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The *Leptobrachium* (Anura: Megophryidae) of the Langbian Plateau, southern Vietnam, with description of a new species

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Abstract

We sampled two forms of *Leptobrachium* in syntopy at the type locality of *L. pullum* at upper elevations on the Langbian Plateau, southern Vietnam. The two forms differed in morphology (primarily in coloration), mitochondrial DNA, and male advertisement calls. One form closely agrees with the type series of *L. pullum* (but not to its original description due to error), and the other is described as new. *Leptobrachium leucops* **sp. nov.** is distinguished from its congeners by having small body size (males with SVL 38.8–45.2), the upper one-third to one-half of iris white, a blue scleral arc, a dark venter, and sexually active males without spines on the upper lip. *Leptobrachium pullum* and *L. mouhoti*, a recently described species from low-elevation slopes of the Langbian Plateau in eastern Cambodia, are morphologically divergent but genetically similar, warranting further investigation into geographic variation in the red-eyed *Leptobrachium* of southern Indochina.

Key words: Langbian Plateau; *Leptobrachium pullum*; Vietnam

Introduction

Smith (1921) described the megophryid frog *Megalophrys hasseltii* var. *pullus*, now known as *Leptobrachium pullum*, based on a series of 20 specimens that he and C. Boden Kloss collected in 1918 on the Langbian Plateau of southern Vietnam (Kloss 1919). Most *Leptobrachium* have conspicuously colored eyes, and this prominent feature is often used to diagnose species (e.g., Dubois & Ohler 1998; Lathrop *et al.* 1998; Matsui *et al*. 1999; Ohler *et al.* 2004; Matsui *et al.* 2010). Unfortunately, this coloration is usually lost over time in fluid preservation, making original field notes (and more recently, color photographs) essential for later determination of eye coloration in preserved specimens. In his brief description, Smith (1921: 440) stated that *L. pullum* had the "upper half of iris (in life) scarlet." Smith's comment is supported by a note from co-collector Kloss found in the jar of paralectotype males BMNH 1972.1465–66 that stated "Black eyeball with scarlet iris. C. B. K." (B. L. Stuart, personal observation, April 2009). Stuart et al. (2006) used Smith's (1921) statement on eye coloration, in addition to body size, to distinguish *L. pullum* from a new species, *L. mouhoti*, collected from low-elevation, western slopes of the Langbian Plateau in eastern Cambodia. Specifically, *L. mouhoti* has scarlet (= bright orange-red) coloration restricted to a scleral arc under the palpebrum (visible in the posterior corner of the eye and when the palpebrum is retracted), whereas *L. pullum* has, according to its original collectors, scarlet on the upper half of the iris.

Our fieldwork in 2008 at upper elevations on the Langbian Plateau, Vietnam, revealed two syntopic forms of *Leptobrachium* that were readily distinguished from one another, but which exhibited a perplexing pattern of adult eye coloration that made assigning one of these forms to *L. pullum* difficult using Smith's (1921) original description. One form had scarlet restricted to a scleral arc under the palpebrum, and the other had white on the upper half of the iris, but neither had scarlet on the upper half of the iris. This means that Smith and Kloss' statements on eye

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coloration were in error (wrong color, wrong location of color, or a composite of both forms), or that we failed to sample true *L. pullum* at its type locality where three forms occur.

Here, we show that the two syntopic forms of *Leptobrachium* that we sampled at upper elevations on the Langbian Plateau are morphologically, genetically, and acoustically distinct, and should be recognized as separate species. We use the type series of *L. pullum* to assign one of the forms to that species, and describe the other as new.

Material and methods

Sampling. Specimens were collected by the authors above 1,500 m elevation on the Langbian Plateau, Vietnam, during March–May 2008 and fixed in 10% buffered formalin after preserving liver in 20% DMSO-salt saturated storage buffer. Specimens were later transferred to 70% ethanol. Specimens and tissue samples were deposited at the North Carolina Museum of Natural Sciences (NCSM), Australian Museum (AMS), and Field Museum of Natural History (FMNH). Some specimens currently at AMS will be deposited at University of Science, Ho Chi Minh City (UNS). These have been cross-cataloged at both institutions, and are reported here as UNS/AMS.

Morphology. Examined specimens are listed in the Appendix. Data were also taken from original descriptions of species (Bourret 1937; Lathrop *et al.* 1998; Orlov 2005). Smith (1921: 440) stated in the original description of *L. pullum* that "altogether 20 specimens were obtained, which I have been able to compare with some 20 examples of the typical form…," but he provided "measurements of type series…" for only eight specimens. Matsui *et al.* (1999) considered only the eight with measurements to be syntypes, but we view all 20 as such. Sixteen of these were located by us at the Natural History Museum, London in April 2009, and they are treated here as a lectotype (following its designation by Matsui *et al.* 1999) and 15 paralectotypes (Appendix).

Measurements were taken to the nearest 0.1 mm with dial calipers: snout-vent length (SVL); head length from tip of snout to rear of jaws (HDL); maximum head width (HDW); snout length from tip of snout to anterior corner of eye (SNT); eye diameter (EYE); interorbital distance (IOD); internasal distance (IND); shank length (SHK); thigh length (TGH); forearm length, from elbow to base of outer palmar tubercle (LAL); manus length from tip of third digit to base of outer palmar tubercle (HND); pes length from tip of fourth toe to base of inner metatarsal tubercle (FTL); inner metatarsal tubercle length (IML); and inner metatarsal tubercle width (IMW).

Molecules. Total genomic DNA was extracted from liver using PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc.). A 546–552 nucleotide basepair fragment of mitochondrial (mt) DNA that encodes part of the 16S rRNA gene was amplified by PCR (the polymerase chain reaction; one cycle of 94ºC 5 min, 35 cycles of 94ºC 45 s, 60ºC 30 s, 72ºC 1 min, one cycle of 72ºC 10 min) using the primers 16Sar-5' and 16Sbr-3' (Palumbi, 1996). PCR products were cleaned using GELase (Epicentre Technologies) or ExoSAP-IT (USB). Cycle sequencing products were sequenced in both directions on a 3730 DNA Analyzer (Applied Biosystems) using the amplifying primers and Big Dye version 3 chemistry (Perkin Elmer). Sequences were edited with Sequencher v. 4.1 (Genecodes) and deposited in GenBank under accession numbers HQ709353-HQ709361. Homologous fragments of 16S obtained by Rao & Wilkinson (2008), Zheng *et al.* (2008), and Matsui *et al.* (2010) were downloaded from GenBank so that types or topotypes of every species of *Leptobrachium* reported from Vietnam, Cambodia, or Laos were included in the analysis (Table 1). Downloaded sequences were trimmed to match the length of the fragment obtained here. The data set was aligned using MUSCLE (Edgar 2004), and pairwise distances were calculated using PAUP* 4.0b10 (Swofford 2002).

Acoustics. Advertisement calls were recorded with an Edirol R-09HR WAVE/MP3 Recorder (44.1 kHz sampling rate and 24-bit encoding) with a Røde NTG-2 condenser shotgun microphone. Calls were recorded at a distance of approximately 0.2–0.5 m and ambient temperatures within 0.15 m of calling sites were taken immediately after recordings using a Kestrel 3500 hand-held weather meter. Calls were analyzed with Raven Pro 1.3© software (http://www.birds.cornell.edu/raven). Audiospectrograms were calculated with fast-Fourier transform (FFT) of 256 points, 50% overlap and 172 Hz grid-spacing, using Hanning windows. We examined oscillograms (waveforms), audiospectrograms and power spectra, and for five individuals per species we measured the call duration (s), intercall interval (s), number of notes per call, note duration (s), internote interval (s), note repetition rate (notes/s), number of pulses per note, pulse repetition rate and dominant frequency (kHz). These temporal and spectral parameters of calls were measured using the definitions of Cocroft & Ryan (1995) except for fundamental frequency, where the definition of Duellman (1970) was used.

Results

Morphology. Our series of *Leptobrachium* from the Langbian Plateau, Vietnam, contained two adult forms: (i) a "red-eyed" form having scarlet (white in preservative) coloration restricted to a scleral arc under the palpebrum that is visible in the posterior corner of the eye and when the palpebrum is retracted; a dorsum lacking large, dark markings; and a white venter, much lighter than the dorsum, with black spotting (Figure 1), and (ii) a "white-eyed" form having white coloration (in life and preservative) on the upper one-third to one-half of the eye; large, dark markings on the dorsum; and a dark venter (Figure 2). The two adult forms are similar in size (Table 1).

TABLE 1. Measurements (mm) of *Leptobrachium* from the Langbian Plateau, southern Vietnam. *Leptobrachium pullum* was previously referred to as the "red-eyed" form and *L. leucops* as the "white-eyed" form. Abbreviations defined in the text.

Measurement	*L. pullum* types		*L. pullum* topotypes		*L. leucops* sp. nov.	
	Males *n* = 15 Range; Mean ± SD	Female (gravid)[a] *n* = 1	Males *n* = 14 Range; Mean ± SD	Female (immature) *n* = 1	Males *n* = 15 Range; Mean ± SD	Females (immature) *n* =2; Range
SVL	42.4–50.6; 46.1 ± 2.0	52.4	41.3–46.1; 44.1 ± 1.5	40.2	38.8–45.2; 43.2 ± 1.6	39.3–41.4
HDL	18.1–21.0; 19.6 ± 1.1	22.8	19.2–22.0; 20.3 ± 0.8	19.2	17.8–21.2; 20.1 ± 0.8	16.9–18.7
HDW	17.5–20.9; 19.4 ± 1.0	21.5	18.8–21.2; 20.2 ± 0.8	16.8	17.4–21.3; 19.7 ± 0.9	15.6–18.0
SNT	7.2–9.3; 8.2 ± 0.6	10.1	7.1–8.4; 7.6 ± 0.4	6.8	6.4–8.3; 7.4 ± 0.5	6.5–7.3
EYE	6.7–7.6; 7.2 ± 0.3	8.1	6.8–7.4; 7.1 ± 0.2	6.5	6.4–8.0; 7.3 ± 0.4	6.5–7.0
IOD	5.3–6.7; 6.0 ± 0.4	6.9	5.4–6.7; 5.9 ± 0.4	4.7	4.9–6.1; 5.5 ± 0.4	5.3–5.4
IND	3.9–4.5; 4.2 ± 0.2	4.4	3.6–4.6; 4.1 ± 0.3	3.9	3.7–4.6; 4.2 ± 0.2	4.2–4.3
SHK	14.3–16.9; 15.3 ± 0.8	18.1	14.6–16.7; 15.9 ± 0.7	14.6	13.4–15.6; 15.0 ± 0.5	15.7–16.1
TGH	16.6–19.1; 17.8 ± 0.8	20.7	18.1–20.4; 19.5 ± 0.8	17.1	16.3–19.1; 18.4 ± 0.7	17.5–18.6
LAL	12.0–14.2; 13.0 ± 0.6	16.1	13.2–15.5; 14.5 ± 0.7	13.5	12.2–14.2; 13.4 ± 0.5	13.4–14.0
HND	9.6–11.7; 10.6 ± 0.5	12.8	9.5–11.0; 10.3 ± 0.5	9.3	9.0–10.9; 10.1 ± 0.5	10.2–10.7
FTL	15.2–17.9; 16.4 ± 0.8	18.9	15.4–18.4; 16.6 ± 0.9	15.2	13.6–16.7; 15.6 ± 0.8	15.5–16.4
IML	2.2–3.7; 2.7 ± 0.4	3.5	2.0–2.6; 2.2 ± 0.2	1.9	1.6–2.5; 2.2 ± 0.2	2.1–2.3
IMW	1.2–1.7; 1.5 ± 0.2	1.8	1.1–1.6; 1.4 ± 0.1	1.3	1.1–1.7; 1.4 ± 0.2	1.2–1.4

a. BMNH 1921.5.5.31 (no. 2108 of Smith, 1921). No. 2093 (now BMNH 1921.5.5.35) and no. 2101 (now BMNH 1921.5.5.33) were listed by Smith (1921) as females, but both are males.

The lectotype and 15 paralectotypes of *L. pullum* that we examined closely agree with our "red-eyed" form in preservative by having eye coloration restricted to a scleral arc, a dorsum lacking large, distinct markings, and a venter that is conspicuously lighter than the dorsum. The *L. pullum* types disagree with our "red-eyed" form only by lacking spotting on the venter. There was no evidence that more than one form was represented among the 16 of 20 types of *L. pullum* that we examined (Appendix).

Molecules. Three individuals of the "white-eyed" form (NCSM 77467, AMS R173164–65) have an uncorrected pairwise divergence of 5.50% in the 16S gene fragment from three individuals of the "red-eyed" form (UNS00113/AMS R173141, UNS00114/AMS R173142, AMS R173143). No sequence variation was observed within either form (Table 2).

Acoustics. The advertisement calls of five individuals of the "white-eyed" form distinctly differed structurally from those of five individuals of the "red-eyed" form, having multiple notes per call compared to the invariably single-note call of the "red-eyed" form (Tables 3–4, Figures 4–5).

Taxonomic conclusions. We assign the "red-eyed" form to *L. pullum* on the basis of similarity in adult morphology, and assume that the absence of ventral spots in the type series of *L. pullum* (the sole difference between them and adults of the "red-eyed" form) is due to fading in preservative over time. Smith (1921: 440) vaguely stated that the types were "below whitish or brownish." It is apparent that scarlet coloration is restricted to the scleral arc in *L. pullum*, and that Smith's (1921) statement that scarlet was on the "upper half of iris " is in error.

TABLE 2. Uncorrected 16S pairwise distances (%) from type or topotype specimens of *Leptobrachium* species that occur in Vietnam, Cambodia, and Laos. *Leptobrachium pullum* was previously referred to as the "red-eyed" form and *L. leucops* as the "white-eyed" form.

Species	*ailaonicum*	*banae*	*buchardi*	*chapaense*	*echinatum*	*leucops*	*mouhoti*	*ngoclinhense*	*promustache*	*pullum*	*smithi*	*xanthospilum*
Locality												
GenBank Accession												
ailaonicum	–											
Ailao Mtn., China												
EU180862												
banae	14.01	–										
Krong Pa, Vietnam												
EF544229												
buchardi	11.19	8.20	–									
Bolaven Plateau, Laos												
HQ709353												
chapaense	4.56	13.97	12.06	–								
Sa Pa, Vietnam												
EF544227												
echinatum	1.45	14.56	11.51	5.27	–							
Sa Pa, Vietnam												
EF544220												
leucops	10.57	9.03	4.58	10.16	10.89	0						
Langbian Plateau, Vietnam												
HQ709354-56 (n = 3)												

Continued next page

TABLE 2. (continued)

Species **Locality** **GenBank Accession**	*ailaonicum*	*banae*	*buchardi*	*chapaense*	*echinatum*	*leucops*	*mouhoti*	*ngoclinhense*	*promustache*	*pullum*	*smithi*	*xanthospilum*
mouhoti Mondolkiri Prov., Cambodia HQ709357	10.99	8.54	4.19	11.14	10.96	5.33	–					
ngoclinhense Ngoc Linh, Vietnam EF544228	11.10	7.02	3.05	11.85	11.27	4.99	3.03	–				
promustache Dawei Mtn., China EU180869	3.64	14.73	12.06	6.55	4.36	11.62	11.69	12.22	–			
pullum Langbian Plateau, Vietnam HQ709358-60 (n = 3)	10.60	7.97	4.00	11.31	10.76	5.50	0.91	2.46	11.12	0		
smithi Khao Chong, Thailand AB530438	14.13	13.97	12.77	13.91	14.52	12.25	13.16	12.02	15.68	12.76	–	
xanthospilum Gia Lai Prov., Vietnam HQ709361	12.66	8.57	6.73	12.79	12.81	7.91	7.11	5.92	13.17	6.19	13.61	–

Leptobrachium pullum and the "white-eyed" form differ from each other in adult morphology, mt DNA, and male advertisement calls. These three independent data sets provide a corroborated hypothesis that these are separate species co-existing in syntopy on the Langbian Plateau. The "white-eyed" form is distinct in morphology and mt DNA (Table 1) and is not assignable to any named species. Additional comments are provided on *L. pullum*, and the "white-eyed" form is described as new, as follows.

Leptobrachium pullum (Smith, 1921)

Megalophrys hasseltii var. *pullus* Smith, 1921: 440.

Expanded description. The adult male lectotype (BMNH 1921.5.5.36; Figure 3) in preservative has a conspicuous, white scleral arc under palpebrum extending from anterior corner of eye at approximately 90° (in right eye), continuing as narrow strip to approximately 270°, becoming wider to its terminus at approximately 240°, visible only in posterior corner of eye when palpebrum is relaxed, covering approximately upper 1/5 of iris. Large, slit-like vocal sac openings on floor of mouth near lateral margin of tongue. Tympanic annulus scarcely visible. Dorsum brown with dark spots over some tubercles, without large, dark markings. Black streak under canthus and supratympanic fold, covering tympanum. Whitish bands on dorsal and anterior surfaces of thigh, posterior surface of shank, and dorsal surface of tarsus, weaker whitish bands on dorsal surface of forelimb, anterior surface of shank, and axial surface of tarsus. Large, whitish spots on flank, more on right side. Venter beige with white spots on tubercles, lighter than dorsum, without darker markings. Ventral surface of thigh darker than belly, with white spots on tubercles. SVL 48.0; HDL 20.7; HDW 20.9; SNT 8.5; EYE 7.1; IOD 6.3; IND 4.2; SHK 16.9; TGH 18.7; LAL 14.2; HND 11.7; FTL 17.9; IML 2.7; IMW 1.4.

Five adult males (NCSM 77458–62) from Bi Doup-Nui Ba National Park, Lac Duong District, Lam Dong Province, Langbian Plateau, Vietnam, have habitus moderately stocky; body tapering to groin. Head broad and depressed; head length and width subequal. Snout rounded or very obtusely pointed in dorsal view, sharply sloping in profile, barely projecting beyond lower jaw in profile; nostril about midway between tip of snout and eye, below canthus, internarial shorter than interorbital distance; canthus rostralis distinct; lores oblique, moderately concave; eye large, slightly projecting from side of head, diameter subequal to snout length, interorbital distance subequal to upper eyelid width; no pineal ocellus; tympanum round, annulus weakly or moderately visible, tympanum diameter about 50% eye diameter and greater than distance between tympanum and eye; tongue heart-shaped, notched posteriorly; large, slit-like vocal sac openings on floor of mouth near lateral margin of tongue; vomerine teeth absent.

Forelimb slender. Fingers moderately slender, without webbing. Tip of fingers blunt, those on fingers I and II slightly swollen; relative finger lengths II = IV<I<III; two oval palmar tubercles in contact, subequal in size, low callous bumps on ventral surface of fingers; nuptial pad absent.

Hindlimb slender and relatively short. Toes moderately slender; webbing on toes I and II to level of distal margin of subarticular tubercle and continuing as a fringe to base of tip, on preaxial side of toe III to level of distal margin of proximal subarticular tubercle continuing as a fringe to base of tip, on postaxial side of toe III to midway between proximal and distal subarticular tubercles continuing as a fringe to base of tip, on toe IV to level of proximal subarticular tubercle continuing as a fringe to base of tip, and on toe V to midway between base and tip. Tips of all toes blunt, slightly swollen; relative toe lengths I<II<V<III<IV; distinct, oval, inner metatarsal tubercle, length about 90% distance between tip of toe I and tubercle; no outer metatarsal tubercle.

Skin above smooth with fine network of ridges; no spines on upper lip; low supratympanic ridge from posterior edge of eye to axilla; ventrally granular, skin smooth on ventral surfaces of limbs; indistinct, round axillary gland on ventrolateral surface slightly posterior to insertion of forelimb with body; oval femoral gland on posteroventral surface of thigh, midway between knee and vent.

In life, dorsum dark brown, dark gray, or black, without distinct markings, some with small black spots on tubercles, some with small, scattered, light orange spots; upper flank like dorsum, lower flank like belly; upper surface of forelimb and hindlimb as dorsum, with light gray bands, some with light orange wash over gray bands; eye black with scarlet scleral arc as described in lectotype (visible in the posterior corner of the eye and when the palpebrum is retracted); no lip bars; narrow black streak under canthus and supratympanic fold, sometimes covering tympanum; chin and belly whitish-gray with dark gray spotting, spotting denser posteriorly; ventral surface of

limbs black with silver-white bands. In preservative, closely resembles color in life, except that scleral arc has faded to light orange, and light orange spotting and wash on dorsal surfaces has faded to white or creamy-white.

Measurements are summarized in Table 1.

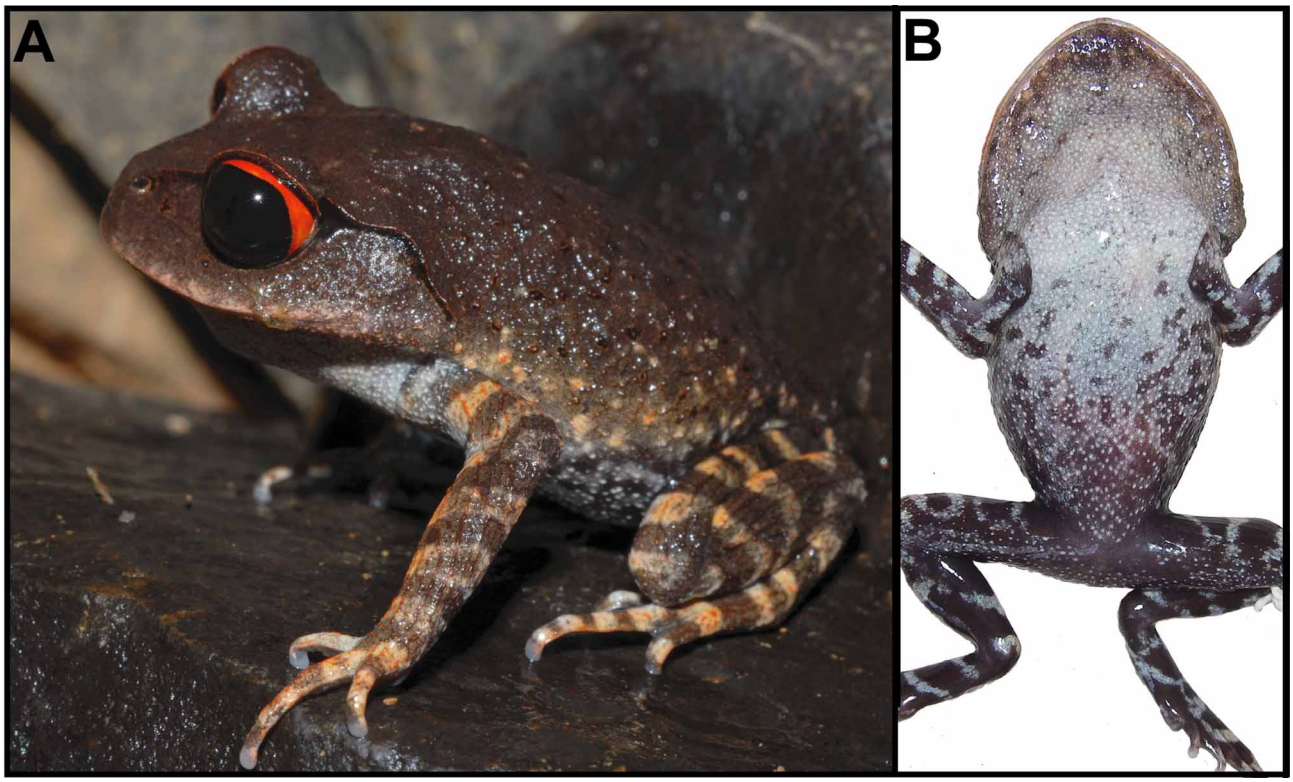

FIGURE 1. (A) Lateral and (B) ventral views of a male *Leptobrachium pullum* (UNS00114/AMS R173142; previously as "red-eyed" form) in life.

TABLE 3. Call parameters of *Leptobrachium pullum* (previously as "red-eyed" form). Parameter values are given as means (ranges). An asterisk (*) refers to the individual in Figure 4.

Parameter	Voucher				
	AMS R173139	NCSM 77458	NCSM 77459	NCSM 77462*	No voucher
Calls recorded	5	3	6	8	9
Notes/call	1	1	1	1	1
Call duration (s)	0.30 (0.29–0.32)	0.44 (0.41–0.46)	0.27 (0.25–0.29)	0.31 (0.27–0.33)	0.29 (0.28–0.30)
Intercall interval (s)	18.7 (9.5–31.1)	45.8 (30.7–60.9)	16.4 (7.0–42.1)	12.9 (7.3–16.0)	13.6 (8.1–23.9)
Call repetition rate	0.053	0.022	0.059	0.076	0.072
Pulses/note (approx.)	40	33	27	35	32
Pulse repetition rate (approx.)	129	72	97	111	105
Dominant frequency (kHz)	1.3	1.1 (1.0–1.2)	1.1	1.3	1.3
Temperature	18.4	13.3	18.4	20.4	19.4

Advertisement call. The following description is based on the calls of five individuals recorded at 13.3–20.4°C (Table 3). The call contains a single, highly-pulsed note lasting 0.25–0.46 s, and repeated relatively infrequently (7.0–60.9 s intercall interval). Each call contained approximately 30 pulses repeated at a near constant rate across the call. Relative amplitude across each call was heterogeneous, with a low and steadily increasing amplitude peaking about two-thirds of the way, followed by a more rapid decline in amplitude (Figure 4). The dominant frequency of calls varied from 1.0–1.3 kHz, and spanned approximately 1 kHz. A low frequency band was present

at around 0.2 kHz, and harmonics were present at approximately 2.4, 3.6, and 5.1 kHz (Figure 4). There was no frequency modulation. Temporal and spectral properties of the call (but not overall structure) may vary with temperature, as call duration, intercall interval, pulse repetition rate and dominant frequency were considerably lower at 13.3°C compared to 18.4–20.4°C (Table 3). To the human ear, the call sounds like a slow, deep bark "waaaaah".

Distribution and natural history. We sampled *L. pullum* from 1,477–1,724 m elevations on the Langbian Plateau in Lam Dong and Khanh Hoa Provinces, Vietnam. The taxonomic identity of red-eyed *Leptobrachium* from elsewhere in southern Vietnam and Cambodia requires evaluation (below). On the Langbian Plateau, *L. pullum* occurs in wet evergreen and cloud forest, where males call from shallow burrows under leaf litter. *Leptobrachium pullum* occurs in syntopy with *L. leucops* **sp. nov.** at some sites; in March, males of both species were heard calling within a few meters of each other.

Leptobrachium leucops sp. nov.

Holotype: NCSM 77465 (field tag BLS 11751; Figures 2, 6), adult male, Vietnam, Langbian Plateau, Lam Dong Province, Lac Duong District, Bidoup-Nui Ba National Park, Hon Giao, 12°11'11.2"N 108°42'53.5"E (Figure 7), 1,627 m elev., coll. 6 March 2008 by Bryan L. Stuart, Jodi J. L. Rowley, Tran Thi Anh Dao, Le Thi Thuy Duong, Hoang Duc Huy, Nguyen Le Xuan Bach, and Nguyen Thi Xuan Phuong.

Paratypes: Fourteen adult males: AMS R173163, same data as holotype; FMNH 280396, NCSM 77463–64, NCSM 77467, UNS00121/AMS R173159, UNS00122/AMS R173160, UNS00123/AMS R173161, AMS R173162, AMS R173165, UNS00124/AMS R173166, AMS R173168, same data as holotype except coll. 4–16 March 2008; NCSM 77466, AMS R173164, Vietnam, Langbian Plateau, Khanh Hoa Province, Khanh Vinh District, 12°11'30.5"N 108°43'03.5"E, 1,558 m elev., coll. 8 March 2008 by Bryan L. Stuart, Jodi J. L. Rowley, Tran Thi Anh Dao, Le Thi Thuy Duong, Hoang Duc Huy, Nguyen Le Xuan Bach, and Nguyen Thi Xuan Phuong. Two immature females: UNS00125/AMS R173167, same data as holotype except 12°11'33.3"N 108°42'41.6"E, 1,900 m elev., coll. 10 March 2008; AMS R173158, same data as holotype except 12°11'24.3"N 108°42'49.0"E, 1,751 m elev., coll. 19 May 2008 by Jodi J. L. Rowley, Tran Thi Anh Dao, Le Thi Thuy Duong, Hoang Duc Huy, Da Du Ha Tien, Vu Hanh Dung, Dinh Binh Phuong, Ly Tri, and Nguyen Thi Xuan Phuong.

Etymology. The specific epithet taken from *leukos* Gr. for white and *ops* Gr. for eye, in reference to the iris color of the new species.

Diagnosis. Assigned to the genus *Leptobrachium* on the basis of having head width larger than shank length; skin above with a network of ridges; large axillary glands; extremities of digits rounded; and upper part of iris colored differently from lower part (Dubois & Ohler 1998). A small-sized *Leptobrachium* having males with SVL 38.8–45.2; upper one-third to one-half of iris white; blue scleral arc; dark venter (purplish-gray, dark gray, or black in life, dark gray in preservative) with minute white spots on tubercles; and sexually active males without spines on the upper lip.

Description of holotype. Habitus moderately stocky; body tapering to groin. Head broad and depressed; head length and width subequal. Snout rounded in dorsal view, sharply sloping in profile, barely projecting beyond lower jaw in profile; nostril closer to tip of snout than to eye, below canthus, internarial shorter than interorbital distance; canthus rostralis distinct; lores oblique, moderately concave; eye large, slightly projecting from side of head, diameter subequal to snout length, interorbital distance subequal to upper eyelid width; no pineal ocellus; tympanum round, annulus weakly visible, tympanum diameter about 40% eye diameter and greater than distance between tympanum and eye; tongue heart-shaped, notched posteriorly; large, slit-like vocal sac openings on floor of mouth near lateral margin of tongue; vomerine teeth absent.

Forelimb slender. Fingers moderately slender, without webbing. Tip of fingers blunt, those on fingers I and II slightly swollen; relative finger lengths II = IV<I<III; two oval palmar tubercles in contact, inner larger than outer, low callous bumps on ventral surface of fingers; nuptial pad absent.

Hindlimb slender and relatively short. Toes moderately slender; webbing on toe I and preaxial side of toe II to level of distal margin of subarticular tubercle, on postaxial side of toe II to base of tip, on preaxial side of toe III to level of proximal subarticular tubercle continuing as a fringe to base of tip, on postaxial side of toe III to midway between proximal subarticular tubercle and tip continuing as a fringe to base of tip, on preaxial and postaxial sides of toe IV to same level as postaxial side of toe III and continuing as a fringe to base of tip, and on toe V to midway

between base and tip. Tips of all toes blunt, slightly swollen; relative toe lengths I<II<III = V<IV; distinct, oval, inner metatarsal tubercle, length about 70% distance between tip of toe I and tubercle; no outer metatarsal tubercle.

Skin above smooth with fine network of ridges, with small granules scattered posteriorly, especially near vent; no spines on upper lip; low supratympanic ridge from posterior edge of eye to axilla; ventrally granular, skin smooth on ventral surfaces of limbs; large, round axillary gland on ventrolateral surface slightly posterior to insertion of forelimb with body; oval femoral gland on posteroventral surface of thigh, midway between knee and vent.

Color of holotype in life. Dorsum dark gray, with distinct dark brown, Y-shaped marking extending from upper eyelids to lower back, becoming narrower posteriorly, edged with cream, with smaller, irregular dark brown to black markings edged with cream on lower back; upper flank with irregular dark brown and black markings edged with cream or white, lower flank dark gray with minute white spots on tubercles; upper surface of forelimb brown with dark gray bands, each flanked with narrower cream bands; upper surface of hindlimb white or creamy-white with dark grey and black bands; eye black with upper one-third of iris white, scleral arc blue (visible in the posterior corner of the eye and when the palpebrum is retracted); brown bar from edge of upper lip to nostril and from edge of upper lip to lower margin of eye; irregular, black streak under canthus and supratympanic fold, covering tympanum; ventral surface of body and limbs purplish-gray to dark gray, black spotting on chin, minute white spots on tubercles on chin, chest, belly, and banding from upper surfaces of limbs extending to outer margins of lower surfaces of limbs; axillary and femoral glands creamy-white.

Color of holotype in preservative. Color in preservative closely resembles color in life, except that cream on dorsal surfaces has faded to white or gray, and purplish-gray on ventral surfaces has faded to dark gray.

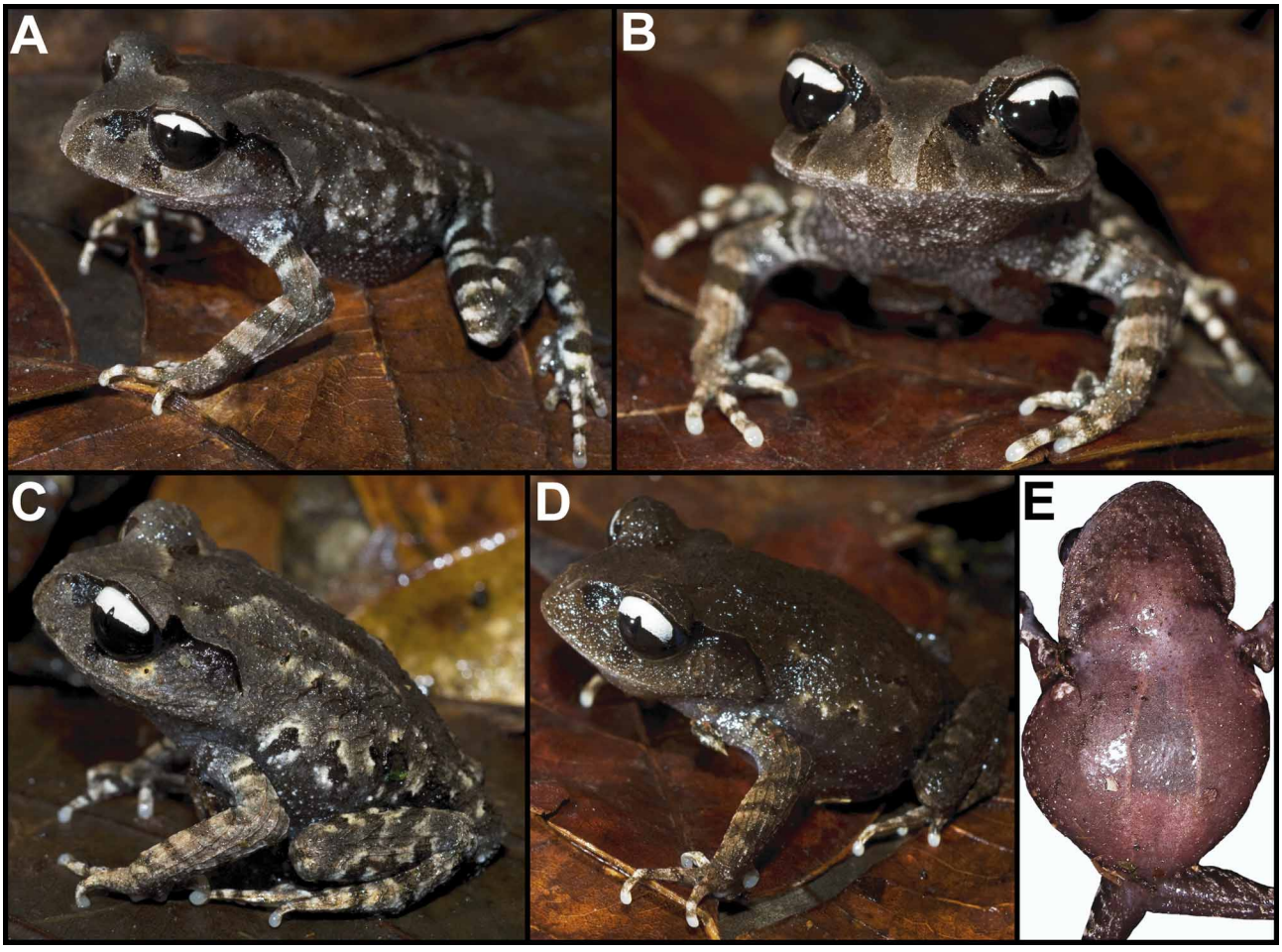

FIGURE 2. (A) Lateral and (B) frontal views of the male holotype (NCSM 77465), (C) lateral view of male paratype UNS00123/AMS R173161, (D) lateral view of male paratype AMS R173163, and (E) ventral view of male paratype NCSM 77463 of *Leptobrachium leucops* **sp. nov.** (previously as "white-eyed" form) in life.

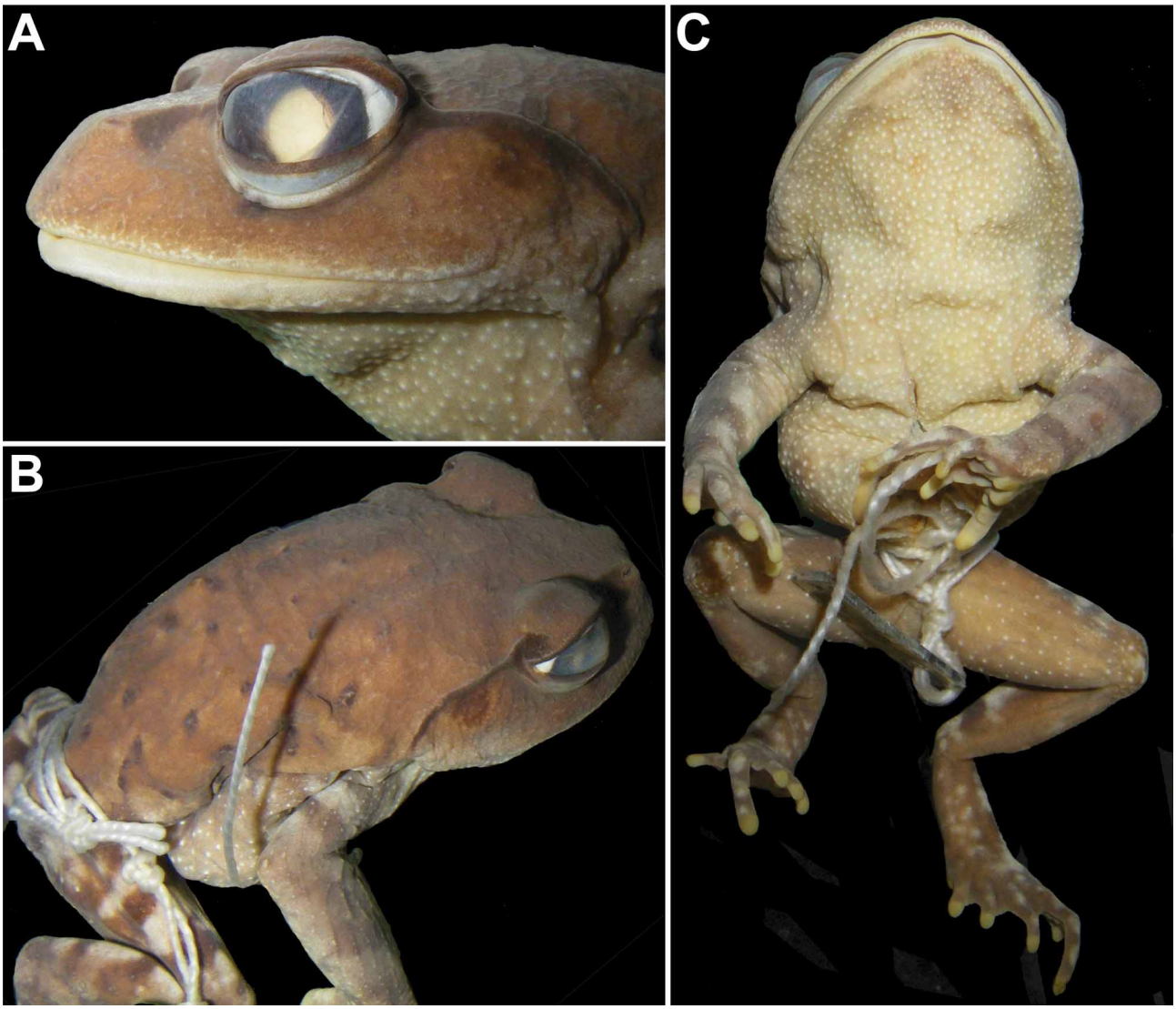

FIGURE 3. (A) Lateral view of head, (B) dorsal view, and (C) ventral view of the lectotype male (BMNH 1921.5.5.36) of *Leptobrachium pullum* in preservative.

Measurements of holotype. SVL 41.2; HDL 19.8; HDW 19.5; SNT 7.0; EYE 7.6; IOD 5.3; IND 4.1; SHK 14.6; TGH 18.3; LAL 13.2; HND 9.7; FTL 15.6; IML 2.0; IMW 1.5.

Variation. The dorsal pattern is highly variable among paratypes (Figure 2). The background color of the dorsum in life varied from brown to light gray to dark gray. The darker markings on the dorsum are variable in size and shape. One (AMS R173165) has the dark markings on back arranged as rounded spots (Figure 6). Some (AMS R173158, UNS00121/AMS R173159, UNS00123/AMS R173161, AMS R173162–64, NCSM 77463, NCSM 77467) have a uniform dark gray dorsum with few or no darker markings (Figure 6). Three paratypes (AMS R173165, UNS00124/AMS R173166, AMS R173168) have more distinct limb banding in preservative than the holotype. The ventral coloration is dark gray to black in some paratypes, and some lack black spotting on the chin. The white coloration in the eye extends to about one half of the iris in some paratypes. Measurements are summarized in Table 1.

Advertisement call. The following description is based on the calls of five individuals recorded at 13.0–18.4°C (Table 4). The call contains 1–5 (but usually 3–4), highly-pulsed notes and lasted 0.10–1.70 s, repeated at a variable interval (6.5–124.1 s). Within a call, notes were relatively evenly spaced and each note contained 8–15 pulses repeated at a near constant rate across the call. Relative amplitude varied symmetrically within each note, gradually rising over the first half of each note and then declining gradually across the second half of the note (Figure 5). The dominant frequency of calls varied from 1.0–1.6 kHz, and spanned approximately 1 kHz (Figure 5). A low frequency band was present at around 0.2 kHz, and a weak harmonic was present at approximately 2.6 kHz.

There was slight frequency modulation within each note, with the first few pulses and occasionally the last few pulses approximately 0.1 kHz lower in frequency. Temporal and spectral properties of the call did not obviously vary with temperature in the five specimens recorded (Table 4). To the human ear, the call sounds like a rapid barking "wah-wah-wah-wah".

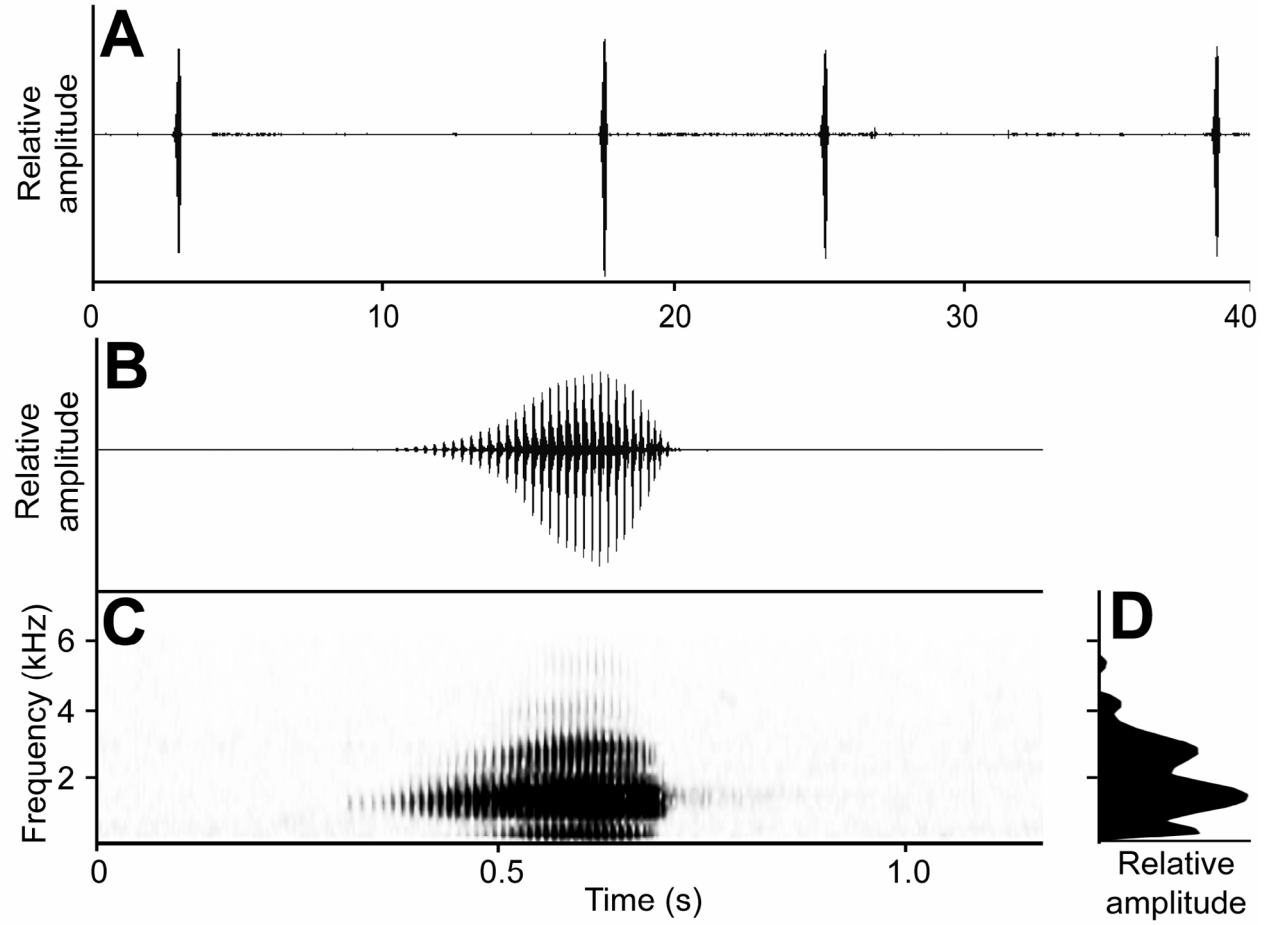

FIGURE 4. Advertisement call of a male *Leptobrachium pullum* (NCSM 77462; previously as "red-eyed" form) recorded at ambient air temperature of 20.4ºC (A) 40 s waveform of relative amplitude, (B) an expanded waveform, and (C) corresponding spectrogram showing a single representative call, and (D) a power spectrum (relative amplitude vs. frequency).

Distribution and natural history. *Leptobrachium leucops* is known only from 1,558–1,900 m elevation on the Langbian Plateau in Lam Dong and Khanh Hoa Provinces, Vietnam. The species occurs in wet evergreen and cloud forest, where males call from shallow burrows under leaf litter. *Leptobrachium leucops* occurs in syntopy with *L. pullum* at some sites; in March, males of both species were heard calling within a few meters of each other.

Comparisons. Only two other named species of *Leptobrachium* that occur in Vietnam, Laos, or Cambodia (Table 2) have the upper part of the iris white: *L. banae* Lathrop, Murphy, Orlov & Ho, 1998 and *L. xanthospilum* Lathrop, Murphy, Orlov & Ho, 1998. Both species are restricted to the Kon Tum Plateau of Vietnam (Lathrop *et al*. 1998), the nearest uplands to the Langbian Plateau (Figure 7). However, both are considerably larger than the new species, with SVL of males in *L. xanthospilum* 62.8–73.4 and *L. banae* 57.2–70.0 (38.8–45.2 in *L. leucops*). *Leptobrachium xanthospilum* further differs by having distinct, large, yellow, glandular spots on the flank (absent in *L. leucops*). *Leptobrachium banae* further differs by having red bands on the limbs (absent in *L. leucops*) and a white scleral arc (blue in *L. leucops*).

Leptobrachium chapaense (Bourret, 1937) has been reported to have a black iris (upper and lower parts not differing) or the upper part of the iris white or blue (Dubois & Ohler 1998; Lathrop *et al.* 1998). Molecular evidence suggests that *L. chapaense* is actually a complex of species across its range (Rao & Wilkinson 2008); at its type locality at Sa Pa in northwestern Vietnam, the eyes are black (Bourret, 1937; Orlov, 2005). *Leptobrachium "chapaense"* at Tam Dao, northern Vietnam, has a white upper iris, but is larger than the new species, with SVL of males 53.5–65.5 (38.8–45.2 in *L. leucops*), and has orange blotches on the sacral region, flank, and dorsal surfaces

of limbs (absent in *L. leucops*; Lathrop *et al.* 1998). The eye color of *L. ngoclinhense* (Orlov, 2005), unreported in its original description (Orlov, 2005), is dark brown to black (no light-colored upper iris) with a white scleral arc (J. J. L. Rowley, unpublished).

TABLE 4. Call parameters of *Leptobrachium leucops* **sp. nov.** (previously as "white-eyed" form). Parameter values are given as means (ranges). An asterisk (*) refers to the individual in Figure 5.

Parameter	Individual				
	AMS R173162	NCSM 77464	NCSM 77465 holotype*	UNS00122/ AMS R173160	No voucher
Calls recorded	5	8	4	5	8
Notes/call	1.7 (1–3)	3.6 (2–5)	4.3 (4–5)	3 (1–5)	3.8 (2–5)
Call duration (s)	0.41 (0.12–1.07)	1.17 (0.46–1.70)	1.21 (1.09–1.53)	0.81 (0.10–1.57)	1.15 (0.42–1.61)
Intercall interval (s)	21.3 (13.9–37.0)	24.6 (8.2–60.1)	10.8 (6.5–15.9)	9.3 (6.7–12.6)	29.9 (10.9–124.1)
Call repetition rate	0.046	0.039	0.08	0.097	0.056
Note duration (s)	0.12 (0.10–0.13)	0.09 (0.07–0.14)	0.09 (0.07–0.11)	0.08 (0.07–0.10)	0.09 (0.07–0.11)
Internote interval (s)	0.37 (0.33–0.39)	0.31 (0.20–0.48)	0.27 (0.21–0.36)	0.28 (0.25–0.37)	0.30 (0.23–0.43)
Note repetition rate	1.7 (1.6–1.9)	2.2 (2.0–2.4)	2.7 (2.6–2.8)	2.5 (2.3–2.6)	2.4 (2.2–2.5)
Pulses/note	11 (10–12)	11 (8–15)	12 (10–15)	10 (7–12)	12 (9–14)
Pulse repetition rate	88 (73–107)	102 (89–116)	124 (111–134)	105 (91–129)	126 (104–139)
Dominant frequency (kHz)	1.2 (1.0–1.2)	1.4	1.4	1.4	1.6
Temperature	16.9	13.0	18.4	15.3	14.4

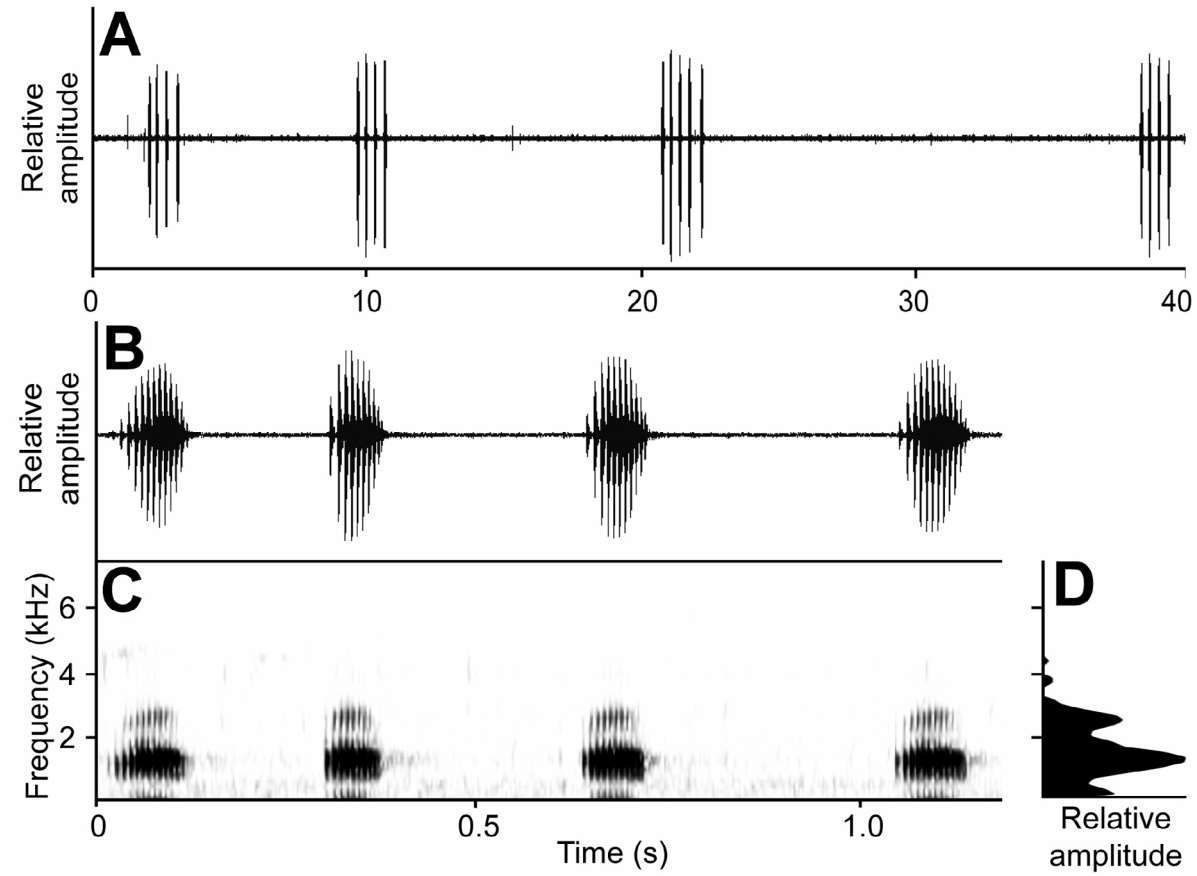

FIGURE 5. Advertisement call of the male holotype (NCSM 77465) of *Leptobrachium leucops* **sp. nov.** (previously as "white-eyed" form) recorded at ambient air temperature of 18.4ºC (A) 40 s waveform of relative amplitude, (B) an expanded waveform, and (C) corresponding spectrogram showing a single representative call, and (D) a power spectrum (relative amplitude vs. frequency).

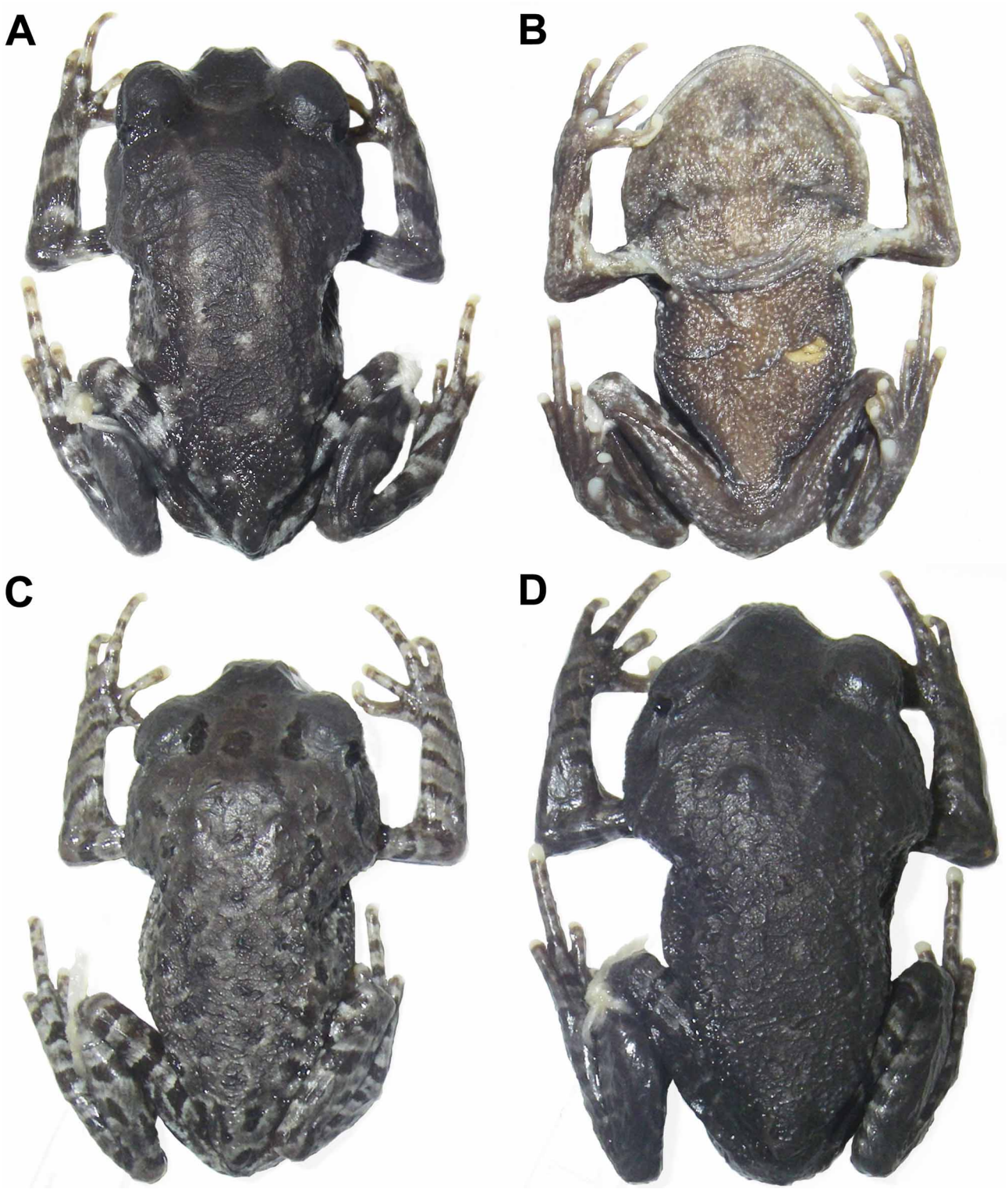

FIGURE 6. (A) Dorsal and (B) ventral views of the male holotype (NCSM 77465), (C) dorsal view of male paratype AMS R173165, and (D) dorsal view of male paratype AMS R173162 of *Leptobrachium leucops* **sp. nov.** in preservative.

Discussion

The Langbian Plateau, southern Vietnam, is the type locality of numerous species of amphibians and reptiles owing to the important collections obtained there during Smith and Kloss' expedition (e.g., Smith 1921, 1923, 1924). However, it is unclear why they failed to sample *L. leucops*. Smith obtained the type series of *L. pullum* at Arbre

Broyé ("5400 ft." = 1,646 m elevation; Kloss 1919) and Camly (between 1,200-1,800 m elevation; Smith 1921) during the month of April (Kloss 1919), an elevational and temporal sampling period that overlaps with our field work on the plateau 90 years later. *Leptobrachium leucops* was as conspicuous and abundant as the syntopic *L. pullum* in March, but was less frequently encountered and not heard calling in May. The new species may have a very narrow window of surface activity, or may be geographically localized on the plateau.

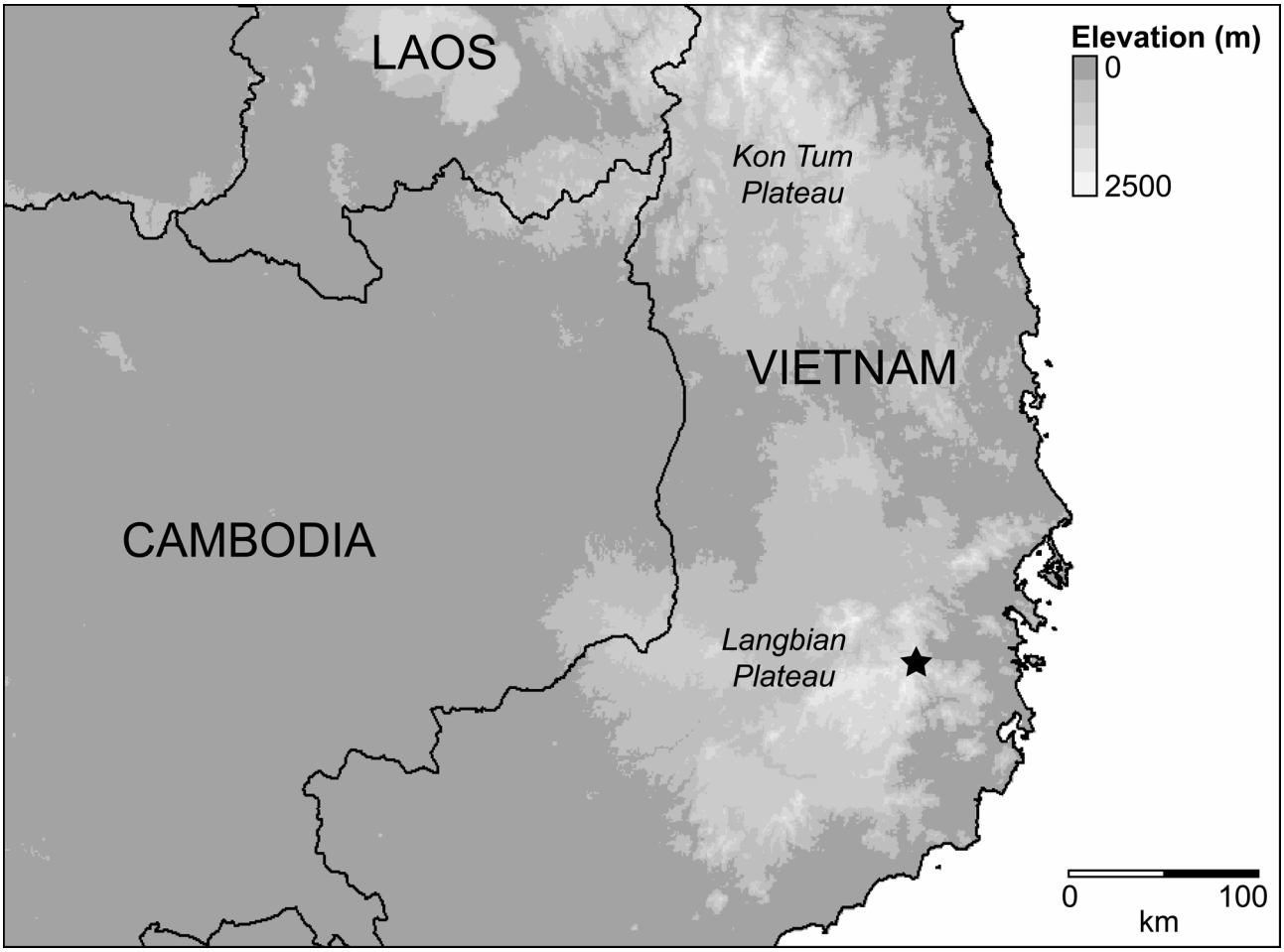

FIGURE 7. Map illustrating the type locality of *Leptobrachium leucops* **sp. nov.** at Bidoup-Nui Ba National Park, Langbian Plateau, Vietnam.

The realization that scarlet coloration is restricted to the scleral arc in *L. pullum*, contrary to Smith's (1921) original description of that species, eliminates one of the morphological characters used by Stuart *et al*. (2006) to distinguish *L. mouhoti* in its original description from *L. pullum*. These two species were also shown here to differ by less than 1% sequence divergence in the 16S mt DNA gene (Table 1). However, the types of *L. mouhoti* (males with SVL 51.6–64.7, mean 58.3 ± 3.5, *n* = 13; Stuart *et al*. 2006) are larger than types and topotypes of *L. pullum* (males with SVL 41.3–50.6, mean 45.1 ± 2.0, *n* = 29; modified from Table 1). Also, the venter of *L. mouhoti* is gray with light spots on tubercles, but that of *L. pullum* is white with black spots. These may represent (i) a single species that exhibits an elevational cline on the Langbian Plateau in body size and ventral coloration, (ii) two species that diverged very recently, and selection has accelerated some changes in morphology, or (iii) two species that exhibit mitochondrial genome introgression via occasional hybridization. The red-eyed *Leptobrachium* from the Kon Tum Plateau of Vietnam (Figure 7), referred to *L. mouhoti* by Bain & Nguyen (2006) but *L. pullum* by Matsui *et al*. (2010), exhibits some genetic divergence from a paratype of *L. mouhoti* (see Matsui *et al.* 2010). Species borders in the red-eyed *Leptobrachium* of southern Indochina are not apparent, and an investigation into the morphological, genetic, and acoustic variation in these frogs is warranted.

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APPENDIX. Comparative specimens examined.

Leptobrachium pullum: BMNH 1921.5.5.36 (lectotype male), BMNH 1921.5.5.32–35, BMNH 1972.1459–64 (paralectotype males), Vietnam, Langbian Plateau, Arbre Broyé. BMNH 1921.5.5.31 (paralectotype female), BMNH 1921.5.5.29–30, BMNH 1972.1465–66 (paralectotype males), Vietnam, Langbian Plateau, Camly. FMNH 280397, NCSM 77458–62, AMS R173138–39, UNS00112/AMS R173140, UNS00113/AMS R173141, UNS00114/AMS R173142, AMS R173143–44 (males), AMS R173147 (female), Vietnam, Langbian Plateau, Lam Dong Province, Lac Duong District, Bi Doup-Nui Ba National Park. AMS R173145 (male), Vietnam, Langbian Plateau, Khanh Hoa Province, Khanh Vinh District.