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Zootaxa 2198: 51–60 (2009)
www.mapress.com/zootaxa/
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Article

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

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

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Abstract

We describe a new species of megophryid frog in the genus *Leptolalax* from central Vietnam. *Leptolalax applebyi* is distinguished from its congeners by a combination of body size (19.6–20.8 mm for five adult males; 21.7 mm for single adult female), uniformly smooth, dark brown dorsum lacking tubercles, dark brownish pink ventral surface with white speckling, an absence of webbing and dermal fringes on fingers, slight basal webbing and no dermal fringes on toes, and short tibia (TIB:SVL 0.466–0.480). The advertisement call of *L. applebyi* consists of 4–5 notes with a dominant frequency of 3962.1–4306.6 Hz, repeated at a rate of approximately 9 notes per second. All specimens were found at the headwaters of rocky streams in evergreen forest above 1300 m elevation. We suggest the species should be considered Data Deficient following IUCN's Red List categories.

Key words: Acoustics, Anura, *Leptolalax applebyi* **sp. nov.**, Quang Nam, Southeast Asia

Introduction

The Southeast Asian megophryid genus *Leptolalax* Dubois is an assemblage of small, cryptic frogs inhabiting the forest floor. The number of species described in the genus has increased rapidly in recent decades, from only four species in 1983 (Dubois 1983), to over 20 species to date (Frost 2009). This increase is a reflection of both intensified field surveys in the region, and the use of advertisement call analysis in delineating species boundaries (Matsui 1997, 2006; Matsui *et al*. 2009).

Frogs of the genus *Leptolalax* breed in small or medium-sized streams in hilly evergreen forests (Inger & Stuebing 2005). With often specific habitat requirements and apparently localised distributions, it is likely that species within *Leptolalax* are vulnerable to habitat loss and degradation. Of the 23 described *Leptolalax* species, six are considered threatened, three are listed as near-threatened, and a further seven species are too poorly known to assess their conservation status and are listed as data deficient (Stuart *et al*. 2008). The most recently described species (Matsui *et al*. 2009) has yet to be assessed.

Six species of *Leptolalax* are currently known from Vietnam, but only two species, *L. pelodytoides* and *L. tuberosus*, have been reported from the central Annamites of Vietnam (Nguyen *et al*. 2009). During a field survey in central Vietnam, we recorded the calls and collected specimens of a small *Leptolalax* species that differs from all described congeners in morphology and advertisement call.

Materials and methods

We recorded morphological data from specimens preserved in 10% formalin and then stored in 70% ethanol. Morphometric data were taken (to the nearest 0.1 mm) with digital calipers. Measurements include snout-vent

Accepted by M. Vences: 6 Jul. 2009; published: 14 Aug. 2009

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length (SVL); head length from tip of snout to rear of jaws (HDL); head width at the commisure 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); foot length from proximal edge of inner metatarsal tubercle to tip of fourth toe (FOT). Mass was recorded in life, using Pesola scales. Comparative morphological characters were taken from references (*L. alpinis* [Fei *et al*. 1991]; *L. arayai* [Matsui 1997]; *L. bouretti* [Dubois 1983; Ohler *et al*. 2000]; *L. dringi* [Dubois 1986]; *L. fulignosus* [Matsui 2006]; *L. gracilis* [Günther 1872; Inger & Stuebing 2005]; *L. hamidi* [Matsui 1997]; *L. heteropus* [Boulenger 1900; Matsui 2006]; *L. kecil* [Matsui *et al*. 2009]; *L. kajangensis* [Grismer *et al*. 2004], *L. lateralis* [Anderson 1871; Humtsoe *et al*. 2008]; *L. liui* [Fei *et al*. 1991]; *L. maurus* [Inger *et al*. 1997]; *L. melanolectus* [Matsui 2006]; *L. nahangensis* [Lathrop *et al*. 1998]; *L. oshanensis* [Lui 1950; Fei *et al*. 2009]; *L. pelodytoides* [Boulenger 1893; Matsui 2006; Fei *et al*. 2009]; *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. tuberosus* [Inger *et al*. 1999]; *L. ventripunctatus* [Fei 1999; Fei *et al*. 2009]). *Leptolalax tuberosus* (AMS R 171714–171722) from Song Thanh Proposed Nature Reserve, Phouc Son district, Quang Nam Province, Vietnam, and colour photographs of the holotype of *L. pluvialis* (MNHN 199.5675) in preservative were also examined.

Advertisement calls were recorded with an Edirol R-09 24-bit WAVE/MP3 Recorder with a Røde NTG-2 condenser shotgun microphone at 44.1 kHz sampling rate and 24-bit encoding. Calls were recorded at a distance of approximately 0.2–0.5 m and ambient temperatures were taken immediately after recordings using a Kestrel 3500 hand-held weather meter. All calls were recorded at the same site and temperature, so no temperature corrections were necessary. 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. We examined oscillograms (waveforms) and audiospectrograms and 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 (Hz). Notes per second were calculated by counting the number of notes within each call, minus one, and dividing that number by the call duration. Comparative advertisement call characters for *Leptolalax* species were taken from references (Jiang *et al*. 2002; Malkmus *et al*. 2002; Matsui 1997, 2006; Matsui *et al*. 2009), and unpublished data (*Leptolalax tuberosus*; Rowley and Cao).

Leptolalax applebyi sp. nov.

Holotype: AMS R171703, an adult male, calling in leaf litter, 2 m from steep, narrow, rocky stream in Song Thanh Proposed Nature Reserve, Phouc Son district, Quang Nam Province, Vietnam (15.27394 ° N, 107.76015 ° E, 1402 m; Figure 1). Collected at 18:15 h on 23 July, 2007 by the authors.

Paratypes: AMS R 171704–171706 adult males from type locality (Figure 1), collected between 18:16–18:30 h on 23 July, 2007. All males were calling. AMS R 171707, one adult female collected at 19:00 h, on 24 July, 2007, from 15.26253 ° N, 107.75759 ° E, 1312 m. All specimens were found 1–2 m from steep, narrow, rocky streams in evergreen forest.

Etymology: specific epithet is a patronym honouring Robert Appleby, an investor in biodiversity conservation and scientific capacity building in Asia.

Diagnosis: Assigned to the genus *Leptolalax* on the basis of the following: small size, the presence of an elevated thenar tubercle not continuous to the thumb, chest glands present but not forming teats, vomerine teeth absent, anterior tip of snout with vertical white bar (Dubois 1980; Lathrop *et al*. 1998). *Leptolalax applebyi* is distinguished from its congeners by a combination of (1) body size (19.6–20.8 mm for five adult males; 21.7 mm for single adult female), (2) uniformly smooth dorsum lacking tubercles, (3) dark brown dorsal surface lacking distinct patterns and dark brownish pink ventral surface with white speckling (4) an absence of webbing and dermal fringes on fingers, (5) slight basal webbing and no dermal fringes on toes (6)

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short tibia (TIB:SVL 0.466–0.480), and (7) unique advertisement call consisting of 4–5 notes with a dominant frequency of 3962.1–4306.6 Hz, repeated at a rate of approximately 9 notes per second.

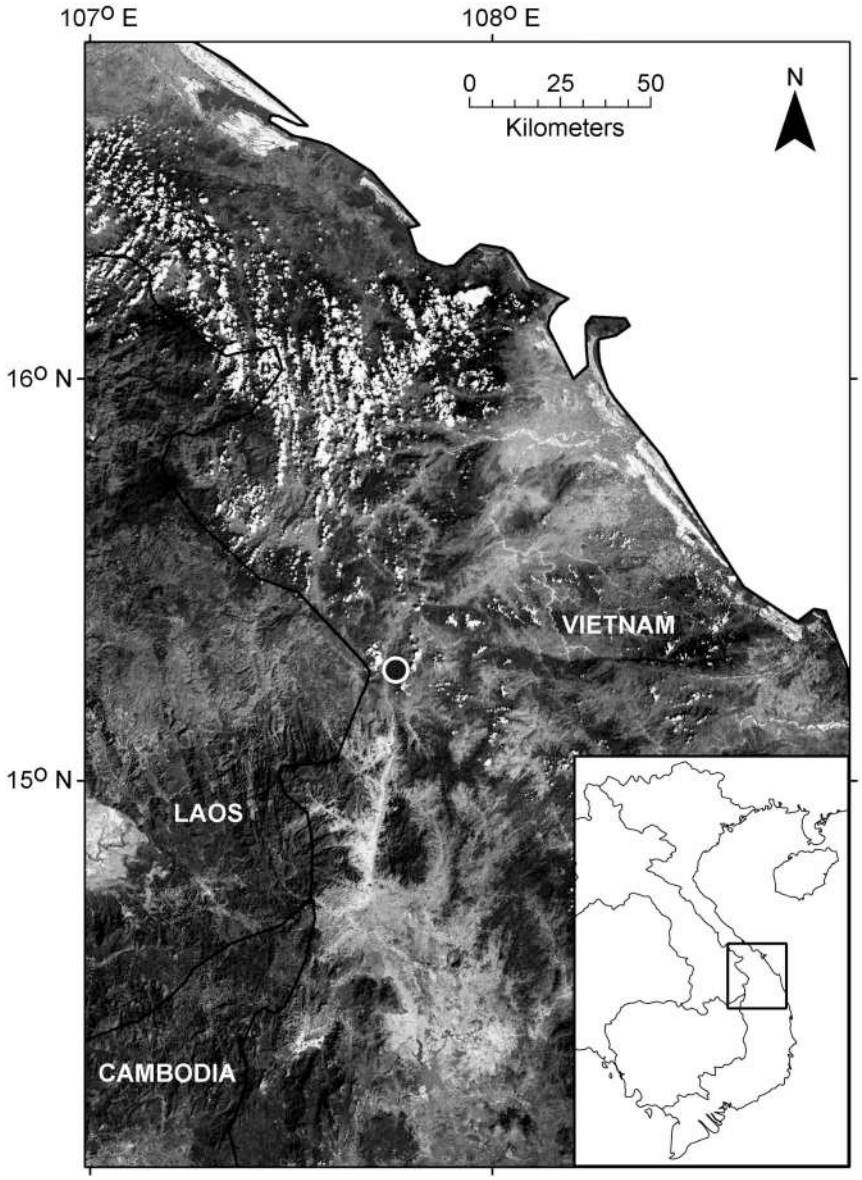

FIGURE 1. Type locality of *Leptolalax applebyi* **sp. nov.** (black circle), central Vietnam.

Description of holotype: Head longer than wide; snout rounded or truncate in profile, projecting slightly over lower jaw; nostril closer to tip of snout than eye; canthi rostralis rounded, constricted; lores sloping, concave; vertical pupil; diameter of eye less than length of snout; tympanum distinct, round, diameter smaller than that of the eye; anterioventral three-quaters of tympanic rim slightly elevated relative to skin of temporal region; vomerine teeth absent; pineal ocellus absent; vocal sac openings absent; tongue large, broad, and weakly notched posteriorly; weak supratympanic ridge running from eye towards axilla. Tips of fingers rounded, slightly enlarged; 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 and fourth toes; small, oval inner metatarsal tubercle pronounced, outer metatarsal tubercle absent; webbing basal, confined to very base of toes; no lateral fringes. Tibia short and stout, width approximately one-third of length; tibiotarsal articulation reaches anterior corner of eye. Skin on dorsal and ventral surfaces smooth, only eyelid with low,

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indistinct tubercles; pectoral gland small, indistinct, circular; femoral gland distinct, small white, on posteroventral surface of thigh, closer to knee than to vent; small gland above axilla; no ventrolateral glandular ridge.

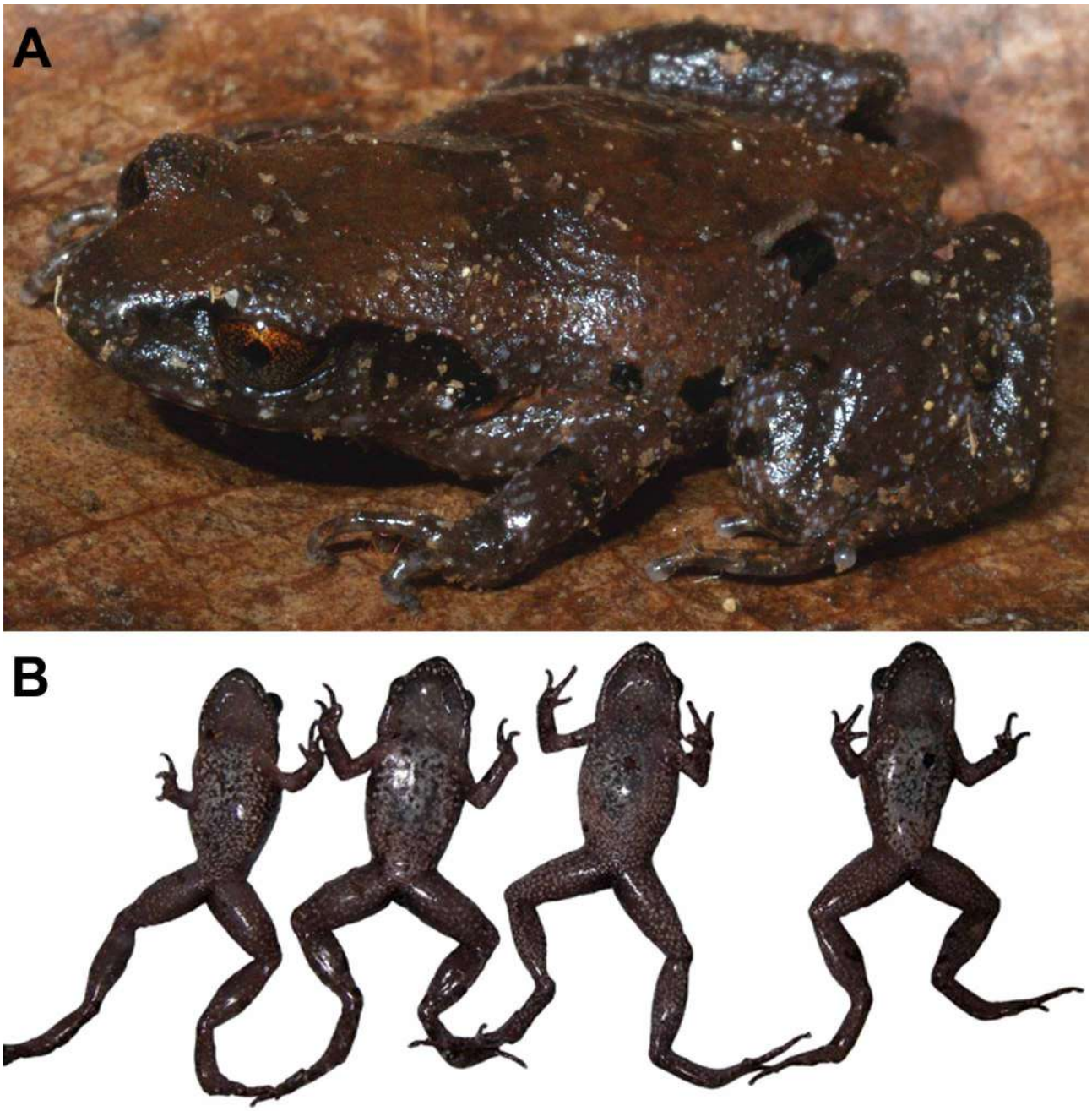

FIGURE 2. (A) Male holotype of *L. applebyi* **sp. nov.** in life (AMS R 171703), and (B) ventral view of male holotype and paratypes of *L. applebyi* **sp. nov.** in life (AMS R 171703–171706, L to R).

Colour of holotype in life: Dorsal surface dark brown with indistinct, diffuse darker brown patch between axillae; vertical darker brown bars on upper lip, indistinct lighter vertical stripes at tip of snout and under eyes; no interorbital bar; black line along canthus rostralis, through eye, and continuing along supratympanic ridge, encompassing most of tympanum, terminating above axilla; transverse darker bars on dorsal surface of limbs; large, black blotch on posterior flank anterior to sacrum and two smaller black spots on lateral flank; ventral surface of elbow and upper arm without dark bars, indistinct paler colouration on elbow; fingers with indistinct transverse barring; ventral surface dark brownish pink, with white speckling

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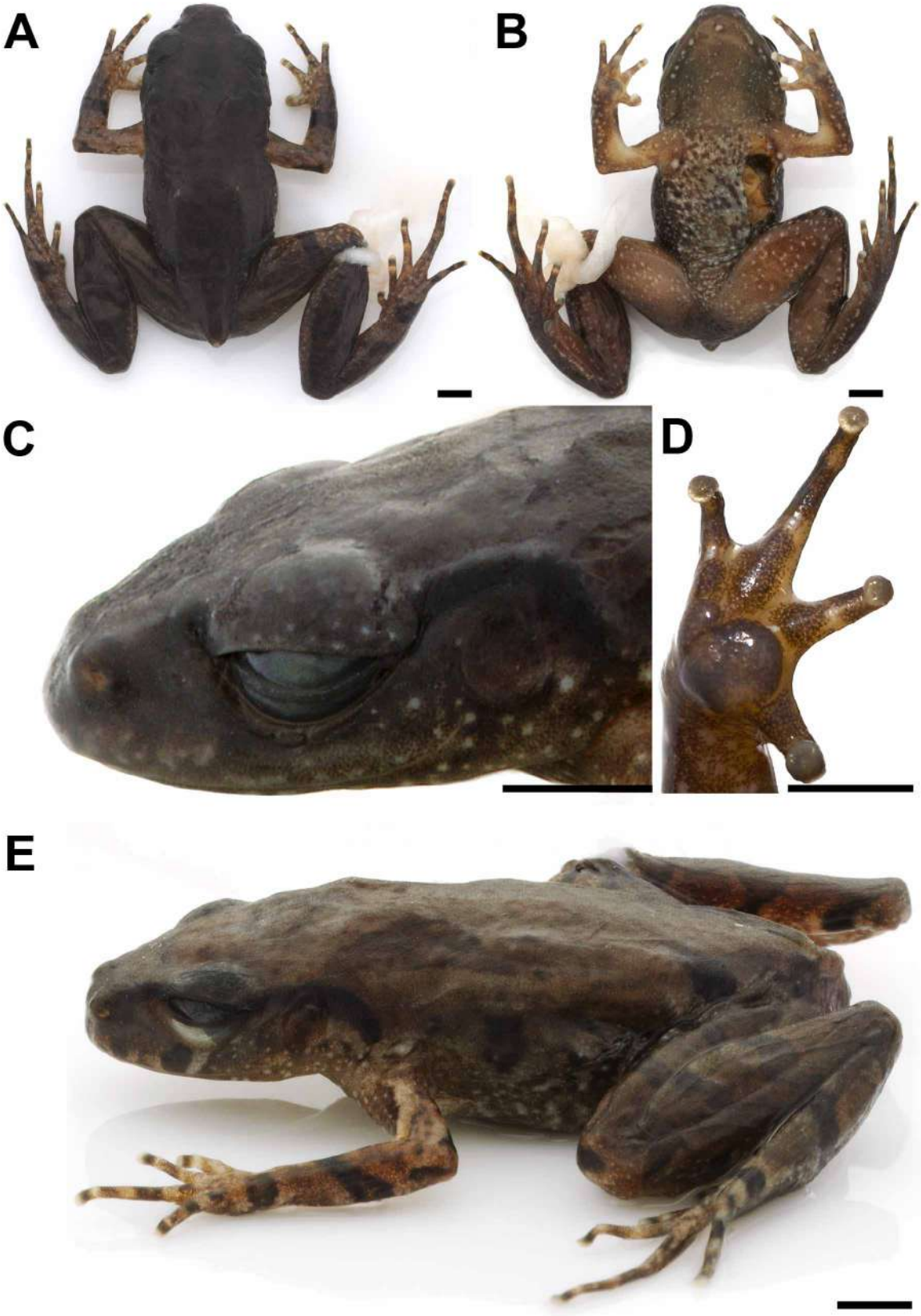

FIGURE 3. (A) Dorsal view, (B) ventral view, and (C) lateral view of head of preserved holotype (AMS R 171703) of *L. applebyi* **sp. nov.**, (D) ventral surface of right hand of preserved male paratype of *L. applebyi* **sp. nov.** (AMS R171705), and (E) lateral view of preserved female paratype of *L. applebyi* **sp. nov.** (AMS R 171707). Scale bar = 2 mm.

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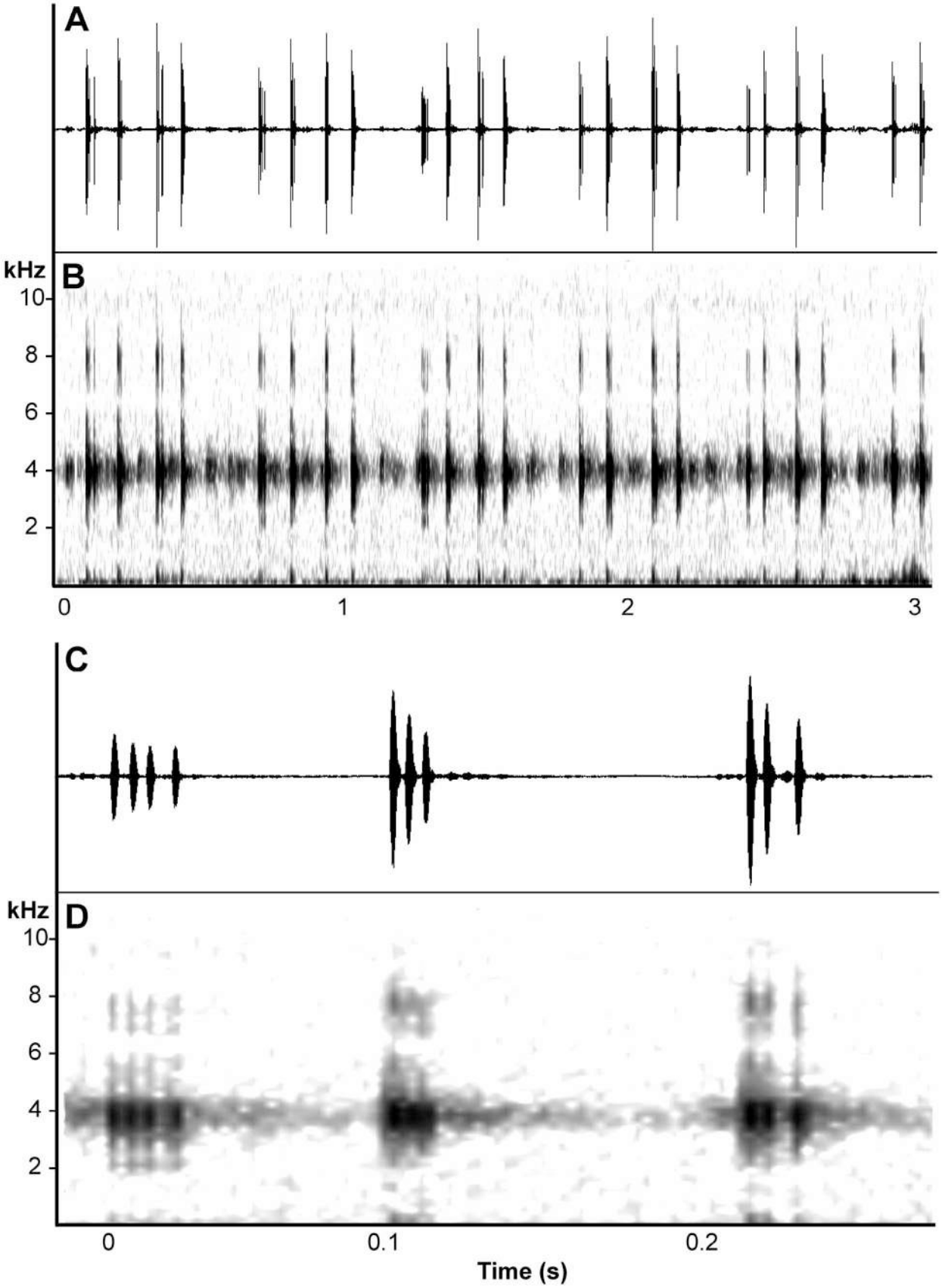

FIGURE 4. Advertisement call of *L. applebyi* **sp. nov.** (holotype AMS R 171703) recorded at ambient air temperature of 21.5º C. (A) a 3 s waveform of relative amplitude, (B) corresponding 3 s spectrogram, (C) expanded, 0.27 s waveform and (D) corresponding spectrogram for same call.

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concentrated on belly, but also on throat, ventral surfaces of arms, tibio-tarsus and feet; fine white speckling extends to ventrolateral surface of belly and on lower margins of head, with a single distinct and slightly larger white spot just posterior to tympanum; ventral margin of throat bearing row of larger white spots. Macroglands white. Iris coppery gold, with minute, black reticulations.

Colour of holotype in preservative: Dark brown dorsal surface. Ventral surface pale brown with white speckling and white macroglands. Colours faded.

Measurements: Holotype: SVL 19.6, HDL 7.4, HDW 6.6, SNT 3.0, EYE 2.0, IOD 2.6, TMP: 1.1, TEY 0.7, TIB 9.2, weight 0.8g

Variation: Specimens vary in dorsal colouration, from uniformly coloured dark brown (AMS R 171706) to weakly mottled (AMS R 171704–5). Dark barring on limbs may be indistinct and diffuse (AMS R 171706). Female (AMS R 171707) pale brown with diffuse darker chevrons on dorsum, above axilla. Paler vertical stripes at tip of snout and under eyes vary from indistinct (AMS R 171706) to distinct (AMS R 171704–5), most distinct in the single female (AMS R 171707). Ventral surface of chest and belly may be white with dark speckling in places, as opposed to all dark with white speckling. Female (AMS R 171707) has slightly paler throat. Elbows of female (AMS R 171707) and one male (AMS R 171705) are distinctly paler. Colouration on dorsal surface of head between the snout and eyes may be slightly paler. Iris colour varies from uniform gold to uniform reddish copper. Black spots on lateral flank variable in number (1–4 spots), size and shape. Female (AMS R 171707) has black spot on left knee. Tibiotarsal articulation reaches between anterior corner of eye and nostril. In life, males weighed 0.8–0.9g. Female, with distinct follicles present in ovary, weighed 1.0 g in life. Measurements of the type series are shown in table 1.

Advertisement call: Call descriptions are based on the calls of the holotype and a non-vouchered individual, both taken at 21.5ºC ambient temperature. Calls consisted of 4–5 notes, repeated at a rate of approximately 9 per second, with each note consisting of between one and five distinct pulses (Table 2, Figures 4A, C). There was little frequency modulation, with the dominant frequency between 3962.1–4306.6 Hz (Figures 4B, D), and harmonics at approximately 7752 Hz. Pulse duration was between 2–7 ms. To the human ear, the call of *L. applebyi* sounds like a faint, rapid rasping, similar to an orthopteran. The calls of *L. applebyi* and *L. tuberosus* differ considerably in structure and frequency (Rowley & Cao, unpublished data).

TABLE 1. Measurements (mm) of adult *Leptolalax applebyi*, **sp. nov.** Abbreviations defined in text.

	Males				Female
	R171703	R171704	R171705	R171706	R171707
SVL	19.6	20.7	20.8	20.8	21.7
HDL	7.4	7.8	7.7	7.6	8.2
HDW	6.6	7.3	7.3	7.2	7.4
SNT	3.0	3.4	3.0	3.2	3.8
EYE	2.0	2.4	2.3	2.4	2.7
IOD	2.6	2.8	3.1	2.8	2.9
TMP	1.1	1.4	1.6	1.5	1.6
TEY	0.7	1.0	0.9	0.6	0.9
TIB	9.2	9.7	10.0	9.7	10.4
HDL:HDW	1.11	1.06	1.05	1.05	1.1
HDL:SVL	0.38	0.38	0.37	0.36	0.38
TIB:SVL	0.47	0.47	0.48	0.47	0.48

Ecology: All specimens were found at the headwaters of rocky streams in medium montane forest (evergreen forest between 1200–1500m; Tordoff *et al*. 2003). Streams were steep, narrow (<2 m wide) and

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had little water flow. Despite intensive surveys, *L. applebyi* was never heard or observed below 1300 m elevation. Male *L. applebyi* were found calling from under leaf litter, always approximately 2 m from the stream. *Leptolalax applebyi* was found in sympatry with *L. tuberosus*. *Leptolalax tuberosus* was more widely distributed in terms of elevation, with individuals collected between 863–1401 m, and was almost always found greater than 5 m distance from streams. The stomach contents of AMS R171707 included remains of a small cockroach (order Blattaria).

TABLE 2. Measurements of advertisement call parameters for *Leptolalax applebyi* **sp. nov.** at 21.5ºC. Parameter values are given as means (and ranges).

Recording	AMS R 171003*	non-vouchered
Number of calls	6	7
Number of notes	24	34
Call duration (ms)	332 (280–358)	405 (340–441)
Intercall interval (ms)	245 (226–287)	287 (234–413)
Notes/call	4	4.8 (4–5)
Note duration (ms)	16.6 (8–32)	14.3 (4–31)
Internote interval (ms)	86.7 (48–140)	83.7 (38–113)
Pulses/note	3.29 (2–4)	2.52 (1–5)
Note repetition rate (notes/s)	9.0 (8.4–10.7)	9.3 (8.6–11.2)
Dominant frequency (Hz)	3962.1	4222.9 (3962.1–4306.6)

*holotype

Conservation status: The five type specimens are the only known representatives of the new species. Given the available information, we suggest the species should be considered Data Deficient following IUCN's Red List categories (IUCN 2001). Being known from only two localities approximately 1.3 km apart, its extent of occurrence is unknown but probably more widespread. The known habitat of *L. applebyi* falls within a protected area and is remote and steep enough that immediate deforestation risk is likely low. The habitat of *L. applebyi* is also contiguous with similar areas of habitat in the central Truong Son landscape (Tordoff *et al*. 2003), including Ngoc Linh Nature Reserve in Kon Tum Province to the south, and Laos in the west, but areas above 1200 m elevation compose only 8.5% of existing forest in the Truong Son landscape and are patchily distributed (Tordoff *et al*. 2003).

Comparisons*: Leptolalax applebyi* is distinguished from all other described *Leptolalax* species by a combination of (1) body size (19.6–20.8 mm for five adult males; 21.7 mm for single adult female), (2) uniformly smooth dorsum lacking tubercles, (3) dark brown dorsal surface lacking distinct patterns and dark brownish pink ventral surface with white speckling (4) an absence of webbing and dermal fringes on fingers, (5) slight basal webbing and no dermal fringes on toes, (6) short tibia (TIB:SVL 0.466–0.480), and (7) unique advertisement call consisting of 4–5 notes with a dominant frequency of 3962.1–4306.6 Hz, repeated at a rate of approximately 9 notes per second.

Leptolalax applebyi is one of the smallest species in the genus. Two species of *Leptolalax* have overlapping male adult body sizes; *L. pluvialis*, known only from northern Vietnam at 21.3–22.3 mm, and the recently described *Leptolalax kecil* from the Malay Peninsula, at 19.3–20.5 mm. Similar body sizes have been reported for *L. alpinis* (24.0–26.4), *L. heteropus* (24.0–33 mm), *L. liui* (23.0–28.7 mm) and *L. tuberosus* (24.4–29.5 mm). All other species have considerably larger male body sizes (*L. arayai* 29.6 mm; *L. bouretti* 27.2–36.2 mm; *L. dringi* 28.7–30.3 mm; *L. fulignosus* 28.2–30.0 mm; *L. gracilis* 31–39 mm; *L. hamidi* 28.7–31.3 mm; *L. kajangensis* 34–35 mm; *L. lateralis* 26.9–28.3 mm; *L. maurus* 26.1 mm; *L. melanolectus* 26.6–28.8 mm; *L. nahangensis* 40.8 mm; *L. oshanensis* 26.6–30.7 mm; *L. pelodytoides* 37 mm; *L. pictus* up to 36 mm; *L. solus* 30–35 mm; *L. sungi* 48.3–52.7 mm; *L. ventripunctatus* 25.5–28.0 mm).

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The single female specimen of *L. applebyi* (21.7 mm) does not fall within the range of female body sizes reported in the genus to date (*L. alpinis* 32.1 mm; *L. bouretti* 42.0–45.0 mm; *L. dringi* 37.5 mm; *L. gracilis* 40–41 mm; *L. hamidi* 36.1–42.8; *L. kecil* 25.0 mm, *L. lateralis* 36 mm; *L. liui* 23.1–28.1 mm; *L. maurus* 31.8 mm; *L. melanolectus* 32.7 mm; *L. oshanensis* 31.6 mm; *L. pictus* up to 47 mm; *L. sungi* 56.7–58.9 mm; *L. tuberosus* 30.2 mm).

The skin texture and colouration of *L. applebyi* differs from all previously described *Leptolalax* species. In terms of skin texture, only *L. applebyi, L. pluvialis*, *L. heteropus*, *L. pictus* and *L. kajangensis* have a smooth dorsum lacking tubercles or dorsal ridges. Of these species, *L. applebyi* is the only species with a dark brown dorsal surface lacking distinct dorsal patterns (*L. heteropus* and *L. pictus* have extensive, distinct dorsal markings or patches, *L. pluvialis* has distinct black interorbital markings and *L. kajangensis* has almost black dorsal surfaces with minute pale spots). Additionally, most *Leptolalax* species lacking a smooth dorsum also differ from *L. applebyi* in terms of dorsal colour and pattern (*L gracilis* and *L. hamidi* have extensive, distinct dorsal markings or patches, *L. arayai, L. fulignosus, L . kecil*, *L. lateralis*, *L. melanoleucus, L. nahangensis*, *L. oshanensis* and *L. sungi* have distinct black interorbital markings, *L. maurus* has almost black dorsal surfaces and *L. tuberosus* has a sandy dorsum with an olive brown hatching pattern).

The dark brownish pink ventral surface with white speckling of *L. applebyi* is also distinctly different from other species in the genus (*L. alpinis, L. arayai, L. bouretti, L. dringi*, *L. fulignosus, L. gracilis, L. hamidi, L. lateralis*, *L. liui, L. nahangensis, L. oshanensis, L. pelodytoides*, *L. pictus, L. solus, L. sungi* and *L. tuberosus* have entirely or mostly white or pale grey venters; *L. pluvialis* has a grey venter with dark grey marbling, uniform pale grey throat with speckling around the border and large whitish grey pectoral glands; *L. melanolectus* 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).

In lacking webbing and dermal fringes on fingers and having only slight basal webbing and no dermal fringes on toes, *L. applebyi* is distinguished further from *L. alpinis, L. bouretti, L. dringi, L. fulignosus, L. hamidi, L. heteropus, L. kecil, L. liui, L. pelodytoides*, *L. pluvalis, L. solus* and *L. sungi*, all of which have dermal fringes or extensive webbing.

The relative tibia length of *L. applebyi* (TIB:SVL 0.47–0.48) distinguishes it from *L. kucil* (males 0.56–0.58) *L. pluvialis* (0.52–0.56) *L. pelodytoides* (0.56), *L. arayai* (0.62), and *L. kajangensis* (0.42).

The advertisement call of *L. applebyi* also differs from the twelve *Leptolalax* species with described calls; *L. arayai, L. dringi, L. fuliginous, L. gracilis, L. hamidi, L. heteropus, L. kecil, L. melanoleucus, L. pelodytoides, L. pictus* and *L. oshanensis, L. solus*. Whereas the advertisement calls of *L. gracilis, L. heteropus, L. kecil, L. pelodytoides,* and *L. solus* overlap with *L. applebyi* in terms of number of notes per call, compared to *L. applebyi*, the calls of *L. gracilis, L. heteropus* and *L. solus* have much lower dominant frequencies (2540–3200Hz) and *L. pelodytoides* has higher dominant frequencies (6350–8100Hz). The advertisement call of *L. kecil* has both a lower dominant frequency, a lower number of pulses per note, and higher intercall interval compared to *L. applebyi*.

Acknowledgements

Field work was supported by ADM Capital Foundation and Conservation International. The National Forestry Protection Department, Diep Thanh Phong and all at Quang Nam Forestry Protection Department gave permission and provided logistical help for the survey. Mr. Otovia, Nguyen Van Hung and several M'nong people from Long Vien village assisted greatly with field-work. Jake Brunner, David Emmett Ben Rawson and all staff at Conservation International Indo-Burma Program and Barney Long, Tran Khanh Duong and staff at World Wildlife Fund Greater Mekong, Tam Ky, provided assistance and logistical support for field work. Photographs of specimens in preservative are by Tandy Rowley, and map is by Michal Elliott. Annemarie Ohler shared photographs of the holotype of *L. pluvialis*. Raoul Bain, Robert Inger, Glenn Shea

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and an anonymous reviewer provided valuable comments on an earlier draft of the manuscript. Ross Sadlier, Glenn Shea and Fred Kraus provided advice and assistance. The Forestry Protection Department (Quang Nam and Hanoi) permitted collection and export of specimens to the AM (permit no. 322/CCKL-QLBVR). For all this assistance we are most grateful.

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