality than the previous ones, is very poorly preserved and characters could not be determined with confidence). Furthermore, the specimen MHNCP 4880 (collected in Cusco, Peru and that we

assign to *H. pellucidum*) has very weak and hardly enameled ulnar, tarsal, and cloacal folds. Duellman & Schulte (1993) assigned KU 217295–7 to *H. munozorum* arguing that the three specimens have snout round in profile. However, these three specimens are poorly preserved and current snout shape is most likely a preservation artifact (i.e. specimens have been compressed at some point during preservation). We assign them to *H. pellucidum* because both share a transparent pericardium, finger webbing formula **III** 2 – 2 **IV** and possibly snout truncate in dorsal view.

Our genetic comparison indicates that in spite of the large geographic distance (~ 1500 km straight line) between the Ecuadorian populations and the southern Peruvian specimens, genetic differences for the studied marker are small (genetic distance 1 %) and both cluster together with high support (bootstrap and BPP of 100%).
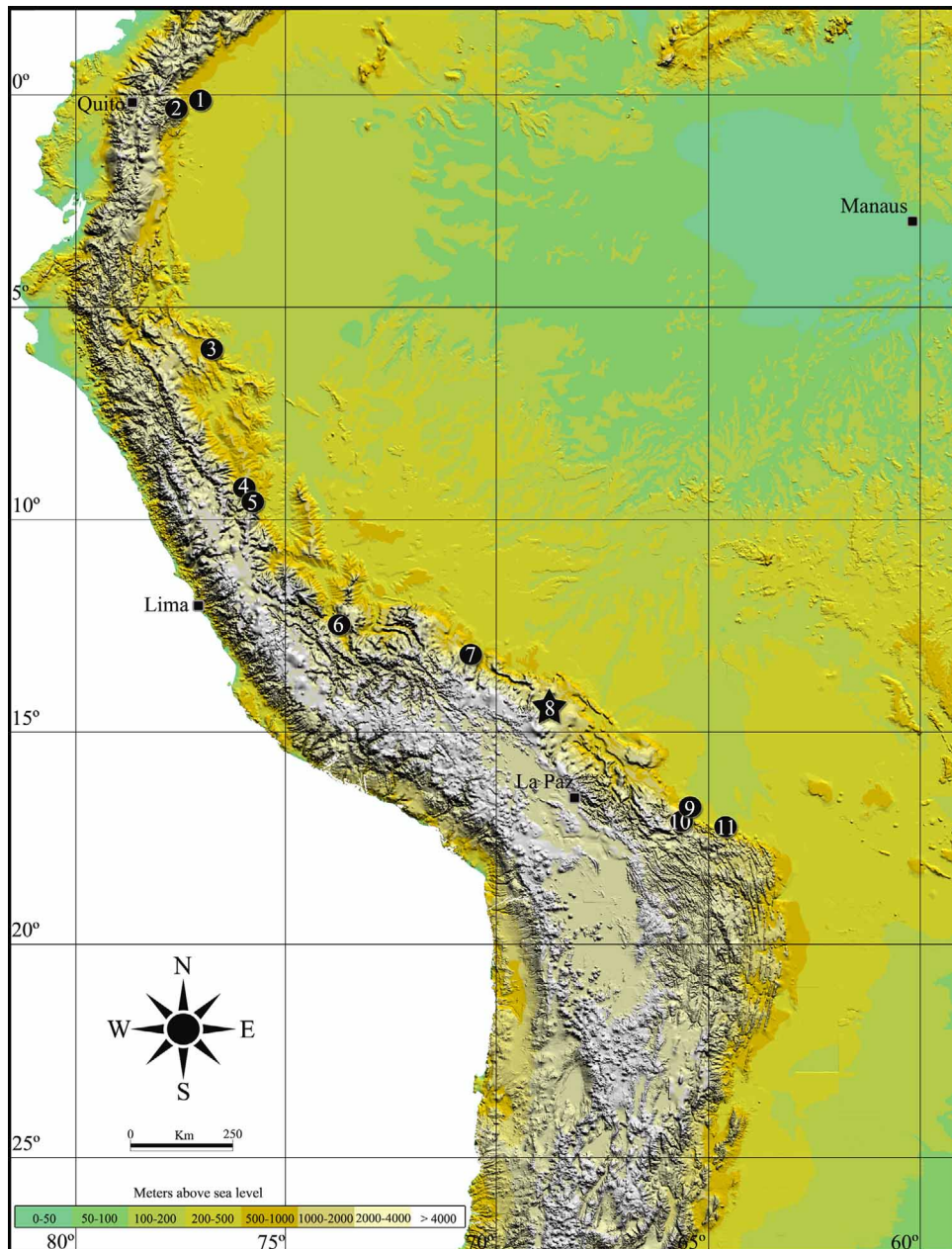
FIGURE 6. Map showing the type localities of *Hyalinobatrachium* species from the eastern slopes of the Andes of Ecuador, Peru and Bolivia and the new locality of *H. pellucidum* in Peru. The star marks the localities of the holotype of *H. carlesvilai* **sp. nov.** 1 = Santa Cecilia; 2 = Río Azuela; 3 = Abra Tangarana; 4 = Cueva de los Guácharos; 5 = Parque Nacional Tingo María; 6 = Río Kimbiri; 7 = Quincemil; 8 = Santa Rosa and San Juan del Oro; 9 = Paractito-los Guácharos; 10 = 58.1 km SW Villa Tunari; 11 = Río Leche.
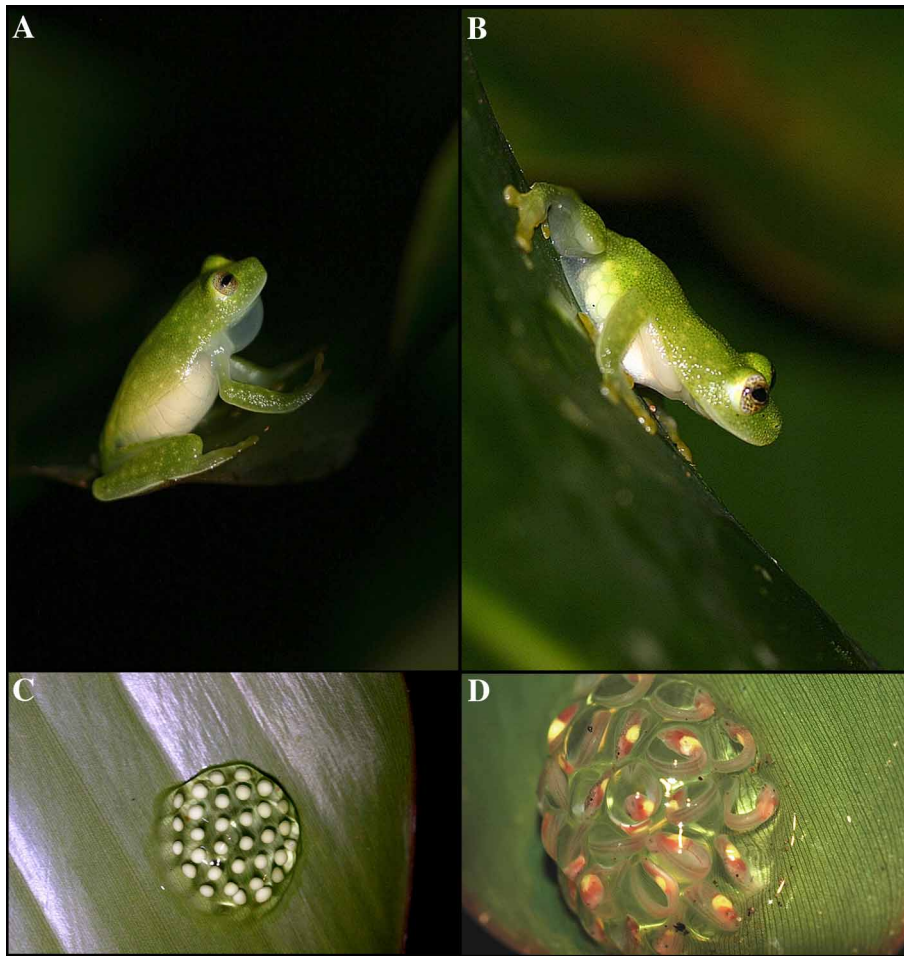
FIGURE 7. Calling male, paratype MNCN 43690 (**A**) and gravid female, paratype MHNCP 5344 (**B**) of *Hyalinobatrachium carlesvilai* **sp. nov.** Both were found in the same leaf together with an egg clutch not collected (**C**). Egg clutch, MNCN/ADN 8999, collected in the same leaf than the holotype (**D**).

Based on the aforementioned evidences, we formally place *Hyalinobatrachium lemur* as a junior synonym of *Hyalinobatrachium pellucidum*. Our results extend the distribution of *H. pellucidum* from a few localities in Napo, Ecuador to the Kimbiri River, Department Cusco, Vilcabamba Mountains, Peru (Fig. 6; Appendix I).

The type series of *Hyalinobatrachium lemur* shows further problems. Duellman & Schultes (1993) designated a gravid female (KU 211769) as paratopotype without further discussion. We studied this specimen and found that it has a completely white pericardium, hand webbing formula **III** $2 - 1^+$ **IV**, and snout truncate in dorsal and lateral view. These characters are not shared with *Hyalinobatrachium pellucidum*. The most similar and geographically close species sharing these characters is *Hyalinobatrachium carlesvilai* **sp. nov.** to which we assign it.

Hyalinobatrachium munozorum has been cited for Peru in different works (Duellman 1976; Duellman & Toft 1979; Cannatella 1980; Cannatella & Duellman 1982; Rodríguez *et al*. 2008). The specimens cited by Duellman (1976) for Kosñipata and Río Piene, Departamento Cusco and Ayacucho respectively, were assigned by Cannatella (1980) to *H. bergeri*. We have collected several specimens of *H. bergeri* at Kosñipata valley that confirm Cannatella's identifications (see Appendix I and II). However, the populations North of Río Urubamba (including those of Río Piene) might belong to a morphological cryptic and non-described species (S. Castroviejo-Fisher, J.M. Guayasamin and J.C. Chaparro unpublished data) referred as *H.* aff. *bergeri* by Guayasamin *et al*. (2008a). Duellman & Toft (1979) cited *H. munozorum* for Departamento Huánuco, in central Peru, at 200 m. Cannatella (1980) and Cannatella & Duellman (1982) compared that material against paratypes of *H*.

munozorum and supported the identification and added a new locality for Peru, Quincemil, Departamento Cusco. Our results indicate that the specimens cited for Quincemil by Cannatella & Duellman (1982) and Rodríguez *et al* (2008) most likely belong to *H. carlesvilai* **sp. nov.** and that it was misidentified on the basis of a shared extent webbing between fingers. After studying the specimens from central Peru, we also assign them to *H. carlesvilai* **sp. nov.** As things stand, we consider that *H. munozorum* is not present in Peru.
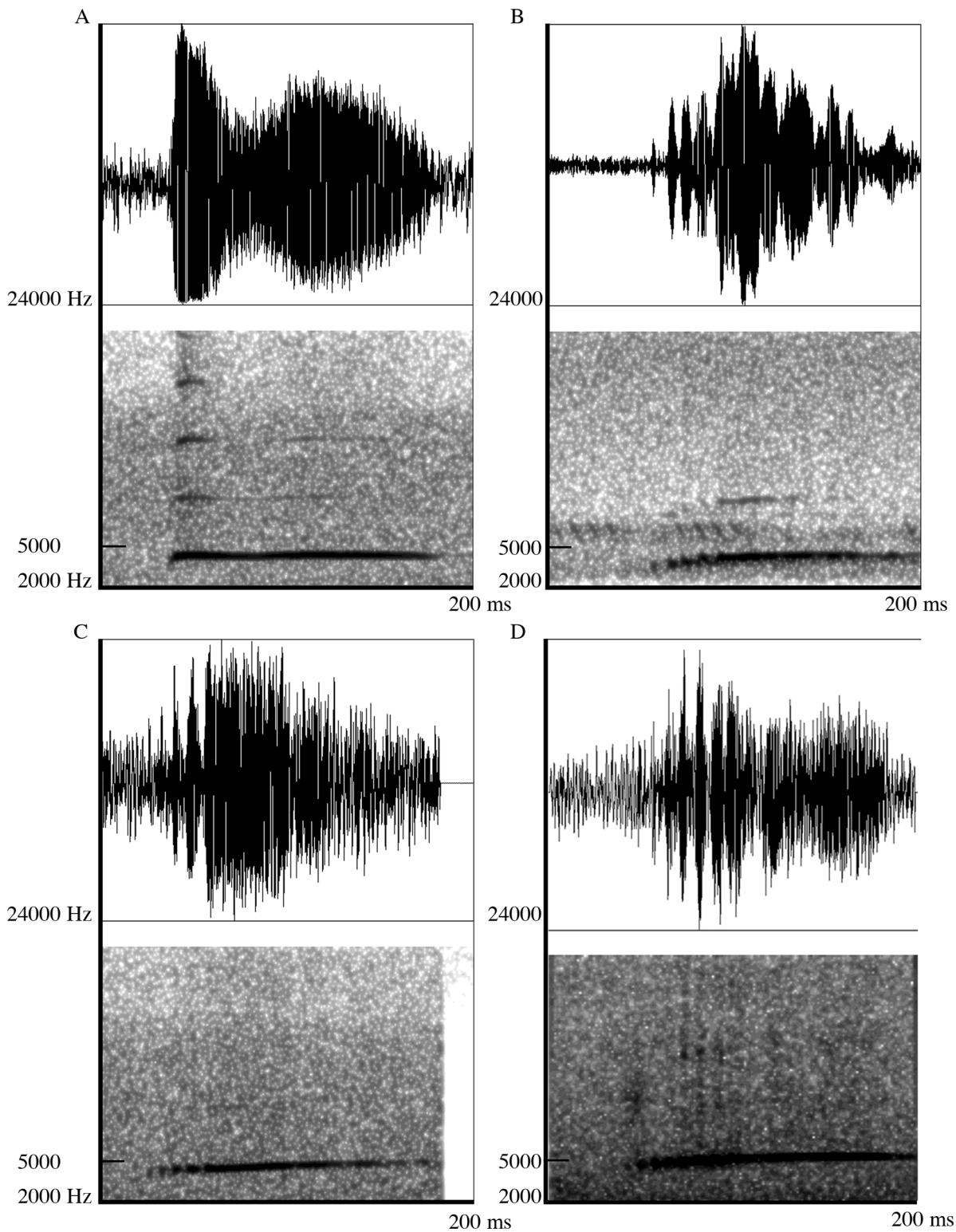
FIGURE 8. Audiospectogram and oscillograms (top and down, respectively) of the advertisement calls of **(A)** *Hyalinobatrachium bergeri*, MHNCP 5394; **(B)** *H. bergeri* sensu Márquez *et al.* (1996) and De la Riva (2002), without voucher; and **(C, D)** *H. carlesvilai* **sp. nov.**, holotype.
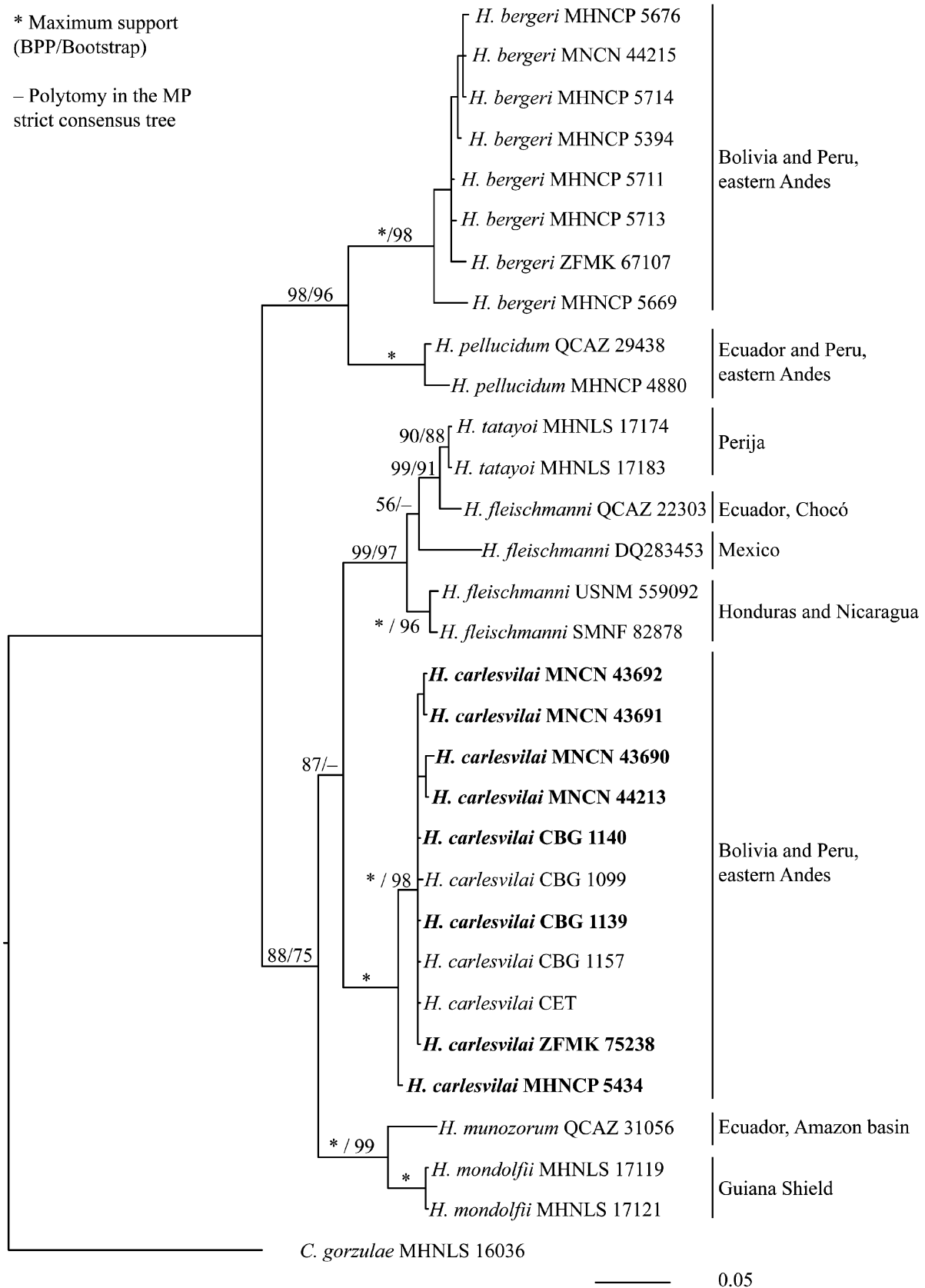
FIGURE 9. Bayesian majority rule consensus gene tree of 850 bp of the 16S mtDNA gene. Numbers on branches indicate Bayesian posterior probabilities and bootstrap support of the Maximum Parsimony analysis respectively. Clades are labeled according to their general distribution (see main text for details). Type specimens of *Hyalinobatrachium carlesvilai* **sp. nov.** are in bold.
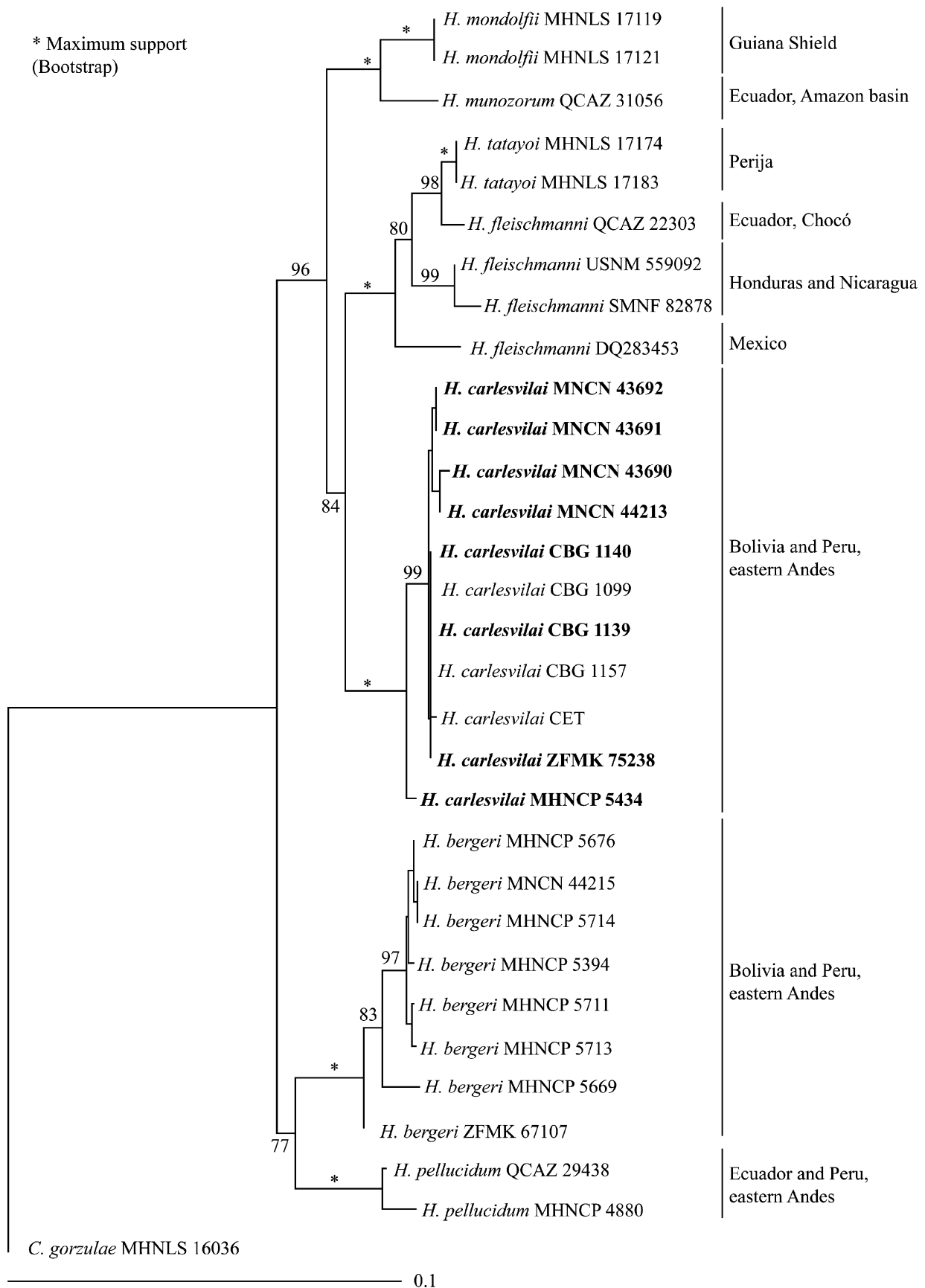
FIGURE 10. Neighbor joining gene tree of 850 bp of the 16S mtDNA gene. Numbers on branches indicate bootstrap support. Clades are labeled according to their general distribution (see main text for details). Type specimens of *Hyalinobatrachium carlesvilai* **sp. nov.** are in bold.

Hyalinobatrachium carlesvilai **sp. nov.** is more similar to the Venezuelan species *H. tatayoi*, in both morphology and bioacoustics (Castroviejo-Fisher *et al.*, 2007). Molecular phylogenetic analyses also suggest that both species are closely related (Figs. 9–10). Nevertheless, the call of *H. tatayoi* presents more pulses at the beginning of the call, 4–12 (7.5 ± 2.5) versus 2–4 (3 ± 0.5). Additionally, all specimens of *H. carlesvilai* **sp. nov.** show a more truncated snout in profile while in *H. tatayoi* the snout is round or semi-round. The distribution of *H. tatayoi* (Serrania de Perijá in Venezuela), the reciprocal monophyly of gene genealogies (Figs. 9–10), and the amount of genetic divergences (4.8–5.5 %) also supports that they are different species.

Hyalinobatrachium fleischmanni is very similar to *H. carlesvilai* **sp. nov.** However, *H. fleischmanni* has a subacuminate snout in profile and is restricted to the Pacific versant of the Andes and Central America. Our genetic results also support them as independent lineages (Figs. 9–10).

Hyalinobatrachium fleischmanni and *H. tatayoi* are also very similar species with slight differences in morphology and acoustic characters (Castroviejo-Fisher *et al.* 2007). Our genetic analysis indicated that *H. tatayoi* from Serrania de Perijá in Venezuela is the sister group of *H. fleischmanni* from the Pacific versant of Ecuador. Considering the Ecuadorian populations as *H. fleischmanni* would render this species paraphyletic for this gene and, hence, it would be non-reciprocally monophyletic to *H. tatayoi* (Figs. 9–10). Three possible explanations stem from those results. First, *H. tatayoi* is a synonym of *H. fleischmanni*. Second, the Ecuadorian populations from the Pacific versant correspond to *H. tatayoi*. Third, the evolutionary information of the marker studied is not appropriate to resolve this problem. The relationships of the *H. fleishmanni* sequences from Central America included in our analyses are problematic. The Bayesian and MP trees fail to resolve the position of the Mexican sequence (Fig. 9). On the other hand, the NJ tree (Fig. 10) shows maximum support for the sequence of the Mexican specimen of *H. fleishmanni* as sister to all the other sequences of *H. fleishmanni* and *H. tatayoi*. Genetic distances between the Mexican specimen and the South American and the other Central American specimens ranges from 2.8–3.6 %, while genetic distances between Central American (excluding Mexico) and South American sequences vary between 2.0–2.3 %. We conclude that the taxonomy of *H. fleischmanni* and *H. tatayoi* should be reevaluated, preferably using a large and geographically widely distributed sample and combining different lines of evidences to detect lineage divergence.

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Appendix I. Additional Specimens Examined

Hyalinobatrachium bergeri: **BOLIVIA, Cochabamba:** 58.1 km SW Villa Tunari (by road) (65°50´W, 17°11´S; 1980 m): KU 182363 (holotype); **La Paz:** Near Coroico: ZFMK 67107. **PERU, Cusco:** Quispicanchis: Between San Miguel and Marcapata (13º28'26.2"S, 70º53'46.2"W; 1612 m): MHNCP 5394; Quispicanchis: 6.1 km from Puente Fortaleza towards Quince Mil (13º11'09.5"S, 70º34'50.1"W; 464 m): MHNCP 5408, 5676; Unión, Valle de Kosñipata (1800 m): MHNCP 5711, 5713–4, MNCN 43693, 44215, KU 162248–9; **Ayacucho:** Tutambaro, Rio Piene (1840 m): KU 162251–5, 162257; **Puno:** Limbani: Santo Domingo de Carabaya (13°49'59.6''S, 69°38'31.8''W; 1650 m): MHNCP 5669.

Hyalinobatrachium carlesvilai: **BOLIVIA, Cochabamba:** Chapare: Repechón (17º06'S, 65º30'W; 500 m): CBG 1098–9; **PERU, Huánuco:** Finca Panguana, Rio Llullapichis, 4–5 Km upstream from Río Pachitea (200 m): KU 154749; **San Martín:** Lamas: West slope of Abra Tangarana, 7 km (by road) northeast of San Juan de Pacaysapa (06°12´S, 76°44´W; 1080 m): KU 211769 (paratopotype of *H. lemur*); **Cusco:** Quispicanchis: 40 Km E of Quincemil, road to Puerto Maldonado, above Río Marcapata: KU 197028–9.

Hyalinobatrachium fleischmanni: **COSTA RICA, San José:** San José: SMF 3760 (lectotype). **NICARAGUA, Atlántico Norte:** Parque Nacional Saslaya: El Padre: SMF 82882, 82878.

Hyalinobatrachium mondolfii: **VENEZUELA, Delta Amacuro:** Slopes of Serranía de Imatáca, first stream of Caño Acoima, tributary of río Grande (08°22'N, 61°32'W; 15 m): MHNLS 12710 (holotype), 17119–22 (topotypes).

Hyalinobatrachium munozorum: **ECUADOR: Napo:** Santa Cecilia (340 m): KU 118054 (holotype), 123225, 105251, 150620 (paratypes).

Hyalinobatrachium pellucidum: **ECUADOR, Napo:** Quito-Lago Agrio road, Río Azuela (1740 m): KU 143298 (holotype); **PERU, San Martín:** Lamas: West slope of Abra Tangarana, 7 km (by road) northeast of San Juan de Pacaysapa (06°12´S, 76°44´W; 1080 m): KU 211768 (holotype of *H. lemur*); 14 Km (by road) noth of Tarapoto, Cataratas Ahuashiyacu (730 m): KU 217295–7; **Cusco:** La Convención: Río Kimbiri, Comunidad Machiguenga Pomoreni (12°35'26.5"S, 73°41'36.8"W; 1100 m): MHNCP 4880.

Hyalinobatrachium tatayoi: **VENEZUELA, Zulia:** a stream near Tokuko (09°50´30.6''N, 72° 49´13.6''W; 301 m): MHNLS 17174 (holotype), MHNLS 17172–73, 17176–7, 17179–84 (paratypes).

Appendix II. Specimens, collection numbers, GenBank accesion numbers and localities of sequences used in this study. GenBank accesion numbers in bold correspond to sequences not published before.

Species	Collection number	GenBank	Locality / Source
C. gorzulae	MHNLS 16036	EU662984	Venezuela: Bolivar: Parque Nacional Canaima, Cuenca alta del río Cucurital, Atapare (05°42'N, 62°33'W) (Guayasamin *et al.* 2008a).
H. bergeri	ZFMK 67107	**GQ142064**	Bolivia: La Paz: near Coroico
H. bergeri	MHNCP 5669	**GQ142063**	Peru: Puno: Limbani: Santo Domingo de Carabaya (13°49'59.6''S, 69°38'31.8''W; 1650 m)
H. bergeri	MHNCP 5711, 5713–4, MNCN 44215	**GQ142061**, **GQ142062**, **GQ142060**, **GQ142059**	Peru: Cusco: Unión, Valle de Kosñipata (1800 m)
H. bergeri	MHNCP 5394	**GQ142058**	Peru: Cusco: Quispicanchis: Between San Miguel and Marcapata (13º28'26.2"S, 70º53'46.2"W; 1612 m)
H. bergeri	MHNCP 5676	EU663033	Peru: Cusco: Ouispicanchis: 6.1 km from Puente Fortaleza towards Quincemil (13°11'09.5"S, 70°34'50.1"W; 464 m) (Guayasamin *et al.* 2008a).
H. carlesvilai	MNCN 43690–2	**GQ142051**, **GQ142050**, **GQ142049**	Peru: Puno: three kilometers towards Santa Rosa from the type locality (14°12'49.1"S, 69º08'09.5"W; 1135 m).
H. carlesvilai	MHNCP 5434	**GQ142056**	Peru: Huánuco: Leoncio: Mariano Damazo Veraun: Parque Nacional Tingo María, first "*chacra*" (crop clearing) after the Puesto de Vigilancia 3 de Mayo (09º25'10.5" S, 75º58'15.0" W; 723 m).
H. carlesvilai	MNCN 44213	**GQ142055**	Peru: Cusco: Ouispicanchis: fifteen kilometers from Quincemil towards Puerto Maldonado (13°12'03.6''S, 70°40'28.9''W; 572 m).
H. carlesvilai	CBG 1139–40	**GQ142053**, **GQ142052**	Bolivia: Cochabamba: Carrasco: Río Leche (17°16'S, 64º45'W; 500 m).
H. carlesvilai	CBG 1099, CET	EU663030, **GQ142054**	Bolivia: Cochabamba: Chapare: Repechón (17º06'S, 65º30'W; 500 m) (Guayasamin *et al.* 2008a).
H. carlesvilai	ZMFK 75238	**GQ142057**	Bolivia: Cochabamba: Chapare: 7 km on road south of Paractito (17°04' S, 65°29' W; 500 m).
H. fleischmanni	JAC 21365	DQ283453	Mexico (Frost *et al.* 2006).
H. fleischmanni	QCAZ 22303	EU663044	Ecuador: Esmeraldas: La Tola (00°24'16.8"N, 79º54'41"W; 31 m) (Guayasamin *et al.* 2008a).
H. fleischmanni	USNM 559092	EU663045	Honduras: Gracias a Dios: Rus Rus Biological Reserve (14°43'N, 82°27'W; 60 m) (Guayasamin *et al.* 2008a).
H. fleischmanni	SMNF 82878	**GQ142048**	Nicaragua: Atlántico Norte: Parque Nacional Saslaya, El Padre.
H. mondolfii	MHNLS 17119, 17121	EU663050, **GQ142046**	Venezuela: Delta Amacuro: Slopes of Serranía de Imatáca, first stream of Caño Acoima, tributary of río Grande (08°22'N, 61°32'W; 15 m) (Guayasamin *et al.* 2008a).
H. munozorum	QCAZ 31056	EU663034	Ecuador: Zamora Chinchipe: Destacamento Militar Shaime (920 m) (Guayasamin *et al.* 2008a).
H. pellucidum	QCAZ 29438	EU663036	Ecuador: Morona Santiago: km 6.6 on the Limón–Macas road (Guayasamin *et al.* 2008a).
H. pellucidum	MHNCP 4880	**GQ142065**	Peru: Cusco: La Convención: Río Kimbiri, Comunidad Machiguenga Pomoreni (12°35'26.5"S, 73°41'36.8"W; 1100 m).
H. tatayoi	MHNLS 17174, 17183	EU663055, **GQ142047**	Venezuela: Zulia: stream near Tokuko (09° 50' 30.6"N, 72° 49' 13.6"W; 301 m) (Guayasamin *et al.* 2008a).

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