

On the occurrence of *Lygosoma siamense* Siler, Heitz, Davis, Freitas, Aowphol, Termprayoon & Grismer, 2018 (Squamata, Scincidae) in China

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Abstract

The genus *Lygosoma* Hardwicke & Gray, 1827 is a group of semi-fossorial supple skink from Southeast Asia, the members of which are still understudied in Indochina. *Lysosoma quadrupes* (Linnaeus, 1766) was once recorded from China based on historical literature; until recent its Indochina specimens were revised and placed into a newly described species, i.e., *L. siamense* Siler, Heitz, Davis, Freitas, Aowphol, Termprayoon & Grismer, 2018. However, *Lygosoma quadrupes* had long been unknown from China but the historical record had not been confirmed. Here we report a vouchered record for *L. siamense* from China for the first time based on four specimens collected in Sanya, Hainan, China. Furthermore, the taxonomic history of *Lygosoma* from China is also reviewed.

Key Words

distribution, supple skink, taxonomic revision, taxonomy, writhing skink

Introduction

The genus *Lygosoma* Hardwicke & Gray, 1827 is generally known as writhing skink or supple skink, which formerly contained more than 30 species distributed across Africa, India, Southeast Asia, the western and southern Philippines, as well as Christmas Island (Australia) (Cogger 2014; Datta-Roy et al. 2014; Geissler et al. 2011; Heitz et al. 2016; Siler et al. 2018). Recently, Freitas et al. (2019) revised the taxonomy of the genus *Lygosoma sensu lato* by using a molecular phylogeny, which indicated that *Lygosoma sensu stricto* is restricted

to Southeast Asia only. Furthermore, the genus *Riopa* was resurrected for a clade of Indian and Southeast Asian species, the genus *Mochlus* Günther, 1864 was expanded to include all African species of writhing skinks, and a new genus, namely *Subdoluseps* Freitas, Datta-Roy, Karanth, Grismer & Siler, 2019, was erected to accommodate Southeast Asian taxa (Freitas et al. 2019). However, only five species were incorporated in the phylogeny of *Lygosoma s. str.* due to the lack of adequate molecular data, and most of the species of *Lygosoma s. l.* from Southeast Asia were still presently recognized as *incertae sedis* (Freitas et al. 2019; Uetz et al. 2021).

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The type species of *Lygosoma, L. quadrupes* (Linnaeus, 1766) was previously known as a widely distributed species, from Indochina, Thailand, southern China (including Hong Kong), Western Malaysia, Indonesia, and the Philippines (Geissler et al. 2011). Until recently, Siler et al. (2018) redescribed true *Lygosoma quadrupes* (Linnaeus, 1766) and restricted its geographic distribution to the island of Java in Indonesia, whereas the Indochina specimens were placed in a newly described species, namely *L. siamense* Siler, Heitz, Davis, Freitas, Aowphol, Termprayoon & Grismer, 2018 based on historical collections from Thailand. However, future studies are also recommended to evaluate the widespread distribution of *L. siamense* across much of Indochina (Siler et al. 2018).

For example, *L. quadrupes* was once recorded from China (including "South China" and "Hong Kong") based on historical literature (Boulenger 1887; Smith 1935; Taylor 1963), but this species was not included in recent Chinese herpetofauna (Wang et al. 2020; Zhao and Adler 1993), probably due to the lack of vouchered specimens. Particularly, although Siler et al. (2018) revised the Indochina record of *L. quadrupes* to *L. siamense*, no vouchered information of Chinese specimen was provided. Therefore, the historical record of "*L. quadrupes*" from China has not been confirmed yet. Furthermore, the coloration in life of *L. siamense* is currently known from a hand drawing only (Chan-Ard et al. 2015).

During a recent field survey from 2019 to 2021, four elongate-bodied skink specimens were collected in Sanya City, Hainan Province, southern China. The results of morphological comparisons and molecular analysis indicated these specimens should be referred to *Lygosoma siamense*, thus we confirm the distribution of *L. siamense* in Hainan Island, southern China. Furthermore, we provide the first detailed description of *L. siamense* in China, the coloration in life, and life history of this semi-fossorial species.

Materials and methods

Taxon sampling

Four specimens of *Lygosoma siamense* were collected from Linchunling Forest Park, Sanya City (18°15.8151'N, 109°31.2985'E; ca 109 m a.s.l.), Hainan Province,

southern China. Muscle or liver samples were dissected from euthanized specimens, preserved in 95% ethanol and stored at -40 °C. The specimens were fixed in 10% buffered formalin and later transferred to 70% ethanol, and deposited in the Museum of Biology, Sun Yat-sen University (SYS) (Guangzhou) and Herpetological Museum, Chengdu Institute of Biology, Chinese Academy of Sciences (CIB) (Chengdu), China.

DNA Extraction, Polymerase Chain Reaction (PCR), and sequencing

Genomic DNA was extracted from muscular tissue using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. We sequenced partial segments of the mitochondrial 16S rRNA gene (16S) of the two samples of Lygosoma siamense from China (specimen voucher number: CIB 118520 and SYS r002573), using primers 16Sar-L (5'- CGCCTGTTTATCAAAAACAT-3') and 16Sbr-H (5'- CCGGTCTGAACTCAGATCACGT') (Palumbi et al. 1991). The PCR procedure was performed with an initial denaturation at 94 °C for 3 min, 40 cycles of 94 °C for 1 min, 51 °C for 1 min and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min (Datta-Roy et al. 2014). PCR products were purified with spin columns and then sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit following the guidelines, on an ABI Prism 3730 automated DNA sequencer by Wuhan Tianyi Huiyuan Bioscience and Technology Inc.

Phylogenetic analysis

Six sequences from five known *Lygosoma* species plus two outgroup sequences from *Subdoluseps bowringii* Günther, 1864, which were used to root the tree, were obtained from GenBank and composed the dataset (Table 1). Sequences were first aligned in Clustal X 2.0 (Larkin et al. 2007), with default parameters. The alignment was then checked and manually revised, if necessary. Trimmed with the gaps partially deleted in MEGA 6.06 (Tamura et al. 2013), while within high variable regions, all gaps were removed. We ran Jmodeltest v2.1.2 (Darriba et al. 2012) with Akaike

Table 1. Localities, voucher information, and GenBank accession numbers for all specimens used in this study.

Species name	Locality	Specimen voucher	168	References		
Ingroup: Lygosoma						
(1) L. isodactylum	Vietnam	FMNH 262154	MK414547	Freitas et al. (2019)		
(2) L. koratense	Thailand	N/A	AB028817	Honda et al. (2000)		
(3) L. quadrupes	Indonesia	ENS 13639	MG367368	Freitas et al. (2019)		
(4) L. siamense	Sanya, Hainan, China	CIB 118520	OM569680	This study		
(5) L. siamense	Sanya, Hainan, China	SYS r002573	OM569681	This study		
(6) L. siamense	Thailand	N/A	AB028818	Honda et al. (2000)		
(7) L. tabonorum	Philippines	PNM9820	MK414557	Freitas et al. (2019)		
(8) L. tabonorum	Philippines	PNM9821	MG367367	Freitas et al. (2019)		
Outgroup: Subdoluseps						
(9) S. bowringii	Cambodia	FMNH 261839	MK414544	Freitas et al. (2019)		
(10) S. bowringii	Malaysia	LSUHC 7993	MK414545	Freitas et al. (2019)		

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and Bayesian information criteria on the alignment, resulting the best-fitting nucleotide substitution models of GTR + I + G. Phylogenetic trees were constructed using maximum likelihood (ML) implemented in RaxmlGUI 1.3 (Silvestro and Michalak 2012), and Bayesian inference (BI) using MrBayes 3.2.4 (Ronquist et al. 2012). For ML analysis, the maximum likelihood tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analyzed. For BI analysis, two independent runs with four Markov Chain Monte Carlo simulations were performed for ten million iterations and sampled every 1,000 iterations. The first 25% of samples were discarded as burn-in. Convergence of the Markov Chain Monte Carlo simulations was assessed with PSRF (average standard deviation of split frequencies) ≤ 0.01 and ESS (effective sample size) value > 200 using Tracer 1.4 (Rambaut et al. 2013). Pairwise distances were also calculated in MEGA 6.06 based on uncorrected *p*-distance (Tamura et al. 2013).

Morphological examination

Measurements were taken following Siler et al. (2018) using digital calipers (Neiko 01407A Stainless Steel 6-Inch Digital Caliper) to the nearest 0.1 mm. Abbreviations of morphological characters are as follows: snout-vent length (SVL: distance from tip of snout to vent), axillagroin distance (AGD: distance between posterior edge of forelimb insertion and anterior edge of hind-limb insertion), total length (TotL: distance from tip of snout to tip of tail), midbody width (MBW: measured from lateral surface to opposing lateral edge at midpoint of axilla-groin region), midbody depth (MBD: measured from ventral surface to dorsal surface at midpoint of axilla-groin region), tail length (TL: measured from posterior margin of vent to tip of tail), tail width (TW: measured at widest section of tail posterior to hemipenis bulge), tail depth (TD: measured from

ventral to dorsal surface of tail at the same point as TW), head length (HL: measured from tip of snout to posterior margin of retroarticular process), head width (HW: widest measure of head width at jaw articulations), head depth (HD: measured from ventral to dorsal surface of head at jaw articulations), eye diameter (ED: at widest point), eye-narial distance (END: from anterior margin of eye to posterior margin of nares), snout length (SNL: from anterior margin of eye to tip of snout), internarial distance (IND: from dorsal aspect between most laterally distal edges of nares), forelimb length (FLL: measured from forelimb insertion to tip of Finger III or longest digit), hind-limb length (HLL: measured from hind-limb insertion to tip of Toe IV or longest digit), midbody scale-row count (MBSR: number of longitudinal scale rows measured around widest point of midbody), paravertebral scale-row count (PVSR: number of scale rows counted between parietals and the base of the tail opposite the vent), axilla-groin scale-row count (AGSR: number of scale rows counted between posterior edge of forelimb insertion and anterior edge of hind-limb insertion), Finger III lamellae count (F3lam: number of enlarged, undivided lamellae beneath Finger III), Toe IV lamellae count (T4lam: number of enlarged, undivided lamellae beneath Toe IV), supralabial count (SL), infralabial count (IFL), superciliary count (SC), and supraocular count (SO).

Sex was determined by dissection. Enlarged gonads were considered evidence of adulthood.

Results

The ML and BI analyses resulted in identical topologies (Fig. 1). Uncorrected pairwise sequence divergences are reported in Table 2. The analysis supported the two specimens (SYS r002573 and CIB 118520) from China and a confirmed specimen of *Lygosoma siamense* from Thailand formed a monophyletic group and with low intrapopulational genetic

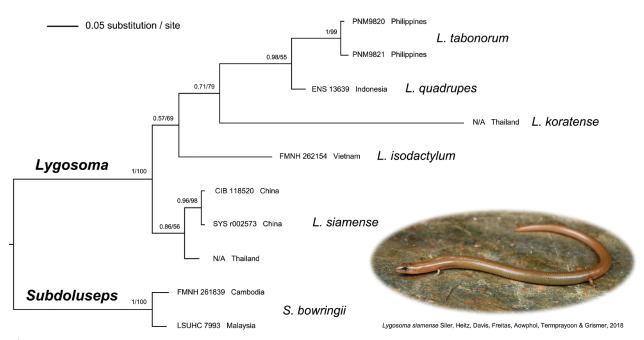


Figure 1. Bayesian inference tree of five species of Lygosoma, based on the partial DNA sequences of the mitochondrial 16S rRNA.

Table 2. Uncorrected p-distance (%) of 16S rRNA fragment among five Lygosoma species used in this study.

Species name	Specimen voucher	1	2	3	4	5	6	7	8
1 Lygosoma isodactylum	FMNH 262154	0.00							
2 Lygosoma koratense	N/A	10.57							
3 Lygosoma quadrupes	ENS 13639	5.85	9.58						
4 Lygosoma siamense	CIB 118520	4.33	9.11	5.19					
5 Lygosoma siamense	SYS r002573	4.33	9.11	5.19	0.00				
6 Lygosoma siamense	N/A	4.12	9.59	4.97	0.99	0.99			
7 Lygosoma tabonorum	PNM 9820	6.97	9.58	1.99	6.26	6.26	6.04		
8 Lygosoma tabonorum	PNM 9821	7.19	9.82	2.20	6.48	6.48	6.26	0.20	0.00

Table 3. Measurements (mm) and scale counts of Lygosoma siamense from China. See materials and methods for abbreviations.

 * indicates dropped or regenerated tail.

	Sex	SVL	AGD	MBW	MBD	TL	TW	TD	HL	HW	HD	ED	END
SYS r002573	Male	72.2	56.0	5.2	4.1	57.9	4.1	3.7	6.1	4.7	3.6	1.3	1.5
CIB 118520	Subadult	49.3	35.7	3.7	2.4	33.7*	2.4	2.0	4.2	3.8	2.0	1.1	1.2
CIB 118521	Subadult	46.2	33.7	3.4	2.4	23.9*	2.3	2.0	4.1	3.7	1.8	1.0	1.1
CIB 118522	Subadult	41.7	28.6	N/A	N/A	2.6*	N/A	N/A	3.5	N/A	N/A	0.8	0.8
	SNL	IND	FLL	HLL	MBSRC	AGSRC	PVSRC	Fin3Lam	Toe4Lam	SuprL	InfrL	SO	SC
SYS r002573	2.6	1.1	3.2	6.1	27	89	119	5	7	6	6	4	6
CIB 118520	1.9	1.0	2.4	4.0	25	94	119	5	6	6	6	4	6
CIB 118521	1.9	0.9	2.2	3.8	27	92	116	5	5	6	7	4	6
CIB 118522	1.2	0.6	2.0	3.2	24	95	115	5	6	7	6	4	6

differentiation (0.99%). Moreover, all of our newly collected specimens are largely similar to the original description of *L. siamense* (Table 3) (Siler et al. 2018). Thus, we herein report the specimens from Sanya City, Hainan Province as a new genus national record of China.

Systematics

Family Scincidae Gray, 1825 Genus *Lygosoma* Hardwicke & Gray, 1828

Lygosoma siamense Siler, Heitz, Davis, Freitas, Aowphol, Termprayoon & Grismer, 2018

Lygosoma siamensis Siler, Heitz, Davis, Freitas, Aowphol, Termprayoon & Grismer, 2018, *J. Herpetol.*, 52 (3): 339, figs 4–5. Holotype: FMNH 177496 (field no. EHT 1390; figs 4, 5), adult male, collected on 7 June 1956 in Thailand, Pattani Province by E. H. Taylor.

Lygosoma chalcides - Boulenger, 1887: 340 (in part).

Lygosoma quadrupes — Smith, 1935: 290 (in part); Taylor, 1963: 1049; Honda et al. 2000: 453; Ziegler et al. 2007: 401; Bourret, 2009: 264 (in part); Wagner et al. 2009: 4; Das, 2010: 240 (in part); Geissler et al. 2011: 1169 (in part); Geissler et al. 2012: 56; Datta-Roy et al. 2016: 165; Grismer et al. 2018: 534.

Lygosoma siamensis — Siler et al. 2018: 339. *Lygosoma siamense* — Freitas et al. 2019: 19.

Etymology. The species name *siamense* was derived from the exonym "Siam", a term formerly used as the name of Thailand. The name was chosen in recognition of Thailand as the center of the species' known distribution (Siler et al. 2018).

The grammatical gender of the generic nomen Lygosoma Hardwicke & Gray, 1827 has been confused

in the literature where it was treated as both feminine (Siler et al. 2018) or neutral (Freitas et al. 2019). The nomen *Lygosoma* is probably based on the Greek nouns "*lygos*" and "*sōma*", meaning "supple-bodied", and the gender of this nomen is neutral. Consequently, the ending of *Lygosoma siamensis* provided by its original description should be modified as "*siamense*".

Common names. Siamese Supple Skink (in English); 泰国蝘蜓 (Tài Guó Yán Tíng) (in Chinese).

Description of specimens from China. Main description according to an original tail adult specimen (SYS r002573, Fig. 2), the scale count data of other three damaged tail subadult specimens (CIB 118520–118522) are given in parentheses. Mensural data of all voucher specimens are listed in Table 3.

Body small, slender, SVL 72.2 mm; head small, weakly differentiated from neck, roughly equal in width to body, HL 6.1 mm, HW 4.7 mm, HD 3.6 mm; snout slightly pointed, rounded in dorsal and lateral profile; head scales large, smooth, SNL 42.6% HL; external ear opening small, transverse diameter slightly larger than longitudinal diameter; eyes small, ED 21.3% HL, 86.7% END; body moderately depressed, nearly uniform in thickness, MBW 126.8% MBD; scales smooth, glossy, imbricate; longitudinal scale rows at midbody 27 (24-27); paravertebral scale rows 119 (115-119); axillagroin scale rows 89 (92-95); limbs short, diminutive, pentadactyl; Finger III lamellae five, Toe IV lamellae seven; FLL 5.7% AGD, 4.4% SVL; HLL 10.9% AGD, 8.4% SVL; original TL 56.0 mm, SVL/TL=1.29, tail nearly as wide as body, gradually tapered towards end, TW 78.8% MBW.

Rostral wider than high, in contact with first supralabials and nasals laterally, frontonasal dorsally; frontonasal wider than long, single, in contact with

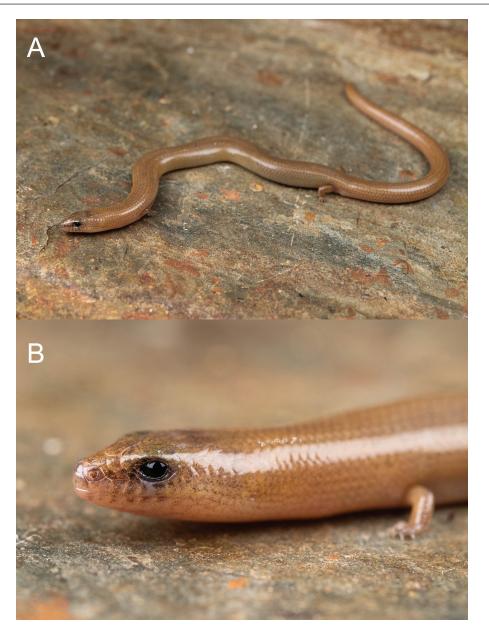


Figure 2. A. The general aspect of the adult male specimen of *Lygosoma siamense* (SYS r002573) from Sanya, Hainan, China in life; **B.** Scalation and coloration characters of the head of the same individual.

nasal and first loreal laterally, prefrontal and frontal posteriorly; nostril ovoid; supranasals present; postnasals small, triangular-shaped; prefrontals small, widely separated, contacting loreals laterally, first superciliary and frontal posteriorly; frontal large, its anterior margin in broad contact with frontonasal, contacting first two supraoculars laterally; four supraoculars; frontoparietals fused into single large scale, in contact with all four supraoculars laterally and parietals and interparietal posteriorly; interparietal somewhat triangular-shaped, eyespot present posteriorly; parietals in broad contact medially behind interparietal; enlarged nuchals absent; two loreals contacting supralabials; supralabials six, first and sixth largest, fourth and fifth subocular; two preoculars, lower much larger than upper; uninterrupted superciliaries six, first one large, in contact prefrontal anteriorly, first supraoculars dorsally; subocular scale row complete, in contact with supralabials; lower eyelid bearing scales; postoculars two; primary temporals two; secondary temporals two, larger than primary temporals; infralabials six, first two contacting postmental, first smaller than second; mental wider than long, in contact with first infralabial laterally, postmental posteriorly; postmental large, single; two pairs of large chin-shields, first pair in contact, second pair narrowly separated by single medial scale; scales on limbs smaller than body scales; single row of supradigitals (Figs 2–4).

Coloration in life. Four specimens (SYS r002573, CIB 118520, CIB 118521, CIB 118522) from China share similar coloration and pattern. Dorsal surface of head, body, limbs and tail brown, dark brown longitudinal lines present on edges of scales and continuing onto tail. Darker pigmentation present on central part of head; ventral surface of body and tail pale pink; iris dark brown.



Figure 3. The general aspect and close-up views of the adult male specimen of *Lygosoma siamense* (CIB 118520) from Sanya, Hainan, China in life. **A.** Dorsolateral view of body; **B.** Close-up view of left head; **C.** Ventral view of body; **D.** Close-up view of fore limb.

Ecological notes. All four specimens were collected during the day, beneath flowerpots, leaf litter substrates or other surface material. Their reduced limbs are folded alongside body during rapid locomotion (Das 2010), whereas they are unfolded when slowly crawling.

Diet and reproductive habits remain poorly known. Nevertheless, a large number of ants were observed where the specimens were collected, and probably served as their main food source, which was similar to the habitats as inferred from Das (2010: 83, plate 33). Smith (1935) reported the reproductive habits in stating "A female kept by me in Bangkok laid three eggs on May 27th measuring 9 by 5 mm. in size. The young were born on June 28th and measured 48 mm. in total length, the head and body measuring 26 mm".

Discussion

The distribution of *Lygosoma chalcides* (now *L. quadrupes*) from Southern China was provided by Boulenger (1887: 341) based on single specimen from Hong Kong, China. No detailed information on the specimen was provided except for its collector's name, Sir J. Bowring. Thus, the solely mentioned specimen of *L. quadrupes* from China can hardly be traced. Although most authors also recorded the distribution of *L. quadrupes* with inclusion of "South China" as well as "Hongkong", seemingly directly followed Boulenger (1887), no further Chinese specimens were reported to our best knowledge (Geissler et al. 2011; Smith 1935; Taylor 1963). Most recently, Siler et al. (2018) restricted true *L. quadrupes* to Java, Indonesia

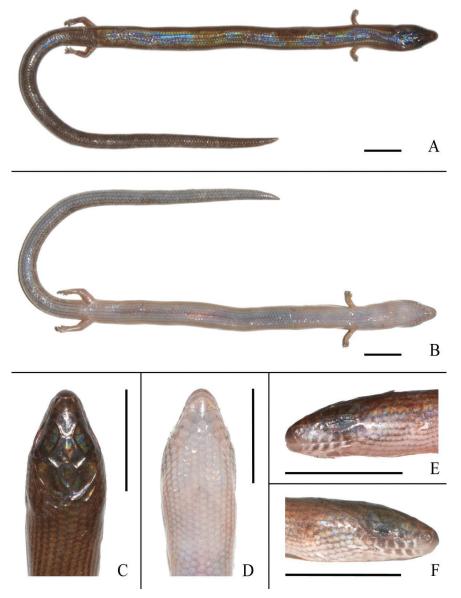


Figure 4. The general aspect and close-up views of the adult male specimen of *Lygosoma siamense* (CIB 118520) from Sanya, Hainan, China, prior to preservation. A. Dorsolateral view of body; B. Ventral view of body; C. Dorsal view of head; D. Ventral view of head; E. Left side view of head; F. Right side view of head. Scale bar: 5 mm.

only, and revised specimens from Thailand to their newly described species, i.e., *L. siamense*. Despite the fact that the distribution of *L. siamense* was given as "Cambodia, Laos, West Malaysia, Thailand, and Vietnam" (Siler et al. 2018: 342), all of their 16 voucher specimens come from Thailand, while the distribution map of *L. siamense* provided by Siler et al. (2018: Figs 1, 3) incorporated the region south to Tropic of Cancer in both Guangdong (including Hong Kong) and Guangxi Province (without Yunnan and Taiwan Province), southern China. Similarly, no voucher specimen from China were provided, and Siler et al. (2018) also recommended that further study is needed to evaluate the populations documented in southern China. Consequently, the historical record of *L. siamense* from China had not been confirmed.

On the other hand, the exact time of the delisting of L. quadrupes from China is also not clear. Although previous studies indeed recorded L. quadrupes in China (1887–1963; Boulenger 1887; Smith 1935; Taylor 1963), the species was not included in recent Chinese herpetofauna (from 1993 to the present study; Wang et al. 2020; Zhao et al. 1999; Zhao and Adler 1993). Intriguingly, no study formally questioned the distribution record of L. quadrupes in China, and no more specimens or detailed information of L. quadrupes were provided to confirm its previous Chinese record (Boulenger 1887). We speculate that the previous record of L. quadrupes in China was not included owing to the lack of a voucher specimen in a long history, but was never given adequate explanation. Nevertheless, we report the distribution of L. siamense in Hainan Island, southern China in the current study, which represents the only record for the species of the genus from China, after the generic revision of Subdoluseps bowringii (Günther, 1864) (Freitas et al. 2019).

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