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A New Species of *Leptolalax* (Anura: Megophryidae) from Southern China

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Abstract A new species, *Leptolalax laui* sp. nov. is described based on specimens collected from Hong Kong and Shenzhen City, Guangdong Province, China. The new species can be distinguished from other known congeners by morphological and molecular data. The new species is characterized by the following characters: 1) small size (adult males SVL 24.8.1 mm−26.7 mm); 2) near immaculate creamy white chest and belly; 3) broad lateral fringes on toes; 4) head longer or as long as wide; 5) distinct dark brown spots in flank; 6) moderate dermal fringes on fingers; 7) brown or reddish-brown dorsum with fine round scattered tubercles; 8) thin traverse brownish-grey bars on the dorsal surface of tibia and lower arms; 9) longitudinal ridges under toes not interrupted at the articulations.

Keywords *Leptolalax laui* sp. nov., morphology, mitochondrial DNA, taxonomy

1. Introduction

The genus *Leptolal*ax Dubois, 1983 within the family Megophryidae Bonaparte, 1850 currently contains 39 species (Dehling and Matsui, 2013; Frost, 2013; Jiang *et al.*, 2013; Rowley *et al*, 2013). Over 25 % of species were described in the last four years, revealing that the diversity of this genus has been underestimated (Ohler *et al.*, 2011; Rowley and Cao 2009; Rowley *et al.*, 2012; Rowley *et al.*, 2010a). Systematic studies of *Leptolalax* are not easy because the species in the genus have a similar appearance and only few diagnostic morphological features (Ohler *et al.*, 2011).

Currently, there are five species of *Leptolalax* confirmed for China: *L. alpinus* from Yunnan and Guangxi, *L. liui* from Fujian, Jiangxi, Guangdong, Guangxi, Hunan and Guizhou Provinces, *L. oshanensis* from Gansu, Sichuan, Chongqing, Guizhou and Hubei Provinces, *L. ventripuntatus* from Yunnan Province and *L. sungi* from Guangxi Province (Fei *et al.*, 2011). *L. pelodytoides*, which had been recorded in China, is now confirmed to be only restricted to the Karin Hills of Myanmar (Ohler *et al.*, 2011). Moreover, Ohler *et al.* (2011) noted that the big-sized *Leptolalax* species found in Yunnan that is currently regarded as *L. pelodytoides* could be in fact *L. eos*; but it still need to be confirmed by further study. Among the five *Leptolalax* species from China, only *L. liui*, which has its type locality at Mt. Wuyi, Fujian, is considered to have a wide distribution in Southern China, including Guangdong and Hong Kong (Fei *et al.*, 2009; Fei *et al.*, 2010).

Herein, we re-evaluate the taxonomic status of the population of *Leptolalax* from Hong Kong and Shenzhen City, Guangdong Province, China based on newly collected specimens during herpetological surveys conducted in 2011–2012. Our results suggest that the population from Hong Kong and Shenzhen belong to the same species, and that this species differs from topotypic *L. liui* and all known species. The new species can be distinguished from its congeners by discrete morphological and genetic divergence. Therefore, we describe it as a new species.

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2. Material and Methods

2.1 Sampling All specimens were deposited at The Museum of Biology, Sun Yat-sen University (SYS), Guangzhou, China. We fixed all specimens in 10 % buffered formalin and then transferred them to 80 % ethanol. Muscle tissues were preserved in 95 % ethanol in advance for genetic analysis.

2.2 Molecular analyses We extracted DNA from muscle tissue using standard phenol-chloroform extraction protocol (Sambrook *et al.*, 1989). A 412 bp fragment of the mitochondrial 16S rRNA gene from all taxon samples was sequenced. We used the primers for ranid frogs following Simons *et al.*, (1994). PCR amplifications were performed in a 60 reaction volume with the following cycling conditions: an initial denaturing step at 94 °C for 1.5 min; 33 cycles of denaturing at 94 °C for 45 s, annealing at 55 °C for 45 s and extending at 72 °C for 90 s, and a final extending step of 72 °C or 10 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 according to the guidelines of the manufacturer. The products were sequenced on an ABI Prism 3730 automated DNA sequencer in Beijing Genomics Institute. All sequences have been deposited in GenBank (Table 1). Sequences of *L. aereus*, *L. applebyi*, *L. arayai*, *L. bidoupensis*, *L. eos*, *L. firthi L. heteropus*, *L. liui*, *L. melicus*, *L. minimus*, *L. pictus*, *L. pluvialis*, and *Xenophrys major* from Genbank were included in the genetic analysis. We used *Xenophrys major* as an outgroup. We conducted the alignments using Clustal X 1.81 (Thompson *et al.*, 1997) and calculated the uncorrected pairwise sequence divergence in MEGA 5.05 (Tamura *et al.*, 2011). We analyzed sequence data and constructed phylogenetic tree using maximum parsimony (MP) in MEGA 5.05 (Tamura *et al.*, 2011). For MP analysis, we used the bootstrap consensus tree inferred from 500 replicates to represent the evolutionary history

Table 1 Species, localities, voucher data and genebank accession numbers for specimens used in the genetic analysis.

ID	Species name	Locality	Specimen voucher No.	Genbank accession No.
Outgroup				
1	*Leptobrachium chapaense*	Vietnam: Thanh Hoa Province, Ben En NP	No Voucher	JN848353
2	*Xenophrys major*	Vietnam: Tam Dao, Vinh Phu	AMNA A 161506	DQ283374
Ingroup				
3	*Leptolalax aereus*	Vietnam: Phong Nha, Quang Binh	No Voucher	JN848440
4	*L. applebyi*	Vietnam: Phouc Son, Quang Nam	AMS R171703	HM133597
5	*L. arayai*	Malaysia: Kinabalu, Borneo	No Voucher	AY523768
6	*L. bidoupensis*	Vietnam: Bidoup-Nui Ba National Park, Lam Dong	AMS R173133	HQ902880
7	*L. bourreti*	Vietnam: Lao Cai, Sa Pa	MNHN 1999.5659	JN848453
8	*L. eos*	Laos: Long Nai, Phongsaly	MNHN 2004.0275	JN848451
9	*L. firthi*	Vietnam: Song Thanh Nature Reserve, Quang Nam	AMS R171714	JQ739203
10	*L. heteropus*	Thailand: Long Nai, Long Nai	No Voucher	JN848354
11	*L. liui*	China: Jinxiu, Guangxi	IZCASH30037	EF544238
12	*L. liui*	China: Wuyishan, Fujian	SYS a001597	KM014547
13	*L. liui*	China: Mt. Tongba, Jiangxi	SYS a001702	KM014548
14	*L. liui*	China: Mt. Huanggang, Jiangxi	SYS a001620	KM014549
15	*L. liui*	China: Mt. Daiyun, Fujian	SYS a001736	KM014550
16	*L. melicus*	Cambodia: Virachey National Park, Ratanakiri	MVZ 258197	HM133599
17	*L. minimus*	Laos: Ban Nong Di, Luang Prabang	MNHN 2006.2323	JN848394
18	*L. oshanensis*	China: Mt. Emei, Sichuan	SYS a001830	KM014810
19	*L. pictus*	Malaysia: Kinabalu , Borneo	No Voucher	DQ642120
20	*L. pluvialis*	Vietnam: Sa Pa, Lao Cai	MNHN 1999.5675	JN848391
21	*L. ventripunctatus*	China: Xishuangbanna, Yunnan	SYS a001768	KM014811
22	*L. zhangyapingi*	Thailand: Chiang Mai, Pang Num Poo	Not provided	JX069979
23	*L. laui* sp. nov.	China: Wutongshan, Shenzhen	SYS a001507	KM014544
24	*L. laui* sp. nov.	China: Wutongshan, Shenzhen	SYS a001515	KM014545
25	*L. laui* sp. nov.	China: Tai Mo Shan, Hong Kong	SYS a002057	KM014546

of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50 % of bootstrap replicates were collapsed.

For our molecular analysis, we included topotypic samples of *L. liui* from Wuyishan City (= Chongan County), Fujian Province (type locality, Figure 1), Mt. Tongba and Mt. Huanggang, Jiangxi Province, China, sample of *L. oshanensis* from Mt. Emei, Sichuan Province (type locality), China and samples of *L. ventripunctatus* from Xishuangbanna, Yunnan Province, China.

2.3 Morphological analyses We took the following measurements to the nearest 0.1 mm from the specimens with digital calipers: Snout-vent length (SVL); head length from tip of snout to rear of jaws (HDL); head width at the commissure of the jaws (HDW); snout length from tip of snout to the anterior corner of eye (SNT); horizontal diameter of the exposed portion of the eyeball (EYE); interorbital distance (IOD); horizontal diameter of tympanum (TMP); distance from anterior edge of tympanum to posterior corner of the eye (TEY); tibia length with the hindlimb flexed (TIB); foot length from proximal edge of inner metatarsal tubercle to tip of fourth toe (FOT), manus length from tip of third digit to proximal edge of inner palmar tubercle (ML), pes length from tip of fourth toe to proximal edge of the inner metatarsal tubercle (PL), length of adpressed first finger from tip to distal edge of the inner palmar tubercle (F1L), length of adpressed second finger from tip to distal edge of inner palmar tubercle (F2L), length of adpressed third finger from tip to distal edge of inner palmar tubercle (F3L), greatest width of femoral gland (FG), greatest width of traverse brownish grey bars closest to body on dorsal surface of tibia (BT; Figure 2) and width of the distal traverse brownish grey bars on dorsal surface of middle section of lower arms (BLA; Figure 2). Comparative data of the new species with other *Leptolalax* were obtained from examination of museum specimens (see Appendix 1) and from the literature: *L. aereus* (Rowley *et al.*, 2010c), *L. alpinus* (Fei *et al.*, 2009; Fei *et al.*, 1990a; Fei *et al.*, 2010), *L. applebyi* (Rowley

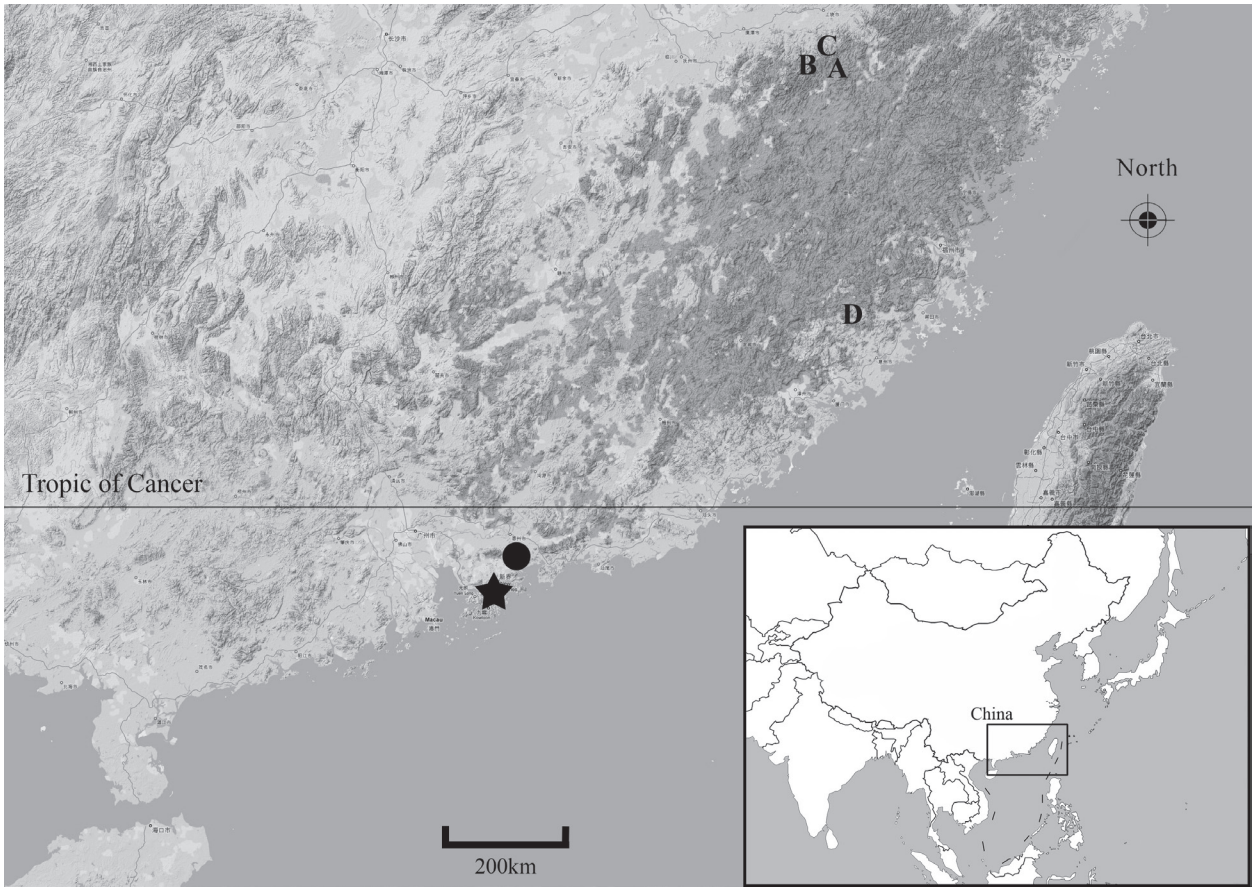

Figure 1 Collection localities of *L. laui* sp. nov. from Tai Mo Shan Country Park, Hong Kong (black star) and Wutongshan National Forest Park (black circle), Guangdong, China, and type locality of *L. liui* in Wuyishan, Fujian, China (A), Mt. Huanggang, Jiangxi, China (B), Mt. Tongba, Jiangxi, China (C) and Mt. Daiyun, Fujian, China (D).

Figure 2 Adult male holotype (SYS a002057) of *L. laui* sp. nov. in life. (A) frontolateral view; (B) ventral view; (C) ventral view of left hand; (D) ventral view of right foot. Adult male holotype of *Leptolalax laui* sp. nov. in preservative (E) dorsal view and (F) ventral view. Red arrows indicates the grey traverse bars for the measurement of width of traverse brownish grey bars closest to body on dorsal surface of tibia (BT) and width of the lowest traverse brownish grey bars on dorsal surface of middle section of lower arms (BLA).

and Cao 2009), *L. bidoupensis* (Rowley *et al.*, 2011), *L. botsfordi* (Rowley *et al.*, 2013b), *L. bourreti* (Dubois 1983; Ohler *et al.*, 2011), *L. croceus* (Rowley *et al.*, 2010a), *L. eos* (Ohler *et al.*, 2011), *L. firthi* (Rowley *et al.*, 2012), *L. fuliginosus* (Matsui, 2006), *L. khasiorum* (Das *et al.*, 2010), *L. lateralis* (Anderson, 1871; Humtsoe *et*

al., 2008), *L. liui* (Fei *et al.*, 2009; Fei *et al.*, 1990a; Fei *et al.*, 2010), *L. melanoleucus* (Matsui, 2006), *L. melicus* (Rowley *et al.*, 2010b), *L. nahangensis* (Lathrop *et al.*, 1998), *L. nokrekensis* (Das and Deuti 2011; Mathew and Sen 2010), *L. nyx* (Ohler *et al.*, 2011), *L. oshanensis* (Fei *et al.*, 2009; Fei *et al.*, 2010; Liu, 1950), *L. pelodytoides* (Boulenger, 1908; Ohler *et al.*, 2011), *L. pluvialis* (Fei *et al.*, 1990a; Ohler *et al.*, 2000), *L. sungi* (Fei *et al.*, 2010; Lathrop *et al.*, 1998), *L. tamdil* (Sengupta *et al.*, 2010), *L. tuberosus* (Inger *et al.*, 1999; Rowley *et al.*, 2010b) and *L. ventripunctatus* (Fei *et al.*, 2009; Fei *et al.*, 1990a; Fei *et al.*, 2010).

3. Results

3.1 Molecular analyses The 412 bp fragments of the 16S rRNA gene strongly supported the monophyly of three individuals of *Leptolalax* collected from Hong Kong and Shenzhen City, China (Figure 3). Uncorrected p distance between these specimens was 0–0.3 %(Table 2). They differed from all other species of *Leptolalax* for which sequences are available by 6.3 %–21.7%, with the lowest values observed in the comparison with the sequences of *L. liui* (6.3%–7.0 %). This value is higher than that observed between two well distinguished species, *L. bourreti* and *L. aereus* (*p* distance 5.1 %). These results show that there is a substantial genetic divergence between the specimens from Hong Kong and Shenzhen City and other *Leptolalax* species, indicating that these populations represent an undescribed species. Morphological evidence corroborates these findings and therefore, we describe the specimens collected from Hong

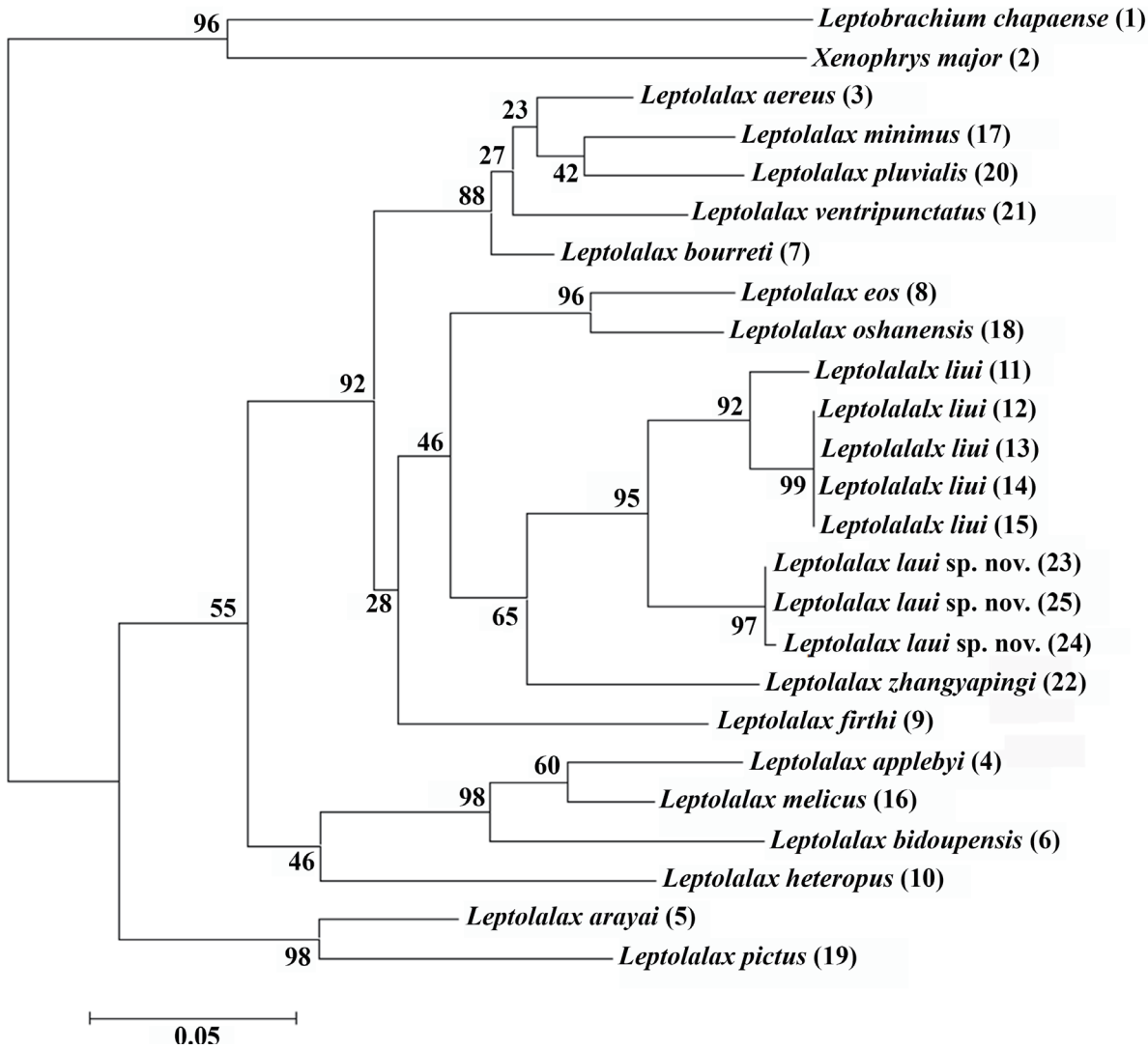

Figure 3 Maximum parsimony tree derived from partial fragments of 16S rRNA genes. The numbers above branches are bootstrap support for maximum likelihood (500 replicates analyses).

Table 2 Genetic divergence of selected *Leptolalax* species with *Xenophrys major* as outgroup, studied, based on uncorrected *P*-distance in a 16S rRNA fragment.

Leptobrachium chapaense (1)																							
Xenophrys major (2)	0.270																						
Leptolalax aereus (3)	0.277	0.272																					
L. applebyi (4)	0.294	0.275	0.172																				
L. arayai (5)	0.274	0.257	0.171	0.166																			
L. bidoupensis (6)	0.296	0.322	0.182	0.107	0.192																		
L. bourreti (7)	0.265	0.273	0.051	0.164	0.183	0.172																	
L. eos (8)	0.285	0.271	0.127	0.173	0.177	0.187	0.120																
L. firthi (9)	0.316	0.311	0.127	0.196	0.181	0.195	0.121	0.149															
L. heteropus (10)	0.240	0.286	0.162	0.151	0.178	0.169	0.160	0.169	0.203														
L. liui (11)	0.254	0.319	0.132	0.181	0.195	0.166	0.118	0.125	0.168	0.169													
L. liui (12)	0.264	0.300	0.121	0.184	0.184	0.163	0.101	0.097	0.157	0.171	0.027												
L. liui (13)	0.264	0.300	0.121	0.184	0.184	0.163	0.101	0.097	0.157	0.171	0.027	0.000											
L. liui (14)	0.264	0.300	0.121	0.184	0.184	0.163	0.101	0.097	0.157	0.171	0.027	0.000	0.000										
L. liui (15)	0.264	0.300	0.121	0.184	0.184	0.163	0.101	0.097	0.157	0.171	0.027	0.000	0.000	0.000									
L. melicus (16)	0.291	0.273	0.131	0.064	0.165	0.106	0.131	0.159	0.175	0.149	0.190	0.182	0.182	0.182	0.182								
L. minimus (17)	0.284	0.300	0.067	0.173	0.195	0.192	0.061	0.142	0.140	0.176	0.130	0.122	0.122	0.122	0.122	0.164							
L. oshanensis (18)	0.283	0.268	0.110	0.166	0.192	0.203	0.100	0.064	0.138	0.162	0.124	0.114	0.114	0.114	0.114	0.149	0.114						
L. pictus (19)	0.296	0.281	0.190	0.202	0.103	0.211	0.187	0.212	0.192	0.214	0.211	0.207	0.207	0.207	0.207	0.189	0.209	0.219					
L. pluvalis (20)	0.259	0.291	0.067	0.178	0.197	0.182	0.074	0.132	0.151	0.161	0.116	0.116	0.116	0.116	0.116	0.158	0.074	0.121	0.213				
L. ventripunctatus (21)	0.281	0.269	0.073	0.193	0.170	0.203	0.064	0.143	0.131	0.187	0.148	0.130	0.130	0.130	0.130	0.157	0.084	0.121	0.204	0.098			
L. zhangyapingi (22)	0.268	0.333	0.124	0.195	0.195	0.190	0.113	0.128	0.158	0.178	0.124	0.113	0.113	0.113	0.113	0.189	0.139	0.128	0.215	0.125	0.143		
L. laui sp. nov. (23)	0.263	0.317	0.134	0.179	0.178	0.183	0.117	0.113	0.148	0.182	0.063	0.066	0.066	0.066	0.066	0.184	0.118	0.103	0.217	0.121	0.132	0.110	
L. laui sp. nov. (24)	0.268	0.311	0.130	0.175	0.174	0.179	0.114	0.110	0.145	0.186	0.067	0.070	0.070	0.070	0.070	0.180	0.114	0.100	0.213	0.125	0.128	0.113	0.003
L. laui sp. nov. (25)	0.263	0.317	0.134	0.179	0.178	0.183	0.117	0.113	0.148	0.182	0.063	0.066	0.066	0.066	0.066	0.184	0.118	0.103	0.217	0.121	0.132	0.110	0.000	0.003

Kong and Shenzhen, China as a new species.

3.2 Description

***Leptolalax laui* sp. nov.**

Holotype: SYS a002057, adult male, from a rocky stream (width 1.5 m) in a secondary forest in Tai Mo Shan Country Park, Hong Kong (22.41057 º N, 114.11794 º E, 680 m a.s.l., Figures 1, 2), collected at 19:30 h on 10 April 2011 by YHS.

Paratypes: SYS a002058, adult male, collected from the same locality as holotype by YHS. SYS a001505, SYS a001507 and SYSa 001515-21, adult males, and SYS a001506, adult female, collected from Wutongshan National Forest Park, Shenzhen City, Guangdong Province, China (22.58481 º N, 114.19875 º E, 178 m a.s.l., Figure 1) at night of 17 and 21 May 2012 by JHY, YYW, and Run-Lin Li.

Etymology: This new species is named in honor of Dr. Michael Wai-Neng Lau from Hong Kong for his long-term herpetological research and conservation in Asia, particularly in South China. As a common name we suggest "Lau's Leaf Litter Toad" (English name).

Diagnosis: The new species is assigned to the subgenus *Lalos* of the genus *Leptolalax* based on the following characteristics: small size, rounded finger tips, presence of an elevated inner palmar tubercle not continuous to the thumb, presence of supra-axillary, pectoral, femoral and ventrolateral glands, absence of vomerine teeth, presence of tubercles on eyelids and vertical bars on anterior tip of snout (Delorme *et al.*, 2006; Dubois, 1983; Lathrop *et al.*, 1998). *Leptolalax laui* sp. nov. is distinguished from its congeners by a combination of 1) medium size (SVL 24.8 mm – 26.7 mm in adult males and 28.1 mm in single adult female), 2) near immaculate creamy-white chest and belly, 3) broad lateral fringes on toes, 4) head longer or as long as wide, 5) distant dark brown spots in flank, 6) moderate dermal fringes on fingers, 7) brown or reddish brown dorsum with fine round scattered tubercles, 8) thin traverse brownish grey bars on the dorsal surface of tibia and lower arms, 9) longitudinal ridges under toes not interrupted at the articulations.

Description of holotype: Head slightly longer than wide; snout rounded in dorsal view but relatively truncate in lateral view; nostril closer to tip of snout than to eye; canthus rostralis distinct, bluntly rounded; lores slightly concave; eye large, diameter shorter than snout length; pupil vertical; tympanum distinct, small round, its diameter smaller than diameter of eye; TMP:EYE ratio 0.45; tympanic rim slightly elevated relative to skin of temporal region; vomerine teeth absent; vocal sac opening slit-like; tongue long and wide with a small notch at posterior tip; supratympanic ridge distinct, running from eye towards axillary gland with raised tubercles. Tips of fingers rounded; relative finger lengths I < IV < II < III; nuptial pad absent; subarticular tubercles absent; large, round inner palmar tubercle and a small laterally compressed outer palmar tubercle; finger webbing absent; moderate lateral dermal fringes on both sides of finger II, III and IV (Figure 2C). Tips of toes rounded; relative toe length I < II < V < III <IV; longitudinal ridges under toes II–V extending on plantar side of phalanges and not interrupted at articulations; subarticular tubercles absent; large oval inner metatarsal tubercle, outer metatarsal tubercle absent; webbing basal; wide lateral fringes present on both sides of all toes (Figure 2D); TIB:SVL ratio 0.48; tibiotarsal articulation reaches anterior margin of eye; dorsal skin shagreened with fine, round, scattered tubercles; ventral skin smooth; femoral gland oval with greatest width 0.8 mm and greatest length 0.9 mm on posteroventral surface of thigh, closer to knee than to vent; axillary gland present, width 1.0 mm, length 1.8 mm; ventrolateral glands present, forming a complete line.

Color of holotype in life: Dorsal surface brown with no distinct darker markings and scattered tiny buff spots, faint transverse brownish grey bars on dorsal surface of fingers, lower arms, tarsus, thigh and tibia (Figure 2A). Fingers, toes and elbow to upper arm are pale copper. Ventral surface of chest and belly opaque cream white with little brown dusting along the margins of ventrolateral glands (Figure 2B); throat transparent pink with brown dusting along anterior margin. Ventral surface of arms and thigh pink with brown dusting along lateral sides; ventral surface of tarsus and tibia brownish pink with creamy white flecks. Axillary, femoral and ventrolateral glands pale copper. Iris uniformly coppery orange with fine black reticulations throughout.

Color of holotype in preservation: Dorsal surface brown (Figure 2E). Ventral surface of belly and chest, and macroglands creamy white (Figure 2F). Brown pigment on belly along the margins of ventrolateral glands have become more obvious. Ventral surface of arms, tarsus, thigh and tibia brown.

Measurements: Holotype: SVL 26.4 mm, HDL 9.7 mm, HDW 9.4 mm, SNT 4.1 mm, EYE 3.6 mm, IOD 2.9 mm, TMP 1.6 mm, TEY 0.9 mm, TIB 12.8 mm, ML 6.3 mm, PL 10.8 mm, F1L 2.5 mm, F2L 2.9 mm, F3L 5.2 mm, FG 0.8 mm, BT 0.7 mm, BLA 0.8 mm.

Variation: Measurements of the type specimens are shown in Table 3. Most individuals of *L. laui* show indistinct darker markings similar to the holotype (Figure

Table 3 Measurements (mm) of *Leptolalax laui* sp. nov. Abbreviations defined in Materials and Methods.

	Male											Female
	SYS a002057	SYS a002058	SYS a001505	SYS a001507	SYS a001515	SYS a001516	SYS a001517	SYS a001518	SYS a001519	SYS a001520	SYS a001521	SYS a001506
SVL	26.4	26.3	26.7	25.4	26.3	25.8	24.8	25.5	25.1	25.8	25.5	28.1
HDL	9.7	9.3	9.7	9.6	9.7	9.5	9.4	9.6	9.6	9.6	9.3	10.7
HDW	9.4	8.9	9.6	9.5	9.7	9.4	9.2	9.5	9.5	9.4	9.3	10.6
SNL	4.1	4.2	3.9	3.3	3.6	4.0	3.3	4.2	4.1	3.6	4.0	3.9
EYE	3.6	3.8	3.6	3.7	3.3	3.6	3.2	4.0	3.2	3.3	3.5	4.0
IOD	2.9	3.0	3.0	2.8	2.7	2.9	2.8	3.0	3.1	2.9	2.7	3.2
TMP	1.6	1.6	1.4	1.7	1.4	1.5	1.7	1.8	1.4	1.6	1.6	2.0
TEY	0.9	1.2	1.4	1.2	1.1	1.1	1.0	1.0	1.2	1.1	1.2	1.6
TIB	12.8	12.1	12.4	12.1	12.1	12.9	11.9	12.5	12.5	12.0	11.7	13.8
ML	6.3	6.5	7.2	7.0	6.8	6.0	6.3	6.5	6.5	6.1	6.7	7.0
PL	10.8	10.7	10.9	10.4	11.3	11.3	10.7	11.2	10.6	11.3	10.1	13.1
F1L	2.5	2.2	2.3	2.5	2.2	2.2	2.1	2.0	2.2	2.3	2.2	2.7
F2L	2.9	2.8	3.3	3.1	2.9	2.6	3.1	3.0	2.8	2.9	2.7	3.7
F3L	5.2	5.0	5.6	5.0	4.9	5.3	5.1	5.3	4.6	4.9	4.5	5.8
TMP:EYE	0.45	0.41	0.40	0.47	0.43	0.42	0.52	0.44	0.43	0.49	0.46	0.49
TIB:SVL	0.48	0.46	0.46	0.48	0.46	0.50	0.48	0.49	0.50	0.46	0.46	0.49
HDL:HDW	0.99	1.04	0.93	0.99	1.00	1.00	0.94	0.85	0.95	0.94	0.98	0.88
HDL:SVL	0.35	0.35	0.34	0.36	0.35	0.35	0.36	0.32	0.35	0.33	0.35	0.33
FG	0.8	1.0	1.1	1.5	1.2	1.2	1.0	1.3	1.0	1.0	0.7	1.1
BT	0.7	0.9	0.8	0.7	0.6	0.9	0.9	0.7	0.9	0.8	0.7	0.8
BLA	0.8	0.5	0.8	0.6	0.7	0.8	0.5	0.8	0.7	0.8	0.7	0.8

2A), but such dorsal markings are more distinctive in some individuals. One of the paratypes (SYS a002058) collected from Hong Kong has a distinctive darker greyish brown triangle between the eyes, w-shaped dark marking on shoulder region and scattered large greyish brown flecks over the iliac region.

Ecology: The species can be found in streams in secondary forests between 100–800 m elevation. Advertisement calls of males of *L. laui* sp. nov. can be heard in streams from February to September in Hong Kong. Calling males were usually observed within two meters from streams.

Distribution and conservation status: *Leptolalax laui* sp. nov. is known to occur in a number of sites, including Tai Mo Shan, Tai Po Kau, Shing Mun, Ho Chung, Kadoorie Farm and Botanic Garden, Sunset Peak, Lantau Peak, in Hong Kong, as well as Wutongshan National Forest Park, Shenzhen City, Guangdong Province; yet the exact distribution of this species in China is unknown. With limited information on the distribution of this species, we recommend the species should be listed as Data Deficient in the IUCN Red List of Threatened Species.

3.3 Comparisons The genus *Leptolalax* contains two subgenera, *Leptolalax* and *Lalos* (Delorme *et al.*, 2006; Ohler *et al.*, 2011). *L. laui* sp. nov. is assigned to the subgenus *Lalos* on the basis of its small size, rounded finger tips, presence of an elevated inner palmar tubercle not continuous to the thumb, presence of supra-axillary, pectoral, femoral and ventrolateral glands, absence of vomerine teeth, presence of tubercles on eyelids and vertical bars on anterior tip of snout and having lateral glandular ridge on the belly (Ohler *et al.*, 2011). Species assigned to the subgenus *Leptolalax* lack these characteristics and differed to *L. laui* sp. nov. Thus, we compare the new species to the known 25 species within the subgenus *Lalos*: *L. aereus*, *L. alpinus*, *L. applebyi*, *L. bidoupensis*, *L. botsfordi*, *L. bourreti*, *L. eos*, *L. firthi*, *L. fuliginosus*, *L. khasiorum*, *L. lateralis*, *L. liui*, *L. melanolecus*, *L. minimus*, *L. melicus*, *L. nahangensis*, *L. nyx*, *L. oshanensis*, *L. pelodytoides*, *L. pluvialis*, *L.*

sungi, *L. tamdil*, *L. tuberosus*, *L. ventripunctatus* and *L. zhangyapingi*.

L. laui sp. nov. (24.8 mm–26.7 mm in adult males and 28.1 mm in adult female) differs from the smaller *L. applebyi* (male 19.6 mm–22.3 mm, females 21.7 mm–25.9 mm), *L. pluvialis* (males 21.3 mm–22.3 mm), *L. nyx* (males 22.4 mm–24.2 mm), and the larger *L. aereus* (male 29.8 mm), *L. botsfordi* (male 29.1 mm–32.6 mm, females 30.0 mm–31.8 mm), *L. bourreti* (male 36.2 mm, females 42 mm–45 mm), *L. eos* (males 33.1 mm–34.7 mm, female 40.7), *L. fuliginosus* (males 28.2 mm–30.0 mm), *L. lateralis* (males 26.9 mm–28.3 mm, female 36.6 mm), *L. nahangensis* (male 40.8 mm), *L. pelodytoides* (males 27.5 mm–32.3 mm, females 35.5 mm–37.8 mm), *L. sungi* (males 48.3 mm–52.7 mm, females 56.7 mm–58.9), and *L. tamdil* (male 32.3 mm, female 31.8 mm), and *L. zhangyapingi* (males 45.8 mm−52.5 mm).

The ventral pattern of *L. laui* sp. nov. (immaculate creamy white with slight brown marbling on sides) distinguishes it from *L. alpinus*, *L. applebyi*, *L. fulignosus*, *L. melanoleucus*, *L. nahangensis*, *L. pluvialis*, *L. tuberosus* and *L. ventripunctatus*, all having dark or maculate chests and/or bellies, and *L. bidoupensis* and *L. botsfordi* that has dark brownish red ventral surface with white speckling.

In having broad lateral fringes on toes, *L. laui* sp. nov. further differs from *L. aereus*, *L. alpinus*, *L. botsfordi*, *L. bourreti*, *L. fuliginosus*, *L. lateralis*, *L. melicus*, *L. minimus*, *L. pelodytoides*, *L. sungi* and *L. tamdil*, all having weak lateral fringes on toes; *L. applebyi*, *L. melanoleucus*, *L. nahangensis*, *L. nyx*, *L. oshanensis*, *L. pluvialis*, *L. tuberosus* and *L. ventripunctatus*, all having no lateral fringes on toes.

In having head longer than or as long as wide, *L. laui* sp. nov. differs from *L. bourreti*, *L. khasiorum*, *L. lateralis* and *L. sungi*, all having head wider than long.

L. laui has distinct dark brown spots on the flank, distinguishing it from *L. eos*, which lacks dark brown spots on the flank. In having moderate dermal fringes on both sides of finger II, III and IV, *L. laui* can be distinguished from *L. firthi*, which has wide dermal fringes on finger II only.

In the appearance, *Leptolalax laui* sp. nov. is most similar to *L. liui*, and it had been regarded as later species for a rather long time. However, it can be distinguished from specimens of *L. liui* collected from its type locality (Wuyishan, Fujian Province, China) by (1) having a brown or reddish-brown dorsal coloration in life (versus grayish-brown in *L. liui*; Figure 4A); (2) skin on dorsum scattered with rounded granular tubercles and absence of glandular folds (versus glandular folds present in *L. liui*; Figure 4A); (3) narrow traverse brownish grey bars on the dorsal surface of tibia and lower arms (versus wider bars in *L. liui*; Table 4); (4) dorsum without light markings in preservatives (versus dorsal large dark markings edged with light margins in *L. liui*; Figure 4B); (5) longitudinal ridges under toes extending on phalanges and not interrupted at the articulations (versus longitudinal ridges interrupted at the articulations in *L. liui*; Figure 4C).

Table 4 Measurements (mm) of type specimens of male *Leptolalax laui* sp. nov. and 13 topotypic specimens of male *Leptolalax liui* (SYS a001571–001578, 001595–001599). Ranges are given in parenthesis.

	Leptolalax laui sp. nov. *n* = 11	*Leptolalax liui* *n* = 13
SVL	26.0 (24.8–26.7)	24.9 (22.6–26.8)
HDL	8.9 (8.9–10.1)	9.6 (8.9–10.1)
HDW	9.5 (8.9–9.7)	9.1 (8.2–9.6)
SNL	3.9 (3.3–4.2)	3.9 (3.0–4.6)
EYE	3.4 (3.0–3.8)	3.5 (3.2–3.7)
IOD	3.0 (2.7–3.2)	3.1 (2.7–3.8)
TMP	1.5 (1.0–1.6)	1.7 (1.4–1.9)
TEY	1.2 (0.9–1.5)	1.2 (0.9–1.6)
TIB	12.4 (11.7–12.9)	12.3 (10.8–13.1)
ML	6.6 (6.0–7.2)	6.2 (5.5–6.9)
PL	11.1 (10.0–11.3)	11.0 (9.6–12.0)
F1L	2.3 (2.0–2.5)	2.3 (1.7–2.7)
F2L	3.0 (2.6–3.3)	2.8 (2.4–3.3)
F3L	5.1 (4.5–5.6)	4.6 (4.2–5.2)
TMP:EYE	0.44 (0.32–0.51)	0.49 (0.40–0.54)
TIB:SVL	0.48 (0.46–0.50)	0.49 (0.40–0.54)
HDL:HDW	0.95 (0.85–1.04)	0.96 (0.86–1.09)
HDL:SVL	0.32 (0.32–0.36)	0.35 (0.31–0.40)
FG	1.1 (0.7–1.5)	1.3 (1.0–1.6)
BT	0.7 (0.6–0.9)	1.3 (1.0–1.7)
BLA	0.7 (0.5–0.8)	1.1 (0.9–1.2)

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Figure 4 Comparison of morphology between male *Leptolalax laui* sp. nov. (left) and topotypic specimens of male *L. liui* (right). (A) Frontolateral view in life of male *L. laui* sp. nov. (SYS a001505) and male *L. liui* (SYS a001571); (B) Frontolateral view in preservative of male *L. laui* sp. nov. (SYS a001518) and male *L. liui* (SYS a001573). (C) Ventral view of left foot in preservative of male *L. laui* sp. nov. (SYS a002057) and male *L. liui* (SYS a001573). Red arrow indicates interruption of longitudinal ridges at the articulations under toes.

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Appendix 1

Specimens examined

Leptolalax liui: China, Fujian Province, Chongan County (SYS a001571-001578, 001595-001599); China, Jiangxi Province, Mt. Tongba (SYS a001702); China, Jiangxi Province, Mt. Huanggang (SYS a001620); China, Fijian Province, Mt. Daiyun (SYS a001736).