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## A new species of *Leptolalax* (Anura: Megophryidae) from the western Langbian Plateau, southern Vietnam

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### Abstract

We describe a new species of megophryid frog from Loc Bac forest in the western part of the Langbian Plateau in the southern Annamite Mountains, Vietnam. *Leptolalax pyrrhops* **sp. nov.** is distinguished from its congeners by a combination of the following morphological attributes: (1) presence of distinct dark brown/black dorsolateral markings, including blackish spots on flanks and dark canthal and/or temporal streaks; (2) rudimentary webbing on toes; (3) tympanum externally distinct; (4) dorsal skin finely shagreened with numerous small tubercles and pustules; (5) medium size for the genus (30.3–33.9 mm in 2 adult males, 30.8–34.3 mm in 7 females); (6) grey-pinkish to dark brownish-violet chest and belly with numerous whitish speckles, also covering the lateral sides of body; (7) ventrolateral glands small, indistinct, do not form a distinct line; (8) pectoral glands comparatively small, comprising 1–3% of adult SVL; (10) iris bicolored, typically bright orange-red in upper two-thirds, fading to silvery green in lower third. The male advertisement call of the new species is also unique among those *Leptolalax* species for which calls are known, with a single long 'introductory' note, consisting of 5–12 pulses, followed by of 4–5 predominantly single-pulsed notes, and an average dominant frequency of 1.91–2.23 kHz. From the morphologically similar *L. applebyi*, *L. melicus* and *L. bidoupsensis*, *Leptolalax pyrrhops* **sp. nov.** can be further distinguished by 13.5%, 13.7% and 10.3% sequence divergence at the 16S rRNA mtDNA gene. At present, the new species is known from montane evergreen forest between 800–1100 m elevation. We suggest the species should be considered as Data Deficient following IUCN's Red List categories. To date our finding represents the southernmost known record of the genus *Leptolalax* from Vietnam.

**Key words:** Indochina, Annamite mountains, Southeast Asia, Truong Son, Da Lat Plateau, taxonomy, new species 16S rRNA, advertisement call, microendemism, *Leptolalax pyrrhops* **sp. nov.**

### Introduction

The Langbian, or Da Lat Plateau, forms the southernmost edge of the Annamite Mountains, or Truong Son Range, a mountain chain spanning the breadth of Indochina, including parts of Vietnam, Laos and Cambodia. The Plateau is known for its high herpetofaunal diversity, a significant portion of which has been discovered only recently (e.g., Rowley *et al.* 2010d, 2011a, 2011b; Stuart *et al.* 2011; Orlov *et al.* 2012; Chan *et al.* 2013; Vassilieva *et al.* 2014; Poyarkov *et al.* 2014). Despite this increase in species discoveries, many areas of the Annamites have received little scientific attention yet. The need for biological exploration in this region is made more urgent given the ongoing loss of natural habitats due to logging and other human activities (Meijer 1973; De Koninck 1999; Laurance 2007; Meyfroidt & Lambin 2008).

The megophryid genus *Leptotalax* Dubois, 1980 occurs widely in Southeast Asia (Frost 2014). 42 nominal species are currently recognized, over 50% (23) of which have been described in only the last 10 years (Frost 2014). Recent research on the genus in Indochina has significantly increased the known diversity of this group in the region (Rowley & Cao 2009; Rowley *et al.* 2010a–c, 2011a, 2012, 2014). However, considering the high morphological similarity of species within the genus and the poor level of biological exploration of many parts of Indochina, additional taxa likely remain undescribed. In order to delineate species within this group, bioacoustic data have proven useful (e.g. Malkmus & Riede 1993; Matsui 1997, 2006; Matsui *et al.* 2009, 2014a, 2014b; Rowley & Cao 2009; Rowley *et al.* 2012). DNA barcoding techniques (e.g., Vences *et al.* 2005a, 2005b; Smith *et al.* 2008; Ohler *et al.* 2011; Jiang *et al.* 2013; Murphy *et al.* 2013) have also facilitated the assessment of cryptic taxonomic diversity in amphibians, including the genus *Leptotalax*.

Three species of morphologically and molecularly similar species of *Leptotalax* are known from central and southern Vietnam and northeastern Cambodia: *Leptotalax applebyi* Rowley & Cao 2009 from central Vietnam, *Leptotalax bidoupensis* Rowley *et al.* 2011 from the Langbian Plateau in southern Vietnam and *Leptotalax melicus* Rowley *et al.* 2010 from northeastern Cambodia. These three species (the ‘*Leptotalax applebyi* group’) are small-bodied and morphologically conserved (Rowley & Cao 2009; Rowley *et al.* 2010, 2011a), and appear to be phylogenetically distinct from other *Leptotalax* (see Jiang *et al.* 2013; Sung *et al.* 2014).

In 2013, during fieldwork in Loc Bac forest on the western edge of the Langbian Plateau, we collected *Leptotalax* specimens morphologically resembling species of the *Leptotalax applebyi* group, but displaying unique morphological characters. Subsequent investigation of morphological variation and partial 16S rRNA mtDNA gene sequences, as well as the study of the advertisement call of the new *Leptotalax* population, confirmed that it represents an yet unknown lineage within the *Leptotalax applebyi* group, clearly distinct both in morphology and mtDNA sequences; we therefore describe it herein as a new species.

## Material and methods

**Sample collection.** All specimens were collected during fieldwork in southern Vietnam, in April 2013 within Loc Bac forest (Lam Dong Province, Bao Lam District, approximate coordinates 11° 44' N, 107° 42' E, nearly 800–1100 m a.s.l.). The geographic position of the surveyed locality and the distribution of the known *Leptotalax* species in the Annamite Mountains (Truong Son) and adjacent regions of southern Indochina (central and southern Vietnam, southern Laos, eastern Cambodia) are shown in Figure 1. These newly collected specimens were deposited in the herpetological collection of the Zoological Museum of Moscow State University (ZMMU) in Moscow, Russia, the Zoological Institute R.A.S. (ZISP) in St. Petersburg, Russia, and the Vietnam National Museum of Nature (VNMN) in Hanoi, Vietnam. Comparative materials examined are stored in herpetological collections of ZMMU and ZISP (see Appendix).

**Morphological analysis.** Specimens were photographed in life, and tissue samples for genetic analysis were taken prior to preservation and stored in 96% ethanol. Sex was determined by direct observation of calling in life, and the presence of internal vocal sac openings and/or gonadal inspection by dissection.

We recorded morphological data from specimens fixed and stored in 75% ethanol. All measurements were taken using digital calipers under the light dissecting microscope to the nearest 0.1 mm; morphometrics follow Rowley *et al.* (2012): snout-vent length (SVL); head length from tip of snout to rear of jaws (HDL); head width at commissure of jaws (HDW); snout length from tip of snout to anterior corner of eye (SNT); diameter of exposed portion of eyeball (EYE); interorbital distance (IOD); horizontal diameter of tympanum (TMP); distance from anterior edge of tympanum to posterior corner of eye (TEY); tibia length with hindlimb flexed (TIB); manus length from tip of third digit to proximal edge of inner metacarpal tubercle (ML); and pes length from tip of fourth toe to proximal edge of the inner metatarsal tubercle (PL). Geographic coordinates were obtained using a Garmin GPSMAP 60CSx GPS receiver and recorded in datum WGS 84.

The diagnosis of the genus *Leptotalax* and morphological characters chosen for comparison were taken from Dubois (1980), Matsui (1997, 2006), Matsui *et al.* (2014a, 2014b), Ohler *et al.* (2011), Rowley & Cao (2009) and Rowley *et al.* (2010a–c, 2011a, 2012, 2014).

We obtained comparative morphological data from museum specimens of *Leptotalax* and photographs of these specimens in life (see Appendix) and from literature: *L. aereus* Rowley, Stuart, Richards, Phimmachak &

Sivongxay (Rowley *et al.* 2010a), *L. alpinus* Fei, Ye, & Li (Fei *et al.* 1991, 2009, 2010), *L. applebyi* Rowley & Cao (Rowley & Cao 2009), *L. arayai* Matsui (Matsui 1997), *L. bidoupensis* Rowley, Le, Tran, & Hoang (Rowley *et al.* 2011a), *L. botsfordi* Rowley, Dau, & Nguyen (Rowley *et al.* 2013), *L. bourreti* Dubois (Dubois 1983; Ohler *et al.* 2011), *L. croceus* Rowley, Hoang, Le, Dau, & Cao (Rowley *et al.* 2010b), *L. dringi* Dubois (Dubois 1987; Inger *et al.* 1995), *L. eos* Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler, & Dubois (Ohler *et al.* 2011), *L. firthi* Rowley, Hoang, Dau, Le, & Cao (Rowley *et al.* 2012), *L. fritinniens* Dehling & Matsui (Dehling & Matsui 2013), *L. fuliginosus* Matsui (Matsui 2006), *L. gracilis* (Günther) (Günther 1872; Inger & Stuebing 2005), *L. hamidi* Matsui (Matsui 1997), *L. heteropus* (Boulenger) (Boulenger 1900), *L. kajangensis* Grismer, Grismer, & Youmans (Grismer *et al.* 2004), *L. kecil* Matsui, Belabut, Ahmad, & Yong (Matsui *et al.* 2009), *L. khasiorum* Das, Tron, Rangad, & Hooroo (Das *et al.* 2010), *L. lateralis* (Anderson) (Anderson 1871; Humtsoe *et al.* 2008), *L. laui* Sung, Yang, & Wang (Sung *et al.* 2014), *L. liui* Fei & Ye (Fei *et al.* 1991, 2009, 2010), *L. marmoratus* Matsui, Zainudin, & Nishikawa (Matsui *et al.* 2014b), *L. maurus* Inger, Lakim, Biun, & Yambun (Inger *et al.* 1997), *L. melanoleucus* Matsui (Matsui 2006), *L. melicus* Rowley, Stuart, Thy, & Emmett (Rowley *et al.* 2010c), *L. minimus* (Taylor) (Ohler *et al.* 2011), *L. nahangensis* Lathrop, Murphy, Orlov, & Ho (Lathrop *et al.* 1998), *L. nokrekensis* (Mathew & Sen) (Mathew & Sen, 2010), *L. nyx* Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler, & Dubois (Ohler *et al.* 2011), *L. oshanensis* (Liu) (Liu 1950; Fei *et al.* 2009, 2010), *L. pelodytoides* (Boulenger) (Boulenger 1893, 1908; Ohler *et al.* 2011), *L. pictus* Malkmus (Malkmus 1992; Malkmus *et al.* 2002), *L. platycephalus* Dehling (Dehling 2012), *L. pluvialis* Ohler, Marquis, Swan, & Grosjean (Ohler *et al.* 2000, 2011), *L. sabahmontanus* Matsui, Nishikawa, & Yambun (Matsui *et al.* 2014a), *L. solus* Matsui (Matsui 2006), *L. sungi* Lathrop, Murphy, Orlov, & Ho (Lathrop *et al.* 1998), *L. tamdil* Sengupta, Sailo, Lalremsanga, Das, & Das (Sengupta *et al.* 2010), *L. tuberosus* Inger, Orlov, & Darevsky (Inger *et al.* 1999; Rowley *et al.* 2010b), *L. ventripunctatus* Fei, Ye, & Li (Fei *et al.* 1991, 2009, 2010) and *L. zhangyapingi* Jiang, Yan, Suwannapoom, Chomdej, & Che (Jiang *et al.* 2013). Due to the high likelihood of undiagnosed diversity within the genus, where available, we relied on examination of topotypic material and/or original species descriptions.

**DNA isolation, PCR and sequencing.** For molecular analysis, total genomic DNA was extracted from ethanol-preserved muscle or liver tissues using standard phenol–chloroform extraction procedures (Hillis *et al.* 1996) followed with isopropanol precipitation. The isolated total genomic DNA was visualized in agarose electrophoresis in presence of ethidium bromide. The concentration of total DNA was measured in 1 µl using NanoDrop 2000 (Thermo Scientific), and consequently adjusted to ca. 100 ng DNA / µL.

We amplified two mtDNA fragments of 16S rRNA mtDNA gene, to obtain a 1083 bp-length fragment of the gene. 16S rRNA is a molecular marker widely applied for biodiversity surveys in amphibians (Vences *et al.* 2005a; 2005b; Vieites *et al.* 2009), and has proven to be particularly useful in studies of megophryid diversity (Matsui *et al.* 2010; Stuart *et al.* 2011, 2012; Hamidy *et al.* 2012 and references therein) including in the genus *Leptolalax* (Rowley *et al.* 2010, 2011a, 2012; Ohler *et al.* 2011; Jiang *et al.* 2013; Matsui *et al.* 2014a, 2014b). Amplification was performed in 25 µl reactions using ca. 50 ng genomic DNA, 10 nmol of each primer, 15 nmol of each dNTP, 50 nmol additional MgCl<sub>2</sub>, Taq PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.1 mM MgCl<sub>2</sub> and 0.01% gelatine) and 1 U of Taq DNA polymerase. Primers used in PCR and sequencing were as follows: forward primers: L1879 (CGTACCTTTTGCATCATGGTC; Matsui *et al.* 2010), L2188 (AAAGTGGGCCTAAAAGCAGCCA; Matsui *et al.* 2006), 16L-1 (CTGACCGTGCAAAGGTAGCGTAATCACT; Hedges, 1994); reverse primers: H1923 (AAGTAGCTCGCTTAGTTTCGG; Matsui *et al.* 2010), H2317 (TTCTTGTTACTAGTTCTAGCAT; Shimada *et al.* 2011), Will6 (CCCTCGTGATGCCGTTGATAC; Wilkinson *et al.* 2002). The PCR conditions followed Matsui *et al.* (2011) and included an initial denaturation step of 5 min at 94°C and 33 cycles of denaturation for 30 s at 94°C, primer annealing for 30 s at 48–50°C, and final extension step for 1 min 30 s at 72°C.

PCR products were loaded onto 1% agarose gels, stained with GelStar gel stain (Cambrex), and visualized in a Dark reader transilluminator (Clare Chemical). If distinct bands were produced, products were purified using 2 µl, from a 1:4 dilution of ExoSapIt (Amersham), per 5 µl of PCR product prior to cycle sequencing. A 10 µl sequencing reaction included 2 µL of template, 2.5 µl of sequencing buffer, 0.8 µl of 10 pmol primer, 0.4 µl of BigDye Terminator version 3.1 Sequencing Standard (Applied Biosystems) and 4.2 µl of water. The cycle-sequencing reaction was 35 cycles of 10 sec at 96°C, 10 s at 50°C and 4 min at 60°C. Cycle sequencing products were purified by ethanol precipitation. Sequence data collection and visualization were performed on an ABI 3730xl automated sequencer (Applied Biosystems). The obtained sequences are deposited in GenBank under the accession numbers KP017573–KP017578 (see Table 1).

**TABLE 1.** Specimens and Genbank sequences of *Leptolalax* used in molecular analyses. AN—Accession number. Numbers of specimens (No. 1–105) correspond to those in Figure 2.

No.	Species	Locality	Voucher ID	Genbank AN	Reference
1	<i>L. minimus</i>	Thailand, Chiangmai, Doi Chiang Dao	THNHM 07418	JN848402	Ohler <i>et al.</i> 2011
2	<i>L. minimus</i>	Thailand, Chiangmai, Doi Chiang Dao	–	JN848403	Ohler <i>et al.</i> 2011
3	<i>L. minimus</i>	Thailand, Chiangmai, Doi Chiang Dao	THNHM 07419	JN848401	Ohler <i>et al.</i> 2011
4	<i>L. minimus</i>	Thailand, Chiangmai, Doi Chiang Dao	THNHM 07420	JN848399	Ohler <i>et al.</i> 2011
5	<i>L. minimus</i>	Thailand, Chiangmai, Doi Chiang Dao	THNHM 07422	JN848400	Ohler <i>et al.</i> 2011
6	<i>L. minimus</i>	Laos, Luang Prabang, Ban Dong Khan	MNHN 2006.2313	JN848385	Ohler <i>et al.</i> 2011
7	<i>L. minimus</i>	Laos, Luang Prabang, Ban Dong Khan	MNHN 2006.2316	JN848384	Ohler <i>et al.</i> 2011
8	<i>L. minimus</i>	Thailand, Chiangmai, Mae Lao Mae Sae	–	JN848369	Ohler <i>et al.</i> 2011
9	<i>L. minimus</i>	Laos, Luang Prabang, Huey Thao	MNHN 2006.2554	JN848367	Ohler <i>et al.</i> 2011
10	<i>L. pluvialis</i>	Vietnam, Lao Cai, Sa Pa	MNHN 1999.5674	JN848391	Ohler <i>et al.</i> 2011
11	<i>L. pluvialis</i>	Vietnam, Lao Cai, Sa Pa	MNHN 1999.5675	JN848390	Ohler <i>et al.</i> 2011
12	<i>L. pluvialis</i>	Vietnam, Lao Cai, Sa Pa	MNHN 1999.5676	JN848389	Ohler <i>et al.</i> 2011
13	<i>L. aereus</i>	Vietnam, Quang Binh, Phong Nha - Ke Bang	ZFMK 71341	AF285194	Ziegler 2000
14	<i>L. aereus</i>	Vietnam, Quang Binh, Phong Nha - Ke Bang	ZFMK 71346	AF285193	Ziegler 2000
15	<i>L. aereus</i>	Vietnam, Quang Binh, U Bo	–	JN848443	Ohler <i>et al.</i> 2011
16	<i>L. aereus</i>	Vietnam, Quang Binh, Cha Noi	ZFMK 86393	JN848442	Ohler <i>et al.</i> 2011
17	<i>L. aereus</i>	Vietnam, Quang Binh, Cha Noi	–	JN848436	Ohler <i>et al.</i> 2011
18	<i>L. aereus</i>	Vietnam, Quang Binh, Cha Noi	–	JN848435	Ohler <i>et al.</i> 2011
19	<i>L. aereus</i>	Vietnam, Quang Binh, Cha Noi	–	JN848441	Ohler <i>et al.</i> 2011
20	<i>L. aereus</i>	Vietnam, Quang Binh, Cha Noi	–	JN848440	Ohler <i>et al.</i> 2011
21	<i>L. aereus</i>	Vietnam, Quang Binh, Cha Noi	ZFMK 86394	JN848439	Ohler <i>et al.</i> 2011
22	<i>L. aereus</i>	Vietnam, no exact locality	TZ819	AF285192	Ziegler 2000
23	<i>L. nyx</i>	Viet Nam, Ha Giang, Tay Conn Linh Mt.	AMNH A163810	DQ283381	Frost <i>et al.</i> 2006
24	<i>L. cf. ventripunctatus</i>	Vietnam, Vinh Phu, Tam Dao	MVZ223642	AY236798	Garcia-Paris <i>et al.</i> 2003
25	<i>L. cf. ventripunctatus</i>	Vietnam, Lao Cai, no exact locality	MVZ223641	AY236797	Garcia-Paris <i>et al.</i> 2003
26	<i>L. cf. ventripunctatus</i>	Vietnam, Vinh Phu, Tam Dao	–	JX564874	Zhang <i>et al.</i> 2013
27	<i>L. cf. ventripunctatus</i>	Vietnam, Vinh Phu, Tam Dao	ROM18282	EF397244	Fu <i>et al.</i> 2007

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TABLE 1. (Continued)

No.	Species	Locality	Voucher ID	Genbank AN	Reference
28	<i>L. ventripunctatus</i>	Laos, Phongsaly, Phongsaly	MNHN 2005.0112	JN848431	Ohler <i>et al.</i> 2011
29	<i>L. ventripunctatus</i>	Laos, Phongsaly, Phongsaly	MNHN 2005.0142	JN848412	Ohler <i>et al.</i> 2011
30	<i>L. ventripunctatus</i>	Laos, Phongsaly, Phongsaly	MNHN 2005.0115	JN848444	Ohler <i>et al.</i> 2011
31	<i>L. ventripunctatus</i>	Laos, Phongsaly, Phongsaly	MNHN 2005.0121	JN848426	Ohler <i>et al.</i> 2011
32	<i>L. ventripunctatus</i>	Laos, Phongsaly, Phongsaly	MNHN 2005.0126	JN848424	Ohler <i>et al.</i> 2011
33	<i>L. ventripunctatus</i>	Laos, Phongsaly, Phongsaly	MNHN 2005.0140	JN848388	Ohler <i>et al.</i> 2011
34	<i>L. ventripunctatus</i>	Laos, Phongsaly, Phongsaly	MNHN 2005.0125	JN848425	Ohler <i>et al.</i> 2011
35	<i>L. ventripunctatus</i>	Laos, Phongsaly, Phongsaly	MNHN 2005.0137	JN848422	Ohler <i>et al.</i> 2011
36	<i>L. ventripunctatus</i>	China, Yunnan, Xishuangbanna	SYS A001768	KM014811	Sung <i>et al.</i> 2014
37	<i>L. firthi</i>	Vietnam, Kon Tum, Ngoc Linh	AMS R176513	JQ739208	Rowley <i>et al.</i> 2012
38	<i>L. firthi</i>	Vietnam, Kon Tum, Ngoc Linh	AMS R176506	JQ739207	Rowley <i>et al.</i> 2012
39	<i>L. firthi</i>	Vietnam, Kon Tum, Ngoc Linh	AMS R171736	JQ739204	Rowley <i>et al.</i> 2012
40	<i>L. firthi</i>	Vietnam, Kon Tum, Ngoc Linh	AMS R173774	JQ739205	Rowley <i>et al.</i> 2012
41	<i>L. firthi</i>	Vietnam, Kon Tum, Ngoc Linh	AMS R176524	JQ739206	Rowley <i>et al.</i> 2012
42	<i>L. firthi</i>	Vietnam, Kon Tum, Ngoc Linh	AMS R171714	JQ739203	Rowley <i>et al.</i> 2012
43	<i>L. liui</i>	China, Fujian, Wuyi-Shan	SYS A001597	KJ579116	Li <i>et al.</i> 2014
44	<i>L. liui</i>	China, Guangxi, Jinxiu	ZYC A100	EF544238	Zheng <i>et al.</i> 2008
45	<i>L. liui</i>	China, Fujian, Daiyun-Shan	SYS A001736	KM014550	Sung <i>et al.</i> 2014
46	<i>L. liui</i>	China, Jiangxi, Huanggang-Shan	SYS A001620	KM014549	Sung <i>et al.</i> 2014
47	<i>L. liui</i>	China, Jiangxi, Tongba-Shan	SYS A001702	KM014548	Sung <i>et al.</i> 2014
48	<i>L. liui</i>	China, Fujian, Wuyi-Shan	SYS A001597	KM014547	Sung <i>et al.</i> 2014
49	<i>L. laui</i>	China, Shenzhen, Wutongshan	SYS A002057	KM014546	Sung <i>et al.</i> 2014
50	<i>L. laui</i>	China, Shenzhen, Wutongshan	SYS A001515	KM014545	Sung <i>et al.</i> 2014
51	<i>L. laui</i>	China, Hong Kong, Tai Mo Shan	SYS A001507	KM014544	Sung <i>et al.</i> 2014
52	<i>L. zhangyapingi</i>	Thailand, Chiang Mai, Phang Num Poo	KJ-2013	JX069979	Jiang <i>et al.</i> 2012
53	<i>L. oshanensis</i>	China, Sichuan, Emei-Shan	ZYC1504	AY561306	Zheng <i>et al.</i> 2004
54	<i>L. oshanensis</i>	China, Sichuan, Emei-Shan	SYS A001830	KM014810	Sung <i>et al.</i> 2014
55	<i>L. cf. oshanensis</i> 1	China, Chongqing, Jinfo-Shan	ZYC799	AY526215	Zheng <i>et al.</i> 2004

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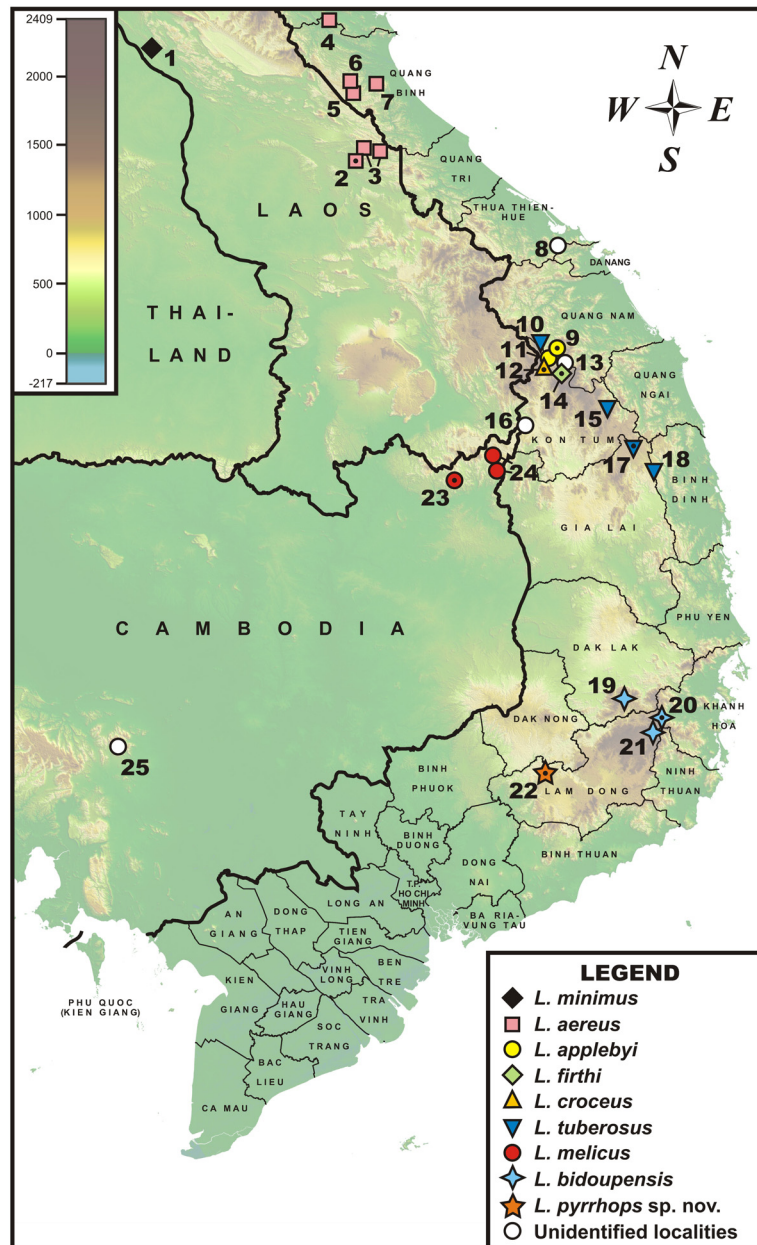
**TABLE 1.** (Continued)

No.	Species	Locality	Voucher ID	Genbank AN	Reference
56	<i>L. cf. oshanensis</i> 2	China, no exact locality	–	NC020610	Xiang <i>et al.</i> 2013
57	<i>Leptolalax</i> sp. 1	Thailand, Chiangmai, Doi Chiang Dao	–	JN848455	Ohler <i>et al.</i> 2011
58	<i>L. bourreti</i>	Vietnam, Lao Cai, Sa Pa	MNHN 1999.5659	JN848453	Ohler <i>et al.</i> 2011
59	<i>L. bourreti</i>	Vietnam, Lao Cai, Sa Pa	MNHN 1999.5660	JN848454	Ohler <i>et al.</i> 2011
60	<i>L. eos</i>	Laos, Phongsaly, Long Nai	MNHN 2004.0276	JN848446	Ohler <i>et al.</i> 2011
61	<i>L. eos</i>	Laos, Phongsaly, Long Nai	MNHN 2004.0279	JN848447	Ohler <i>et al.</i> 2011
62	<i>L. eos</i>	Laos, Phongsaly, Long Nai	MNHN 2004.0277	JN848448	Ohler <i>et al.</i> 2011
63	<i>L. eos</i>	Laos, Phongsaly, Long Nai	MNHN 2005.0147	JN848449	Ohler <i>et al.</i> 2011
64	<i>L. melicus</i>	Cambodia, Ratanakiri, Virachey	MVZ 258197	HM133599	Rowley <i>et al.</i> 2010c
65	<i>L. melicus</i>	Cambodia, Ratanakiri, Virachey	MVZ 258198	HM133600	Rowley <i>et al.</i> 2010c
66	<i>L. melicus</i>	Cambodia, Ratanakiri, Virachey	MVZ 258199	HM133601	Rowley <i>et al.</i> 2010c
67	<i>L. applebyi</i>	Vietnam, Quang Nam, Song Thanh	AMS R171703	HM133597	Rowley & Cao 2009
68	<i>L. applebyi</i>	Vietnam, Quang Nam, Song Thanh	AMS R171704	HM133598	Rowley & Cao 2009
69	<i>L. bidouppensis</i>	Vietnam, Lam Dong, Hon Giao	NCSM 77320	HM902882	Rowley <i>et al.</i> 2011
70	<i>L. bidouppensis</i>	Vietnam, Lam Dong, Hon Giao	NCSM 77321	HM902883	Rowley <i>et al.</i> 2011
71	<i>L. bidouppensis</i>	Vietnam, Lam Dong, Hon Giao	AMS R 173133	HM902880	Rowley <i>et al.</i> 2011
72	<i>L. bidouppensis</i>	Vietnam, Lam Dong, Hon Giao	AMS R 173134	HM902881	Rowley <i>et al.</i> 2011
73	<i>L. bidouppensis</i>	Vietnam, Lam Dong, Bi Doup	ZMMU NAP-01453	KP017573	this paper
74	<i>L. bidouppensis</i>	Vietnam, Lam Dong, Bi Doup	ZMMU NAP-01458	KP017574	this paper
75	<i>Leptolalax</i> sp. 2	Thailand, Phang Nga, Phang Nga	P987	JN848354	Ohler <i>et al.</i> 2011
76	<i>Leptolalax</i> sp. 2	Thailand, Phang Nga, Phang Nga	P316	JN848355	Ohler <i>et al.</i> 2011
77	<i>Leptolalax</i> sp. 2	Thailand, Phang Nga, Phang Nga	MNHN 2000.2412	JN848356	Ohler <i>et al.</i> 2011
78	<i>Leptolalax</i> sp. 2	Thailand, Phang Nga, Phang Nga	MNHN 2000.2414	JN848357	Ohler <i>et al.</i> 2011
79	<i>Leptolalax</i> sp. 2	Thailand, Phang Nga, Phang Nga	P973	JN848358	Ohler <i>et al.</i> 2011
80	<i>Leptolalax</i> sp. 2	Thailand, Phang Nga, Phang Nga	LEL8	JN848359	Ohler <i>et al.</i> 2011
81	<i>L. arayai</i>	Malaysia, Sabah, Kinabalu, Mesilau	BORN 22931	AB847558	Matsui <i>et al.</i> 2014a
82	<i>L. arayai</i>	no locality	–	AY523768	Roelants & Bossuyt 2005
83	<i>L. arayai</i>	no locality	AE100/S9	DQ642119	unpublished

.....continued on the next page

TABLE 1. (Continued)

No.	Species	Locality	Voucher ID	Genbank AN	Reference
84	<i>L. gracilis</i>	Malaysia, Sarawak, Mulu, camp 1	KUHE 55624	AB847560	Matsui <i>et al.</i> 2014a
85	<i>L. maurus</i>	Malaysia, Sabah, Kinabalu	SP 21450	AB847559	Matsui <i>et al.</i> 2014a
86	<i>L. pictus</i>	Malaysia, Sabah, Kinabalu, Poring	KUHE 39298	AB847556	Matsui <i>et al.</i> 2014a
87	<i>L. pictus</i>	no locality	AE102/S32	DQ642120	unpublished
88	<i>L. fritiniensis</i>	Malaysia, Sarawak, Mulu, base camp	KUHE 55371	AB847557	Matsui <i>et al.</i> 2014a
89	<i>L. dringi</i>	Malaysia, Sarawak, Mulu, Sg. Tapin	KUHE 55612	AB847554	Matsui <i>et al.</i> 2014a
90	<i>L. dringi</i>	Malaysia, Sarawak, Mulu, Sg. Tapin	KUHE 55613	AB847555	Matsui <i>et al.</i> 2014a
91	<i>L. sabahmontanus</i>	Malaysia, Sabah, Kinabalu, Sg. Silau-Silau	BORN 12454	AB847550	Matsui <i>et al.</i> 2014a
92	<i>L. sabahmontanus</i>	Malaysia, Sabah, Kinabalu, Sg. Silau-Silau	BORN 12632	AB847551	Matsui <i>et al.</i> 2014a
93	<i>L. sabahmontanus</i>	Malaysia, Sabah, Kinabalu, Sg. Silau-Silau	BORN 22784	AB847552	Matsui <i>et al.</i> 2014a
94	<i>L. heteropus</i>	Malaysia, Perak, Larut	KUHE 15487	AB530453	Matsui <i>et al.</i> 2014a
95	<i>L. heteropus</i>	Malaysia, Perak, Larut	KUHE 15490	AB847561	Matsui <i>et al.</i> 2014a
96	<i>L. heteropus</i>	Malaysia, Perak, Larut	–	AB719263	Hamidy <i>et al.</i> 2012
97	<i>L. marmoratus</i>	Malaysia, Sarawak, Padawan, Annah Rais	KUHE 53192	AB969292	Matsui <i>et al.</i> 2014b
98	<i>L. marmoratus</i>	Malaysia, Sarawak, Padawan, Annah Rais	KUHE 53191	AB969291	Matsui <i>et al.</i> 2014b
99	<i>L. marmoratus</i>	Malaysia, Sarawak, Padawan, Gn. Penrissen	KUHE 53925	AB969287	Matsui <i>et al.</i> 2014b
100	<i>L. hamidi</i>	Malaysia, Sarawak, Bukit Lanjak	KUHE 17545	AB969286	Matsui <i>et al.</i> 2014b
101	<i>L. pyrrhops</i> <b>sp. nov.</b>	Vietnam, Lam Dong, Loc Bac	ZMMU ABV-00148	KP017575	this paper
102	<i>L. pyrrhops</i> <b>sp. nov.</b>	Vietnam, Lam Dong, Loc Bac	ZMMU ABV-00176	KP017576	this paper
103	<i>L. pyrrhops</i> <b>sp. nov.</b>	Vietnam, Lam Dong, Loc Bac	ZMMU ABV-00177	KP017577	this paper
104	<i>L. pyrrhops</i> <b>sp. nov.</b>	Vietnam, Lam Dong, Loc Bac	ZMMU ABV-00213	KP017578	this paper
105	<i>Brachytarsophrys carinense</i>	Myanmar, no exact locality	–	JX564854	Zhang <i>et al.</i> 2013



**FIGURE 1.** Distribution of *Leptotalax* species in southern Indochina: Annamite Mountains and adjacent regions. Black dots in the center of an icon indicate the type locality of new species. **Locality information.** **Laos:** 1—Khammouan Province, Huey Kun Luang (Ohler *et al.* 2011); Savannakhet Province: 2—Vilabouli, Sepon Mining Tenement (Rowley *et al.* 2010); 3—Vilabouli, Sangi (Rowley *et al.* 2010); **Vietnam:** 4—Ha Tinh Province (Ohler *et al.* 2011); Quang Binh Province: 5—Phong Nha - Ke Bang NP (Ohler *et al.* 2011); 6—U Bo (Ohler *et al.* 2011); 7—Cha Noi (Ohler *et al.* 2011); 8—Da Nang, Ba Na N.P. (ZMMU A-5221); 9—Quang Nam Province, Song Thanh Proposed Nature Reserve (Rowley *et al.* 2010a); Kon Tum Province: 10—Dac Lei (Rowley *et al.* 2010b); 11, 12,—Dac Lei environs, Ngoc Linh Nature Reserve (Rowley *et al.* 2010b); 13—Dac Lei environs, Ngoc Linh Nature Reserve, Ngoc Linh Mt. (ZISP 12051–12058, ZISP 12091–12093; ZISP 12086–12088; ZISP 12089–12090; ZMMU A-5210; ZMMU A-5217–A-5218); 14—Ngoc Linh Nature Reserve (Rowley *et al.* 2012); 15—Kon Plong Nature Reserve (ZMMU A-4110; ZMMU A-5213; ZISP 12094–12095); 16—Chu Mom Ray Nature Reserve (Jestrzemiński *et al.* 2013; the authors report two species of *Leptotalax*, preliminary identified as *L. cf. applebyi* and *L. cf. firthi*); Gia Lai Province: 17—Kon Cha Rang Nature Reserve (Inger *et al.* 1999); 18—Binh Dinh Province, An Toan Nature Reserve (Hong Minh Duc, personal communication); 19—Dak Lak Province, Chu Yang Sin National Park (ZMMU A-5215); Lam Dong Province: 20—Bidoup—Nui Ba National Park, Hon Giao Mt. and environs of Giang Ly station (Rowley *et al.* 2011a; ZMMU A-4717; ZMMU A-4797); 21—Bidoup—Nui Ba National Park, Bidoup Mt. (Paiarkov [Poyarkov] & Vasilieva 2012; ZMMU A-4797; ZMMU A-5211); 22—Loc Bac forestry (this paper); **Cambodia:** 23, 24—Ratanakiri Province, Virachey National Park (Rowley *et al.* 2010c); 25—Kampong Speu Province, Phnom Aural Wildlife Sanctuary, Phnom Aural Mt. (Ohler *et al.* 2002; authors record unidentified *Leptotalax* larvae and metamorphs).



**Phylogenetic analysis.** 16S rRNA partial sequences of 105 specimens (104 representatives of ca. 29 *Leptolalax* species and 1 outgroup full mitogenomic sequence of *Brachytarsophrys carinense* (Boulenger) (JX564854), the data on voucher specimens and GenBank sequences used in phylogenetic analyses are summarized in Table 1) with a total length of up to 1083 bp were included in the final alignment and subjected to phylogenetic analyses. Sequences were also submitted to a BLAST search in GenBank to confirm that the intended sequences had been amplified. The forward and reverse sequences were checked visually in Chromas Pro software (Technelysium Pty Ltd., Tewantin, Australia) and a consensus sequence was compiled with BioEdit 5.0.9 (Hall 1999). Nucleotide sequences were initially aligned using ClustalX 1.81 (Thompson *et al.* 1997) with default parameters, and then optimized manually in BioEdit 7.0.5.2 (Hall 1999) and MEGA 6.0 (Tamura *et al.* 2013). Mean uncorrected genetic distances (*p*-distances) between sequences were determined with MEGA 6.0 (Tamura *et al.* 2013). Uncorrected genetic distances (*p*-distance) between sequences were calculated in MEGA 6.0. MODELTEST v.3.06 (Posada & Crandall 1998) was used to estimate the optimal evolutionary models to be used for the data set analysis. The best-fitting model as suggested by the Akaike Information Criterion (AIC) was the general time-reversible (GTR) model of DNA evolution with a gamma shape parameter (G).

Phylogenetic analyses were conducted in PAUP version 4.0b4a (Swofford 1998), Treefinder (Jobb *et al.* 2004) and MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) software. The Maximum Parsimony (MP) analyses were conducted with the program PAUP version 4.0b4a (Swofford 1998), using a heuristic search and the closest step-wise sequence addition algorithm. Most-parsimonious trees were generated with 100 random-addition sequences and the tree-bisection-reconnection (TBR) algorithm, for branch swapping. Transitions and transversions were equally weighted. The Maximum Parsimony tree was reconstructed with random addition, 20 replicates with CNI (Close Neighbor Interchange) value = 3. The Maximum Likelihood (ML) analyses were conducted using Treefinder (Jobb *et al.* 2004). Transitions and transversions were equally weighted, and gaps were treated as missing data. Confidence in tree topology was tested by non-parametric bootstrap analysis (Felsenstein 1985) with 1000 replicates, and by posterior probability (PP) for Bayesian inference (BI) in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). We *a priori* regarded tree nodes with bootstrap values 70% or greater and posterior probabilities values over 0.95 as sufficiently resolved, those between 75% and 50% (0.95 and 0.90 for BI) were regarded as tendencies, those below 50% (0.90 for BI) were considered to be non-resolved (Huelsenbeck & Hillis 1993).

**Acoustic analysis:** Advertisement calls of the newly discovered *Leptolalax* population were recorded *in situ* on 15th April 2013 from 22:00 to 23:30 h at a temperature of 25 °C (temperature was measured at the calling site immediately after recording with a digital thermometer KTJ TA218A Digital LCD Thermometer-Hydrometer), using a Nikon D 600 digital SLR camera (Nikon Corporation, Japan) in video mode with default sound settings (16-bit encoding, 48 kHz sampling rate, linear PCM audio format and auto sound level control); in total, 7 advertisement calls from a single individual were recorded. The total duration of the recording was 51.88 s. The audio track was removed from video recording using Avisoft SASLab Pro software v. 5.2.05 (Avisoft Bioacoustics, Germany) with a 22.05 kHz sampling frequency and 16-bit precision. The records of advertisement calls were deposited at the Fonoteca Zoologica and are available at the website <http://www.fonozoo.com> (under the accession number 9198).

Calls were analyzed using Avisoft SASLab Pro software v. 5.2.05; spectrograms for analysis were created using Hamming window, FFT-length 1024 points, frame 100%, and overlap 93.75%. Figure spectrograms were created using Hamming window, FFT-length 512 points, frame 50%, and overlap 93.75%. To facilitate the interspecific comparison of acoustic parameters of the advertisement calls among the species of the genus *Leptolalax* we followed the terminology applied in Rowley & Cao 2009, Rowley *et al.* 2010a, Rowley *et al.* 2010b, Rowley *et al.* 2010c, Rowley *et al.* 2011, Rowley *et al.* 2013. We measured the duration of each call, the intercall interval, the number of notes per call, the duration of each note, the dominant frequency (frequency of maximum amplitude) and the amplitude of the first and following notes of each call. For the first note of each call we also measured the number of pulses and the pulse rate. Notes (or pulses) per second were calculated by counting the number of notes (or pulses) within each call (or in the first note), minus one, and dividing that number by the call duration (or duration of the first note; following Rowley & Cao 2009). The call analysis was complicated because of the background noise created by nocturnal insects. Thus, for precise measurement of temporal characteristics of the calls we applied a high-pass filter (down to 3 kHz), not affecting values of the main call parameters (such as the dominant frequency).

Comparative advertisement call characters for *Leptotalax* species were taken from references, with advertisement calls known for 24 of the 42 known species of *Leptotalax* (Matsui 1997, 2006; Jiang *et al.* 2002; Malkmus *et al.* 2002; Matsui *et al.* 2009, 2014b; Xu *et al.* 2005; Rowley & Cao 2009; Rowley *et al.* 2010a, 2010b, 2010c, 2011a, 2012, 2014; Sukumaran *et al.* 2010).

All numeral parameters are given as mean  $\pm$  SE, the minimum and maximum values are given in parentheses (min–max).

## Results

**Molecular differentiation of Vietnamese *Leptotalax*. Sequence and statistics:** The studied 16S rRNA mtDNA fragment consisted of 1083 sites, where 571 sites were conserved and 492 sites were variable, of which 417 were found to be parsimony-informative. The transition–transversion bias (R) was estimated as 1.349 (all data given for ingroup only). Substitution rates were estimated under the Kimura (1980) 2-parameter model (+G+I). Nucleotide frequencies were A = 32.53%, T = 23.35%, C = 25.73%, and G = 18.38%.

**Preliminary phylogenetic relationships of Vietnamese *Leptotalax*:** The results of the phylogenetic analysis of the 16S rRNA gene are shown in Figure 2. Phylogenetic relationships between the accessed taxa of *Leptotalax* are poorly resolved with major basal nodes in the tree having low (BS < 75%) or insignificant levels (BS < 50%; BPP < 0.95) of support, whereas monophyly of species-level groups and species complexes is significantly supported (BS > 90%; BPP  $\geq$  0.95). ML, MP and Bayesian analyses resulted in essentially similar topologies (see Fig. 2). The MP-trees differed from ML and BI topologies only in associations at poorly supported nodes.

The ML tree of the (Fig. 2) infers the following set of phylogenetic relationships among studied *Leptotalax* species:

General topology of the Bayesian tree suggests that the genus *Leptotalax* is divided into two major groups: the first group joins taxa from Sundas, peninsular Malaysia and adjacent southern Thailand and corresponds to the nominative subgenus *Leptotalax* *sensu stricto*, while the second group comprises taxa from mainland Southeast Asia including Indochina and corresponds to the subgenus *Lalos* *sensu* Dubois *et al.* (2010). However, the monophyly of both groups received low support in all types of phylogenetic analyses. Phylogenetic structuring within *Leptotalax* requires further study, including the incorporation of additional nuclear DNA markers.

Within the group of Sundanese *Leptotalax* species (*Leptotalax* *sensu stricto*) the species indicated as *L. heteropus* by Ohler *et al.* (2011) from southern Thailand (from Phang Nga in Phang Nga province, but erroneously indicated as “Long Nai, Long Nai Province” in Table 2 by Ohler *et al.* 2011: 8) forms a sister clade to all other species in this group and does not form a monophyletic clade with *L. heteropus* from Perak, Malaysia (topotype, sequences from Matsui *et al.* 2014a). Phylogenetic relationships of Bornean taxa of *Leptotalax* in general correspond well to the results of Matsui *et al.* (2014a, 2014b).

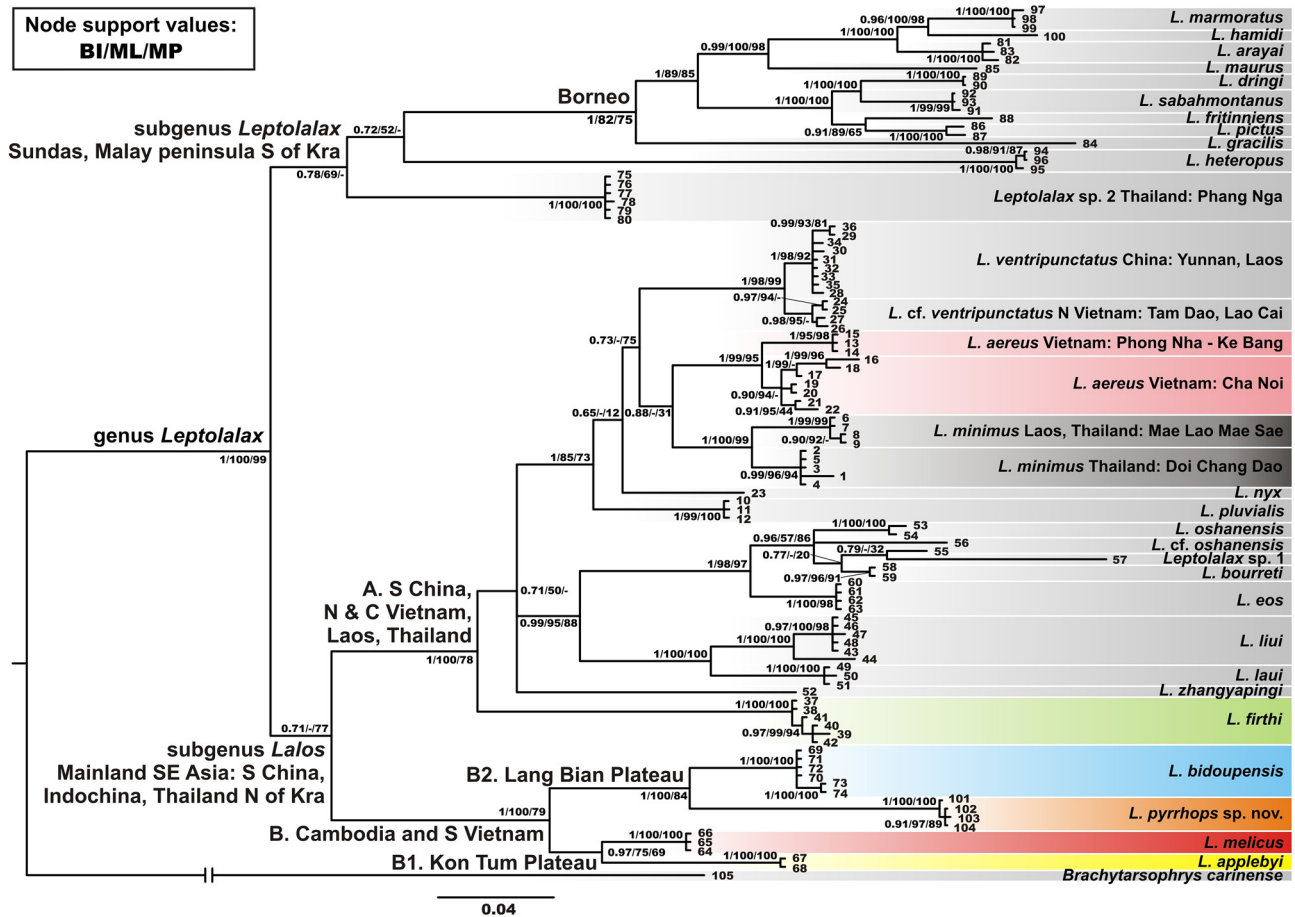
Within the group of mainland Southeast Asian *Leptotalax* species (*Lalos* *sensu* Dubois *et al.* 2010) two major monophyletic groups are revealed with high levels of node support: (A) a clade joining *Leptotalax* species from southern China, northern and central Vietnam, Laos and northern Thailand (1.0/100/78 for BI/ML/MP support values); (B) a clade joining small-bodied *Leptotalax* species (the *L. applebyi* group) from the Kon Tum Plateau in Cambodia and central Vietnam and the Langbian Plateau in southern Vietnam (1.0/100/79) (see Fig. 2).

Phylogenetic relationships within the clade A in general correspond to the results of Ohler *et al.* (2011), Jiang *et al.* (2013) and Sung *et al.* (2014) with the inclusion of *L. firthi* Rowley, Hoang, Dau, Le et Cao, 2012, which has an unresolved position in the clade.

*L. oshanensis*—*L. bourreti* species complex appears to be a sister clade of group joining *L. liui* and *L. laui* with high level of node support (0.99/95/88) and includes a number of mtDNA lineages with poorly resolved phylogenetic relationships: *L. bourreti*, *L. oshanensis*, *L. eos*, undescribed species *Leptotalax* sp. 1 from Doi Chiang Dao, Thailand, and two lineages identified as *L. cf. oshanensis* from southern China. The latter, however do not form a monophyletic group with nominative *L. oshanensis* from the type locality (Emei-Shan, Sichuan, sample 45, see Table 1) and appear to be closely related to *L. bourreti* from Lao Cai, northern Vietnam; thus, the taxonomy and diagnostics of members of *L. oshanensis*—*L. bourreti* from southern China should be reconsidered.

Within clade B, including populations from the central and southern Annamites (Truong Son mountains), two subclades are further recognized: subclade B1 (0.97/75/69) joins two small species from Kon Tum Plateau—*L. applebyi* (central Vietnam) and *L. melicus* (north-east Cambodia), and subclade B2 (1.0/100/84), joining *L.*

*bidouensis* from highland forests of Bidoup and Hon Giao mountains on the north-eastern edge of the Langbian Plateau (altitudes >1500 m a.s.l.) and a population of a new form of *Leptolalax* from Loc Bac forest on the westernmost edge of the Langbian Plateau (altitudes <1000 m a.s.l.). The monophyly of both lineages received absolute levels of node support (1.0/100/100), see Fig. 2.



**FIGURE 2.** Bayesian inference dendrogram of *Leptolalax* derived from the analysis of 1083 bp of 16S rRNA mtDNA gene fragment. Voucher specimen IDs and GenBank accession numbers are given in Table 1. *Brachytarsophrys carinensis* used as an outgroup. Numbers near branches represent posterior probability (PP) or bootstrap support values (BS, 1000 replicates) for BI/ML/MP inferences respectively. Color of clade labels for south Indochinese species corresponds to icon colors on Figure 1. Numbers of specimens (No. 1–92) correspond to those in Table 1.

**Sequence divergence:** The uncorrected *p*-distances among and within the 16S rRNA gene fragment of the studied *Leptolalax* taxa are shown in the Table 2.

The observed interspecific distances within the genus *Leptolalax* studied varied from  $p = 4.4\%$  to  $p = 23.6\%$  of substitutions. The values of uncorrected genetic *p*-distances in ingroup and outgroup comparisons notably overlapped. The minimal interspecific *p*-distance between recognized nominal species in the analysis was found between the two closely related species from Borneo: *L. marmoratus* and *L. arayai* ( $p = 4.6\%$ ). The maximum *p*-distance was observed between *L. bourreti* from northern Indochina and *L. heteropus* from Malay Peninsula ( $p = 23.5\%$ ).

Intraspecific distances within *Leptolalax* species in our analysis varied from  $p = 0\%$  (in a number of taxa: *L. applebyi*, *L. arayai*, *L. bourreti*, *L. dringi*, *L. eos*, *L. heteropus*, *L. melicus*, *L. pluvialis* and *L. sp. 2* cf. “*heteropus*” from Thailand) to  $p = 1.2\%$  in *L. liui* and  $p = 2.6\%$  in *L. minimus*. In some cases, genetic distances between lineages of uncertain taxonomic status approached intraspecific genetic distances. Examples include *Leptolalax* populations of Tam Dao and surrounding areas in northern Vietnam, which were previously regarded as “*L. pelodytoides*” (Garcia-Paris *et al.* 2003; Fu *et al.* 2007; Zhang *et al.* 2013; herein we indicate this lineage as *L. cf.*

*ventripunctatus*). From the mtDNA phylogenetic perspective, this lineage is recovered as a sisterclade to *L. ventripunctatus* from southern Yunnan, northern Laos and adjacent Vietnam; uncorrected genetic *p*-distance between these lineages is low ( $p = 0.8\%$ ) and indicates that the Tam Dao population might be conspecific with *L. ventripunctatus*. A complicated situation is observed in the *L. bourreti*—*L. oshanensis* species complex from central and southern China and northern Vietnam, where two lineages, preliminary identified as “*L. oshanensis*” (Zheng *et al.* 2004; Xiang *et al.* 2013) from Jinfo-Shan Mt., Chongqing (here as “*L. cf. oshanensis* 1”) and unidentified locality in southern China (here as “*L. cf. oshanensis* 2”) proved not to form a monophyletic group with nominal *L. oshanensis* from Emei-Shan, Sichuan. These three lineages form a poorly-resolved group with *L. bourreti* from Lao Cai, northern Vietnam, and *L. eos* from northern Laos and Vietnam; genetic *p*-distances between these lineages vary from  $p = 3.0\%$  to  $p = 6.2\%$ . An observed overlap between inter- and intraspecific genetic distances in the latter case can be explained by the insufficient taxonomy of the group and other lines of evidences, including morphological and acoustic data, appear to be required for better understanding of species boundaries in the *L. bourreti*—*L. oshanensis* species complex.

The newly discovered population of *Leptolalax* from Loc Bac Forest in Langbian plateau was found to be most closely related to *L. bidoupensis* at the gene fragment examined ( $p = 10.3\%$ ). This value is higher than observed between several species of *Leptolalax* (Table 2).

## Taxonomy

Based upon the phylogenetic analysis of 16S rRNA mtDNA gene sequences, the *Leptolalax* from Loc Bac Forest represents a highly divergent mtDNA lineage, clearly distinct from all other *Leptolalax* species for which comparable 16S mtDNA sequences are available. The observed differences in mtDNA sequences are congruent with other lines of evidences, including diagnostic morphological characters and differences in advertisement calls (see below in “Comparisons”). These results support our hypothesis that the recently discovered and morphologically distinct population of *Leptolalax* from Loc Bac represents a previously undescribed species which we describe below:

### *Leptolalax pyrrhops* sp. nov.

**Holotype.** ZMMU A-5208 (field number ABV-00148), adult female from Loc Bac forest (operated by Loc Bac Forest Enterprise), Loc Bao Commune, Bao Lam District, Lam Dong Province, Vietnam (coordinates 11°44' 17" N, 107° 42' 25" E, elevation 830 m. a.s.l.), collected by E.A. Galoyan and A.B. Vassilieva on 10 of April 2013.

**Paratypes.** ZMMU A-4873, two adult males (individual field numbers ABV-00213 and ABV-00215) collected by E.A. Galoyan and A.B. Vassilieva on 15 of April 2013 in mountain forest approximately 1700 m from the area of holotype collection, within the Loc Bac forest, Lam Dong Province, Vietnam (11°44'07" N, 107° 43' 17" E, elevation 1100 m. a.s.l.), and four adult females collected in the same area as male paratypes on 13 of April 2013 (individual field numbers ABV-00157–00158) and on 15 of April 2013 (individual field numbers ABV-00176 and ABV-00214). ZISP 12041 (field number ABV-00212), adult female from Loc Bac forest, Loc Bao Commune, Bao Lam District, Lam Dong Province, Vietnam (coordinates 11°44' 17" N, 107° 42' 25" E, elevation 830 m. a.s.l.), collected by E.A. Galoyan and A.B. Vassilieva on 15 of April 2013. VNMN A2015.02 (field number ABV-00177), adult female from Loc Bac forest, Loc Bao Commune, Bao Lam District, Lam Dong Province, Vietnam (coordinates 11°44' 17" N, 107° 42' 25" E, elevation 830 m. a.s.l.), collected by E.A. Galoyan and A.B. Vassilieva on 15 of April 2013.

**Etymology:** The specific epithet is a noun in the nominative case, derived from Greek “*pyrrhos*” for “fire-colored” and Greek “*ops*” for “eye”, in reference to the iris color of the new species.

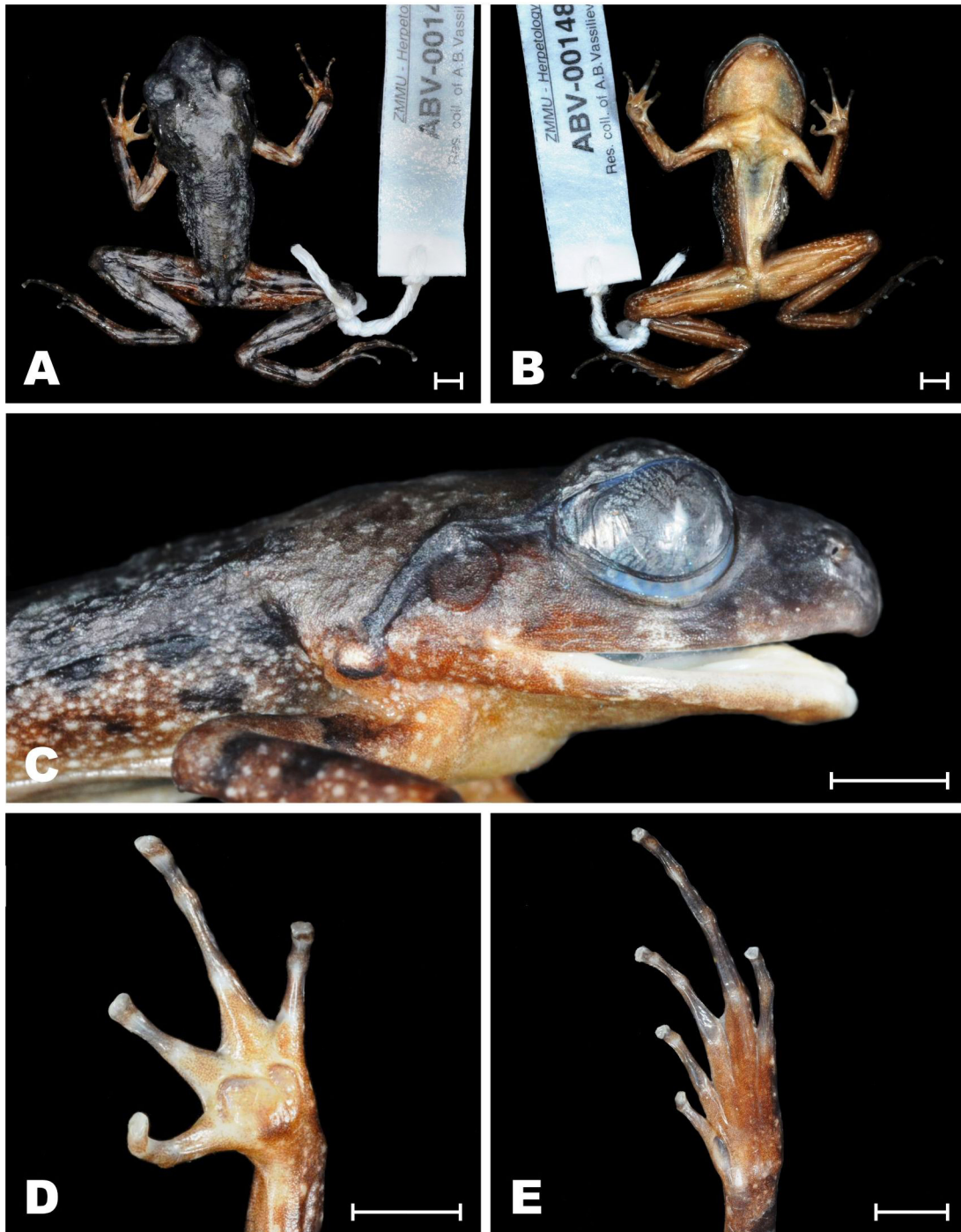
**Recommended vernacular name.** The recommended common name in English is “*Orange-eyed litter frog*”, referring to the beautiful iris coloration of the new species. The recommended common name in Vietnamese is “*Cóc Mày Mắt Cam*”.

**Diagnosis.** The species is assigned to the genus *Leptolalax* based on the following characters considered to be diagnostic for the genus: (1) comparatively small size; (2) rounded finger tips, the presence of an elevated inner

metacarpal tubercle not continuous on to the thumb; (3) presence of macroglands on body (including supra-axillary, pectoral, femoral and ventrolateral glands, chest glands are present but they do not form teats); (4) vomerine teeth absent; (5) tubercles on eyelids and (6) anterior tip of snout with whitish vertical bar (Inger 1966; Dubois 1980, 1983; Matsui 1997, 2006; Lathrop *et al.* 1998; Delorme *et al.* 2006). *Leptolalax pyrhhops* **sp. nov.** is distinguished from its congeners by a combination of the following morphological attributes: (1) presence of distinct dark brown/black distinct dorsolateral markings including blackish spots on the flank and dark canthal and/or temporal streaks; (2) rudimentary webbing on toes; (3) externally distinct tympanum, (4) dorsal skin finely shagreened with numerous small tubercles and pustules; (5) medium size for the genus (30.3–33.9 mm in 2 adult males, 30.8–34.3 mm in 7 females); (6) grey-pinkish to dark brownish-violet chest and belly with numerous whitish speckles also covering the lateral sides of body; (7) ventrolateral glands small, indistinct, do not form a distinct line; (8) comparatively small pectoral glands, comprising 1–3% of adult SVL; (10) a bicolored iris, typically bright orange-red in upper two-thirds, fading to silvery green in lower third. The new species is also markedly distinct from all congeners for which comparable sequences are available (16S rRNA mitochondrial gene; uncorrected genetic distance >10.3%). The advertisement call of the new species, consisting of a single long 'introductory' note, comprising 5–12 pulses, followed by of 4–5 predominantly single-pulsed notes, and with an average dominant frequency of 1.91–2.23 kHz, also distinguishes the new species from *Leptolalax* species for which calls are known.

**Description of holotype.** Medium-sized specimen in good state of preservation for head and body and partially dehydrated limbs due to ethanol preservation; habitus slender (Figs. 3, 4A, 4B). The holotype is dissected on left side of trunk, dissection length 9.3 mm; left ovary with small oocytes (diameter 0.85–0.95 mm) clearly seen in the dissection. The ventral side of the right femur is also dissected, dissection length 11.9 mm. **Head.** Head slightly longer than wide (HDW/HDL 0.90), flattened, triangular in dorsal view; top of head flat; snout comparatively short (SNT/HDL 0.37), obtusely rounded in dorsal view (Fig. 3A) and slightly truncate but gently rounded in profile (Fig. 3C), snout slightly projecting beyond margin of the lower jaw; nostril oval-shaped, vertical, located much closer to tip of snout than to eye (Fig. 3C); loreal region slightly concave; canthus rostralis distinct, bluntly rounded; eyes large (EYE/HDL 0.36), eye diameter almost equal to snout length (EYE/SNT 0.98), notably protuberant in dorsal view and in profile, pupil vertical, diamond-shaped; tympanum distinct, round with vertical diameter slightly exceeding the horizontal diameter (vertical diameter to horizontal diameter (TMP) ratio 1.14), tympanum comparatively small with diameter over two times smaller than that of the eye (TMP/EYE 0.46); tympanic rim notably elevated relative to skin of temporal region; vomerine teeth absent; pineal ocellus absent; vocal sac and vocal sac openings absent; tongue long, wide, with free posterior end, heart-shaped with a shallow medial notch at posterior tip; supratympanic fold forming a distinct wide ridge, running from the posterior corner of eye posteriorly towards dorsal edge of tympanum, sharply curving down towards axillary gland posteriorly to corner of mouth, supratympanic ridge comparatively smooth with few flat tubercles (Fig. 3C). **Forelimbs.** Forelimbs thin, slender; tips of fingers in life rounded and somewhat swollen, but appear to be truncate, notably flattened in preservative, slightly widened distally (wider than distal finger articulation) (Fig. 3D); relative finger lengths  $IV < I = II < III$ ; nuptial pad absent; subarticular tubercles absent, replaced by low dermal ridges, distinct on toes II–IV; a large, flat oval-shaped inner metacarpal tubercle not separated from small, laterally compressed drop-shaped outer metacarpal tubercle (inner metacarpal tubercle is about three times wider than the outer metacarpal tubercle, width ratio 0.39), they touch each other on almost full length of the outer metacarpal tubercle, but the borders of both tubercles are clearly marked by a thin medial groove; fingers completely free of webbing, in life with no distinct fringing, no signs of fringes on fingers I, II and IV; in preservative all fingers look somewhat flattened (Fig. 3D). **Hindlimbs.** Hindlimbs slender, tibia half of snout-vent length (TIB/SVL ratio 0.5); tibiotarsal articulation of adpressed limb reaching snout, but not beyond snout tip, posterior edge of tibiotarsal articulation reaches well beyond anterior margin of eye. Tips of toes rounded and slightly swollen in life, but look truncate, notably flattened in preservative possibly to dehydration in ethanol, slightly widened distally, similar to that of fingers; relative toe length  $I < II < V < III < IV$ ; subarticular tubercles absent, replaced by clear, well-developed dermal ridges, distinct on all toes and continuing to metatarsus; large, oval-shaped inner metatarsal tubercle well pronounced, 2.3 times longer than wide, outer metatarsal tubercle absent; basal webbing present between all five toes, web especially clear between toes II–III and III–IV, greatly reduced between toes I–II and IV–V; in life no clear lateral fringes seen on any toe (Fig. 3E). **Skin texture and skin glands.** Skin shagreened with numerous small tubercles and pustules finely and relatively evenly scattered on dorsal surfaces of trunk, head and limbs,

tubercles absent on sides (Fig. 3A, Fig. 4A), upper eyelid with numerous small tubercles (Fig. 3C); skin on ventral surfaces of trunk, head and limbs smooth (Fig. 3B, Fig. 4B); pectoral gland located at forelimb basis on ventral surface of axillary region, rounded, 0.78 mm in diameter, distinct in preservative and in life (Fig. 3B, Fig. 4B); femoral gland oval, elongated, on posteroventral surface of thigh, approximately three times closer to knee than to vent, approximately 1.62 mm in diameter, more distinct in life than in preservative (Fig. 3B, Fig. 4B); supra-axillary gland located in axillary region dorsally from insertion of forelimb, rounded, raised, 1.54 mm in diameter; ventrolateral glands small (diameter less than 0.7 mm), indistinct, rounded and flat, irregularly scattered on flanks, not forming a distinct line.



**FIGURE 3.** Holotype *Leptolalax pyrrhops* sp. nov. (ZMMU A-5208, female; filed number ABV-00148) in preservative: (A) dorsal view, (B) ventral view (C) head, lateral view, (D) volar view of the left hand, (E) plantar view of the left foot. Scale bar 3 mm. Photos by N.A. Poyarkov.



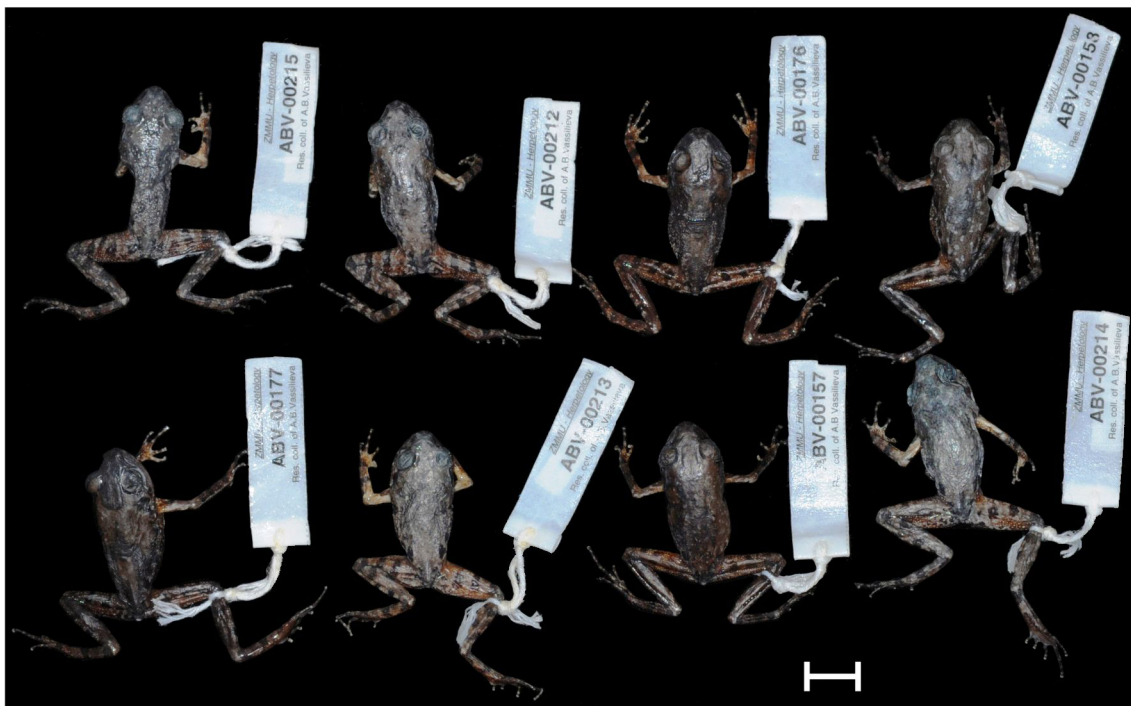
**FIGURE 4.** Coloration of the *Leptolalax pyrrhops* sp. nov. in life: female holotype (ZMMU A-5208; filed number ABV-00148): (A) dorsolateral view, (B) ventral view; female paratype (ZMMU A-4873, filed number ABV-00158) in life: (C) dorsolateral view, (D) ventral view. Photos by E.A. Galoyan.

**Colour of holotype in life.** Dorsal surfaces of head and trunk dark reddish-brown with no distinct darker markings, but several dark-ochre blotches of irregular shape and indistinct borders located at base of head and on the posterior part of the dorsum. Dorsal surfaces of forelimbs (lower arms) and hindlimbs somewhat lighter brownish, forearms much lighter with orange to light reddish-brown background color. Faint transverse brownish grey bars are darker than the background color and are located on the dorsal surface of the thighs, tibia, tarsus, lower arms, fingers and toes (Fig. 4A). Tiny whitish to light bluish flecks scattered on dorsolateral sides of body, dorsal surfaces of lower arms, thighs, tibia, tarsus, and limb insertion areas; flecks are especially numerous on lateral sides of body, being densely scattered (Fig. 4A). On lateral sides of body several distinct blackish spots (1–2 mm in diameter) located in two lines running from axilla to groin (5 large spots on each side of body; the most posterior black spot located in the groin area is about 1.5 times larger than the other spots) and ventrally of this line (one larger and one smaller spot on each side of the body). Two smaller blackish elongated spots located on the sacrum. The dorsolateral surfaces of body, fingers, toes and elbow to upper arm bluish-grey to dusty pinkish. A distinct creamy-white spot on the edge of the lower jaw under the eye and a smaller indistinct whitish blotch located anteriorly to the latter; tiny white stripe on the snout creamy-white, with irregular borders. Tympannic area dark blackish-brown from the supratympanal ridge to the ventral edge of tympanum; the dark spot has indistinct borders and extends from the posterior corner of eye towards the supra-axillary gland. Ventral surfaces of forelimbs, hindlimbs and belly opaque pinkish to light bluish-purple; throat transparent pinkish, somewhat warmer

in color compared to the belly (Fig. 4B). Ventral surface of chest bluish-violet (due to transparent skin and underlying liver). Ventral surfaces of head, limbs, chest and belly covered with irregular bluish-white speckles and dusting; speckles larger and more densely scattered on belly and chest, on head speckles are larger along the anterior margin of throat. Ventral surface of arms pinkish-purple with whitish dusting along lateral margins; ventral surface of thighs, tibia, and tarsus brownish-purple with faint bluish-white flecking. Metatarsal tubercle bluish with darker distal edge (Fig. 4B). Supra-axillary gland pale copper-red; pectoral glands bluish-white; femoral glands whitish edged with purplish-brown color; ventrolateral glands indistinct, bluish white, indistinct from whitish lateral flecking. Iris bicolored: bright orange-gold in upper half, fading to greenish-silver in lower third; orange color brighter in upper half, lower half showing silvery greenish speckles; fine black reticulations throughout the iris, but denser on its periphery. Anterior part of iris bearing a notably darker (brownish-orange) stripe, running from middle of pupil to anterior corner of eye. Iris periphery lined with black (Fig. 4B). Sclera light whitish-cream.

**Colour of holotype in preservative.** In preservative coloration fades to dark grey-brown on dorsum and flanks, with slightly paler limbs and yellowish-grey to whitish on the venter; reddish and pinkish tints, as well as iris coloration, fades completely; other features remain without significant change (Fig. 3A). Banding on limbs are less pronounced, white flecks on body flanks and ventral sides are more pronounced (Fig. 3C). Ventral surface of chest, belly, throat, interior portions of arms and thighs are pale greyish brown; macrogrands turn creamy white (Fig. 3B).

**Measurements of the holotype (all in mm).** SVL 33.8; HDL 14.1; HDW 12.6; SNT 5.2; EYE 5.1; IOD 4.0; TMP 2.4; TEY 1.6; TIB 17.0; ML 8.9; PL 16.2.



**FIGURE 5.** Paratypes of *Leptotalax pyrrops* sp. nov. in preservative. Scale bar 10 mm. Photo by N.A. Poyarkov.

**Variation.** All individuals in the type series are generally similar in morphology and body proportions (see Fig. 5); variation of the type series in morphometric characters is shown in Table 3. There is no clear difference in body size between the sexes based upon the series examined. Specimens vary in the number and size of black ventrolateral blotches. In life, both sexes of the new species show much lighter coloration of belly and throat nocturnally (Fig. 4B). Diurnally the belly is much darker, coloured brown-violet to purple with white speckles. Whitish gonads can be easily seen through the semitransparent skin on the belly of gravid females (Fig. 4D). Compared to the nocturnal coloration, the diurnal coloration of the dorsum has more brownish, ochre and orange tints, and the dark brownish and blackish blotches on the dorsum are more obvious (Fig. 4C; Fig. 6A). At night the dorsum appears almost uniformly dark grayish or blackish with contrasting bluish-white speckling on flanks (Fig. 4A; Fig. 6B). Iris coloration varies slightly in the balance of coppery-orange versus pale greenish-silver; in one



individual (ZMMU A-4873, field number ABV-00213), however, the lower silvery-green third of iris looks almost the same uniformly golden-orange as the upper two thirds (Fig. 6B). The new species shows no significant variation in skin structure among sexes (see Fig. 6); in preservative large and elongated tubercles become flatter than in life, but remain distinct; smaller pustules become less distinct in preservative.



**FIGURE 6.** *Leptotalax pyrhhops* sp. nov. *in situ*: (A) a pair in amplexus (male ZMMU A-4873, field number ABV-00215; female ZISP 12041, field number ABV-00212); (B) calling male (ZMMU A-4873, field number ABV-00213) showing night coloration. Photos by E.A. Galoyan.

**Advertisement call.** In total we measured 7 advertisement calls (40 notes) from one individual; calls were recorded at 25.0° C ambient temperature. The calls varied from 208 to 297 ms in duration and consisted of 5–6 notes (Table 4, Fig. 8). The interval between successive calls in one individual varied from 3.26 to 5.63 s. For 7

successive calls the calling rate was 0.13 calls per second. Note duration varied from 11 to 67 ms. The first ‘introductory’ note always was longer than successive notes ( $45 \pm 4$  [32–67] ms versus  $15 \pm 0.1$  [11–20] ms). The first note of each call consisted of 5–12 pulses repeated at a rate of approximately 123–165 pulses per note (Table 4, Fig. 8). In most cases, first note had lower amplitude than other notes, the difference between amplitude values of the first ‘introductory’ note and successive notes was  $80.2 \pm 0.8$  (45.7–97.9) mV (Table 4). The frequency modulation was absent and the harmonics were not clearly visible in the recording (Fig. 8).

**TABLE 4.** Measurements of advertisement call parameters for *Leptotalax pyrrhops* sp. nov. Parameter values are given as means  $\pm$  SE (and ranges).

Recording	Parameter	Registration No. ZMMU A-4873, ABV-00213
	Temperature (°C)	25
	Number of calls	7
	Call duration (ms)	$254 \pm 11$ (208–297)
	Intercall interval (s)	$4.42 \pm 0.43$ (3.26–5.63)
	Notes/call	$5.71 \pm 0.18$ (5–6)
	Internote interval (ms)	$29 \pm 1$ (16–36)
	Call repetition rate (calls/s)	0.13
Introductory note	Note duration (ms)	$45 \pm 4$ (32–67)
	Pulses/note	$7.43 \pm 0.90$ (5–12)
	Dominant frequency (Hz)	$1990 \pm 20$ (1910–2060)
	Amplitude (mV)	$356.6 \pm 0.8$ (298.6–440)
	Pulse repetition rate (pulses/s)	$141.45 \pm 6$ (123.5–164.9)
Clicks/Notes 2–X	Note duration (ms)	$15 \pm 0.1$ (11–20)
	Pulses/note	1
	Dominant frequency (Hz)	$2070 \pm 20$ (1930–2230)
	Amplitude (mV)	$436.8 \pm 0.8$ (375.1–485.8)
	Note repetition rate (notes/s)	$18.61 \pm 0.37$ (16.84–19.38)

**Position in mtDNA phylogeny and sequence divergence.** Uncorrected genetic *p*-distances between *Leptotalax pyrrhops* sp. nov. 16S rRNA sequences and all homologous sequences available on GenBank included in the analysis (see Table 1) varied from 13.5% (with *L. applebyi*) to 20.8% (with *L. bourreti*), with the exception of the sister species *L. bidouensis*, which showed an uncorrected sequences divergence of 10.3% (see Table 2). This degree of pairwise divergence in the 16S rRNA gene is greater than that usually representing differentiation at the species level in anura (Vences *et al.* 2005a, 2005b; Vieites *et al.* 2009). Intraspecific variation in this gene fragment for *Leptotalax pyrrhops* sp. nov. sampled from the type locality was <0.31%.

**Distribution.** The new species has been so far recorded from only two sites (<2 km from each other) in the mountain forests of the Loc Bac forest, Bao Lam District, Lam Dong Province, Vietnam; on the western edges of the Langbian Plateau at altitudes from 800 to 1100 m a.s.l. The distribution of the new species may be quite narrow, possibly restricted to a small mountain ridge on the borders of Loc Bao and Loc Lam communes, Bao Lam District; less than 10 km in length. The known localities of the new species are located ca. 40 km southwards from the southernmost locality of *L. bidouensis* (Bidoup Mt., Bidoup—Nui Ba N.P., Lam Dong Province), thus marking the southernmost record of the genus *Leptotalax* from Vietnam and Indochina known to date.

**Ecology.** All specimens were collected at night after heavy rains along the small intermittent, rocky streams on the limited parcels of primary montane high polydominant evergreen tropical forest with a high abundance of large rocks and the predominance of trees of the families Magnoliaceae, Sapindaceae, Podocarpaceae, Euphorbiaceae, Fagaceae, Theaceae, Sapotaceae, Caesalpiniaceae, Anacardiaceae, Altingiaceae, Rhodoleiaceae, Elaeocarpaceae, Lauraceae, Sterculiaceae and Dipterocarpaceae (Fig. 7). *Leptotalax pyrrhops* sp. nov. occurs in syntopy with *Leptobranchium pullum* (Smith, 1921), *Ophryophryne* sp., *Limnonectes limborgi* (Sclater, 1892), *Limnonectes* sp.,

*Kurixalus* sp., *Polypedates megacephalus* Hallowell, 1861, *Hylarana milleti* (Smith, 1921). Calling males were found along the stream, sitting on large mossy rocks, some specimens were hiding under the rocks and stones and were difficult to locate (Fig. 6B). Females were found hiding under rocks on the edge of water, or in the dry riverbed of a mountain stream filled with forest litter and leaves. The ovaries of all females contained well-developed, unpigmented eggs of a diameter of approximately 1.6–2.1 mm. Reproductive activity and calling males were recorded in April (10–15 of April). Amplexus occurred while keeping males and females in a plastic container after capture (Fig. 6A). Despite our intensive searches we could not find the tadpoles of the new species; larvae of *Limnonectes* sp. were found in the same streams where the new species was observed.



**FIGURE 7.** Natural habitat of *Leptotalax pyrrhops* sp. nov. in Loc Bac woodland, Lam Dong Province, typical breeding site at small forest stream. Photo by E.A. Galoyan.

**Conservation status.** *Leptotalax pyrrhops* sp. nov. is presently known from two closely located sites in Loc Bac forest (currently operated by Loc Bac Forest Enterprise) in Lam Dong Province. The actual distribution of the new species is unknown but probably is quite narrow. Given the available information, we suggest *Leptotalax pyrrhops* sp. nov. to be considered as a Data Deficient species following IUCN's Red List categories (IUCN 2001).

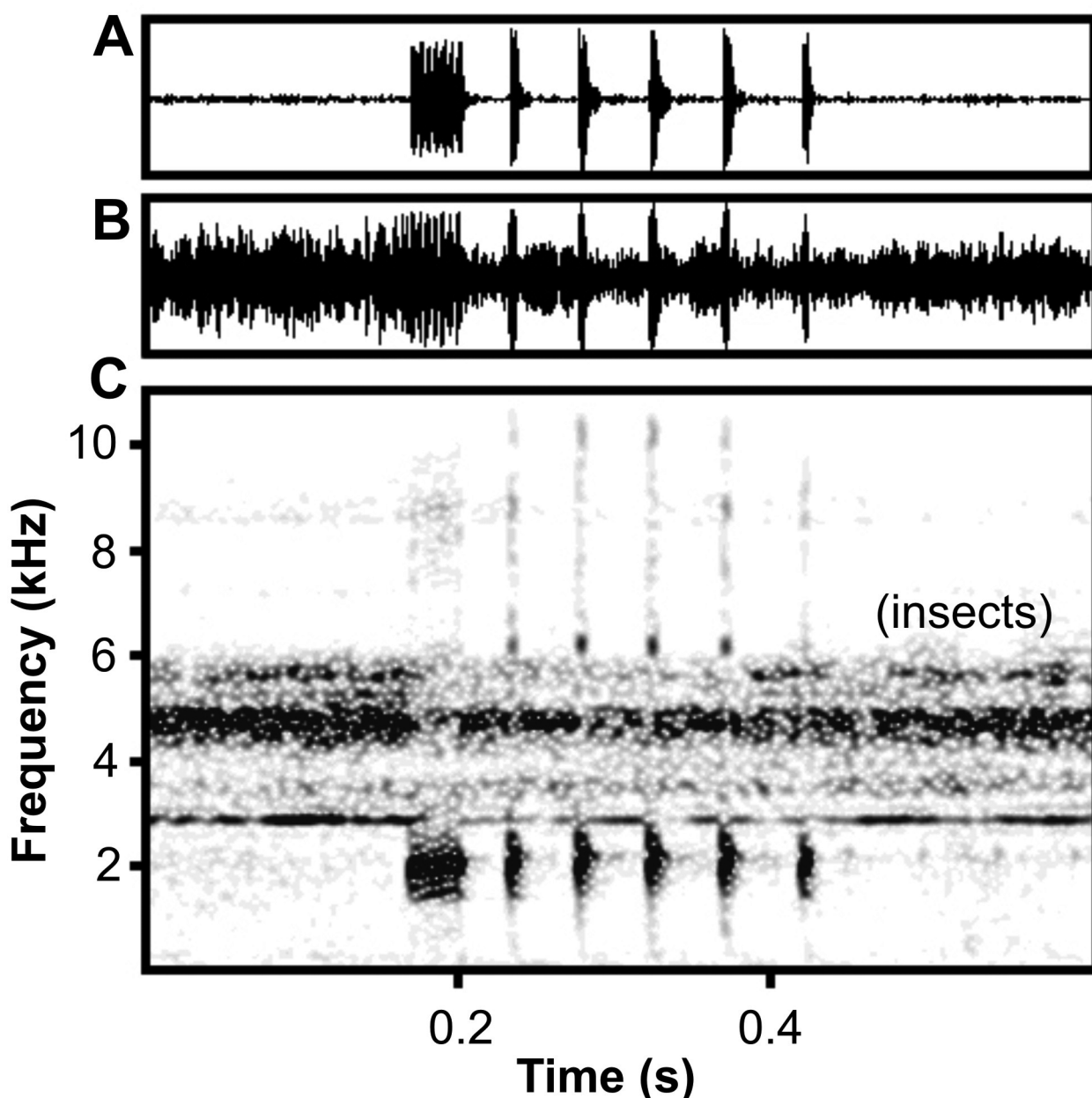
**Comparisons.** *Leptotalax pyrrhops* sp. nov. is both most morphologically and molecularly similar to small-bodied *Leptotalax* species from southern and central Vietnam and northeastern Cambodia: *L. applebyi*, *L. bidoupenensis* and *L. melicus* (Clade B), and *L. botsfordi* from northern Vietnam. In having a dark brownish red ventral surface with white speckling, *Leptotalax pyrrhops* sp. nov. can be differentiated from all other *Leptotalax* in mainland Southeast Asia, except for *L. applebyi*, *L. bidoupenensis*, *L. melicus* and *L. botsfordi* (*L. aereus*, *L. alpinis*, *L. arayai*, *L. bourreti*, *L. dringi*, *L. firthi*, *L. fuliginosus*, *L. fritinniensis*, *L. gracilis*, *L. hamidi*, *L. khasiorum*, *L. lateralis*, *L. laui*, *L. liui*, *L. marmoratus*, *L. minimus*, *L. nahangensis*, *L. nokrekensis*, *L. oshanensis*, *L. pelodytoides*, *L. pictus*, *L. playcephalus*, *L. sabahmontanus*, *L. solus*, *L. sungi*, *L. tamdil*, *L. tuberosus* and *L. zhangyapingi* have mostly white or pale greyish or brownish venters, with or without dark spots or mottling; *L. croceus* has a bright orange venter; *L. pluvialis* has a dirty white or grey belly with dark brown or grey marbling, and uniform pale dirty white or grey throat with pale speckling only around the margins; *L. melanoleucus* and *L.*

*ventripunctatus* show large patches of distinct brown or black and white marbling, *L. heteropus* displays a grey venter, speckled with black; *L. maurus* has a black or dark grey brown venter, with indistinct small light patches, and *L. kecil* displays a uniformly dark venter with large, dark orange pectoral glands).

In having an externally distinct tympanum the new species can be easily distinguished from two Vietnamese species *L. sungi* and *L. tuberosus* (versus tympanum externally indistinct in *L. sungi* and *L. tuberosus*).

In having toes with rudimentary webbing and poor lateral fringing, *Leptolalax pyrrhops* **sp. nov.** can also be readily differentiated from *L. alpinus*, *L. firthi*, *L. laui* and *L. liui*, which have wide lateral fringing on toes, and from *L. pelodytoides*, which has more extensive webbing and wide lateral fringes between toes.

In typically having a bicoloured iris, with the upper half bright orange-red and the lower half fading to greenish-silver, *Leptolalax pyrrhops* **sp. nov.** can be further distinguished from *L. aereus*, *L. applebyi*, *L. botsfordi*, *L. croceus*, *L. kajangensis*, *L. kecil*, *L. laui*, *L. liui*, *L. maurus*, *L. melicus*, *L. nahangensis*, *L. sungi* and *L. tuberosus*, all of which typically have a uniform iris coloration with black reticulations.



**FIGURE 8.** Advertisement call of the male paratype advertisement calls of *Leptolalax pyrrhops* **sp. nov.** (ZMMU A-4873, field number ABV-00213) recorded at air temperature of 25°C. (A) waveform of high-pass filtered call, (B) waveform and (C) corresponding spectrogram of the same call, unfiltered.



**FIGURE 9.** Members of *Leptolalax applebyi* species group. *L. applebyi* (Ngoc Linh N.P., Kon Tum Province, Vietnam): (A) adult male AMS R173635; (B) AMS R 173777; *L. melicus* (Virachey N.P., Ratanikiri Province, Cambodia): (C) adult male holotype, MVZ258198; (D) adult male paratype MVZ258199; *L. bidoupensis*: (E) ZMMU A-5215, Chu Yang Sin N.P., Dak Lak Province, Vietnam; (F) adult female paratype NCSM 77320, Bidoup—Nui Ba N.P., Lam Dong Province, Vietnam (photos A–D and F by J.J.L. Rowley; E—photo by N.A. Poyarkov).

In having the dorsal skin finely shagreened with numerous small tubercles and pustules finely and relatively evenly scattered on dorsum, the new species can be differentiated from *L. arayai*, *L. croceus*, *L. khasiorum*, *L. lateralis*, *L. maurus*, *L. minimus*, *L. solus*, *L. tamdil*, *L. tuberosus* and *L. ventripunctatus* (roughly granular or tuberculate skin texture with skin ridges and large tubercles) and from *L. alpinus*, *L. applebyi*, *L. bidoupensis*, *L. bourreti*, *L. eos*, *L. fuliginosus*, *L. melanoleucus* and *L. pluvialis* (smooth or otherwise less tuberculate dorsal skin in these species).

**TABLE 2.** Uncorrected *p*-distance (percentage) between 16S rRNA sequences of *Leptolalax* species included in phylogenetic analyses (below the diagonal) and standard error estimates (above the diagonal). The ingroup mean uncorrected *p*-distances are shown on the diagonal and shaded with grey (continues next page).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 <i>L. minimus</i>	2.6	1.2	1.2	1.1	1.3	1.3	1.7	1.6	1.6	1.7	1.4	1.6	1.4	1.6	1.7	1.6	1.9
2 <i>L. pluvialis</i>	5.4	0.0	1.2	1.3	1.4	1.3	1.7	1.7	1.7	1.6	1.5	1.7	1.6	1.7	1.9	1.6	1.9
3 <i>L. aereus</i>	7.0	5.7	1.1	1.1	1.4	1.3	1.8	1.7	1.8	1.7	1.3	1.6	1.2	1.5	1.7	1.5	1.9
4 <i>L. nyx</i>	6.0	6.0	5.8	—	1.3	1.2	1.7	1.6	1.8	1.8	1.4	1.7	1.4	1.6	1.8	1.6	2.0
5 <i>L. ventripunctatus</i>	7.8	7.2	7.7	6.2	0.5	0.4	1.6	1.6	1.9	1.5	1.6	1.9	1.5	1.8	1.9	1.8	2.2
6 <i>L. cf. ventripunctatus</i>	7.4	6.5	7.3	5.9	0.8	0.0	1.6	1.5	1.8	1.5	1.5	1.8	1.5	1.8	1.9	1.7	2.1
7 <i>L. firthi</i>	13.9	13.8	13.5	11.8	12.1	11.8	0.4	1.8	2.2	1.8	1.9	2.1	1.9	2.0	2.3	2.1	2.1
8 <i>L. lui</i>	12.2	11.6	12.1	10.7	13.8	12.5	15.0	1.2	1.5	1.8	1.8	1.9	1.7	1.9	2.3	1.8	2.1
9 <i>L. laui</i>	11.5	11.3	13.4	11.8	14.1	12.8	16.7	6.9	0.2	1.7	1.7	1.8	1.8	1.7	2.3	1.7	2.2
10 <i>L. zhangyapingi</i>	12.7	10.7	12.1	11.1	12.1	11.7	14.3	12.9	12.1	—	1.7	1.8	1.8	1.8	2.1	1.7	2.0
11 <i>L. oshanensis</i>	11.7	11.8	11.4	10.5	11.9	11.6	15.8	12.1	12.5	13.7	1.1	0.7	1.0	1.4	1.0	1.3	2.0
12 <i>L. cf. oshanensis</i> 1	11.3	11.2	11.0	9.9	12.3	11.7	15.2	11.3	11.5	13.0	3.6	—	1.2	1.4	1.1	1.4	2.1
13 <i>L. cf. oshanensis</i> 2	11.0	10.7	9.5	10.3	11.8	11.2	14.3	9.5	11.9	12.4	5.3	4.4	—	1.3	1.0	1.3	2.0
14 <i>Leptolalax</i> sp. 1	11.6	11.6	12.7	11.8	12.9	12.5	16.0	12.9	13.5	14.0	9.0	7.8	5.9	—	1.8	1.5	2.0
15 <i>L. bourreti</i>	12.1	11.6	11.4	11.2	13.6	12.7	16.6	12.7	14.4	15.4	5.1	3.4	3.0	8.3	0.0	1.5	2.4
16 <i>L. eos</i>	12.1	11.7	12.1	11.4	13.4	12.7	15.5	10.8	12.1	12.0	7.3	6.2	4.7	8.7	5.6	0.0	2.1
17 <i>L. applebyi</i>	15.4	14.2	14.9	14.9	16.4	16.1	18.4	17.4	17.4	15.2	16.3	15.8	13.7	13.4	18.1	14.9	0.0
18 <i>L. melicus</i>	13.4	12.7	11.4	11.2	13.7	13.4	17.2	18.1	18.4	15.5	15.2	15.2	12.5	13.5	16.6	14.6	7.1
19 <i>L. bidouensis</i>	15.5	15.4	15.8	14.8	17.5	17.3	17.9	16.3	17.7	17.3	17.8	18.2	15.2	15.9	20.6	15.7	9.3
20 <i>L. pyrrophops</i> sp. nov.	16.8	15.9	15.5	15.7	17.2	16.7	18.2	16.5	16.6	17.8	18.7	17.8	17.2	16.1	20.8	16.8	13.5
21 <i>Leptolalax</i> sp. 2	14.9	14.5	15.5	14.5	17.3	17.0	18.9	18.2	17.8	17.3	16.1	15.2	14.9	16.3	18.1	15.8	14.0
22 <i>L. arayai</i>	15.2	16.6	15.6	16.0	15.9	16.0	19.7	18.4	18.3	18.7	17.8	17.8	14.8	15.6	19.0	16.3	14.5
23 <i>L. gracilis</i>	20.6	20.6	18.8	21.4	22.3	21.7	22.4	23.8	22.0	21.7	21.1	19.9	19.4	19.1	23.4	20.5	18.1
24 <i>L. maurus</i>	16.9	17.6	15.7	17.0	17.1	17.3	19.2	19.7	20.3	17.3	17.3	17.0	14.6	15.6	20.2	17.0	15.2
25 <i>L. pictus</i>	17.8	17.7	16.3	16.2	17.6	17.1	16.6	18.6	20.4	19.0	19.0	18.1	16.0	17.6	19.9	16.5	17.5
26 <i>L. frititiniens</i>	16.4	16.1	15.4	15.8	16.3	15.8	18.4	18.5	19.8	19.3	18.0	17.7	16.6	16.6	20.0	15.5	18.1
27 <i>L. dringi</i>	16.3	16.6	15.7	16.1	16.4	16.1	18.0	18.3	17.8	18.5	17.8	17.6	16.8	16.2	19.9	16.7	16.4
28 <i>L. sabahmontanus</i>	15.6	16.5	16.0	16.3	16.0	15.5	17.8	18.3	18.6	18.1	17.6	16.8	15.3	15.8	19.2	15.5	14.6
29 <i>L. heteropus</i>	17.3	17.2	17.7	17.2	18.7	18.4	22.2	20.9	21.9	20.9	21.5	21.5	18.8	20.6	23.5	20.6	16.0
30 <i>L. marmoratus</i>	16.1	15.7	15.8	16.4	15.6	15.1	18	18.2	19.3	17	18.1	17.6	14.6	15.3	18.7	15.1	13.6
31 <i>L. hamidi</i>	16.6	17	16.1	16.7	16.6	16.1	18.4	19.1	17.8	17	17.4	16.7	15.2	15.9	19.5	14.5	15.2
32 <i>Brachytarsophrys</i>	22.4	21.9	20.4	20.1	19.5	19.8	23.3	21.7	22.1	21.7	21.7	21.1	21.2	23.1	23.3	21.4	22.4

.....continued on the next page

TABLE 2. (Continues).

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
<b>Species</b>															
1	1.7	2.0	1.9	1.8	1.7	1.9	1.8	1.7	1.9	1.7	1.6	1.9	1.5	1.6	2.2
2	1.7	2.0	1.9	1.9	1.8	2.0	1.9	1.8	1.9	1.8	1.7	1.8	1.5	1.8	2.3
3	1.7	2.2	2.0	1.8	1.8	1.9	1.8	1.8	1.9	1.9	1.6	1.9	1.7	1.8	2.2
4	1.6	2.1	2.0	1.8	1.9	2.1	1.8	1.8	2.0	2.0	1.9	2.1	1.7	1.8	2.2
5	2.0	2.3	2.1	2.0	1.7	2.1	1.7	1.7	2.0	1.8	1.8	2.1	1.5	1.8	2.2
6	1.9	2.2	2.0	2.0	1.7	2.0	1.7	1.7	2.0	1.7	1.7	2.0	1.4	1.7	2.2
7	2.1	1.9	2.3	2.1	2.0	2.4	2.2	2.0	2.0	2.0	1.9	2.3	1.9	2.1	2.2
8	1.9	1.9	2.1	2.1	2.0	2.2	2.1	2.0	2.2	2.2	2.2	2.2	1.8	2.0	2.2
9	2.1	2.1	2.1	2.3	2.0	2.1	2.0	2.2	2.3	2.2	2.3	2.4	2.0	2.0	2.5
10	2.1	2.2	2.3	2.0	2.1	2.3	2.0	2.1	2.3	2.0	2.0	2.4	1.9	1.9	2.2
11	1.7	2.1	2.0	1.8	1.9	1.9	1.8	1.9	2.0	1.9	1.9	2.1	1.9	1.9	2.1
12	1.9	2.4	2.1	1.9	2.0	2.0	1.9	2.0	2.1	2.0	2.0	2.3	2.0	2.0	2.3
13	1.7	2.1	2.1	2.0	1.8	2.0	1.7	1.9	2.1	2.1	2.0	2.2	1.8	1.8	2.1
14	1.8	2.0	1.9	1.9	2.0	2.2	2.0	2.1	2.0	2.0	2.0	2.1	1.9	1.9	2.1
15	2.1	2.6	2.3	2.2	2.3	2.4	2.2	2.5	2.5	2.4	2.4	2.6	2.3	2.4	2.4
16	2.0	2.0	2.1	1.9	1.9	2.1	1.9	2.0	2.0	2.0	1.9	2.3	1.7	1.7	2.3
17	1.3	1.8	2.1	1.9	2.0	2.0	2.0	2.1	2.2	2.0	1.9	2.0	1.8	1.8	2.1
18	<b>0.0</b>	1.8	2.0	1.9	1.9	2.1	1.9	2.1	2.2	2.1	1.8	2.1	1.7	1.9	2.3
19	9.6	<b>0.0</b>	1.8	2.3	2.3	2.3	2.2	2.0	2.0	2.1	2.1	2.2	2.0	2.1	2.4
20	13.7	10.3	<b>0.3</b>	2.3	2.2	2.3	2.3	2.1	2.1	2.1	2.0	2.0	2.1	2.3	2.2
21	13.4	16.0	16.3	<b>0.0</b>	2.1	2.1	1.9	2.1	2.0	2.1	1.8	2.3	1.9	2.0	2.3
22	13.9	16.3	17.2	14.6	<b>0.0</b>	1.8	1.6	1.7	1.9	1.8	1.7	2.1	1.1	1.2	2.4
23	18.1	19.9	18.8	18.1	11.8	—	1.7	1.7	2.0	2.0	1.9	2.1	1.8	1.6	2.3
24	15.2	17.0	19.0	15.3	9.8	11.2	—	1.7	1.6	1.7	1.7	2.1	1.6	1.7	2.2
25	16.0	17.8	17.9	15.9	11.3	13.0	12.0	<b>0.6</b>	1.3	1.3	1.1	2.1	1.6	1.7	2.2
26	16.3	15.8	16.9	15.6	10.6	14.1	10.9	6.2	—	1.5	1.3	2.0	1.7	1.6	2.3
27	15.5	16.7	17.1	15.2	11.7	14.0	12.0	9.0	8.4	<b>0.0</b>	1.3	2.0	1.4	1.7	2.4
28	14.0	15.8	17.5	13.8	10.5	13.8	10.3	6.4	7.7	7.4	<b>0.4</b>	2.1	1.5	1.6	2.3
29	16.4	17.6	16.8	19.7	18.0	21.1	18.4	18.6	17.9	18.7	18.7	<b>0.2</b>	2.0	2.2	2.4
30	14.3	16.4	17.8	15.8	4.6	11.5	9.0	9.6	9.3	9.9	9.1	17.2	<b>0.0</b>	1.2	2.3
31	14.9	16.7	18.1	15.2	6.2	11.8	9.3	10.7	9.6	11.7	9.5	17.8	4.9	—	2.2
32	21.8	24.5	22.1	21.7	21.0	24.3	21.7	21.8	21.8	22.6	22.2	22.2	22.6	21.7	—

**TABLE 3.** Measurements of the type series of *Leptolalax pyrrohops* sp. nov. For abbreviations see Material and methods. All measurements are given in mm.

Specimen ID; field ID	Sex	SVL	HDL	HDW	SNT	EYE	IOD	TMP	TEY	TIB	ML	PL	Type status
ZMMU A-4873; ABV-00213	♂	30.3	12.0	11.1	4.8	4.5	3.8	1.9	1.4	15.5	7.9	14.3	Paratype
ZMMU A-4873; ABV-00215	♂	33.9	14.2	11.2	5.2	4.8	4.0	2.0	1.7	16.0	8.9	15.8	Paratype
	Mean	<b>32.10</b>	<b>13.09</b>	<b>11.14</b>	<b>4.99</b>	<b>4.62</b>	<b>3.89</b>	<b>1.95</b>	<b>1.58</b>	<b>15.78</b>	<b>8.41</b>	<b>15.03</b>	
ZMMU A-5208; ABV-00148	♀	33.8	14.1	12.6	5.2	5.1	4.0	2.4	1.6	17.0	8.9	16.2	Holotype
ZMMU A-4873; ABV-00157	♀	32.3	13.3	11.7	5.2	5.1	4.0	2.2	1.7	16.2	8.7	15.6	Paratype
ZMMU A-4873; ABV-00158	♀	30.8	13.7	12.1	4.4	5.1	4.5	2.3	1.3	15.9	8.6	15.6	Paratype
ZMMU A-4873; ABV-00176	♀	33.6	13.6	12.0	5.0	5.0	3.9	2.2	1.8	17.0	8.7	16.1	Paratype
VNMN A2015.02; ABV-00177	♀	34.3	13.2	11.2	4.5	5.4	4.1	2.2	2.0	16.3	7.8	15.0	Paratype
ZISP 12041; ABV-00212	♀	33.9	15.0	12.0	4.7	4.8	4.0	2.2	1.9	16.2	8.1	15.6	Paratype
ZMMU A-4873; ABV-00214	♀	33.5	15.9	12.1	5.7	5.3	4.3	2.1	1.7	17.2	8.3	16.3	Paratype
	Mean	<b>33.16</b>	<b>14.10</b>	<b>11.96</b>	<b>4.97</b>	<b>5.12</b>	<b>4.10</b>	<b>2.20</b>	<b>1.71</b>	<b>16.55</b>	<b>8.44</b>	<b>15.76</b>	
	St. dev.	1.23	1.01	0.44	0.46	0.17	0.20	0.10	0.23	0.50	0.37	0.44	
	Max	34.3	15.9	12.6	5.7	5.4	4.5	2.4	2.0	17.2	8.9	16.3	
	Min	30.8	13.2	11.2	4.4	4.8	3.9	2.1	1.3	15.9	7.8	15.0	



The presence of black markings (spots or blotches) on the flanks further distinguishes the new species from *L. aereus*, *L. arayai*, *L. croceus*, *L. eos*, *L. firthi*, *L. laui*, and *L. tuberosus* (no distinct black lateral markings on the flanks), *L. zhangyapingi* (indistinct brown lateral spots and flecks).

In having an indistinct ventrolateral glandular line, *Leptotalax pyrrhops* **sp. nov.** is differentiated from *L. alpinus*, *L. botsfordi*, *L. fuliginosus*, *L. khasiorum*, *L. liui*, *L. oshanensis*, *L. pelodytoides*, *L. pluvialis* and *L. tamdil*, which all have distinct, more complete ventrolateral glandular lines.

The new species can be further easily distinguished from *L. botsfordi* (Lao Cai Province, northern Vietnam), also with dark brownish red ventral surface with white speckling, by the following: (1) comparatively larger head size (HDL:SVL 0.41–0.47 versus 0.34–0.36 in *L. botsfordi*), (2) dense white speckling on the belly (versus only faint white spotting on the belly in *L. botsfordi*) and (3) relatively small femoral glands (1.0–3.0 mm, 2–4% SVL versus 2.4–4.3 mm, 7–14% SVL in *L. botsfordi*).

*Leptotalax pyrrhops* **sp. nov.** can be further differentiated from the three most morphologically similar species from Kon Tum and Langbian Plateaus with dark reddish brown ventral surfaces (Clade B), *L. applebyi*, *L. bidoupensis* and *L. melicus*, in the following ways. From *L. applebyi* (central Vietnam, Kon Tum Plateau, see Fig. 9A, B) the new species is markedly different by having (1) shagreened skin on dorsum with numerous small tubercles (versus more smooth skin on dorsum lacking large tubercles, only scarcely scattered small tubercles in *L. applebyi*, see Fig. 9A, B), (2) bicolored orange-silvery iris (versus uniformly coppery red iris in *L. applebyi*), (3) notably larger body size in adults (30.3–33.9 mm in males; 30.8–34.3 mm in females) (versus 19.6–22.3 mm for males; 21.7–26.4 for females in *L. applebyi*), and (4) by comparatively longer tibia in the new species (male TIB:SVL 0.48–0.52 versus 0.42–0.48 in *L. applebyi*).

From *L. melicus* (Kon Tum Plateau in Ratanakiri, north-eastern Cambodia, see Fig. 9C, D) the new species can be diagnosed by the following: (1) in having bicolored orange-silvery iris (versus uniformly dark golden iris in *L. melicus*), (2) by a larger body size in adults (30.3–33.9 mm in males; 30.8–34.3 mm in females) (versus 19.5–22.7 mm for males in *L. melicus*), (3) by a slightly longer tibia in the new species (male TIB:SVL 0.48–0.52 versus 0.46–0.48 in *L. melicus*), and (4) smaller relative pectoral gland size (PEC/SVL 1–3% versus 3–6% in *L. melicus*).

From its sister species, *L. bidoupensis* (southern Vietnam, eastern and northern edges of Langbian Plateau, see Fig. 9E, F), *Leptotalax pyrrhops* **sp. nov.** can be distinguished by having (1) shagreened skin on dorsum with numerous small tubercles (versus mostly smooth skin texture with no skin ridges or obvious tubercles in *L. bidoupensis*), (2) comparatively larger head (HDL:SVL 0.41–0.47 versus 0.36–0.42 in *L. bidoupensis*), (3) notably larger body size in adults (30.3–33.9 mm in males; 30.8–34.3 mm in females) (versus 18.5–25.4 mm for males; 28.3–29.4 mm for females in *L. bidoupensis*), (4) longer tibia in the new species (male TIB:SVL 0.48–0.52 versus 0.42–0.50 in *L. bidoupensis*), and (5) smaller relative pectoral gland size (PEC/SVL 1–3% versus 3–7% in *L. bidoupensis*). The two sister species can also be distinguished by mtDNA (see above) and acoustic properties (see below).

The advertisement call of *Leptotalax pyrrhops* **sp. nov.** differs structurally from all 24 *Leptotalax* species with described calls: *L. aereas*, *L. alpinis*, *L. applebyi*, *L. arayai*, *L. bidoupensis*, *L. botsfordi*, *L. croceus*, *L. dringi*, *L. firthi*, *L. fuliginosus*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kecil*, *L. liui*, *L. marmoratus*, *L. melanoleucus*, *L. melicus*, *L. oshanensis*, *L. pictus*, *L. solus*, *L. sabahmontanus* and *L. tuberosus*. Although our comparisons are based upon only a single individual, *Leptotalax pyrrhops* **sp. nov.** is the only species of *Leptotalax* with a call containing a single long introductory note containing 5–12 pulses approximately 82% the amplitude of successive notes, followed by 4–5 single-pulsed notes ('clicks'). Compared to species in the *L. applebyi* group, the advertisement call of *Leptotalax pyrrhops* **sp. nov.** most closely resembles that of *L. melicus*, in having a distinct introductory note, but differs by having 4–6 notes/call (versus 4–11 in *L. melicus*), and 5–12 (mean 7.4) relatively high amplitude pulses in a introductory note (versus 8–50 [mean 22.7 and 26.5]) relatively low-amplitude pulses in *L. melicus*). *Leptotalax pyrrhops* **sp. nov.** also appears to differ from *L. melicus* in terms of dominant frequency (1.9–2.2 kHz versus 2.6–4.0 kHz in *L. melicus*), but this may be at least partially related to body size (eg. Rowley *et al.* 2010a). More detailed comparisons between *Leptotalax pyrrhops* **sp. nov.** and *L. melicus* are not possible given our sample size (n=1 in *L. pyrrhops* **sp. nov.** and n=2 in *L. melicus*). Differences in the advertisement call between the new species and both *L. applebyi* and *L. bidoupensis* are much clearer. The advertisement call of *Leptotalax pyrrhops* **sp. nov.** differs from *L. applebyi* in having a long introductory note and relatively high number of pulses (versus no introductory note in *L. applebyi*), 5–6 notes call (versus 4–5 notes/call in *L. applebyi*), a single pulse in all non-introductory notes (versus 4–5 pulses/note in *L. applebyi*), and a dominant frequency of 1.9–2.2

kHz (versus 4.0–4.3 kHz for *L. applebyi*; although this may at least partially reflect body size differences). The advertisement call of *Leptotalax pyrrhops* **sp. nov.** differs from the call of its sister species, *L. bidouensis*, by having a long introductory note and relatively high number of pulses (versus no introductory note in *L. bidouensis*), and 4–5 notes/call (versus 5–9 notes/call in *L. bidouensis*).

Finally, the new species is markedly distinct from all other congeners for which comparable sequences are available, including its closest relative—the sister species *L. bidouensis*—by relatively large genetic distances in 16S rRNA mtDNA gene fragment ( $p = 10.3\%$ ). The only known locality of the new species is 100 km from the closest known locality of *L. bidouensis*; the ranges of the two species appear to be confined to different mountain massifs within the Langbian Plateau. In addition, *L. pyrrhops* **sp. nov.** and *L. bidouensis* may inhabit different altitudinal ranges (the new species is known from 800 to 1100 m a.s.l.; *L. bidouensis* has been reported from 1550 to 2100 m a.s.l., our data). Based on this data we assume that the two species have allopatric distributions.

## Discussion

Our phylogenetic hypothesis of the evolutionary relationships within the genus *Leptotalax* (Fig. 2) corresponds well to the preliminary phylogenies of the genus reported by Ohler *et al.* (2011), Jiang *et al.* (2013) and Sung *et al.* (2014). Our results indicate division of the genus *Leptotalax* into two major groups: the Sundanese group (taxa from southern Thailand to Malaysia and Indonesia; subgenus *Leptotalax* sensu Dubois *et al.* 2010) and the mainland Southeast Asian group, which includes species from Indochina and corresponds to the subgenus *Lalos* sensu Dubois *et al.* (2010). However, both phylogenetic analyses carried out failed to support the monophyly of either group, and available mtDNA datasets appear insufficient for resolving the species-level phylogeny of the genus *Leptotalax*. Evolutionary relationships within *Leptotalax* require further investigation, including additional phylogenetic markers and taxon sampling. Thus, to date there is no firm phylogenetic support for splitting *Leptotalax* into two subgenera as recommended by Dubois *et al.* (2010) and we use the recommended subgenus *Lalos* cautiously until a more well-supported phylogenetic study is published.

Within the group of Sundanese *Leptotalax* species (*Leptotalax* sensu stricto), the two lineages from southern Thailand both indicated as *L. heteropus* form two most basal radiations in the group and tend to join with the clade joining Bornean species of *Leptotalax* (see Fig. 2) though with low node support values. The *Leptotalax* species from Phang Nga in Phang Nga province of Thailand indicated as *L. heteropus* by Ohler *et al.* (2011) and *L. heteropus* from Perak, Malaysia (identified by Matsui *et al.* 2014a) do not form a clade. We assume that the observed paraphyly may be explained by an assumption that the identification of Phang Nga *Leptotalax* population by Ohler *et al.* (2011) as “*L. heteropus*” is erroneous. Matsui (2006) reviewed *Leptotalax* species of southern and central Thailand and suggested that *L. heteropus* is restricted to peninsular Malaysia, whereas *Leptotalax* species from southern Thailand previously identified as *L. heteropus* or *L. gracilis* (Chan-ard, 2003) may correspond to distinct species *L. solus* Matsui 2006 or *L. melanoleucus* Matsui 2006 (see discussion in Matsui 2006). The Phang Nga population of *Leptotalax* may belong to one of these species or may represent a new species, thus herein we tentatively refer to the Phang Nga species as *Leptotalax* sp. 2.

Our data support monophyly of the *L. applebyi* species group, consisting of two reciprocally monophyletic clades, joining species from the Kon Tum (*L. applebyi* and *L. melicus*) and Langbian (*L. bidouensis* and *Leptotalax pyrrhops* **sp. nov.**) Plateaus. According to our data, the *L. applebyi* species group is phylogenetically distinct from all other *Leptotalax* species inhabiting central and northern Indochina and adjacent parts of China and Thailand, evolutionary relationships within the latter clade are poorly resolved. In general, the phylogenetic patterns in the *Leptotalax* included in our study demonstrate strong geographic structuring, with certain lineages being restricted to separate mountain massifs. With larval development in flowing water, *Leptotalax* are associated with mountain streams and are assumed to exhibit limited dispersal capabilities (Orlov & Ananjeva 2007; Rowley & Cao 2009, Rowley *et al.* 2010a, Rowley *et al.* 2010b, Rowley *et al.* 2010c, Rowley *et al.* 2011, Rowley *et al.* 2013). A number of studies indicated a key role of mountainous areas for speciation of amphibians (see review in Wollenberg *et al.* 2008; see also Orlov & Ananjeva 2007; Poyarkov *et al.* 2012, 2014; Geissler *et al.* 2015), which is also likely the case of the genus *Leptotalax*. This also implies that further sampling of *Leptotalax* in mountain areas of Indochina might lead to discovery of new lineages and species.

To date, *Leptotalax pyrrhops* is known only from Loc Bac (or Loc Bao) forest in Lam Dong Province,

presently belonging to the Loc Bac Forestry Enterprise; this is the southernmost record of the genus in Vietnam and in Indochina. Recently, a new frog species *Kalophrynus cryptophonus* Vassilieva, Galoyan, Gogoleva & Poyarkov, 2014 (Microhylidae) was described from the same woodland area (Vassilieva *et al.* 2014), and is assumed to be endemic to the area. Our field surveys in 2012 and 2013 indicate that the area of Loc Bac forest hosts a considerable herpetofaunal diversity, including some rare and narrow-ranged species of amphibians and reptiles (such as *Theلودerma bambusicola* Orlov, Poyarkov, Vassilieva, Ananjeva, Nguyen, Sang & Geissler, 2012, *Kaloula indochinensis* Chan, Blackburn, Murphy, Stuart, Emmett, Ho et Brown, 2013, *Cyrtodactylus bugiamapensis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang et Ziegler, 2012, *Cyrtodactylus irregularis* Smith, 1921; see Orlov *et al.* 2012; Chan *et al.* 2013; Nazarov *et al.* 2012). Nevertheless, the surveyed territory does not belong to any protected area at present and is under immediate threat from intensive logging undertaken by the Loc Bac Forestry Enterprise. Given the apparently significant biodiversity of the area, much of which is likely to remain undiscovered, we urge that the area be considered for protected area status.

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**APPENDIX.** Examined material, museum IDs given in bold.

- Leptolalax aereus*: **ZISP 12042** (ROM 28761), **ZISP 12043** (ROM 28872), **ZISP 12044** (ROM 28823), **ZISP 12045** (ROM 29022) (Vietnam, Quang Binh Pr., Phong Nha—Ke Bang N.P.; 4 sp.); **ZMMU A-5214** (Vietnam, Quang Binh Pr., Phong Nha—Ke Bang N.P.; ROM 28773, ROM 29023; 2 sp.).
- Leptolalax bidoupensis*: **ZMMU A-4717** (Vietnam, Lam Dong Pr., Bidoup—Nui Ba N.P., Bidoup Mt., Giang Ly St.; NAP-01740, NAP-01870; 2 sp.); **ZMMU A-4797** (Vietnam, Lam Dong Pr., Bidoup—Nui Ba N.P., Bidoup Mt., Hon Giao Mt.; NAP-01453–01454, NAP-01457–01458; 4 sp.); **ZMMU A-5211** (Vietnam, Lam Dong Pr., Bidoup—Nui Ba N.P., Bidoup Mt.; NAP-00583; 1 sp.).
- Leptolalax cf. bidoupensis*: **ZMMU A-5215** (Vietnam, Dak Lak Pr., Chu Yang Sin N.P., Chu Yang Sin Mt.; ABV-00582, ABV-00652; 2 sp.).
- Leptolalax bourreti*: **ZISP 12046** (ROM 36080), **ZISP 12048** (ROM 36096) (Vietnam, Lao Cai Pr., Sa Pa, Hoang Lien N.P.; 2 sp.); **ZMMU A-5220** (Vietnam, Lao Cai Pr., Sa Pa, Hoang Lien N.P.; ROM 36104; 1 sp.); **ZISP 12048** (ROM 36229), **ZISP 12049** (ROM 36230), **ZISP 12050** (ROM 36258) (Vietnam, Lao Cai Pr., Van Ban N.R.; 3 sp.); **ZMMU A-5219** (Vietnam, Lao Cai Pr., Van Ban N.R.; ROM 36257; 1 sp.); **ZMMU A-5031** (Vietnam, Lao Cai Pr., Sa Pa, Tram Don, Phansipan Mt., Hoang Lien N.P.; adults: NAP-01910, NAP-03312–03314; juveniles: NAP-03318–03320; 6 sp.).
- Leptolalax cf. bourreti*: **ZMMU A-4255** (Vietnam, Ninh Binh Pr., Cuc Phuong; no ID; 2 sp.).
- Leptolalax cf. firthi*: **ZISP 12091** (ROM 27673), **ZISP 12058** (ROM 27698), **ZISP 12051** (ROM 28605), **ZISP 12052** (ROM 28624), **ZISP 12053** (ROM 28647), **ZISP 12054** (ROM 28654), **ZISP 12055** (ROM 28658), **ZISP 12056** (ROM 28659), **ZISP 12057** (ROM 28669), **ZISP 12092–12093** (2 sp. with no ID) (Vietnam, Kon Tum, Ngoc Linh N.P., Dac Glei; 11 sp.); **ZMMU A-5210** (Vietnam, Kon Tum, Ngoc Linh N.P., Dac Glei; ROM 28686, ROM 28687, ROM 28657, ROM 28685, ROM 28604; 5 sp.).
- Leptolalax nyx*: **ZISP 12059–12061** (Vietnam, Ha Giang Pr., Ha Giang; no ID; 3 sp.).
- Leptolalax phuvialis*: **ZISP 12075–12081** (Vietnam, Lao Cai Pr., Sa Pa, Hoang Lien N.P., Tram Don; no ID; 7 sp.); **ZMMU A-5209** (Vietnam, Lao Cai Pr., Sa Pa, Hoang Lien N.P., Tram Don; no ID; 4 sp.); **ZMMU A-5222** (Vietnam, Lao Cai Pr., Sa Pa, Tram Don, Phansipan Mt.; no ID; 8 sp.).
- Leptolalax pyrrhops* sp. nov.: **ZMMU A-5208** (Vietnam, Loc Bac Forest Enterprise, Loc Bao Comm., Bao Lam Distr., Lam Dong Pr., Vietnam; ABV-00148; female holotype); **ZMMU A-4873** (Vietnam, Loc Bac Forest Enterprise, Loc Bao Comm., Bao Lam Distr., Lam Dong Pr., Vietnam; 2 adult males ABV-00213, ABV-00215, 4 adult females ABV-00157–00158, ABV-00176, ABV-00214; 6 sp., paratypes); **ZISP 12041** (Vietnam, Loc Bac Forest Enterprise, Loc Bao Comm., Bao Lam Distr., Lam Dong Pr., Vietnam; ABV-00212; female paratype); **VNMIN A2015.02** (Vietnam, Loc Bac Forest Enterprise, Loc Bao Comm., Bao Lam Distr., Lam Dong Pr., Vietnam; ABV-00177; female paratype).
- Leptolalax sungi*: **ZMMU A-4349** (Vietnam, Ha Giang Pr., Ha Giang; no ID; 3 sp.).
- Leptolalax tuberosus*: **ZMMU A-4110** (Vietnam, Kon Tum Pr., Kon Plong; RAN-634; 1 sp.); **ZISP 12094** (ROM 39335), **ZISP 12095** (ROM 39492) (Vietnam, Kon Tum Pr., Kon Plong; 2 sp.); **ZMMU A-5213** (Vietnam, Kon Tum Pr., Kon Plong; ROM 39897; 1 sp.).
- Leptolalax ventripunctatus*: **ZMMU A-5223** (Vietnam, Dien Bien Pr., Muong Nhe N.R., Sin Hau St.; NAP-04386–04387, NAP-04400, NAP-04416–04417, NAP-04440, NAP-05001–05004, NAP-05007–05008; 12 sp.); **ZMMU A-5156** (Vietnam, Dien Bien Pr., Muong Nhe N.R., Sin Hau St.; NAP-05016–05020; 5 sp.); **ZMMU A-5225** (Vietnam, Phu Tho, Xuan Son N.P.; ABV-00731, ABV-00760, ABV-00763; 3 sp.); **ZMMU A-5224** (Vietnam, Phu Tho, Xuan Son N.P.; ABV-00758; 1 sp.); **ZISP 12062** (N-105), **ZISP 12063** (N-115), **ZISP 12064** (N-515), **ZISP 12065** (N-519), **ZISP 12066** (V-520), **ZISP 12067** (V-352), **ZISP 12068** (V-437), **ZISP 12069** (V-447), **ZISP 12070** (V-439), **ZISP 12071** (V-488), **ZISP 12072** (V-945), 11 males; **ZISP 12073** (N-61), **ZISP 12074** (N-516), 2 females (Vietnam, Vinh Phuc Pr., Tam Dao N.P.; 13 sp.); **ZMMU A-5212** (Vietnam, Vinh Phuc Pr., Tam Dao N.P.; N-105, V-446, V-443, ROM-20955 (3 males), N-514, N-518 (2 females); 5 sp.).
- Leptolalax* sp.: **ZMMU A-5221** (Vietnam, Da Nang, Ba Na N.R.; ROM 03980; 1 sp.).
- Leptolalax* sp.: **ZISP 12082** (ROM 21524), **ZISP 12083** (ROM 21526), **ZISP 12084** (ROM 21527), **ZISP 12085** (ROM 21529) (Vietnam, Lai Chau Pr., Ma Ham N.R.; 4 sp.); **ZMMU A-5216** (Vietnam, Lai Chau Pr., Ma Ham N.R.; ROM 21563–21564; 2 sp.).
- Leptolalax* sp.: **ZISP 12086** (ROM 28670), **ZISP 12087** (ROM 27671), **ZISP 12088** (ROM 27672) (Vietnam, Kon Tum, Ngoc Linh N.P., Dac Glei; 3 sp.); **ZMMU A-5217** (Vietnam, Kon Tum, Ngoc Linh N.P., Dac Glei; 2 sp. no ID; 2 sp.).
- Leptolalax* sp.: **ZISP 12089** (ROM 29124), **ZISP 12090** (ROM 29125) (Vietnam, Kon Tum, Ngoc Linh N.P., Dac Glei; 2 sp.); **ZMMU A-5218** (Vietnam, Kon Tum, Ngoc Linh N.P., Dac Glei; ROM 29126–29127; 2 sp.).