



A new species of the genus *Leptolalax* (Anura: Megophryidae) from Mt. Gaoligongshan of western Yunnan Province, China

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Abstract

A new species of the genus *Leptolalax* is described from the Tengchong Section of Gaoligongshan National Nature Reserve, Tengchong County, Yunnan Province, China. The new species, *Leptolalax tengchongensis* sp. nov., can be distinguished from its congeners by a combination of the following characters: (1) small size (SVL 23.9–26.0 mm in males, 28.8–28.9 mm in females); (2) dorsal skin shagreened and scattered with fine, round reddish tubercles; (3) toes with rudimentary webbing and narrow lateral fringes; (4) tympanum distinctly discernible, almost entirely black; (5) ventrolateral glands indistinct; (6) flanks with several distinct and large dark blotches; (7) ventral surfaces white, scattered with distinct irregular dark speckling; (8) iris not bicolored, uniformly dark brown and scattered with minute, coppery reticulations throughout. To date, the new species has only been found at its type locality in evergreen broadleaf forests at elevations between 2000–2100 m.

Key words: Megophryidae, taxonomy, *Leptolalax tengchongensis* sp. nov., Tengchong, China

Introduction

The megophryid genus *Leptolalax* Dubois, 1983 is widely distributed from southern China west to northeastern India and Myanmar, through Indochina mainland to peninsular Malaysia and the island of Borneo. Forty-four nominal species within the genus are recognized to date, with more than half (25) described only in the past ten years (Frost 2015; Rowley *et al.* 2015a). With the description of *Leptolalax laui* (Sung *et al.* 2014), seven species of *Leptolalax* are now known from China, i.e., *L. alpinus* from Yunnan and Guangxi Provinces, *L. laui* from Southern Guangdong including Hong Kong, *L. liui* from Fujian, Jiangxi, Guangdong, Guangxi, Hunan and Guizhou Provinces, *L. oshanensis* from Gansu, Sichuan, Chongqing, Guizhou and Hubei Provinces, *L. cf. pelodytoides* from southern Yunnan Province, *L. ventripuntatus* from Yunnan Province and *L. sungi* from Guangxi (Fei *et al.* 2009, 2012; Ohler *et al.* 2011; Sung *et al.* 2014).

In 2014 and 2015, during field surveys in the southwestern part of Mt. Gaoligongshan in Tengchong County, Yunnan Province, a series of specimens belonging to the genus *Leptolalax* was collected from high altitude (2000–2100 m) evergreen broadleaf forest. Subsequent studies on morphological characters and 16S rRNA mtDNA gene sequences revealed that it represents an undescribed species and can be distinguished from all recognized congeners by a combination of morphological characters and molecular divergence; we therefore describe it herein as a new species.

Materials and methods

Sampling. All specimens were collected during fieldwork in the Tengchong Section of Gaoligongshan National Nature Reserve, Tengchong County, Yunnan Province between April 2014 and May 2015. Specimens were fixed and preserved in 80% ethanol and deposited at The Museum of Biology, Sun Yat-sen University (SYS). The geographic position of the surveyed locality and the distribution of the four known *Leptolalax* species in Yunnan Province are shown in Figure 1.

DNA extraction and sequencing. Genomic DNA was extracted from the muscle tissue using TIANamp Genomic DNA Kit. We amplified a fragment of the mitochondrial 16S rRNA gene from *Leptolalax* specimens using the primer pairs L3975 and H4551 (Simon *et al.* 1994). PCR amplifications were performed in a reaction volume of 25 μ l containing 100 ng of template DNA, 0.3 mM of each PCR primer and 10 μ l Premix EX Taq™ (Takara). The PCR conditions were an initial denaturing step at 95 °C for 4 min, followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 52 °C for 30 s an extension step at 72 °C for 1 min, and a final extension of 72 °C for 7 min. PCR products were purified with spin columns. The purified products were sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit (Thermo Fisher Scientific Inc., Waltham, Mass., USA) according to the guidelines of the manufacturer. The products were sequenced on an ABI Prism 3730 automated DNA sequencer at the ShangHai Majorbio Bio-pharm Technology Co.,Ltd.

Phylogenetic analyses. In addition to the newly collected specimens, sequences of all species of the genus *Leptolalax* for which 16S mtDNA were available from the GenBank were included in the genetic analysis (Table 1). We used *Brachytarsophrys carinense* and *Xenophrys parva* as outgroups. The resulting 32 sequences were first aligned using the ClustalW algorithm with default parameters (Thompson *et al.* 1994) in MEGA6.06 (Tamura *et al.* 2013). The alignment was further checked and manually revised if necessary. Indels were removed before phylogenetic analyses. The General Time Reversible model assuming a gamma-shaped distribution across sites (Felsenstein 2004) was selected as the best-fit nucleotide substitution model using Akaike's Information Criterion (Akaike 1974) in jModelTest 1.0 (Posada & Buckley 2004). The alignment was analyzed using maximum likelihood (ML) implemented in MEGA 6.06 (Tamura *et al.* 2013), and Bayesian inference (BI) using MrBayes 3.12 (Ronquist & Huelsenbeck 2003). For ML analysis, the bootstrap consensus tree inferred from 1000 replicates was used to represent the evolutionary history of the analyzed taxa. Branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates were collapsed. For BI analysis, two independent runs, each comprising four Markov Chain Monte Carlo simulations were performed for one million iterations and sampled every 1000th step. The first 25% of the samples were discarded as burn-in. Convergence of the Markov Chain Monte Carlo simulations was assessed by checking the average standard deviation of split frequencies between two runs using Tracer v.1.4 (<http://tree.bio.ed.ac.uk/software/tracer/>). Apart from phylogenetic tree-based methods, we also calculated uncorrected pairwise distances between the 32 sequences in MEGA 6.06.

Morphological characters. Measurements followed Fei *et al.* (2009) and Rowley *et al.* (2013), and were taken with digital callipers to the nearest 0.1 mm: 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); internasal distance (IND); upper eyelid width measured as greatest width of the upper eyelid (UEW); nostril-eyelid length (NEL); nostril-snout length (NSL); 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 palmar tubercle (ML); length of lower arm and hand (LAHL); pes length from tip of fourth toe to proximal edge of the inner metatarsal tubercle (PL); and hindlimb length from tip of fourth toe to vent (HLL). Sex was determined by direct observation of calling in life and the presence of internal vocal sac openings, and the presence of eggs in abdomen. Comparative morphological data of *Leptolalax* species were obtained from examination of museum specimens (see Appendix) and from the literature: *L. aereus* (Rowley *et al.* 2010c), *L. alpinus* (Fei *et al.* 2009), *L. applebyi* (Rowley & Cao 2009), *L. arayai* (Matsui 1997), *L. bourreti* (Ohler *et al.* 2011), *L. bidoupensis* (Rowley *et al.* 2011), *L. botsfordi* (Rowley *et al.* 2013); *L. croceus* (Rowley *et al.* 2010a), *L. dringi* (Inger *et al.* 1995; Matsui & Dehling 2012), *L. eos* (Ohler *et al.* 2011), *L. firthi* (Rowley *et al.* 2012), *L. fritinniens* (Dehling & Matsui 2013), *L. fuliginosus* (Matsui 2006), *L. gracilis* (Günther 1872; Dehling 2012b), *L. hamidi* (Matsui 1997), *L. heteropus* (Boulenger 1900), *L. isos* (Rowley *et al.* 2015a), *L. kajangensis* (Grismer *et al.* 2004), *L. kecil* (Matsui *et al.* 2009), *L. khasiorum* (Das *et al.* 2010), *L. lateralis* (Anderson 1871; Humtsoe *et al.* 2008), *L. laui* (Sung *et al.* 2014), *L. liui* (Fei *et al.* 2009, Sung *et al.* 2014), *L. marmoratus* (Matsui *et al.* 2014b), *L. maurus* (Inger *et al.* 1997),

L. melanoleucus (Matsui 2006), *L. melicus* (Rowley *et al.* 2010b), *L. minimus* (Taylor 1962; Ohler *et al.* 2011), *L. nahangensis* (Lathrop *et al.* 1998), *L. nokrekensis* (Mathew & Sen 2010), *L. nyx* (Ohler *et al.* 2011), *L. oshanensis* (Fei *et al.* 2009), *L. pelodytoides* (Boulenger 1893; Ohler *et al.* 2011), *L. pictus* (Malkmus 1992), *L. platycephalus* (Dehling 2012a), *L. pluvialis* (Ohler *et al.* 2000, 2011), *L. pyrrhops* (Poyarkov *et al.* 2015), *L. sabahmontanus* (Matsui *et al.* 2014a), *L. solus* (Matsui 2006), *L. sungi* (Lathrop *et al.* 1998), *L. tamdil* (Sengupta *et al.* 2010), *L. tuberosus* (Inger *et al.* 1999), *L. ventripunctatus* (Fei *et al.* 2009), *L. zhangyapingi* (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.

TABLE 1. Samples and sequences used in the phylogenetic analysis in this study.

Species	Locality	Voucher No.	GenBank No.
(1) <i>Leptolalax tengchongensis</i> sp. nov.	China: Tengchong County, Yunnan Prov.	SYS a004596	KU589208
(2) <i>Leptolalax tengchongensis</i> sp. nov.	China: Tengchong County, Yunnan Prov.	SYS a004598	KU589209
(3) <i>Leptolalax tengchongensis</i> sp. nov.	China: Tengchong County, Yunnan Prov.	SYS a004600	KU589210
(4) <i>Leptolalax aereus</i>	Vietnam: U Bo, Quang Binh Prov.	ZFMK 86362	JN848409.1
(5) <i>Leptolalax applebyi</i>	Vietnam: Song Thanh, Quang Nam	AMS R171704	HM133598.1
(7) <i>Leptolalax bidoupensis</i>	Vietnam: Hon Giao, Lam Dong	AMS R 173133	HQ902880.1
(8) <i>Leptolalax bourreti</i>	Vietnam: Sa Pa, Lao Cai Prov.	1999.5660	KR827860.1
(9) <i>Leptolalax dringi</i>	Malaysia: Borneo	KUHE:55610	AB847553
(10) <i>Leptolalax eos</i>	Laos: Long Nai, Phongsaly Prov.	MNHN 2004.0274	JN848452
(11) <i>Leptolalax firthi</i>	Vietnam: Ngoc Linh NR, Kon Tum Prov.	AMS:R 176524	JQ739206.1
(12) <i>Leptolalax fritinniens</i>	Borneo	NMBE1056267	KJ831292.1
(13) <i>Leptolalax gracilus</i>	Malaysia: Borneo	KUHE 55624	AB847560
(14) <i>Leptolalax hamidi</i>	Malaysia: Borneo	KUHE 17545	AB969286
(15) <i>Leptolalax heteropus</i>	Malaysia, Peninsula	KUHE 15487	AB530453
(16) <i>Leptolalax isos</i>	Vietnam: Gia Lai Province	AMS R 176480	KT824769
(17) <i>Leptolalax laui</i>	China: Tai Mo Shan, Hong Kong	SYS a002057	KM014546
(18) <i>Leptolalax liui</i>	China: Wuyishan, Fujian	SYS a001597	KM014547
(19) <i>Leptolalax marmoratus</i>	Malaysia: Borneo	KUHE 53227	AB969289
(20) <i>Leptolalax maurus</i>	Malaysia: Borneo	SP 21450	AB847559
(21) <i>Leptolalax melicus</i>	Cambodia: Virachey, Ratanakiri Prov.	MVZ 258198	HM133600.1
(22) <i>Leptolalax minimus</i>	Laos: Houey Thao, Luang Prabang Prov.	MNHN 2006.2553	JN848374
(23) <i>Leptolalax nyx</i>	Vietnam: Cao Bo Commune, Ha Giang Prov.	AMNH A163810	DQ283381
(24) <i>Leptolalax oshanensis</i>	China: Mt. Emei, Sichuan Prov.	SYS a001830	KM014810
(25) <i>Leptolalax pictus</i>	Malaysia: Borneo	UNIMAS 8705	KJ831295
(26) <i>Leptolalax pyrrhops</i>	Vietnam: Loc Bao, Lam Dong Prov	ZMMU ABV-00148	KP017575
(27) <i>Leptolalax pluvialis</i>	Vietnam: Mt. Fan Si Pan, Lao Cai Prov.	MNHN:1999.5675	JN848391.1
(28) <i>Leptolalax sabahmontanus</i>	Malaysia: Borneo	BORNEENSIS 12632	AB847551
(29) <i>Leptolalax ventripunctatus</i>	China: Meng-La, Yunnan Prov.	SYS a001768	KM014811
(30) <i>Leptolalax zhangyapingi</i>	Thailand: Chiang Mai, Pang Num Poo	Not provided	JX069979
(31) <i>Brachytarsophrys carinense</i>	Myanmar: no exact locality	Not provided	JN848360.1
(32) <i>Xenophrys parva</i>	Vietnam: Sa Pa, Lao Cai Prov.	Not provided	JN848362.1

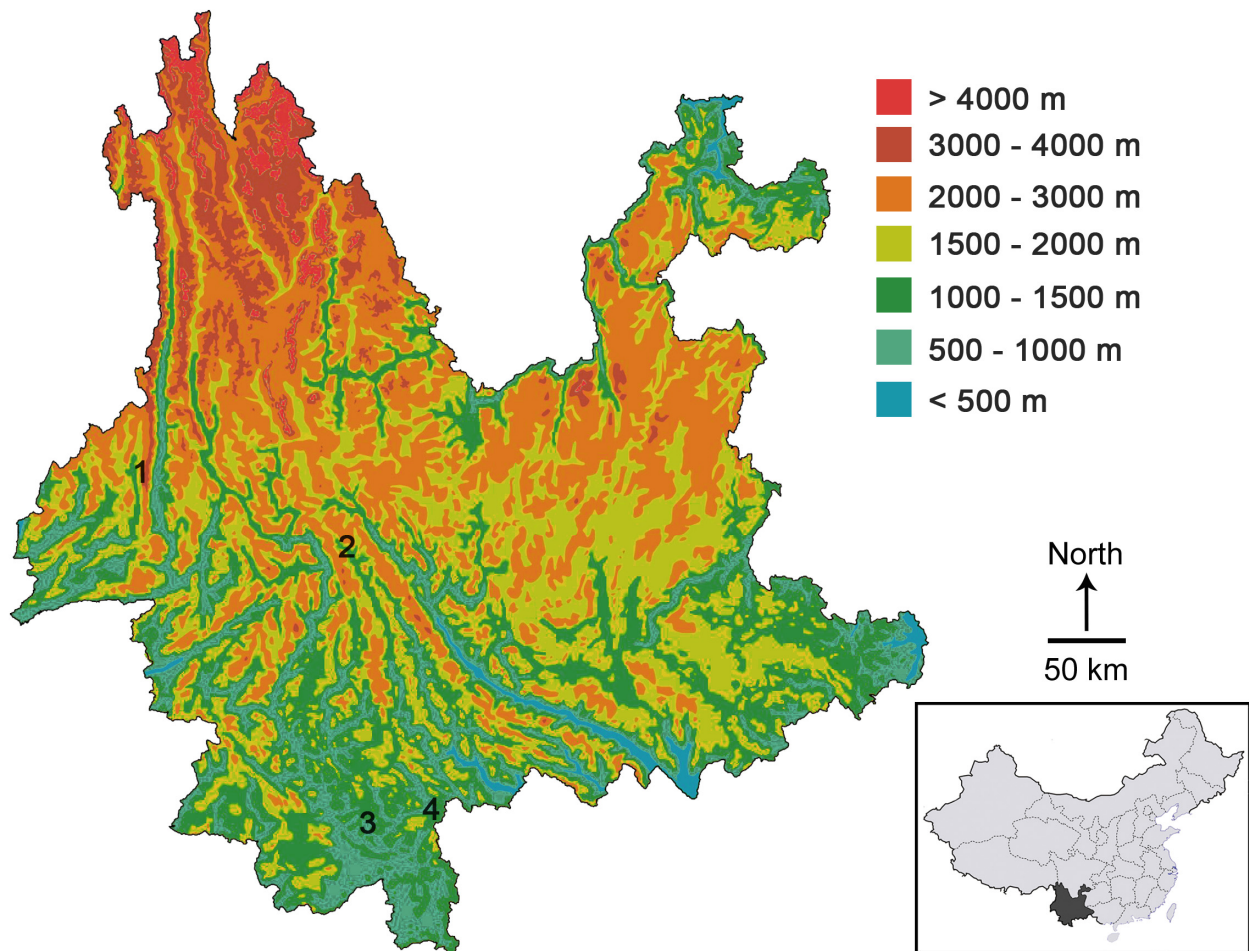


FIGURE 1. Map showing known type localities of four species of *Leptotalax* in Yunnan Province, China (refers to Fei *et al.* 2009). 1: type locality of *Leptotalax tengchongensis* sp. nov., Linjiapu substation of the Tengchong Section of Gaoligongshan National Nature Reserve, Tengchong County; 2: type locality of *L. alpinus*, Mt. Wuliangshan, Jingdong County; 3 *L. cf. pelodytoides*, Mengyang Town; 4: type locality of *L. ventripuntatus*, Mengla County.

Results

Molecular relationships. For the aligned characters, 13 base pairs were deleted as a result of omitting indels; and there were 211 potentially informative base pairs between the in-group and outgroup, 198 among the in group taxa. Based upon the phylogenetic analysis of 16S rRNA mtDNA gene sequences, our newly collected specimen of *Leptotalax* from Mt. Gaoligongshan represents a highly divergent mtDNA lineage, with a sister relationships to the clade comprising *L. oshanensis*, *L. bourreti* and *L. eos* (Fig. 2). For the uncorrected *p*-distances among and within the 16S rRNA mtDNA gene fragments of the studied *Leptotalax* taxa, the observed interspecific distances range from *p* = 4.9% (between *L. hamidi* and *L. marmoratus* from Borneo) to 27.6% (between *L. gracilis* from Borneo and *L. liui* from southern China). The *Leptotalax* species from Mt. Gaoligongshan differs from all other congeners by remarkably high genetic distances 9.3% to 25.2%, with the lowest value *p* = 9.3% observed in the comparison with the sequences of *L. bourreti* from northern Vietnam. This value is higher than those observed between several pairs of well-distinguished species of *Leptotalax*, as well as that usually representing differentiation at the species level in frogs (Vences *et al.* 2005). As the observed molecular differences are corroborated by differences in morphology (see below), we therefore consider the *Leptotalax* sp. from Mt. Gaoligongshan to be an undescribed species.

TABLE 2. Uncorrected *p*-distances (%) among the *Leptolalax* species, *Brachytarsophrys carinense*, and *Xenophrys parva*, based on a 16S rRNA fragment.

	(1-3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)	(27)	(28)	(28)	(30)	(31)	(32)							
(1-3) <i>Leptolalax</i> sp.	0.0																																				
(4) <i>L. aereus</i>	14.6	-																																			
(5) <i>L. applebyi</i>	20.3	16.3	-																																		
(6) <i>L. arayai</i>	19.1	17.9	17.1	-																																	
(7) <i>L. bidoupenensis</i>	17.5	15.4	9.3	17.5	-																																
(8) <i>L. bourreti</i>	9.3	14.2	19.1	19.9	19.1	-																															
(9) <i>L. dringi</i>	19.5	18.7	19.9	14.2	18.3	20.7	-																														
(10) <i>L. eos</i>	12.2	14.6	17.9	19.1	16.7	7.7	20.3	-																													
(11) <i>L. firrhi</i>	13.0	15.4	21.1	20.7	18.7	14.6	19.9	17.9	-																												
(12) <i>L. fritiniensis</i>	20.7	17.5	21.5	13.4	18.3	21.5	8.9	19.9	20.7	-																											
(13) <i>L. gracilis</i>	25.2	20.3	20.3	13.8	22.4	23.6	15.9	24.4	24.4	16.7	-																										
(14) <i>L. hamidi</i>	19.5	19.1	18.7	6.9	19.9	21.1	13.0	18.7	21.1	10.2	13.4	-																									
(15) <i>L. heteropus</i>	24.8	19.5	19.5	22.4	20.3	26.0	21.1	24.8	25.6	22.0	24.4	22.0	-																								
(16) <i>L. isos</i>	13.0	15.0	19.1	17.5	16.3	12.6	20.3	14.6	14.2	22.0	23.2	20.3	24.4	-																							
(17) <i>L. laui</i>	13.0	13.4	19.5	20.3	17.1	14.2	20.3	12.2	16.3	21.1	23.2	21.5	23.6	15.4	-																						
(18) <i>L. liui</i>	13.8	13.4	20.3	21.5	15.4	13.4	21.1	10.6	16.3	21.1	27.6	23.6	24.0	16.7	6.5	-																					
(19) <i>L. marmoratus</i>	19.5	18.7	17.1	5.7	18.7	19.5	11.4	19.1	20.3	11.4	13.0	4.9	21.5	19.5	22.0	22.4	-																				
(20) <i>L. maurus</i>	20.7	17.5	17.9	12.2	19.1	20.3	13.8	20.3	21.1	13.4	12.6	10.6	22.0	17.9	21.1	22.4	10.6	-																			
(21) <i>L. melicus</i>	19.1	13.8	7.3	16.7	9.3	18.7	18.7	18.3	19.5	19.5	19.5	17.9	19.9	19.1	21.1	20.7	16.7	17.1	-																		
(22) <i>L. minutus</i>	11.8	7.3	17.9	18.3	17.5	13.8	19.9	13.8	14.6	18.7	23.2	20.3	21.5	16.3	12.6	13.4	18.7	19.5	16.3	-																	
(23) <i>L. nyx</i>	11.8	6.5	16.3	18.3	15.0	13.0	18.3	12.2	12.2	17.9	23.2	19.5	19.9	14.2	12.2	18.3	18.7	13.0	6.5	-																	
(24) <i>L. oshimensis</i>	12.6	13.4	17.9	21.5	19.1	6.9	19.9	6.9	17.1	20.3	23.2	21.5	24.8	13.4	11.4	12.2	21.5	20.7	18.3	12.6	11.0	-															
(25) <i>L. pictus</i>	20.3	18.7	19.5	13.0	19.1	22.0	8.1	21.1	19.1	7.3	15.4	11.0	22.0	22.0	22.8	22.8	10.6	13.4	18.3	19.1	17.9	22.0	-														
(26) <i>L. pyrrhops</i>	18.3	16.3	14.2	19.5	10.2	19.9	18.3	18.3	19.1	18.3	19.9	19.1	20.3	17.9	17.5	17.9	18.7	20.3	14.2	17.9	16.7	18.7	18.3	-													
(27) <i>L. pluvialis</i>	13.4	5.7	17.1	19.5	15.9	13.0	19.1	13.0	15.0	19.5	23.2	21.1	20.3	16.3	11.8	11.8	19.9	20.7	15.4	6.9	6.1	12.6	19.5	17.1	-												
(28) <i>L. sabahmontanus</i>	21.1	19.1	17.9	13.0	18.3	22.0	8.1	20.7	19.5	8.5	15.4	10.2	23.6	20.7	22.0	22.8	10.6	11.8	16.7	18.7	18.3	21.1	5.7	17.5	20.7	-											
(29) <i>L. ventripunctatus</i>	13.4	7.7	17.5	16.3	16.7	15.4	17.9	14.2	13.4	16.7	23.2	18.3	20.3	12.6	11.8	13.0	17.5	17.9	15.0	7.3	5.7	13.4	18.3	16.7	7.7	17.9	-										
(30) <i>L. zhangyapingi</i>	13.0	14.6	18.7	22.0	19.1	14.6	22.8	13.8	15.9	23.6	25.6	22.8	24.4	15.9	14.2	14.6	22.4	21.5	19.1	14.6	12.2	14.6	22.2	19.1	12.6	22.8	13.8	-									
(31) <i>B. carinense</i>	26.4	22.4	21.1	22.4	23.6	22.8	23.6	22.0	26.0	25.6	25.6	25.2	24.0	22.0	25.6	24.8	23.2	23.6	21.1	24.0	22.8	23.2	24.4	25.2	23.6	20.7	26.8	-									
(32) <i>X. parva</i>	28.0	23.6	24.4	25.2	27.6	24.8	26.8	24.0	30.5	26.8	26.4	26.4	26.4	26.0	25.2	26.0	24.8	23.2	25.2	26.0	24.8	23.2	26.0	24.8	23.2	26.0	25.6	26.0	22.8	26.4	12.2	-					

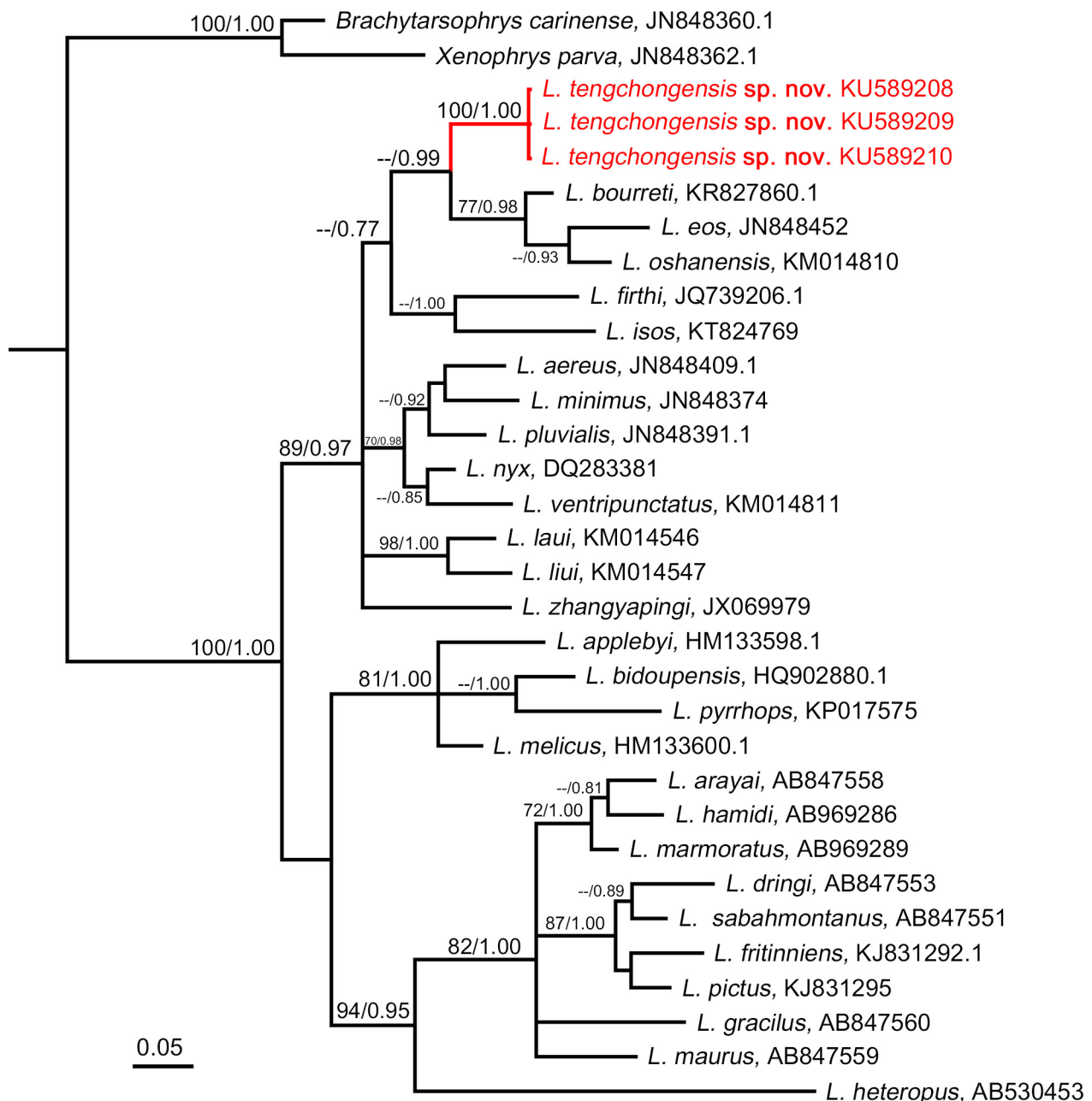


FIGURE 2. Bayesian inference (BI) tree derived from partial sequences of the mitochondrial 16S rRNA gene. Numbers above branches are bootstrap support values for maximum likelihood analyses (>70 retained) and numbers below branches indicate Bayesian posterior probabilities (> 70% retained).

Leptolalax tengchongensis sp. nov.

Holotype. SYS a004600, adult male, calling at the base of a shrub about 3 m away from a rocky stream. The stream was 3–4 m wide, flowing through a montane evergreen broadleaf forest at Linjiapu substation of the Tengchong Section of Gaoligongshan National Nature Reserve, Tengchong County, Yunnan Province, China (25°17'51.26" N, 98°42'03.93" E, 2100 m above sea level), collected at 23:40 h on 16 May 2015 by J.H. Yang.

Paratypes. SYS a004596, adult male, calling under rock by the side of the stream, from the same locality as holotype, collected by J.H. Yang on 29 April 2014; SYS a004597, a004599 and a004601, three adult males, data identical to holotype; SYS a004598 and a004602, two adult females, collection data identical to holotype.

Etymology. The specific epithet, “*tengchongensis*”, is in reference to the type locality, Tengchong County of Yunnan Province, China. For the common name, we suggest “Tengchong Leaf Litter Toad”.



FIGURE 3. Holotype of *Leptolalax tengchongensis* **sp. nov.** (SYS a004600) in preservative: (A) dorsal view; (B) ventral view; (C) plantar view of the left foot; (D) volar view of the left hand; (E) lateral view of head. Photos by J.H. Yang.

Diagnosis. The new species is assigned to the genus *Leptolalax* by the possession of the following morphological characters considered to be diagnostic for the genus: (1) comparatively small size; (2) rounded finger tips, the presence of an elevated inner palmar tubercle not continuous to the thumb; (3) presence of macroglands on body including supra-axillary, pectoral, femoral and ventrolateral glands; (4) vomerine teeth absent; (5) tubercles on eyelids present and (6) anterior tip of snout with whitish vertical bar (Dubois 1983; Matsui 1997, 2006; Lathrop *et al.* 1998; Delorme *et al.* 2006; Das *et al.* 2010); and it is further assigned to the subgenus *Lalos* based on the presence of a ventrolateral gland (Ohler *et al.* 2011). *Leptolalax tengchongensis* **sp. nov.** can be distinguished from its congeners by a combination of the following characters: (1) small size (SVL 23.9–26.0 mm in males, 28.8–28.9 mm in females); (2) dorsal skin shagreened and scattered with fine, round reddish tubercles; (3) toes with rudimentary webbing and narrow lateral fringes; (4) tympanum distinctly discernible, almost entirely

black; (5) ventrolateral glands indistinct; (6) flanks with several distinct and large dark blotches; (7) ventral side whitish, scattered with distinct irregular dark speckling; (8) iris not bicolored, uniformly dark brown and scattered with minute, coppery reticulations throughout.



FIGURE 4. Holotype of *Leptolalax tengchongensis* sp. nov. (SYS a004600) in life; inset image illustrates the iris coloration of holotype in life. Photos by J.H. Yang.

Description of holotype. SYS a004600 (Fig. 3, 4), adult male, body size small (SVL 26.0 mm), head width equal to head length; snout slightly protruding, projecting slightly beyond margin of the lower jaw; nostril equidistant between snout and eye; canthus rostralis gently rounded; loreal region slightly concave; interorbital space flat, larger (IOD 3.2 mm) than upper eyelid (2.5 mm in width) and internarial distance (2.6 mm); pineal ocellus absent; vertical pupil; eye diameter smaller than snout length; tympanum distinct, round, diameter (TMP 1.5 mm) smaller than that of the eye (EYE 3.3 mm), and larger than tympanum-eye distance (TEY 0.9 mm); tympanic rim slightly elevated relative to skin of temporal region; vomerine teeth absent; vocal sac openings slit-like, located posterolaterally on floor of mouth in close proximity to the margins of the mandible; tongue long, wide, with a small shallow notch at posterior tip; supratympanic ridge distinct, extending from eye to supra-axillary gland; tubercles present on supratympanic ridge. Tips of fingers rounded, slightly swollen; relative finger lengths I = II = IV < III; nuptial pad absent; subarticular tubercles absent; a large, round inner palmar tubercle distinctly separated from small, round outer palmar tubercle; finger webbing and dermal fringes absent (Fig. 3D). Tips of toes like fingers; relative toe length I < II < V < III < IV; subarticular tubercles absent; distinct dermal ridges present under the 3rd to 5th toes; large, oval inner metatarsal tubercle present, outer metatarsal tubercle absent; toe webbing rudimentary; narrow lateral fringes present on all toes (Fig. 3C). Tibia 47% of snout-vent length; tibiotarsal articulation reaches to middle of eye. Skin on dorsum shagreened and scattered with fine, round tubercles; ventral skin smooth; pectoral gland medium size, round, 0.8 mm diameter; femoral gland large, oval, 1.8 mm diameter, situated on posteroventral surface of thigh, closer to knee than to vent; supra-axillary gland raised, 1.1 mm diameter. Ventrolateral gland faintly visible as small white dots forming an incomplete line.

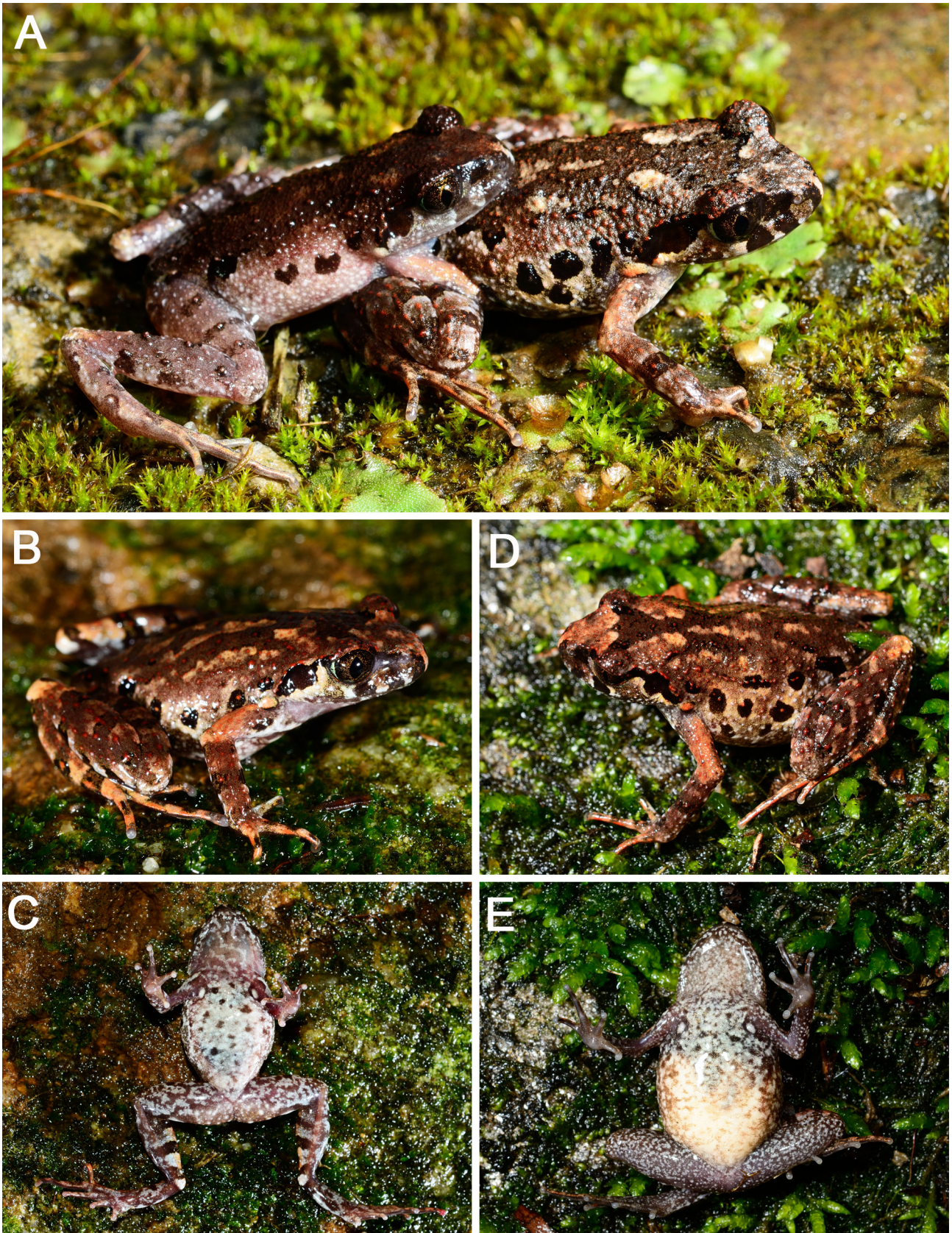


FIGURE 5. *Leptolalax tengchongensis* sp. nov.: (A) A pair in amplexus (male SYS a004601; female SYS a004602); (B & C) dorsal and ventral views of male paratype SYS a004596 in life; (D & E) dorsal and ventral views of female paratype SYS a004598 in life. Photos by J.H. Yang.



FIGURE 6. Paratypes of *Leptolalax tengchongensis* sp. nov. in preservative, note the variation in dorsal and ventral patterns: SYS a004596, SYS a004597, SYS a004599, SYS a004601, SYS a004598, SYS a004602 (from left to right). Photos by J.H. Yang.

Coloration of holotype in life. Dorsal surface brown with distinct darker brown markings and scattered with light coppery and irregular spots (Fig. 4); V-shaped interorbital marking connected to the W-shaped marking between axillae, and continuously connected to the inverted V-shaped, irregular marking above sacrum; fine, distinct reddish tubercles on upper eyelids, snout, head, dorsal surfaces of body and limbs, those on lower flanks somewhat whitish; anterior upper lip with distinct blackish brown patches; blackish brown line along canthus rostralis, through eye, and continuing below supratympanic ridge, terminating above arm insertion, encompassing posterior edge of nare and most of tympanum; a blackish brown spot present on loreal region; transverse dark brown bars on dorsal surface of limbs; large, black blotches on flanks from groin to axilla; elbow and upper arms without dark bars but with distinct coppery orange coloration; fingers and toes with transverse bars. Ventral surface white, with distinct irregular and moderate dark brown spots on chest and belly, and dark marbling on throat; lateral lower lip dark brown scattered with irregular white spots. Supra-axillary gland coppery orange; femoral and pectoral glands white and distinct; ventrolateral glands white, somewhat indistinct from the white venter. Iris not bicolored, uniform dark brown, scattered with minute, coppery reticulations throughout.

Coloration of holotype in preservative. Dorsum dark brown with slightly paler limbs (Fig. 3A, B). Ventral surface dull white, with dark brown spots on chest and belly, and marbling on throat. Dorsal color pattern turns to shades of gray and macroglands on the ventral surfaces become less distinct.

Variation. All six paratypes match the overall characters of the holotype (for measurements of the type series see Table 3). All type specimens possess a dull white venter scattered with distinct dark blotches and marbling except that the dark patterns on venter is somewhat indistinct in the smallest male paratype, SYS a004597 (Fig. 6). No significant differences between males and females were revealed (Fig. 5), however females are slightly larger than males (SVL 28.8–28.9 mm in females versus 23.8–26.0 mm in males).

Distribution and natural history. *Leptolalax tengchongensis* sp. nov. is so far only known from its type locality, near Linjiapu substation (a rangers' station) of the Tengchong Section of Gaoligongshan National Nature Reserve, Tengchong County, Baoshan City, Yunnan Province, China. The new species was found along a clear-water rocky stream (ca. 3–4 m in width and ca. 30 cm in depth) and small nearby seeps in well-preserved montane evergreen broadleaf forest at elevations between 2000–2100 m (Fig. 7); calling males were found mainly hidden under leaf litter, and some were found calling under rocks by the side of the stream.

We conducted three rapid field surveys in the type locality of the new species between April 2014 and July 2015. During the first survey, 28–30 April 2014, very few males of *Leptolalax tengchongensis* sp. nov. were heard calling and only one male was found and collected, but during the third survey between 15–18 May 2015, calling

males of the new species were ubiquitous along the main stream and riparian forest, and five calling adult males and two gravid adult females were collected. No calls and specimens were detected during the second survey on 9–10 December 2014.

TABLE 3. Measurements (in mm) of type specimens of *Leptolalax tengchongensis* **sp. nov.** Abbreviations defined in text.

	SYS a004596	SYS a004597	SYS a004599	SYS a004600	SYS a004601	Mean ± SD (N=5)	SYS a004598	SYS a004602	Mean ± SD (N=2)
Sex	Male	Male	Male	Male	Male		Female	Female	
SVL	24.9	23.9	25.6	26.0	23.8	24.84±0.99	28.9	28.8	28.85±0.07
HDL	8.5	8.3	8.8	8.8	8.4	8.56±0.23	9.0	9.9	9.45±0.64
HDW	8.1	7.8	8.5	8.7	8.1	8.24±0.36	9.0	9.2	9.10±0.14
SNT	3.8	3.7	3.9	3.8	3.8	3.80±0.07	4.3	4.3	4.30±0.00
EYE	3.0	3.1	3.3	3.3	3.2	3.18±0.13	3.4	3.5	3.45±0.07
IOD	3.0	2.9	3.1	3.2	3.0	3.04±0.11	3.2	3.3	3.25±0.07
TMP	1.4	1.5	1.6	1.5	1.5	1.50±0.07	1.9	1.8	1.85±0.07
TEY	0.7	0.8	0.8	0.9	0.8	0.80±0.07	1.1	1.1	1.10±0.00
TIB	12.0	10.3	11.8	12.1	11.8	11.60±0.74	13.8	13.0	13.40±0.57
ML	5.8	5.7	6.0	6.1	6.0	5.92±0.16	7.1	7.0	7.05±0.07
PL	11.0	10.4	11.7	11.9	11.4	11.28±0.60	12.4	12.8	12.60±0.28
LAHL	12.3	11.2	12.2	12.5	11.9	12.02±0.51	14.3	13.8	14.05±0.35
HLL	37.9	34.9	39.1	39.7	39.1	38.14±1.93	46.2	44.8	45.50±0.99
HDL/HDW	1.05	1.06	1.04	1.01	1.04	1.04±0.02	1.00	1.08	1.04±0.05
HDL/SVL	0.34	0.35	0.34	0.34	0.35	0.34±0.01	0.31	0.34	0.33±0.02
SNT/HDL	0.45	0.45	0.44	0.43	0.45	0.44±0.01	0.48	0.43	0.46±0.03
SNT/ED	1.27	1.19	1.18	1.15	1.19	1.20±0.04	1.26	1.23	1.25±0.03
EYE/TMP	2.14	2.07	2.06	2.20	2.13	2.12±0.06	1.79	1.94	1.87±0.--11
TMP/EYE	0.47	0.48	0.48	0.45	0.47	0.47±0.01	0.56	0.51	0.54±0.03
TEY/TMP	0.50	0.53	0.50	0.60	0.53	0.53±0.04	0.58	0.61	0.60±0.02
TIB/SVL	0.48	0.43	0.46	0.47	0.50	0.47±0.02	0.48	0.45	0.46±0.02
LAHL/SVL	0.49	0.47	0.48	0.48	0.50	0.48±0.01	0.49	0.48	0.49±0.01
HLL/SVL	1.52	1.46	1.53	1.53	1.64	1.54±0.07	1.60	1.56	1.58±0.03
TIB/HLL	0.32	0.30	0.30	0.30	0.30	0.30±0.01	0.30	0.29	0.29±0.01

Comparison. By the presence of supra-axillary and ventrolateral glands, *Leptolalax tengchongensis* **sp. nov.** can be allocated into the subgenus *Lalos* Dubois, Grosjean, Ohler, Adler & Zhao, 2010, and distinctly differs from the 14 known species of the subgenus *Leptolalax*, i.e., *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmoratus*, *L. maurus*, *L. pictus*, *L. platycephalus*, *L. sabahmontanus* and *L. solus*, all of which occur south of the Isthmus of Kra and lack supra-axillary and ventrolateral glands (Dubois *et al.* 2010; Dehling & Matsui 2013; Matsui *et al.* 2014b).

Compared to the 30 recognized species in the subgenus *Lalos*, the relatively small size of *Leptolalax tengchongensis* **sp. nov.** (SVL 23.8–26.0 mm in five adult males and 28.8–28.9 mm in two adult females) differs from the larger *L. alpinus* (females 31.7–32.5 mm), *L. botsfordi* (males 29.1–32.6 mm, females 30.0–31.8 mm), *L. bourreti* (males 28.0–36.2 mm, females 42.0–45.0 mm), *L. eos* (males 33.1–34.7 mm, female 40.7 mm), *L. firthi* (males 26.4–29.2 mm), *L. fuliginosus* (males 28.2–30.0 mm), *L. lateralis* (males 26.9–28.3 mm, female 36.6 mm), *L. melanoleucus* (males 26.6–28.8 mm, female 32.7 mm), *L. minimus* (males 25.7–31.4 mm, females 31.6–37.3 mm), *L. nahangensis* (male 40.8 mm), *L. nyx* (males 26.7–32.6 mm, females 37.0–41.0 mm), *L. oshanensis* (males 26.6–30.7 mm, female 31.6 mm), *L. pelodytoides* (males 27.5–32.3 mm, females 35.5–37.8 mm), *L. pyrrhop*

(males 30.3–33.9 mm, females 30.8–34.3 mm), *L. sungi* (males 48.3–52.7 mm, females 56.7–58.9 mm), *L. tamdil* (male 32.3 mm, female 31.8 mm), *L. ventripunctatus* (females 31.5–35.0 mm), and *L. zhangyapingi* (males 45.8–52.5 mm), and the smaller *L. applebyi* (males 19.6–20.8 mm, female 21.7 mm), and *L. melicus* (males 19.5–22.7 mm), *L. pluvialis* (males 21.0–22.0 mm).



FIGURE 7. Habitat of *Leptotalax tengchongensis* sp. nov.: (A) microhabitat of calling male holotype SYS a004600 at type locality, near the rocky main stream; (B) microhabitat of calling males along the seeps at type locality. Photos by J.H. Yang.

In having an iris of uniform coloration, *L. tengchongensis* **sp. nov.** differs from *L. alpinus*, *L. bidoupensis*, *L. eos*, *L. firthi*, *L. fuliginosus*, *L. isos*, *L. khasiorum*, *L. melanoleucus*, *L. minimus*, *L. pluvialis*, *L. pyrrhop*, *L. tamdil*, *L. ventripunctatus* and *L. zhangyapingi*, all of which have a distinctly bicolored iris.

In having toes with rudimentary webbing and narrow lateral fringes on toes, *L. tengchongensis* **sp. nov.** differs from *L. applebyi*, *L. croceus*, *L. melanoleucus*, *L. melicus*, *L. minimus*, *L. nahangensis*, *L. nyx*, *L. oshanensis*, *L. pluvialis*, *L. pyrrhop*, *L. tuberosus* and *L. ventripunctatus*, all of which lack lateral fringes on toes; *L. eos*, *L. khasiorum*, *L. laui*, *L. liui* and *L. zhangyapingi*, all of which have wide fringes on toes.

In having a distinct tympanum *L. tengchongensis* **sp. nov.** further differs from *L. croceus*, *L. sungi* and *L. tuberosus*, all of which have an indistinct tympanum.

In having distinct large black blotches on flanks, *L. tengchongensis* **sp. nov.** differs from *L. botsfordi*, *L. croceus*, *L. eos*, *L. firthi* and *L. isos* (without black markings on flanks); and further differs *L. aereus*, *L. alpinus*, *L. applebyi*, *L. laui*, *L. liui*, *L. pluvialis*, *L. sungi* and *L. tamdil*, all of which have lesser, smaller and/or indistinct dark spots on flanks.

In having distinct dark brown speckling on the chest and belly, and marbling on the throat, *L. tengchongensis* **sp. nov.** differs from *L. aereus*, *L. bourreti*, *L. eos*, *L. firthi*, *L. isos*, *L. khasiorum*, *L. laui*, *L. liui*, *L. minimus*, *L. nahangensis*, *L. nys*, *L. oshanensis*, *L. pelodytoides*, *L. sungi*, *L. tamdil* and *L. zhangyapingi*, all of which have an immaculate white or pale grey belly; and from *L. applebyi* (brownish pink ventral surface with white speckling), *L. bidoupensis* (dark brownish red ventral surface with white speckling), *L. botsfordi* (dark brownish red ventral surface with white speckling), and *L. croceus* (bright orange belly in life, fades to cream in preservative).

Discussion

The discovery of *Leptolalax tengchongensis* **sp. nov.** brings the number of *Leptolalax* recorded from China up to eight. The new species is also the third known species in the genus inhabit elevations above 2000 m; the other high-elevation two species are *L. alpinus* (1150–2400 m) from Yunnan Province of China (Fei *et al.* 2010) and *L. botsfordi* (2795–2815 m) from northern Vietnam (Rowley *et al.* 2013).

Species of the genus *Leptolalax* are small frogs inhabiting the forest floor in montane evergreen forest, and cryptic in both coloration and behavior (Rowley *et al.* 2015b). Intensified survey efforts in recent years and the use of an integrated approach incorporating morphological, molecular and acoustic data have resulted in continued discovery new species in the region, in particularly from Indochina (eg. Poyarkov *et al.* 2015a and Rowley *et al.* 2015b). Future herpetological exploration and study in southern China will likely continue uncovering new *Leptolalax* species from the region.

Acknowledgements

This study was supported by Kadoorie Farm and Botanic Garden, Hong Kong. We are grateful to the management of Gaoligongshan National Nature Reserve, particularly the Tengchong Section, for permission to conduct fieldwork and logistics support. Special thanks to Mr. Xiang-Yuan Huang of the Nature Reserve in arranging the fieldwork; we also thank other staff and wardens of the Nature Reserve, as well as colleagues from KFBG and The Museum of Biology, Sun Yat-sen University who helped in the surveys; Prof. Yue-Zhao Wang of Chengdu Institute of Biology for permission to examine specimens of *Leptolalax* deposited at CIB; Jodi J.L. Rowley and two anonymous reviewers for their useful comments and suggestions.

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APPENDIX. Specimens examined.

Institutional abbreviations used are CIB = Chengdu Institute of Biology, the Chinese Academy of Sciences; SYS = The Museum of Biology, Sun Yat-sen University.

Leptolalax alpinus: China, Yunnan Province, Mt. Wuliangshan: **CIB 24353** (Holotype), **CIB 24354**; **SYS a 003915–3917, 003927**.

Leptolalax laui: China, Hong Kong: **SYS a002057** (Holotype), **SYS a002058**; China, Guangdong Province, Shenzhen: **SYSa 001505–001507, 001515–001521**.

Leptolalax liui: China, Fujian Province, Mt. Wuyishan: **CIB 24355** (Holotype), **CIB 24356**, **SYS a001571–001578, 001595–001599**.