Zootaxa 2796: 15–28 (2011)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

A new species of *Leptolalax* (Anura: Megophryidae) from southern Vietnam

JODI J. L. ROWLEY[1,4], DUONG THI THUY LE[2], DAO THI ANH TRAN[2,3], HUY DUC HOANG[2]

[1]*Australian Museum, 6 College St, Sydney, NSW, 2010, Australia.*
[2]*Faculty of Biology, University of Science-Ho Chi Minh City, 227 Nguyen Van Cu, District 5, Ho Chi Minh City, Vietnam*
[3]*Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, D-53113 Bonn, Germany*
[4]*Corresponding author. E-mail: Jodi.Rowley@austmus.gov.au*

Abstract

We describe a new species of small megophryid frog from the Langbian Plateau in southern Vietnam, the southernmost record of *Leptolalax* from Vietnam. *Leptolalax bidoupensis* **sp. nov.** is distinguished from its congeners by a combination of a dark brownish red ventral surface with white speckling on entire ventral surface including throat, arms and legs, small size (23.6–24.6 mm in four adult males and 29.2–29.4 mm in two adult females), bicoloured iris (coppery red upper half, fading to pale silver ventrally), a mostly smooth skin texture with no skin ridges, and relatively short tibia (male TIB:SVL 0.44–0.46). The male advertisement call of the new species, consisting of 6–9 single-pulsed notes with a dominant frequency of 1.9–3.8 kHz, is also unique among *Leptolala*x species for which calls are known. From the morphologically similar *L. applebyi* and *L. melicus*, *L. bidoupensis* **sp. nov.** differs by 9.3% and 9.6% sequence divergence at the 16S mtDNA gene. At present, the new species is known from montane evergreen forest between 1620–1730 m elevation, within an area of 1 km^2. We suggest the species should be considered Data Deficient following IUCN's Red List categories.

Key words: Acoustics, Anura, Da Lat Plateau, *Leptolalax bidoupensis* **sp. nov.**, Lam Dong Province, Langbian Plateau, Southeast Asia, Vietnam

Introduction

The genus *Leptolalax* (Dubois 1983) currently contains 29 species of small frogs of the forest floor, distributed in rocky streams in hilly evergreen forest throughout Southeast Asia, southern China and northeastern India (Frost 2010, Rowley *et al*. 2010a, 2010b, 2010c). There has been a rapid increase in the number of known *Leptolalax* species in recent years, resulting from a combination of increased field surveys in the region and the incorporation of acoustic and molecular data in delineating species boundaries (eg. Das *et al*. 2010; Rowley *et al*. 2010a, 2010b, 2010c; Sengupta *et al*. 2010).

To date, eleven species of *Leptolalax* have been reported from Indochina; *L. aereus*, *L. applebyi*, *L. bourreti*, *L. croceus*, *L. melicus*, *L. nahangensis*, *L. oshanensis*, *L. pelodytoides*, *L. pluvialis*, *L. sungi* and *L. tuberosus* (Frost 2010; Rowley *et al*. 2010a, 2010b, 2010c). All but *L. aereus* and *L. melicus* have been reported from Vietnam (Nguyen *et al*. 2009; Rowley *et al*. 2010a, 2010c). Within Vietnam, *Leptolalax* are known throughout suitable habitat in northern and central Vietnam, with the southernmost record of *Leptolalax* to date from the Kon Tum Plateau in central Vietnam (Gia Lai Province; Nguyen *et al*. 2009).

Here we describe a new *Leptolalax* species collected during recent herpetological surveys on the Langbian Plateau in southern Vietnam. Although important amphibian collections were made on the Langbian Plateau by Smith (1921, 1924), *Leptolalax* were not reported from the Plateau, and the new species represents the southernmost record of *Leptolalax* from Vietnam. The new species is most morphologically similar to *L. applebyi* and *L. melicus*, both recently discovered from the Kon Tum Plateau to the north, but can be distinguished from these and all other *Leptolalax* species on the basis of morphological, acoustic and molecular differences.

Accepted by M. Vences: 9 Feb. 2011; published: 21 Mar. 2011

Material and methods

We recorded morphological data from specimens fixed in 10% formalin and then stored in 70% ethanol. Specimens were deposited at the Australian Museum (AMS) and the North Carolina Museum of Natural Sciences (NCSM). Some specimens currently at the AMS will be deposited at the University of Science, Ho Chi Minh City (UNS) and have been cross-cataloged at both institutions. In these instances, voucher numbers are reported as UNS/AMS. Morphometric data were taken (to the nearest 0.1 mm) with digital callipers. Measurements include snout-vent length (SVL); head length from tip of snout to rear of jaws (HDL); head width at the commissure of the jaws (HDW); snout length from tip of snout to the anterior corner of eye (SNT); diameter of the exposed portion of the eyeball (EYE); interorbital distance (IOD); horizontal diameter of tympanum (TMP); distance from anterior edge of tympanum to posterior corner of the eye (TEY); tibia length with the hindlimb flexed (TIB), manus length from tip of third digit to base of inner palmar tubercle (ML), pes length from tip of fourth toe to base of the inner metatarsal tubercle (PL), length of adpressed first finger from tip to distal edge of the inner palmar tubercle (F1L), length of adpressed second finger from tip to distal edge of inner palmar tubercle (F2L), and length of adpressed third finger from tip to distal edge of inner palmar tubercle (F3L). Sex was determined by direct observation of calling in life, the presence of internal vocal sac openings and/or gonadal inspection. Mass was recorded in life (to the nearest 0.1 g), using Pesola scales. We obtained comparative morphological data from museum specimens of *Leptolalax* and photographs of these specimens in life (Appendix) and from the literature: *L. aereus* (Rowley *et al*. 2010c), *L. alpinis* (Fei *et al*. 1991, 2009, 2010), *L. arayai* (Matsui 1997), *L. bourreti* (Dubois 1983), *L. croceus* (Rowley *et al.* 2010a), *L. dringi* (Dubois 1986; Inger & Stuebing 2005), *L. fuliginosus* (Matsui 2006), *L. gracilis* (Günther 1872; Malkmus *et al*. 2002; Inger & Stuebing 2005), *L. hamidi* (Matsui 1997), *L. heteropus* (Boulenger 1900), *L. kecil* (Matsui *et al*. 2009), *L. khasiorum* (Das *et al*. 2010), *L. kajangensis* (Grismer *et al*. 2004), *L. lateralis* (Anderson 1871; Humtsoe *et al.* 2008), *L. liui* (Fei *et al.* 1991, 2009, 2010), *L. maurus* (Inger *et al*. 1997), *L. melanoleucus* (Matsui 2006), *L. melicus* (Rowley *et al*. 2010b), *L. nahangensis* (Lathrop *et al*. 1998), *L. oshanensis* (Liu 1950; Fei *et al.* 2009, 2010), *L. pelodytoides* (Boulenger 1893, 1908), *L. pictus* (Malkmus 1992; Malkmus *et al*. 2002), *L. pluvialis* (Ohler *et al.* 2000), *L. solus* (Matsui 2006), *L. sungi* (Lathrop *et al.* 1998), *L. tamdil* (Sengupta *et al*. 2010), *L. tuberosus* (Inger *et al*.1999; Rowley *et al*. 2010a), *L. ventripunctatus* (Fei *et al*. 1991, 2009, 2010). Due to the undiagnosed diversity within genus, where available we relied exclusively on descriptions of specimens reported in the original species descriptions. We also examined colour photographs of the holotype of *L. pluvialis* (MNHN 1999.5675) in preservative.

Advertisement calls were recorded with an Edirol R-09 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 0.1–0.3 m and ambient temperatures were taken immediately after recordings using a Kestrel 3500 handheld weather meter. Calls were analysed with Raven Pro 1.3© software (http://www.birds.cornell.edu/raven). Audiospectrograms in figures were calculated with fast-Fourier transform (FFT) of 256 points, 50% overlap and 172 Hz grid-spacing, using Hanning windows. Comparisons of advertisement calls among species within the genus *Leptolalax* are hindered by the limited nature of many published call descriptions, with call measurements and visual representations of calls insufficient for meaningful comparisons between species. Comparisons of anuran advertisement calls in general are also complicated by inconsistent use of terms and a lack of clear definitions in terminology, particularly with respect to the units of a call, a note or a pulse (Gerhardt & Huber 2002). Here we use the definitions of Duellman (1970), except that we define a single call as vocalisations produced during a single expiration (Brown & Richards 2008). 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. For each call recording, we measured the call duration (ms), intercall interval (ms), number of notes per call, note duration (ms), internote interval (ms), number of pulses per note, note repetition rate (notes/s) and dominant frequency (kHz). Comparative advertisement call characters for *Leptolalax* species were taken from references, with advertisement calls known for 18 of the 29 known species of *Leptolalax* (Jiang *et al*. 2002; Malkmus *et al*. 2002; Matsui 1997, 2006; Matsui *et al*. 2009; Xu *et al*. 2005; Rowley & Cao 2009; Rowley *et al.* 2010a, 2010b, 2010c; Sukumaran *et al.* 2010). To maintain consistency and facilitate meaningful comparisons, we have used the terminology defined above to compare calls, regardless of terms used in these references.

We analyzed approximately 550 base pairs (bp) of mitochondrial 16S ribosomal RNA for four individuals of the new species and compared them with two *Leptolalax applebyi* (GenBank accession numbers HM133597–

HM133598) and three *Leptolalax melicus* (GenBank accession numbers HM133599–HM133601), the most morphologically similar and least geographically distant species. DNA was extracted using DNeasy tissue extraction kits (Qiagen). We used the primers 16SAR and 16SBR of Palumbi *et al.* (1991) to amplify the 16S rRNA gene. Standard PCR protocols were used and PCR products were purified using ExoSap-IT (USB Corporation, OH, USA). Purified templates were sequenced directly by Macrogen (Seoul, Korea). Sequences were validated using Sequencher 4.10 (Gene Codes, Ann Arbor, MI), aligned using the Clustal option in MEGA 4 and refined by eye. Kimuara Uncorrected pairwise sequence divergence was calculated using MEGA 4. DNA sequences for the new species were deposited in GenBank under the accession numbers HQ902880– HQ902883.

Leptolalax bidoupensis sp. nov.

Holotype: AMS R 173133, adult male, calling on clay bank 0.2 m from 1–4 m wide, medium-high gradient, rocky stream in montane evergreen forest in Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam (12.19225º N, 108.71494º E, 1730 m, Figure 1). Collected at 23:55 h on 19 May 2008 by J. J. L. Rowley, Hoang D. H., Le T. T. D., and Tran T. A. D.

Paratypes: UNS 00101/AMS R 173135, adult male, calling on tree root 0.2 m above 2–5 m wide, medium-high gradient, rocky stream in montane evergreen forest in Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam (12.19106º N, 108.71703º E, 1641 m), collected at 20:25 h on 20 May 2008. UNS 00102/AMS R 173137, metamorph, in water of swampy area adjacent to a swift, rocky stream in cloud forest in Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam (12.18644º N, 108.71486º E, 1627 m), collected at 19:45 h on 18 May 2008. AMS R 173134, adult female, on clay bank 0.2 m from 1–4 m wide, medium-high gradient, rocky stream in montane evergreen forest in Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam (12.19225º N, 108.71494º E, 1730 m), collected at 23:50 h on 19 May 2008, in close proximity to holotype. AMS R 173136, adult male, calling on leaf litter 0.2 m from 2–5 m wide, medium-high gradient, rocky stream in montane evergreen forest in Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam (12.19106º N, 108.71703º E, 1641 m), collected at 21:40 h on 20 May 2008. NCSM 77320, adult female, in water of swampy area off swift, rocky stream in montane evergreen forest in Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam (12.18644º N, 108.71486º E, 1627 m), collected at 19:50 h on 18 May 2008. NCSM 77321, adult male, sitting in upright posture (previously calling?) in leaf litter, 0.1 m from swift, rocky stream in montane evergreen forest in Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam (12.18644º N, 108.71486º E, 1627 m), collected at 21:45 h on 18 May 2008. NCSM 77322, metamorph, on clay bank 0.5 m from 2–5 m wide, medium-high gradient, rocky stream in montane evergreen forest in Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam (12.19106º N, 108.71703º E, 1641 m), collected at 21:30 h on 20 May 2008. All specimens were collected by J. J. L. Rowley, Hoang D. H., Le T. T. D., and Tran T. A. D.

Etymology. specific epithet is in reference to the type locality of Bidoup-Nui Ba National Park.

Diagnosis. Assigned to the genus *Leptolalax* on the basis of the following: small size, rounded finger tips, the presence of an elevated inner palmar tubercle not continuous to the thumb, presence of macroglands on body (including supra-axillary, pectoral, femoral and ventrolateral glands), vomerine teeth absent, tubercles on eyelids, anterior tip of snout with vertical white bar (Dubois 1983; Lathrop *et al*. 1998; Delorme *et al*. 2006). *Leptolalax bidoupensis* is distinguished from its congeners by a combination of (1) a dark brownish red ventral surface with white speckling on entire ventral surface including throat, arms and legs, often forming distinct marbling on chest and belly, (2) small size (23.6–24.6 mm in four adult males and 29.2–29.4 mm in two adult females), (3) bicoloured iris (coppery red upper half, fading to pale silver ventrally), (4) a mostly smooth skin texture with no skin ridges, and (5) relatively short tibia (male TIB:SVL 0.44–0.46). The male advertisement call of the new species, consisting of 6–9 single-pulsed notes with a dominant frequency of 1.9–3.8 kHz, is also unique among *Leptolala*x species for which calls are known.

Description of holotype. Head slightly longer than wide; snout bluntly rounded in dorsal view and in profile, projecting slightly beyond margin of the lower jaw; nostril closer to tip of snout than eye; canthus rostralis distinct, gently rounded; lores sloping; vertical pupil; eye diameter smaller than snout length; tympanum distinct, round, diameter smaller than that of the eye; tympanic rim elevated relative to skin of temporal region; vomerine teeth absent; pineal ocellus absent; vocal sac openings oval, located posteriolaterally on floor of mouth; tongue long,

moderate width, with slight notch at posterior tip; raised supratympanic ridge running from eye towards axillary gland. Tips of fingers rounded, not swollen; relative finger lengths I < II = IV < III; nuptial pad absent; subarticular tubercles absent; a large, round inner palmar tubercle distinctly separated from small, laterally compressed outer palmar tubercle; no finger webbing or lateral fringes. Tips of toes like fingers; relative toe length I < II < V < III < IV; subarticular tubercles absent, replaced by dermal ridges, distinct on second, third, fourth and fifth toes; large, oval inner metatarsal tubercle pronounced, outer metatarsal tubercle absent; basal webbing; weak lateral fringes. Tibia relatively short and stout, width approximately one third of length, tibia 46% of snout-vent length; tibiotarsal articulation reaches eye. Skin on dorsum mostly smooth, with fine, scattered tubercles concentrated on eyelids, ventrolateral surfaces and on upper surfaces on tibiotarsus; ventral skin smooth; pectoral gland oval, 1.2 mm diameter; femoral gland oval, 0.8 mm diameter, on posteroventral surface of thigh, closer to knee than to vent; supraaxillary gland raised, 1.2 mm diameter. Ventrolateral glands present, dorsolaterally compressed, forming an incomplete line that is difficult to distinguish from white marbling.

TABLE 1. Measurements (mm) of *Leptolalax bidoupensis* **sp. nov.** Abbreviations defined in text. Tympana were indistinct in metamorphs.

	Males				Females		Metamorphs	
	AMS R 173133*	UNS 00101/ AMS R 173135	AMS R 173136	NCSM 77321	AMS R 173134	NCSM 77320	UNS 00102/ AMS R 173137	NCSM 77322
SVL	23.6	24.3	24.6	24.6	29.4	29.2	17.3	17.1
HDL	9.2	9.2	9.6	9.6	10.1	10.5	5.4	5.7
HDW	8.9	8.8	9.2	9.0	9.6	9.8	5.5	5.5
SNT	3.6	3.5	3.6	3.6	3.8	3.8	2.3	2.3
EYE	2.5	2.6	2.8	2.8	2.8	2.7	1.6	1.7
IOD	2.9	3	2.9	2.9	3.1	3.1	2.1	2.4
TMP	1.4	1.5	1.9	1.5	1.8	1.6	–	–
TEY	1.3	0.4	0.9	1.0	0.6	1.5	–	–
TIB	10.4	10.7	11.2	11.3	12.2	11.3	7.6	7.9
ML	5.2	5.5	5.5	5.2	6.4	5.5	3.8	4.1
PL	9.9	10.3	10.3	9.8	11.9	10.9	6.8	7.2
F1L	2.0	2.0	2.3	2	2.3	2.3	1.1	1.4
F2L	2.3	2.5	2.5	2.3	2.5	2.5	1.3	1.6
F3L	3.8	4.1	4.3	4.3	5.0	4.5	2.7	2.9
TIB:SVL	0.44	0.44	0.45	0.46	0.41	0.39	0.44	0.46
HDL:HDW	1.04	1.05	1.04	1.07	1.06	1.07	1.00	1.02
HDL:SVL	0.36	0.37	0.39	0.39	0.34	0.36	0.31	0.33
Weight (g)	1.1	1.2	1.4	1.2	2.2	1.4	0.5	0.6

*holotype.

Colour of holotype in life. Dorsal surface brown with distinct darker brown markings; V-shaped interorbital marking, W-shaped marking between axillae and inverted V-shaped, irregular marking above sacrum; fine, pale greyish-blue tubercles on upper eyelids, snout, lateral margins of dorsum and upper surfaces of legs; blackish brown patch on upper lip under eye; blackish brown line along canthus rostralis through eye, and continuing below supratympanic ridge, terminating above axilla, encompassing nare and most of tympanum; transverse dark brown bars on dorsal surface of limbs; large, black blotch on posterior flank joining with inverted V-shaped marking above sacrum and numerous, smaller black spots on sides from groin to axilla; elbow and upper arms without dark bars but with distinct copper colouration; fingers and toes with transverse barring. Dark brownish red ventral surface with white speckling on entire ventral surface including throat, arms and legs, forming distinct marbling on

chest and belly; ventral margin of throat bearing broken row of slightly larger white spots. Supra-axillary gland copper; femoral glands white; pectoral glands white, lined with dark brown. Iris bicoloured, with upper half coppery red, fading to pale silver in lower half; minute, black reticulations throughout.

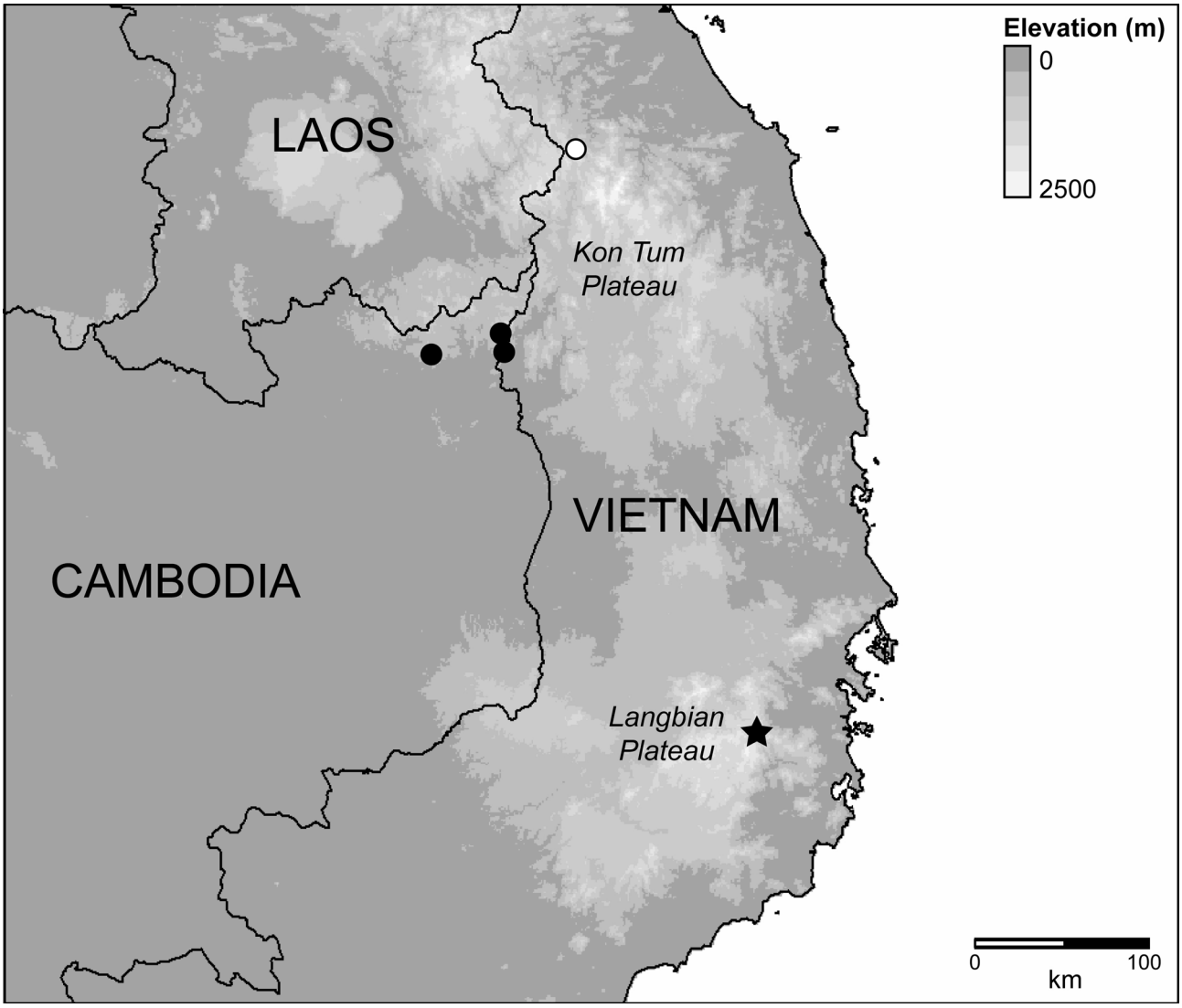

FIGURE 1. Collection site of *Leptolalax bidoupensis* **sp. nov.** from Bidoup-Nui Ba National Park (black star) and type localities of *Leptolalax applebyi* (white circle) and *Leptolalax melicus* (black circles).

Colour of holotype in preservative. Dorsum dark brown with slightly paler limbs. Ventral surface brown, with white speckling and marbling. Macroglands white. Patterns on dorsal surface less distinct (Figure 3).

Measurements. Holotype: SVL 23.6, HDL 9.2, HDW 8.9, SNT 3.6, EYE 2.5, IOD 2.9, TMP 1.4, TEY 1.3, TIB 10.4, ML 5.2, PL 9.9, F1L 2.0, F2L 2.3, F3L 3.8, weight 1.1g

Variation. Specimens vary slightly in colour in life. AMS R 173135 and NCSM 77320 have a paler brown dorsum compared to the holotype, and dorsal patterns lined with diffuse, paler brown. AMS R 173136 and NCSM 77321 also have paler brown dorsal surfaces, but with only indistinct, diffuse dorsal markings; in NCSM 77321 the only distinct dark markings are over the tympanum, below the supratympanic ridge and as a single patch on the flank; AMS R 173136 has larger and more numerous brownish black patches on the flanks. AMS R 173134 and UNS 00101/AMS R 173135 have more mottled dorsal patterns. The ventral surfaces of specimens in the type series vary in their degree of white speckling and in the presence or absence of white marbling on parts of the chest and belly; UNS 00101/AMS R 173135 and NCSM 77320–77321 have less intense white speckling on ventral surfaces and only slight white marbling on parts of their chest; AMS R 173133 has slightly less intense white speckling and no white marbling. Pectoral glands are more distinct in specimens with less marbling. Ventrolateral glands are most

easily detected in the holotype AMS R 173133, and more widely spaced and difficult to distinguish from white ventral patterning on all other specimens in the type series. UNS 00102/AMS R 173137 and NCSM 77322 are metamorphs with incompletely reabsorbed tails (tail length 2 mm in UNS 00101/AMS R 173137 and 8 mm in NCSM 77322), almost uniformly dark brown dorsal surfaces, dark brown ventral surfaces with white speckling, and indistinct tympana. Iris colouration varies slightly in the balance of coppery red versus pale silver (Figure 5A–C).

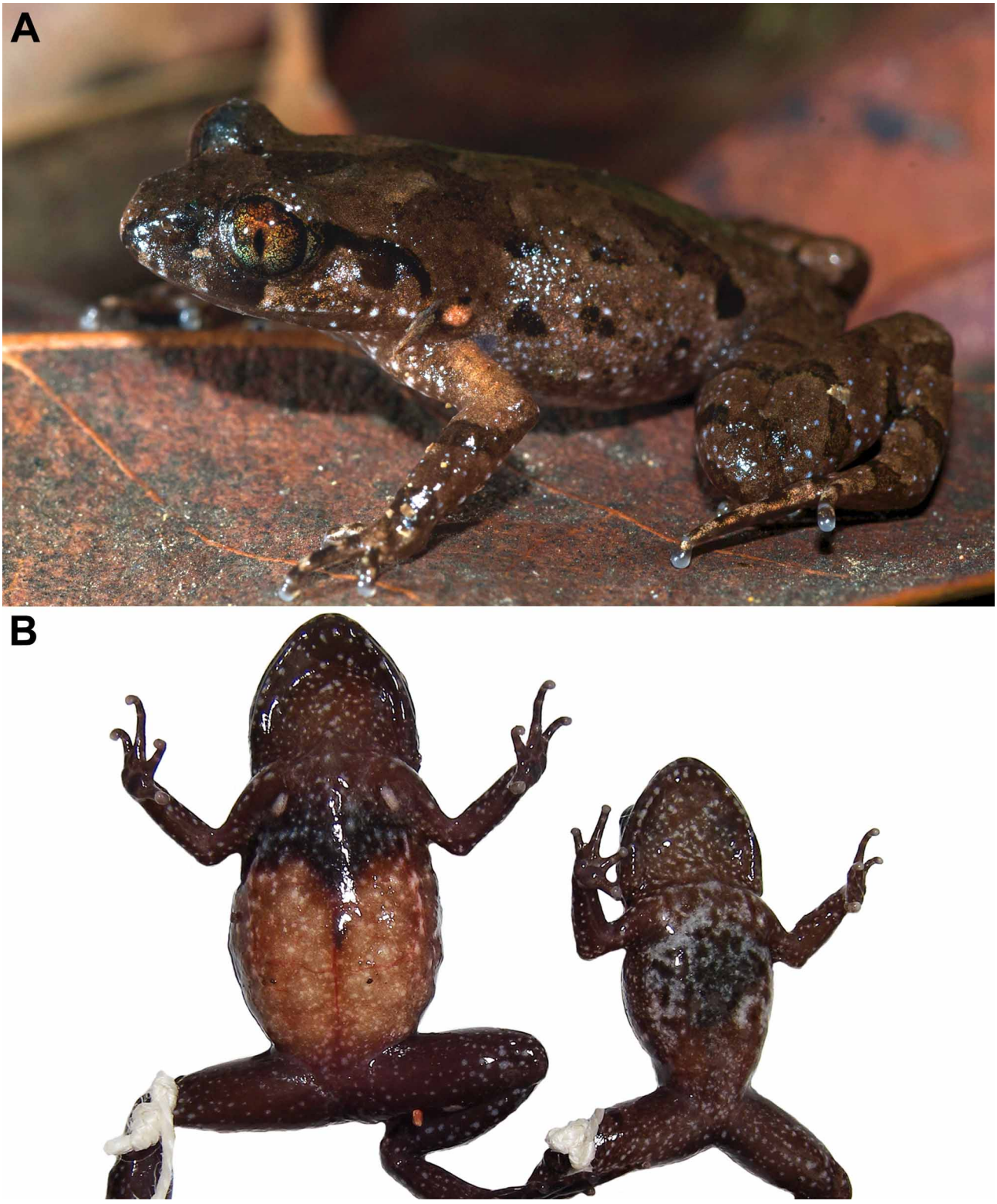

FIGURE 2. (A) Lateral view of male holotype (AMS R 173133) of *L. bidoupensis* **sp. nov**. in life, and (B) ventral views of gravid female paratype (AMS R 173134) and male holotype (AMS R 173133) of *L. bidoupensis* **sp. nov.** in life.

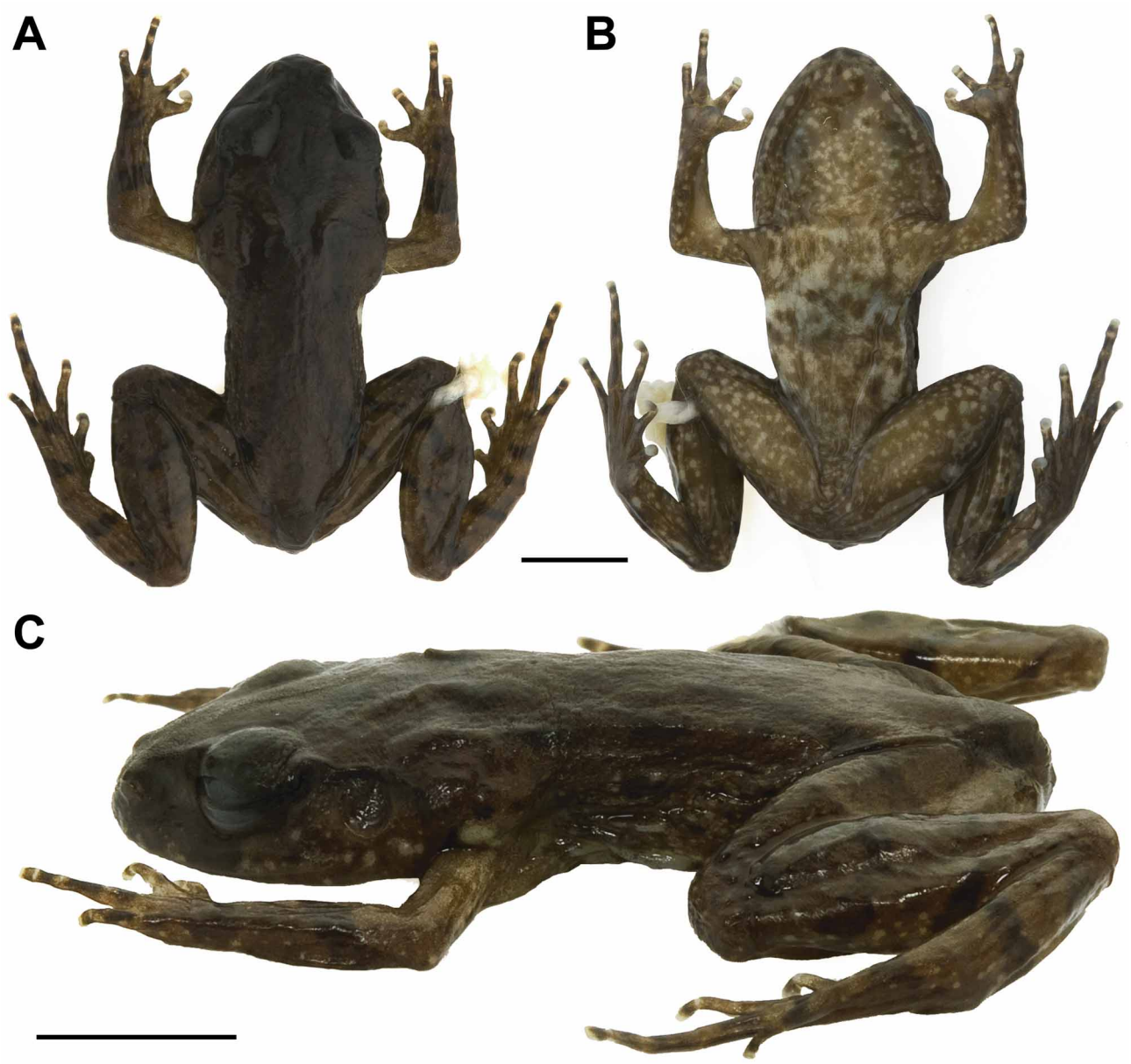

FIGURE 3. (A) Dorsal, (B) ventral and (C) lateral view of preserved holotype (AMS R 173133) of *L. bidoupensis* **sp. nov**. Scale bars = 5 mm.

Advertisement call. Call descriptions are based on the calls of the holotype, taken at 19.0 ºC ambient temperature. Calls were an average of 479 ms in duration and consisted of 6–9 (usually 7) singly pulsed notes, or clicks, of variable (1–13 ms) duration, repeated at a rate of 18–21 notes per second (Table 2, Figures 4A, C). Calls were highly amplitude modulated, with amplitude peaking towards the middle of the call. The dominant frequency was 2.3–2.6 kHz, and harmonics were weak or absent. Slight frequency modulation was present within each call, with lower amplitude notes, particularly the last note, often slightly higher or lower frequency. Calls were repeated at a rate of approximately 0.2 calls per second, and had an average intercall interval of 4 s. Call repetition rate, intercall interval, and the number of notes per call varied slightly within calling bouts and among individuals (Table 2, Figure 4). To the human ear, the advertisement call of *L. bidoupensis* is a slow rasping, similar to an orthopteran, with each note distinctly discernable. The call is extremely faint and difficult to locate, even when compared to other species within the genus.

TABLE 2. Measurements of advertisement call parameters for *Leptolalax bidoupensis* **sp. nov.** Parameter values are given as means (and ranges).

	AMS R 173133*	UNS 00101/ AMS R 173135	AMS R 173136	Non-vouchered 1	Non-vouchered 2
Number of calls	10	10	10	8	10
Number of notes	85	53	67	50	72
Call duration (ms)	479 (326–543)	320 (278–343)	334 (234–391)	394 (380–411)	401 (277–463)
Call repetition rate (calls/s)	0.22	0.17	0.13	0.14	0.1
Intercall interval (ms)	4014 (3590–4521)	5537 (4393–7430)	7071 (4984–13106)	6689 (5153–8492)	9511 (7560–12284)
Notes/call	8.2 (6–9)	6.7 (6–7)	6.7 (5–8)	7 (7)	7.2 (5–8)
Note duration (ms)	8.4 (1–13)	6.3 (2–9)	8.3 (4–11)	7.9 (4–12)	7.4 (4–11)
Internote interval (ms)	55 (18–70)	49 (42–59)	49 (9–76)	56 (46–76)	55 (22–66)
Note repetition rate (notes/s)	18–21	21	14–21	18	18
Dominant frequency (kHz)	2.1(2.3–2.6)	2.1 (2.1–3.6)	2.5 (1.9–3.4)	2.0 (1.9–2.3)	2.5 (2.3–3.8)
Temperature (°C)	19.0	20.4	21.0	20.2	20.4

*holotype

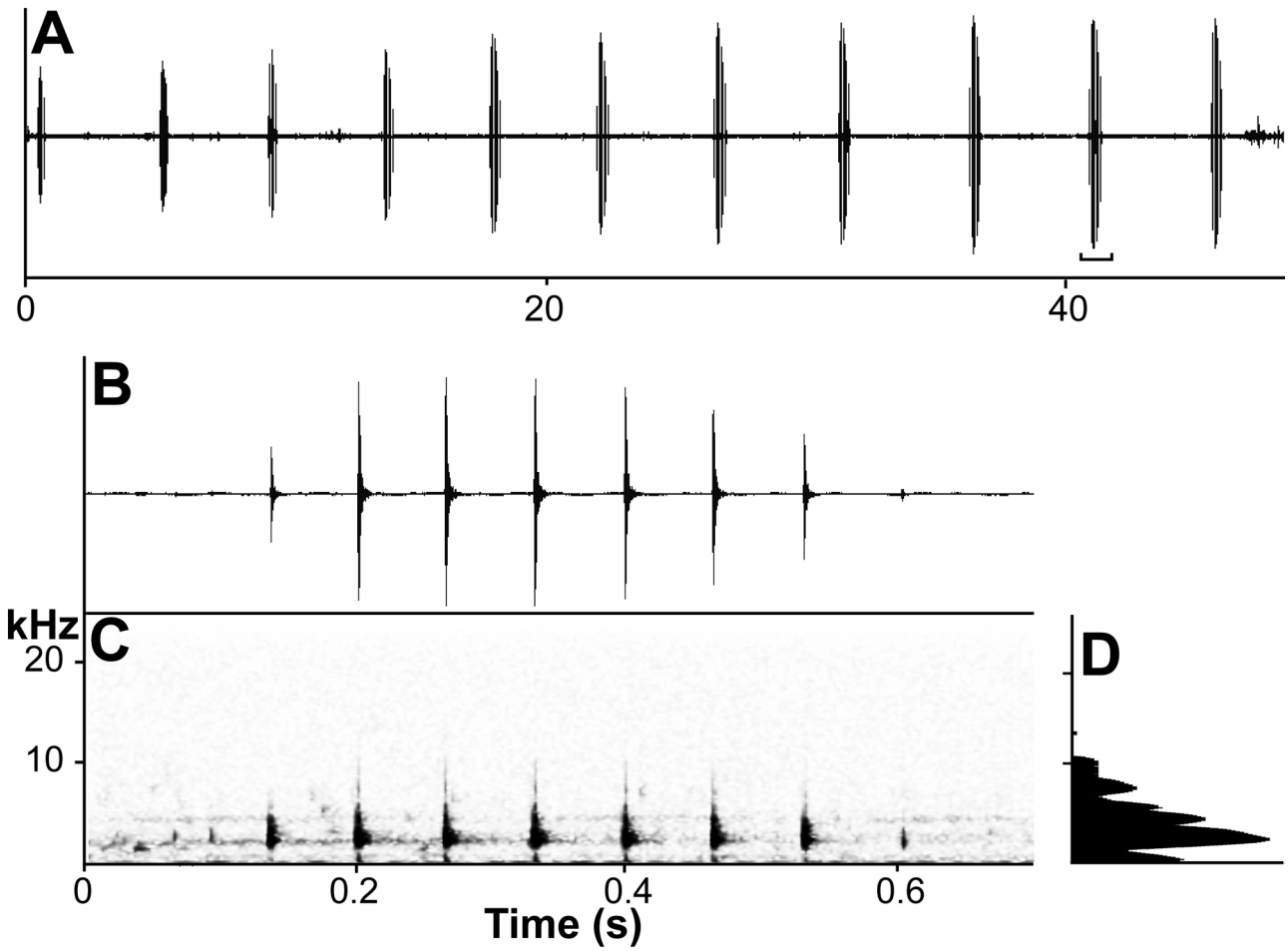

FIGURE 4. Advertisement call of *Leptolalax bidoupensis* **sp. nov.** (holotype AMS R 173133) recorded at ambient air temperature of 19.0º C. (A) 48 s waveform of relative amplitude over time for 11 calls, (B) waveform and (C) corresponding spectrogram of single representative call containing eight notes, expanded from section shown in A, and (D) power spectrum (relative amplitude vs. frequency) of fourth note shown in B–C.

Sequence divergence. Uncorrected sequence divergences between *L. bidoupensis* (NCSM 77320–77321, AMS R 173133–173134) and *L. applebyi* (holotype AMS R 171703, paratype AMS R 171704) collected from approximately 350 km away were 9.3% at the 16S rRNA gene. Uncorrected sequence divergences between *L. bidoupensis* and *L. melicus* (MVZ 258197–258199) collected from approximately 300 km away were 9.6% at the 16S rRNA gene. This degree of pairwise divergence in the 16S rRNA gene in frogs usually represents differentiation at the species level (Vences *et al.* 2005), and is greater than that between *L. applebyi* and *L. melicus* (6.6%). There was no intraspecific variation in this gene fragment for *L. bidoupensis*, *L. applebyi*, and *L. melicus*.

Ecology. All specimens of the new species were found in montane evergreen forest between 1620–1730 m elevation. Males were observed calling on stream banks, less than 0.5 m from small (< 5 m wide) rocky streams in May and July. The new species was not heard or observed during surveys in the month of March, when conditions were cooler and drier.

Conservation status. *Leptolalax bidoupensis* is known only from an area of approximately 1 km^2 in Bidoup Nui Ba National Park. The actual distribution of the new species is unknown but probably extends to adjacent forested areas in the Langbian Plateau, including Chu Yang Sin National Park in Dak Lak Province, and Phuoc Binh National Park in Ninh Thuan Province, both of which contain montane evergreen forest at similar elevations and continuous with Bidoup-Nui Ba National Park. Given the available information, we suggest the species should be considered Data Deficient following IUCN's Red List categories (IUCN 2001).

Comparisons. *Leptolalax bidoupensis* is distinguished from all other species in the genus except for *L. applebyi* by having a dark brownish red ventral surface with white speckling on entire ventral surface including throat, arms and legs, forming distinct marbling on chest and belly (*L. aereus*, *L. alpinis*, *L. arayai*, *L. bourreti*, *L. dringi*, *L. fuliginosus*, *L. gracilis*, *L. hamidi*, *L. khasiorum*, *L. lateralis*, *L. liui*, *L. nahangensis*, *L. oshanensis*, *L. pelodytoides*, *L. pictus*, *L. solus*, *L. sungi*, *L. tamdil* and *L. tuberosus* have mostly white or pale grey/brown venters, with or without dark spots or mottling; *L. croceus* has a bright orange belly; *L. melicus* has an off-white to pale pink ventral surface with diffuse dark brown blotches and distinct white speckling on the chest, belly and throat; *L. pluvialis* has a dirty white/grey venter with dark brown/grey marbling, and uniform pale dirty white/grey throat with pale speckling only around the margins; *L. melanoleucus* and *L. ventripunctatus* display large patches of distinct black and white marbling, *L. heteropus* has a grey venter, speckled with black; *L. maurus* has a black or dark grey brown venter, with indistinct small light areas, and *L. kecil* has a uniformly dark venter with large, dark orange pectoral glands).

The small size of *Leptolalax bidoupensis* (23.6–24.6 mm in four adult males and 29.2–29.4 mm in two adult females) further distinguishes it from the larger *L. arayai* (male 29.6 mm), *L. bourreti* (male 36.2 mm, females 42–45 mm). *L. dringi* (males 28.7–30.3 mm, female 37.5 mm), *L. fuliginosus* (males 28.2–30.0 mm), *L. gracilis* (males 30–36 mm), *L. hamidi* (males 28.7–31.3 mm, females 36.1–42.8 mm), *L. heteropus* (male 33 mm), *L. kajangensis* (males 34–35 mm), *L. nahangensis* (male 40.8 mm), *L. pelodytoides* (male ~30 mm, female ~37 mm), *L. pictus* (males 31–34 mm, female 47 mm), *L. sungi* (males 48.3–52.7 mm, females 56.7–58.9), and *L. tamdil* (male 32.3 mm, female 31.8 mm). The remaining, small-sized congeners may have overlapping body-sizes for either or both sexes (*L. aereus* males 25.1–28.9 mm, females 27.1–38.6 mm; *L. alpinis* males 24.0–26.4 mm, females 31.7–32.5 mm; *L. applebyi* males 19.6–20.8 mm, females 21.7 mm; *L. croceus* males 22.2–27.3 mm; *L. kecil* males 19.3–20.5 mm, female 20.5 mm; *L. khasiorum* males 24.5–27.3 mm, females 31.2–33.5 mm; *L. lateralis* males 26.9–28.3 mm, female 36 mm; *L. liui* males 23.0–28.7 mm, females 23.1–28.1 mm; *L. maurus* male 26.1 mm, female 31.8 mm; *L. melanoleucus* males 26.6–28.8 mm, female 32.7 mm; *L. melicus* males 19.5–22.7 mm; *L. oshanensis* males 26.6–30.7 mm, female 31.6 mm; *L. pluvialis* males 21.3–22.3 mm; *L. solus* male 27.6 mm; *L. tuberosus* males 24.4–29.5 mm, female 30.2 mm; and *L. ventripunctatus* males 25.5–28.0 mm).

In having a bicoloured iris, with the upper half coppery red and the lower half fading to pale silver, *L. bidoupensis* differs from at least *L. aereus*, *L. applebyi*, *L. croceus*, *L. kajangensis*, *L. kecil*, *L. maurus*, *L. melicus*, *L. nahangensis*, *L. sungi* and *L. tuberosus*, all of which have uniform iris colouration under black reticulations (Table 3; Figure 5). *Leptolalax bidoupensis* is also differentiated from *L. arayai*, *L. croceus*, *L. khasiorum*, *L. lateralis*, *L. maurus*, *L. solus*, *L. tamdil*, *L. tuberosus* and *L. ventripunctatus* in having mostly smooth (versus tuberculate) skin texture with no skin ridges, and from *L. pictus* and *L. pluvialis* in having relatively short tibia (male TIB:SVL 0.44–0.46, versus 0.55–0.61 in *L. pictus* and 0.52–0.56 in *L. pluvialis*).

Leptolalax bidoupensis can be further differentiated from the two most morphologically similar species, *L. applebyi* and *L. melicus*, by having slight lateral fringing on feet, and by being approximately 19% larger than *L.*

applebyi (Wilcoxin rank-sum test, W= 16, p = 0.0284; N=8) and 14% larger than *L. melicus* (Wilcoxin rank-sum test, W= 28, p = 0.0106; N=11). In life, *L. bidoupensis* also weighs more than either species (*L. bidoupensis* males 1.1–1.4 g, N=4; *L. applebyi* males 0.8–0.9 g, N=7; *L. melicus* males 0.7–0.75 g, N=2).

TABLE 3. Iris colouration in life for species within the genus *Leptolalax*.

Species	Iris bi-coloured	Iris colouration	Source
*L. aereus**	No	Bronze with minute, black reticulations.	Rowley *et al.* 2010c
L. alpinis	No?	Copper/gold with black reticulations.	Fei *et al.* 2010 (from figures on p. 169)
*L. applebyi**	No	Coppery gold to gold with black reticulations.	Rowley & Cao 2009
L. arayai	Yes	Upper 100–120° bright red-brown, rest whitish grey to light greyish green, with black reticulations.	Malkmus *et al.* 2002
L. bidoupensis **sp. nov**.*	Yes	Upper half coppery red, lower half fading to pale silver. Extensive black reticulations.	Present paper
L. bourreti	?	?	–
*L. croceus**	No	Pale gold with distinct, black reticulations encircling the pupil.	Rowley *et al.* 2010a
L. dringi	Yes	Upper section reddish, rest pale golden grey. Black reticulations.	Inger & Stuebing 2005 (from Fig. 19)
*L. fuliginosus**	Yes	Upper half reddish orange, lower half gold. Black reticulations.	Matsui 2006
L. gracilis	Yes	Upper section reddish orange, rest pale golden grey. Black reticulations.	Inger & Stuebing 2005 (from Fig. 20)
L. hamidi	Yes	Upper half reddish, rest pale golden grey, with black reticulations.	Inger & Stuebing 2005 (from Fig. 21)
*L. heteropus**	?	?	–
*L. kajangensis**	No	Metallic gold with black reticulations.	Grismer *et al.* 2004
*L. kecil**	No	Dark red.	Matsui *et al.* 2009
*L. khasiorum**	Yes	Upper third bright orange, rest yellowish cream. Black reticulations.	Das *et al.* 2010
L. lateralis	?	?	–
L. liui	?	?	–
L. maurus	No	Reddish brown.	Malkmus *et al.* 2002
*L. melanoleucus**	Yes	Upper half orange, lower half silver. Black reticulations.	Matsui 2006
*L. melicus**	No	Dark gold. Black reticulations.	Rowley *et al.* 2010b
*L. nahangensis**	No	Gold with black reticulations.	Lathrop *et al.* 1998
L. oshanensis	No?	Copper/gold with black reticulations.	Fei *et al.* 2010 (from figures on p. 172)
L. pelodytoides	?	?	–
*L. pictus**	Yes	Upper 120° with segments of dark brown, rest golden-grey. Black reticulations	Malkmus *et al.* 2002
*L. pluvialis**	No?	Brown?	Ohler *et al.* 2000 (from Fig. 2)
*L. solus**	Yes	Upper half dark red, lower half dark brown	Matsui 2006
*L. sungi**	No	Gold-green. Black reticulations only at edges	Lathrop *et al.* 1998
*L. tamdil**	Yes	Upper third bright orange, rest greyish cream, edged with black reticulations.	Sengupta *et al.* 2010
L. tuberosus	No	Pale gold with minute black reticulations.	Rowley *et al.* 2010a
L. ventripunctatus	?	?	–

* documented for type specimens.

TABLE 4. Summary of male advertisement call parameters for species of *Leptolalax* with documented calls.

Species	Notes/ call	Call duration (ms)	Call repetition rate (calls/s)	Variable note structure	Pulses/note	Note duration (ms)	Dominant frequency (kHz)	Frequency modulation	Temp °C	Source
*aereus**	2–16	13–130	2.4–8.0	Slight	1–2	3–30	6.2–7.9	No	22.4–25.7	Rowley *et al.* 2010c
alpinis	9.45±2.73	270±69	?	No?	1?	3	6.7±0.1	No?	16	Xu *et al.* 2005
*applebyi**	4–5	280–441	1.7	No	1–5	4–32	4.0–4.3	No	21.5	Rowley & Cao 2009
*arayai**	2	29	9.0–9.3	Note 1 shorter	1	?	5.4–5.8	Slight decrease	17.4	Matsui *et al.* 1997
*bidoupensis**	6–9	277–543	0.1–0.2	No	1	1–13	1.9–3.8	Very slight	19–21	Present paper
*croceus**	4–6	164–249	0.1–0.7	Yes	1–31 (variable note type)	Variable	4.6–3.0	No	21.6–25.1	Rowley *et al.* 2010a
dringi	2–4	47	11.0–11.5	No	?	?	7.6–8.1	Strong decrease	24.3	Matsui *et al*. 1997
*fuliginosus**	6–9	51–80	2.9	No?	?	?	2.3–2.4	No	19.3–19.6	Matsui 2006
gracilis	3–4	47	6.9–7.2	No	1?	?	2.5–2.7	Slight	20.0	Matsui *et al.* 1997
hamidi	2–3	33	9–9.3	Note 1 longer?	?	?	6.7–7.3	Strong decrease	22.9	Matsui et al. 1997
heteropus	3–6	207–513	?	Yes	Note 1: c. 20 Notes 2–3: 5–6	Note 1: 80 Notes 2–3: 28	2.8	Slight	21.0	Matsui *et al*. 1997
*kecil**	4	110–130	c. 0.2	No?	1–2	?	3.2	Very weak	18.5–21.4	Matsui *et al.* 2009
maurus	4	85		No	1	8	5.2	No	?	Sukumaran *et al.* 2010
*melanoleucus**	3–4	40–63	6.1	No?	?	?	3.1–3.3	No	23.9	Matsui 2006
*melicus**	4–11	168–484	0.7–0.8	Yes	Note 1: 8–50 Clicks: 1–7	Note 1: 24–132 Clicks: 2–24	2.6–4.0	No	26.1–26.2	Rowley *et al.* 2010b
oshanensis	3	?	c. 2–3	No?	?	29–67	4.4–4.6	No?	14	Jiang *et al.* 2002
pictus	1	30–35	11–13	No	?	na	6.8–7.2	Strong decrease	19–22	Malkmus *et al*. 2002
*solus**	4–8	130–260	2.1–2.3	No	?	?	3.1–3.2	No	24.2–24.3	Matsui 2006
tuberosus	1	54–78	0.1–0.5	No	Indistinctly pulsed	na	2.6–2.8	No	22.5–24.5	Rowley *et al.* 2010a

* documented from type specimens.

The advertisement call of *L. bidoupensis* differs in terms of structure and/or frequency from all other *Leptolalax* with described calls (Table 4). In addition, uncorrected sequence divergences between *L. bidoupensis* and all homologous 16S rRNA sequences available on GenBank (from individuals assigned to *L. applebyi*, *L. arayai*, *L. bourreti*, *L. liui*, *L. melicus*, *L. oshanensis*, *L. pelodytoides*, and *L. pictus*) were >9%.

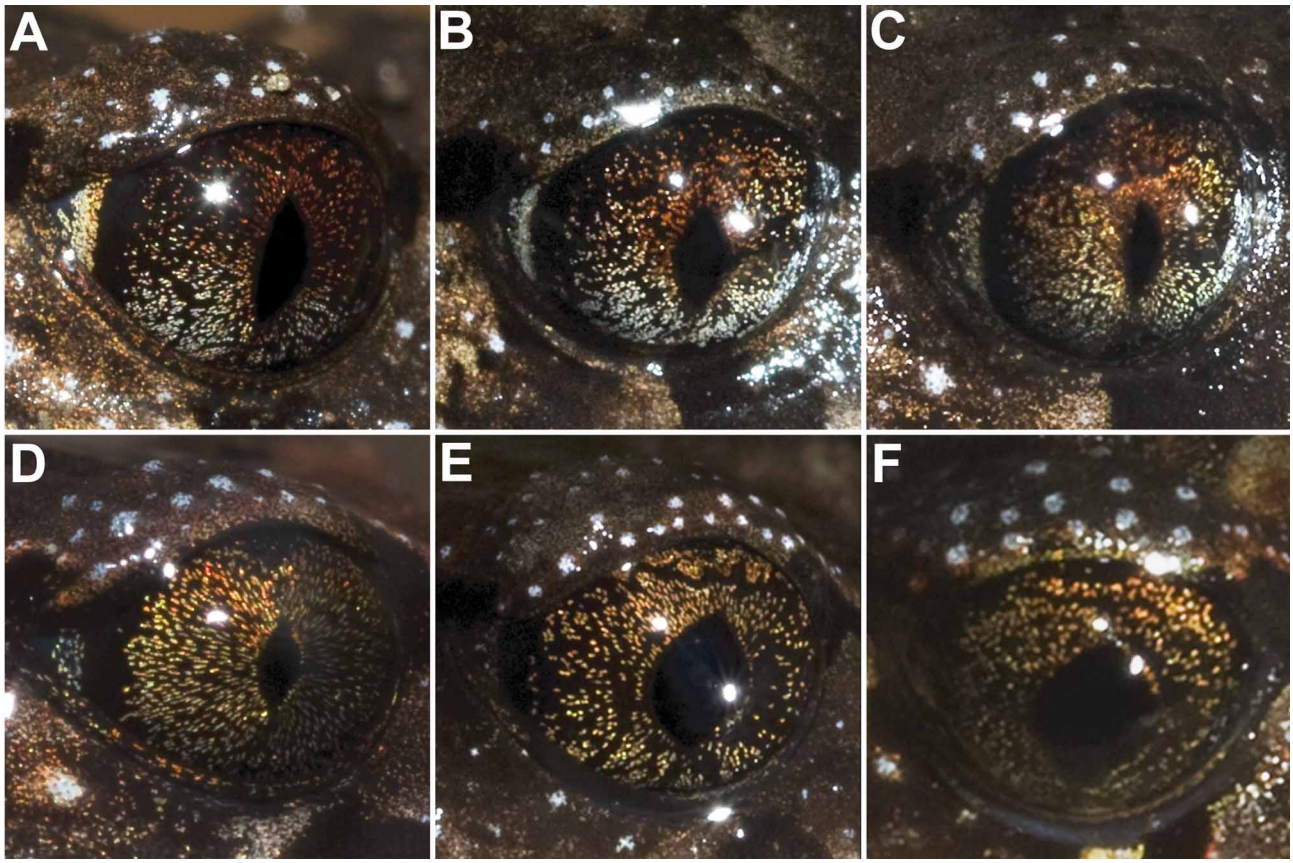

FIGURE 5. Iris colouration in life of (A) *Leptolalax bidoupensis* **sp. nov.** NCSM77320, (B) *Leptolalax bidoupensis* **sp. nov.** AMS R 173134, (C) *Leptolalax bidoupensis* **sp. nov.** AMS R 173133, (D–E) *Leptolalax applebyi*, and (F) *Leptolalax melicus* MVZ 258199.

Acknowledgements

The Vietnamese Ministry of Agriculture and Rural Development and staff at Bidoup-Nui Ba National Park kindly facilitated surveys and issued permission to collect (Permit number 3023/GT-BNN-KL). Da Du Ha Tien, Vu Hanh Dung, Nguyen Thi Xuan Phuong, Ly Tri, and Dinh Binh Phuong assisted with fieldwork. The research was supported by grants from the National Geographic Conservation Trust, ADM Capital Foundation, Conservation International, and The John D. and Catherine T. MacArthur Foundation. Bryan L. Stuart kindly loaned specimens and tissues in his care and provided valuable comments on the manuscript. Ross Sadlier, Cecilie Beatson, Rebecca Johnson, Karen Gray and Robert Mason provided assistance with laboratory work. Indraneil Das provided valuable input on *Leptolalax* advertisement calls. Tandy Rowley provided photographs of *L. bidoupensis* specimens in preserve and Annemarie Ohler shared photographs of the holotype of *L. pluvialis*. For all this assistance we are most grateful.

References

Anderson, J. (1871) A list of the reptilian accession to the Indian Museum, Calcutta, from 1865 to 1870, with a description of some new species. *Journal of the Asiatic Society of Bengal*, 40, 12–39.

Boulenger, G.A. (1893) Concluding report on the reptiles and batrachians obtained in Burma by Signor L. Fea, dealing with the

collection made in Pegu and the Karin Hills in 1887-88. *Annali del Museuo Civico di Genoa,* 13, 304–347.
Boulenger, G.A. (1900) Descriptions of new batrachians and reptiles from the Larut Hills, Perak. *Annals and Magazine of Natural History,* 6 (series 7) 186–194.
Boulenger, G.A. (1908) A revision of the Oriental pelobatid batrachians (genus *Megalophrys*). *Proceedings of the Zoological Society of London,* 1908, 407–430.
Brown, R.M. & Richards, S.J. (2008) Two new frogs of the genus *Platymantis* (Anura: Ceratobatrachidae) from the Isabel Island group, Solomon Islands. *Zootaxa,* 1888, 47–68.
Cocroft, R.B. & Ryan, M.J. (1995) Patterns of advertisement call evolution in toads and chorus frogs. *Animal Behaviour,* 49, 283–303.
Das, I., Lyngdoh Tron, R.K., Rangad, D., Hooroo, R.N.K. (2010) A new species of *Leptolalax* (Anura: Megophryidae) from the sacred groves of Mawphlang, Meghalaya, north-eastern India. *Zootaxa,* 2339, 44–56.
Dubois, A. (1983) Note preliminaire sur le genre *Leptolalax* Dubois, 1980 (Amphibiens, Anoures), avec diagnose d'une espece novella du Vietnam. *Alytes*, 2, 147–153.
Dubois, A. (1987 "1986") Miscellanea taxinomica batrachologia (I). *Alytes,* 5, 7–95.
Duellman, W.E. (1970) The Hylid Frogs of Middle America. *Monograph of the Museum of Natural History, University of Kansas*. 1, 1–427.
Fei, L., Hu, S., Ye, C. & Huang, Y. (2009) *Fauna Sinica. Amphibia Vol. 2 Anura.* Science Press, Beijing. 957 pp.
Fei, L., Ye, C. & Huang, Y. (1991 "1990") *Key to Chinese Amphibia.* Chongqing Branch Science and Technology Literature Press, Chongquing. 364 pp.
Fei, L., Ye, C. & Jiang, J. (2010). *Colored Atlas of Chinese Amphibians*. Sichuan Publishing Group. Sichuan Publishing House of Science & Technology, Sichuan, 519 pp.
Frost D.R. (2010) *Amphibian Species of the World: an online reference. Version 5.4 (8 April, 2010).* American Museum of Natural History, New York, USA. Available from http://research.amnh.org/vz/herpetology/amphibia/ (accessed 25 January 2011).
Gerhardt, H.C., & Huber, F. (2002) *Acoustic Communication in Insects and Anurans : Communication Problems and Diverse Solutions*. University of Chicago Press, Chicago and London. 516 pp.
Grismer, L.L., Grismer, J.L. & Youmans, T.M. (2004) A New Species of *Leptolalax* (Anura: Megophryidae) from Pulau Tioman, West Malaysia. *Asiatic Herpetological Research,* 10, 8–10.
Günther, A. (1872) On the reptiles and amphibians of Borneo. Proceedings of the Zoological Society of London. 1872, 586–600.
Humtsoe, L.N., Bordoloi, S., Ohler, A. & Dubois, A. (2008) Rediscovery of a long known species, *Ixalus lateralis* Anderson, 1871. *Zootaxa*, 1921, 24–34.
Inger, R.F., Lakim, M., Biun, A. & Yambun, P. (1997) A New species of *Leptolalax* (Anura: Megophryidae) from Borneo. *Asiatic Herpetological Research,* 7, 48–50.
Inger, R.F., Orlov, N. & Darevsky, I. (1999) Frogs of Vietnam: a report on new collections. *Fieldiana Zoology,* 92, 1–46.
Inger, R.F. & Stuebing, R.B. (2005) *A Field Guide to the Frogs of Borneo*. Natural History Publications, Kota Kinabalu. 201 pp.
IUCN. (2001) *IUCN Red List Categories and Criteria: Version 3.1.* IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK. ii + 30 pp.
Jiang, J.-P., Xie, F., Fei, L., Ye, C. & Zheng, M-Q. (2002) Mating calls of six forms of pelobatid in Wawu Mountain national forest park, Sichuan, China (Anura: Pelobatidae). *Zoological Research,* 23, 89–94.
Lathrop, A., Murphy, R.W., Orlov, N. & Ho, C.T. (1998) Two new species of *Leptolalax* (Anura: Megophryidae) from northern Vietnam. *Amphibia-Reptilia,* 19, 253–267.
Liu, C-C. (1950) Amphibians of western China. *Fieldiana: Zoology Memoirs,* 2, 1–400.
Malkmus, R. (1992) *Leptolalax pictus sp.n.* (Anura: Pelobatidae) vom Mount Kinabalu/Nord-Borneo. *Sauria,* 14, 3–6.
Malkmus, R., Manthey, U., Vogel, G., Hoffman, P. & Kosuch, J. (2002) *Amphibians and Reptiles of Mount Kinabalu (North Borneo).* ARG Ganter Verlag Kommanditgesellschaft, Ruggell. 424 pp.
Matsui, M. (1997) Call characteristics of Malaysian *Leptolalax* with a description of two new species (Anura: Pelobatidae). *Copeia,* 1997, 158–165.
Matsui, M. (2006) Three new species of *Leptolalax* from Thailand (Amphibia, Anura, Megophryidae). *Zoological Science,* 23, 821–830.
Matsui, M., Belabut, D.M., Ahmad, N. & Yong, H-S. (2009) A New species of *Leptolalax* (Amphibia, Anura, Megophryidae) from peninsular Malaysia. *Zoological Science*, 26, 243–247.
Nguyen, V. S., Ho, T.C. & Nguyen, Q. T. (2009) *Herpetofauna of Vietnam*. Edition Chimaira, Frakfurt am Main. 768 pp.
Rowley, J.J.L, & Cao, T.T. (2009) A new species of *Leptolalax* (Anura: Megophryidae) from central Vietnam. *Zootaxa*, 2198, 51–60.
Rowley, J.J.L., Hoang, D.H., Le, T.T.D., Dau, Q.V. & Cao, T.T. (2010a) A new species of *Leptolalax* (Anura: Megophryidae) from Vietnam and further information on *Leptolalax tuberosus*. *Zootaxa*, 2660, 33–45.
Rowley, J.J.L., Stuart, B.L., Neang, T., Emmett, D.A. (2010b) A new species of *Leptolalax* (Anura: Megophryidae) from north-eastern Cambodia. *Zootaxa,* 2567, 57–68.

Rowley, J.J.L., Stuart, B.L., Richards, S.J., Phimmachak, S. & Sivongxay, N. (2010c) A new species of *Leptolalax* (Anura: Megophryidae) from Laos. *Zootaxa*, 2681, 35–46.
Sengupta, S., Sailo, S., Lalremsanga, H.T., Das, A. & Das, I. (2010) A new species of *Leptolalax* (Anura: Megophryidae) from Mizoram, North-eastern India, *Zootaxa*, 2406, 57–68.
Smith, M.A. (1921) New or little-known reptiles and batrachians from southern Annam (Indo-China). *Proceedings of the Zoological Society of London*, 1921, 423–440.
Smith, M.A. (1924) New tree-frogs from Indo-China and the Malay Peninsula. *Proceedings of the Zoological Society of London*, 1924, 225–234.
Sukumaran, J., Das, I. & Haas, A. (2010) Descriptions of the advertisement calls of some Bornean frogs. *Russian Journal of Herpetology*, 17, 189–194.
Xu, J-X., Xie, F., Jiang, J-P., Mo, Y-M. & Zheng, Z-H. (2005) The acoustic features of the mating call of 12 anuran species. *Chinese Journal of Zoology*, 40, 12–19.

APPENDIX. Comparative material examined.

Leptolalax aereus: Laos, Savannakhet Province, Vilabouli District (NCSM 76038–76057, 76061–76071; SAMA R64234–64242 type specimens)
Leptolalax applebyi: Vietnam, Quang Nam Province, Song Thanh Proposed Nature Reserve (AMS R 171703–171707; type specimens)
Leptolalax croceus: Vietnam, Kon Tum Province, Ngoc Linh Nature Reserve (AMS R 173738–173748, UNS 00108/ AMS R 173750, AMS R 173751, UNS 00109/AMS R 173752, UNS 00110/AMS R 173775, UNS 00111/AMS R 173779; type specimens)
Leptolalax melicus: Cambodia, Ratanakiri Province, Virachey National Park (MVZ 258074–258077, MVZ 258197– 258199; type specimens)
Leptolalax oshanensis: China, Sichuan Province, Hongya Xian (FMNH 232907–232921; c. 50 km from type locality)
Leptolalax tuberosus: Vietnam, Quang Nam Province, Song Thanh Proposed Nature Reserve (AMS R 171714–171722)