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Source: Current Herpetology, 42(1) : 83-97

Published By: The Herpetological Society of Japan

URL: <https://doi.org/10.5358/hsj.42.83>

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## Two New Species of *Leptobranchella* from Northern Thailand (Amphibia, Anura, Megophryidae)

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Zoobank LSID: 3132258D-C1A1-4CDF-BDE7-21179D726E7A

**Abstract:** Phylogenetic analyses using mtDNA gene sequences revealed the presence of two genetic clades in the genus *Leptobranchella* (former *Leptotalax*) from northern Thailand. One formed a clade including *L. ventripunctata* and *L. aerea*, while another included *L. bourreti* and *L. laoi*. The first clade contained *L. cf. minima* from Doi Suthep, while the second clade contained *L. murphyi* and two undescribed species, one from Doi Inthanon, at a high altitude of 2,300 m asl, and another from Mae Hong Son, at Myanmar border. These form a clade, that is sister to *L. murphyi*. Morphologically, the first species is generally similar to *L. murphyi*, while the second species resembles *L. cf. minima*. However, they are clearly distinguished from those described species by great genetic differences, and we describe them as *L. korifi* sp. nov. and *L. sinorensis* sp. nov., respectively.

Key words: *Lalos*; *Leptobranchella korifi* sp. nov.; *Leptobranchella sinorensis* sp. nov.; *Leptotalax*; mtDNA phylogeny; Thailand

### INTRODUCTION

Medium- to small-sized, forest-floor dwelling megophryids from southern China and Vietnam through Myanmar and Thailand to Peninsular Malaysia and Borneo were formerly called *Leptotalax* Dubois, 1980, but are now synonymized with Bornean *Leptobranchella* (Smith, 1925; see Discussion for problems with this classification). Now the genus includes nearly 100 species, of which one-third

have been described within the last half-decade (e.g., Shi et al., 2021; Luo et al., 2022). This is chiefly because many of the members of this genus are morphologically very similar and were thus difficult to identify. However, acoustic analyses of their calls have detected some cryptic members (Matsui, 1997, 2006), and more recently, molecular phylogenetic analyses rapidly clarified the high species diversity of this genus (e.g., Chen et al., 2018).

Compared with neighboring Vietnam (e.g., Nguyen et al., 2021) and China (e.g., Chen et al., 2021), known species diversity of this genus in Thailand is low. Historically, Taylor (1962, as *Leptobranchium*) listed *L. pelody-*

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*toides* (Boulenger, 1893) and described *L. minima*. Then, in a publication of an illustrated guidebook on the amphibian fauna, Chan-ard (2003) listed four species (as *Leptolalax*: *L. bourreti* (Dubois, 1983), *L. gracilis* (Günther, 1872), *L. heteropus* (Boulenger, 1900), and *L. pelodytoides*). However, these records are doubtful, without sources of data or the bases for identification (Matsui, 2006). Matsui (2006) added three species (as *Leptolalax*: *L. melanoleuca*, *L. fuliginosa*, and *L. sola*) from southern region, while Jiang et al. (2013) described *L. zhangyapingi* (as *Leptolalax*) from Doi Saket, northern region. More recently, Niyomwan et al. (2019) used the generic name *Leptobranchella* and listed *L. cf. bourreti*, *L. fuliginosa*, *L. heteropus*, *L. melanoleuca*, *L. minima*, *L. cf. pelodytoides*, *L. sola*, and *L. zhangyapingi* as the Thai members, and Chen et al. (2021) described *L. murphyi* from the northern region. Thus, only seven species have been recorded as valid from the country.

During our field survey in Thailand nearly 30 years ago, we collected *Leptobranchella* or ascertained its occurrence without collection in northeastern (Loei Province), northern (Mae Hong Son and Chiang Mai Provinces), southwestern (Kanchanaburi and Prachuap Khiri Khan Provinces), and southern (Surat Thani and Narathiwat Provinces) regions of that country between 1994 and 2004 (regional divisions following Nabhitabhata et al., 2000). We failed to find specimens of this genus in other parts of Thailand. Specimens from the southern region were already described as three distinct species, *L. melanoleuca*, *L. fuliginosa*, and *L. sola* (Matsui, 2006, all as *Leptolalax*), but details of the more northern samples have been left unreported. Recently, *L. murphyi* Chen, Suwannapoom, Wu, Poyarkov, Xu, Pawangkhanant, and Che, 2021 was described from Chiang Mai Province, northern region. We also had this species in our collection. We examined molecular variation of northern specimens and found two species that are significantly different genetically from *L. cf. minima* (see below) and *L. murphyi*. In this paper, we describe these two species as new to science.

## MATERIALS AND METHODS

We collected *Leptobranchella* during our field survey between 1994 and 1995. We collected specimens at the type locality of *L. minima* (Doi Suthep: Taylor, 1962), but our specimens differed slightly from the original description and the holotype now stored in the FMNH (M. Matsui, unpublished data). We thus call our specimens *L. cf. minima* in this paper. We took tissues for subsequent biochemical analysis, and fixed the specimens for vouchers. Specimens, fixed in 10% formalin and later preserved in 70% ethanol, are stored at Graduate School of Human and Environmental Studies, Kyoto University (KUHE).

We obtained sequence data of mitochondrial DNA from muscle/liver samples preserved in 99% ethanol. We reconstructed phylogenetic trees from approximately 2,000 base pairs (bp) of the partial sequences of mitochondrial 12S and 16S rRNA genes to clarify the genetic structure of specimens representing *Leptobranchella* species and outgroup species, *Leptobranchium hasseltii* Tschudi, 1838 (Table 1). Methods for DNA extraction, and amplification and sequencing of the mtDNA fragments are the same as those reported by Hamidy et al. (2011). The resultant new sequences were deposited in GenBank (Accession numbers LC741025–741038; Table 1). The alignment matrices were subjected to estimate phylogenetic relationships using maximum likelihood (ML) and Bayesian inference (BI). Pairwise comparisons of uncorrected sequence divergences (p-distance) were also calculated. Details for these procedures are given in Matsui et al. (2010) and Hamidy et al. (2011). Pairwise comparisons of uncorrected sequence divergences (p-distance) were also calculated for 16S rRNA.

For morphological analyses, 19 body measurements were taken, mainly following Matsui (1984): (1) snout-vent length (SVL); (2) head length (HL); (3) nostril-eyelid length (N-EL); (4) snout length (SL); (5) eye length (EL, including eyelid); (6) tympanum-eye length (T-EL); (7) tympanum diameter (TD); (8) head

TABLE 1. Samples of *Leptobrachella* frogs and an outgroup species used for DNA analysis in this study, together with the information on voucher specimens, collection locality, and GenBank accession numbers. Voucher abbreviations; CIB: Chengdu Institute of Biology; KUHE: Kyoto University, Human and Environmental Studies; MNHN: Muséum National d'histoire Naturelle, Paris; VNMN: Vietnam National Museum of Nature.

	Species	Voucher	Locality		GenBank
1	<i>Leptobrachella</i> cf. <i>minima</i>	KUHE 19200	Thailand	Doi Suthep	LC741025
2	<i>Leptobrachella</i> cf. <i>minima</i>	KUHE 19201	Thailand	Doi Suthep	LC201981
3	<i>Leptobrachella</i> cf. <i>minima</i>	KUHE 19210	Thailand	Doi Suthep	LC741026
4	<i>Leptobrachella</i> cf. <i>minima</i>	KUHE 23733	Thailand	Changdao	LC201980
5	<i>Leptobrachella murphyi</i>	KUHE 19113	Thailand	Doi Inthanon	LC741027
6	<i>Leptobrachella murphyi</i>	KUHE 19131	Thailand	Doi Inthanon	LC741028
7	<i>Leptobrachella murphyi</i>	KUHE 19132	Thailand	Doi Inthanon	LC741029
8	<i>Leptobrachella murphyi</i>	KUHE 19220	Thailand	Doi Pui	LC741030
9	<i>Leptobrachella murphyi</i>	KUHE 19221	Thailand	Doi Pui	LC741031
10	<i>Leptobrachella murphyi</i>	KUHE 19222	Thailand	Doi Pui	LC741032
11	<i>Leptobrachella korifti</i> sp. nov.	KUHE 19134	Thailand	Doi Inthanon	LC741033
12	<i>Leptobrachella sinorensis</i> sp. nov.	KUHE 19809	Thailand	Mae Hong Son	LC741034
13	<i>Leptobrachella sinorensis</i> sp. nov.	KUHE 19815	Thailand	Mae Hong Son	LC741035
14	<i>Leptobrachella sinorensis</i> sp. nov.	KUHE 19816	Thailand	Mae Hong Son	LC741036
15	<i>Leptobrachella ventripunctata</i>	CIB 201307-062	China	Yunnan	LC201978
16	<i>Leptobrachella aerea</i>	VNMN 2013.70	Vietnam	Quang Nam	LC741037
17	<i>Leptobrachella sungi</i>	KUHE 55242	Vietnam	Tam Dao	LC741038
18	<i>Leptobrachella bourreti</i>	MNHNP 5659	Vietnam	Sa Pa	LC201983
19	<i>Leptobrachella laui</i>	KUHE 21705	China	Hongkong	LC201982
20	<i>Leptobrachella melanoleuca</i>	KUHE 19720	Thailand	Srat Thani	LC201991
21	<i>Leptobrachella fuliginosa</i>	KUHE 20174	Thailand	Phetchaburi	LC201987
22	<i>Leptobrachella sola</i>	KUHE 23261	Thailand	Hala Bala	LC202007
23	<i>Leptobrachella heteropus</i>	KUHE 15490	Malaysia	Larut	AB847561
24	<i>Leptobrachella gracilis</i>	KUHE 55624	Malaysia	Sarawak	AB847560
25	<i>Leptobrachium hasseltii</i>	KUHE 42820	Indonesia	Java	AB530424

width (HW); (9) internarial distance (IND); (10) interorbital distance (IOD); (11) upper eyelid width (UEW); (12) forelimb length (FLL); (13) lower arm and hand length (LAL); (14) inner palmar tubercle length (IPTL); (15) hindlimb length (HLL); (16) tibia length (TL); (17) foot length (FL); (18) inner metatarsal tubercle length (IMTL); and (19) first toe length (1TOEL). We made all measurements to the nearest 0.1 mm with dial calipers under a binocular dissecting microscope.

In describing coloration, we adopted the terminology on dorsal pattern shown by Ohler et

al. (2011). Sex and maturity of specimens were determined by observation of gonads and secondary sexual characters such as vocal opening. A single specimen from near the peak of Doi Inthanon proved to be a female, but we had no comparable females of *L. murphyi* at hand. We thus compared our data for *L. murphyi* with those shown in Table 3 of Chen et al. (2021). We chose 12 characters that could be reliably compared: SVL, HL (HDL in Chen et al., [2021]), N-EL (NEL in Chen et al., 2021), SL (SNT in Chen et al. [2021]), T-EL (TEY in Chen et al. [2021]), TD (TMP in Chen

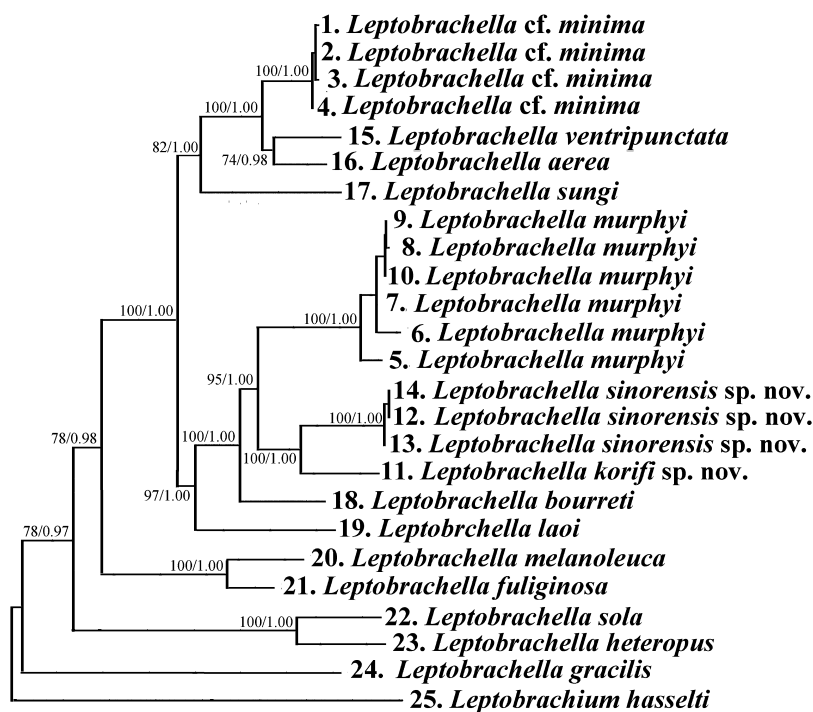


FIG. 1. Bayesian tree from a ~2,849 bp sequence of mitochondrial 12S and 16S rRNA genes for samples of *Leptobrachella*. Numbers above or below branches represent bootstrap supports for ML inference and Bayesian posterior probability (ML-BS/BPP).

et al. [2021]), HW (HDW in Chen et al. [2021]), IND, IOD, UEW, TL (TIB in Chen et al. [2021]), and IMTL (IMT in Chen et al. [2021]). We found that the two datasets for male *L. murphyi* overlap with each other in terms of the ranges of variation relative to SVL. In the data of Chen et al. (2021), sexual dimorphism in *L. murphyi* was found only in SVL (23.2–24.9 mm in males and 29.3–32.1 mm in females) and HL relative to SVL (RHL 35.3–39.1% in males and 31.5–34.5% in females). We thus compared the single female specimen from near the peak of Doi Inthanon with eight male specimens of *L. murphyi* at hand, excluding SVL and RHL for possible presence of sexual dimorphism.

For comparison, we also examined specimens of *L. pelodytoides* stored at the Museo Civico di Storia Naturale, Genova (MSNG 29845, designated lectotype); Natural History Museum, London (BM 1947.2.2514, syntype);

and Zoologisches Museum, Universität Humboldt, Berlin (ZMB 11588), and the holotype of *L. minima* stored at the Field Museum of Natural History, Chicago (FMNH 178228).

## RESULTS

In the molecular phylogenetic analyses, all samples, including outgroups, yielded 2,849 bp of concatenated fragments of mtDNA genes. The best substitution model selected was GTR with gamma shape parameter (G) of 0.396 for ML and 0.416 for BI, with the likelihood values (-lnLs) of 17,469.2785 and 17,497.847, respectively. Two different optimality criteria yielded identical relationships in the phylogenetic analyses employed. As shown in the BI tree (Fig. 1), specimens of *Leptobrachella*, including those from northern Thailand (MLBS=100, BPP=1.00), were clearly split into two well-supported major clades, A and B.

Major Clade A (MLBS=82, Bayesian posterior probability [BPP]=1.00) included *L. sungi* and a clade (MLBS=100, BPP=1.00), in which a subclade of *L. ventripunctata* and *L. aerea* (MLBS=74, BPP=0.98) and a subclade of *L. cf. minima* from Doi Suthep and Chiang Dao (MLBS=100, BPP=1.00) were recognized. Major Clade B (MLBS=97, BPP=1.00) included *L. laoi* and a clade of *L. bourreti* and northern Thai samples (MLBS=100, BPP=1.00). The latter (MLBS=95, BPP=1.00) was split into a subclade of *L. murphyi* (MLBS=100, BPP=1.00), and another subclade (MLBS=100, BPP=1.00) including the lineage from near the peak of Doi Inthanon, and the lineage from Mae Hong Son.

Uncorrected p-distances for a short fragment of 16S rRNA (516 bp) between the two clades, A and B, were substantially large, ranging from 9.3–13.6%. Distances in Clade B ranged from 7.2% between the lineage from near the peak of Doi Inthanon and the lineage from Mae Hong Son, to 11.7% between the lineage from Mae Hong Son and *L. laoi* (Table 2).

The specimens of *Leptobranchella* from northern Thailand are similar to each other, and their morphological separation was not easy. However, the values for p-distances were sufficiently larger than the minimum values reported in allied species (e.g., 5.5%: Luo et al., 2020). This indicates that each of two lineages in the Clade B, one from the high site of Doi Inthanon and another from Mae Hong Son, is a distinct species. Because these two lineages are separated not only genetically, but also morphologically (see comparisons below) from other Thai species of *Leptobranchella*, we conclude that each of these lineages represents a distinct species, which we describe as follows:

#### SYSTEMATICS

##### *Leptobranchella korifi* sp. nov.

(Fig. 2A, B)

##### Diagnosis

A small-sized form of the genus *Leptobranchella*; adult female 22.7 mm in SVL (n=1);

ventral side dusty; tibiotarsal articulation reaching anterior corner of eye; pectoral gland very large; lateral fringe on toes weak.

##### Etymology

The specific epithet, *korifi*, is from the Greek “koreefee,” meaning summit, in reference to the high elevation of the habitat of the new species.

##### Holotype

KUHE 19134, an adult female from near the summit of Doi Inthanon, Chom Thong, Chiang Mai Province, Thailand (18°35' N, 98°29' E, 2,300 m asl), collected on 6 August 1994 by Kunio Araya.

##### Description of holotype (measurements in mm)

SVL 22.7; habitus moderately slender; head longer (HL 9.2, 40.5%SVL) than broad (HW 8.5, 37.4%SVL); snout round in dorsal view, rounded in profile, projecting beyond lower jaw; eye length (EL 3.7, 16.3%SVL) slightly larger than snout length (SL 3.6, 15.9%SVL); canthus distinct, slightly constricted; lore oblique, slightly concave; nostril lateral, below canthus, distinctly closer to tip of snout than to eye; internarial distance (IND 2.8, 12.3%SVL) equaling to interorbital distance (IOD 2.8, 12.3%SVL), both wider than upper eyelid (UEW 2.1, 9.3%SVL); pineal spot absent; tympanum visible, diameter (TD 1.7, 7.3%SVL) less than half that of eye, and separated from eye by one-third of tympanic diameter (T-EL 0.6, 2.6%SVL); vomerine teeth absent; tongue notched, without papillae (Table 3).

Forelimb long (FLL 16.7, 73.6%SVL, LAL 11.7, 51.5%SVL) and slender; fingers slender, unwebbed; finger length formula I<II<IV<III; tips slightly swollen; inner palmar tubercle large (IPTL 1.6, 6.9%SVL), not extending onto first metacarpal and much smaller than outer palmar tubercle; subarticular tubercles indistinct.

Hindlimb moderately long (HLL 37.0, 163.0%SVL); tibia relatively long (TL 11.6, 51.1%SVL), heels overlapping when limbs are

TABLE 2. Uncorrected p-distances (%) among samples of *Leptobrachella* frogs and other species for fragments of 16S rRNA.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 <i>Leptobrachella</i> cf. <i>minima</i>	—													
2 <i>Leptobrachella ventripunctata</i>	6.35	—												
3 <i>Leptobrachella aerea</i>	5.09	5.04	—											
4 <i>Leptobrachella sungi</i>	8.28	9.88	8.33	—										
5 <i>Leptobrachella murphyi</i>	11.74	12.27	11.98	12.37	—									
6 <i>Leptobrachella korifi</i> sp. nov.	12.94	12.60	12.79	13.57	8.62	—								
7 <i>Leptobrachella sinorensis</i> sp. nov.	11.74	12.27	11.11	12.47	8.69	7.24	—							
8 <i>Leptobrachella bourreti</i>	9.50	10.85	9.30	10.85	7.72	9.69	8.79	—						
9 <i>Leptobrachella laui</i>	9.74	11.05	10.47	11.24	11.01	11.43	11.69	9.11	—					
10 <i>Leptobrachella melanoleuca</i>	11.72	13.57	11.82	12.60	14.50	15.31	14.21	12.60	13.95	—				
11 <i>Leptobrachella fuliginosa</i>	11.58	12.60	11.43	11.82	13.47	15.50	14.79	13.37	13.57	6.20	—			
12 <i>Leptobrachella sola</i>	16.33	16.86	15.50	18.22	17.76	18.80	18.09	17.83	18.80	16.86	14.34	—		
13 <i>Leptobrachella heteropus</i>	15.02	16.47	15.70	16.28	17.15	18.02	17.70	18.02	17.64	16.67	14.92	6.78	—	
14 <i>Leptobrachella gracilis</i>	17.30	18.41	16.47	17.83	16.51	18.02	18.48	17.64	17.44	16.47	16.09	18.22	17.83	—
15 <i>Leptobrachium hasseltii</i>	19.23	19.57	19.38	20.16	20.06	20.54	19.64	20.74	20.93	18.41	19.77	20.93	21.12	18.99

TABLE 3. Measurements of *Leptobrachella*. SVL (mean±SD, in mm) and medians of ratios (R) of other characters relative to SVL, followed by ranges in parentheses. See text for character abbreviations.

	<i>L. korifi</i> sp. nov. Doi Inthanon 1 Female	<i>L. murphyi</i> Doi Inthanon 5 Males	<i>L. sinorensis</i> sp. nov. Mae Hong Son 3 Males	<i>L. cf. minima</i> Doi Suthep 5 Males	<i>L. pelodytoides</i> Myanmar 2 Males
SVL	22.7	22.8±0.6 (22.1–23.6)	26.8±0.3 (26.6–27.1)	28.3±0.5 (27.7–28.9)	26.7 (24.7–28.6)
RHL	40.5	39.4 (37.3–42.0)	38.7 (35.8–39.0)	40.8 (38.9–41.2)	38.7 (38.1–39.3)
RHW	37.4	36.5 (35.6–36.6)	34.6 (33.9–35.6)	36.3 (34.0–36.5)	36.3 (35.3–37.2)
RIND	12.3	12.0 (11.1–12.2)	9.3 (9.0–9.7)	10.2 (9.6–11.2)	10.3 (10.1–10.5)
RIOD	12.3	12.2 (11.4–13.6)	11.4 (10.0–11.7)	11.0 (10.3–12.7)	10.9 (10.5–11.2)
RUEW	9.3	9.5 (9.2–10.9)	9.2 (8.9–9.9)	10.2 (9.3–10.4)	10.3 (10.1–10.5)
RSL	15.9	15.4 (14.8–15.8)	15.0 (14.4–15.1)	14.9 (14.2–16.4)	15.4 (15.4–15.4)
REL	16.3	16.3 (15.1–17.8)	15.9 (15.4–16.8)	16.4 (14.7–17.1)	14.8 (13.8–15.8)
RTD	7.3	7.4 (7.2–8.2)	6.3 (5.5–7.0)	7.0 (6.6–7.3)	6.8 (6.6–6.9)
RT-EL	2.6	2.4 (1.9–3.5)	3.0 (2.3–3.7)	2.7 (2.2–3.4)	4.0
RN-EL	7.1	6.8 (6.2–8.1)	7.2 (6.4–10.0)	7.2 (6.0–8.3)	—
RFL	73.6	71.9 (67.4–73.5)	71.5 (70.7–72.0)	70.2 (68.8–71.1)	—
RLAL	51.5	54.0 (51.3–56.1)	51.3 (50.8–55.1)	51.2 (49.8–53.4)	51.8 (51.0–52.6)
RIPTL	6.9	5.4 (4.8–7.2)	6.2 (5.7–6.4)	5.5 (2.6–5.7)	—
RTL	51.1	53.5 (50.8–54.6)	49.2 (46.9–49.4)	46.7 (46.5–47.0)	49.7 (49.7–49.8)
RFL	48.5	51.1 (47.9–52.6)	45.4 (43.2–46.1)	43.3 (40.6–45.6)	49.9 (49.4–50.3)
RHLL	163.0	177.7 (167.8–179.1)	160.5 (154.9–164.8)	155.0 (148.3–159.0)	165.3 (165.0–165.6)
RIMTL	4.4	3.8 (2.9–4.8)	4.5 (4.4–5.4)	4.2 (3.6–4.8)	5.3
RITOEL	10.0	9.5 (8.9–11.0)	9.7 (9.2–9.9)	8.3 (7.0–8.8)	—

held at right angles to body; tibiotarsal articulation of adpressed limb reaching anterior corner of eye; foot (FL 11.0, 48.5%SVL) slightly shorter than tibia; toe length formula I<II<V<III<IV; toe tips similar to those of finger; webbing confined to bases of toes; toes weakly fringed laterally; subarticular tubercles at base of each toe, distally replaced by elongate, low keratinous dermal ridges that are interrupted at the articulations; inner metatarsal tubercle low, length (IMTL 1.0, 4.4%SVL) about half of first toe (ITOEL 2.2, 10.0%SVL); no outer metatarsal tubercle.

Skin nearly smooth, with tubercles and glandular folds above; a low, supratympanic ridge from eye to axilla; sides more tuberculate than dorsum; chest and abdomen smooth; pectoral gland prominent and very large, femoral gland indistinct and relatively small, at middle of posterior thigh; a pair of larger glands below cloaca; a round, white gland just above axilla; ventrolateral glandular ridges distinct; skin of gular region not modified.

#### Color

In preservative, light brown dorsally on head and body, with clear dark spots: spot on snout, triangle between eyes, and spots on shoulder region continuous; spots on upper part of iliac region present, but triangle on lower part of iliac region absent; dorsal portion of tympanum included in brown mask; black band below canthus and supratympanic fold; lips barred with black; blackish spots on flanks small and scattered, restricted to anterior half; groin without dark spot; limbs marked dorsally with dark brown crossbars, especially wide on lower arm and tibia; elbow and upper arm without dorsal bars; posterior side of thigh dark brown; femoral and circum-cloacal glands forming elongated oval white spots; throat, chest, and abdomen dusted with fine brown network; ventral surfaces of legs dusky, dotted with light brown; color of iris in life not recorded.



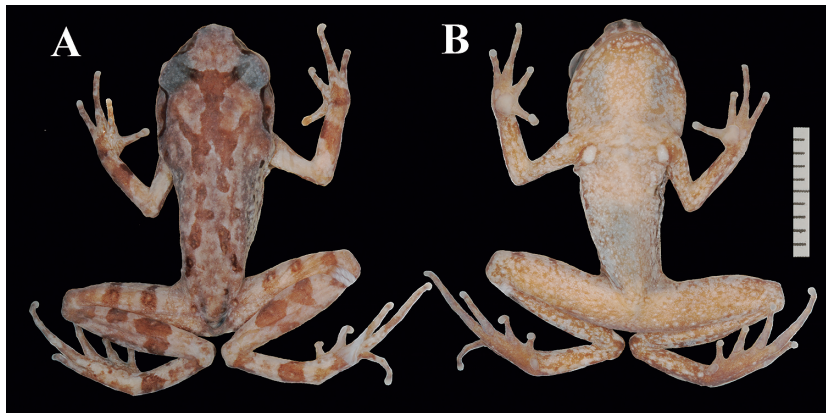


FIG. 2. Dorsal (A) and ventral (B) views of female holotype of *Leptobranchella korifi* sp. nov. (KUHE 19134) in preservative. Scale bar=10 mm.



FIG. 3. Dorsolateral view of a male paratype of *Leptobranchella sinorensis* sp. nov. (KUHE 19809).

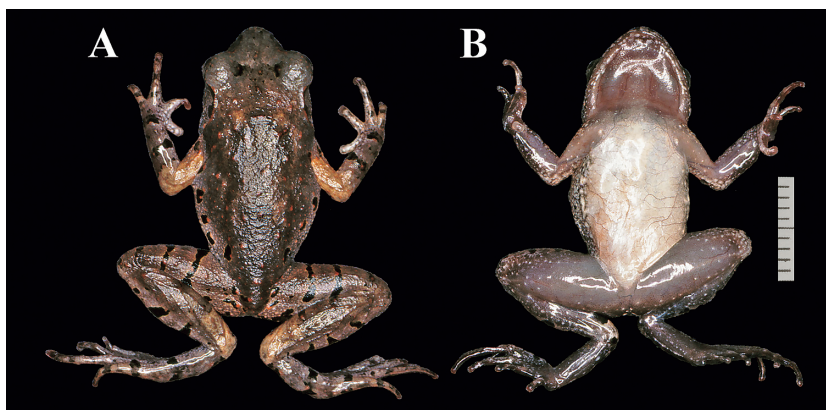


FIG. 4. Dorsal (A) and ventral (B) views of male holotype of *Leptobranchella sinorensis* sp. nov. (KUHE 19816). Scale bar=10 mm.

### Variation

Unknown.

### Range

Known only from the type locality, near the summit of Doi Inthanon, Chiang Mai Province, northern Thailand.

### Natural history

The holotype was found in the daytime, under a stone on a mountain trail in the pine forest, far from streams. No other frog species were observed. In this female, half-developed ova, uniformly cream in color and with a diameter of about 0.54 mm, were seen in ovaries. This indicates that the female was found during the interval between breeding seasons.

### Call characteristics

Calls unknown.

### Comparisons

Compared with female *L. murphyi* (Chen et al., 2021), *L. korifi* sp. nov. is much smaller in SVL (22.7 mm vs. 29.3–32.1 mm in *L. murphyi*). It has larger values relative to SVL than male *L. murphyi* in head width (RHW 37.4% vs. 35.6–36.6%), internarial distance (RIND 12.3% vs. 11.1–12.2%), snout length (RSL15.9% vs. 14.8–15.8%), forelimb length (RFL 73.6% vs. 67.4–73.5%) and smaller value in hindlimb length (RHLL 163.0% vs. 167.8–179.1%). Also, the new species has pectoral gland distinct and very large (vs. small and indistinct in *L. murphyi*), lateral fringes on toes poorly developed (vs. well developed in *L. murphyi*), and ventral surfaces dusted with light brown (vs. abdomen creamy white in *L. murphyi*).

*Leptobranchella korifi* sp. nov. is smaller than *L. sinorensis* sp. nov., described below, in SVL (22.7 mm vs. 26.6–27.1 mm in *L. sinorensis* sp. nov.), but has larger relative values than *L. sinorensis* sp. nov. in head length (RHL 40.5% vs. 35.8–39.0%), head width (RHW 37.4% vs. 33.9–35.6%), internarial distance (RIND 12.3% vs. 9.0–9.7%), interorbital distance (RIOD 12.3% vs. 10.0–11.7%), snout length

(RSL15.9% vs. 14.4–15.1%), tympanum diameter (RTD 7.3% vs. 5.5–7.0%), forelimb length (RFL 73.6% vs. 70.7–72.0%), inner palmer tubercle length (RIPTL 6.9% vs. 5.7–6.4%), tibia length (RTL 51.1% vs. 46.9–49.4%), foot length (RFL 48.5% vs. 43.2–46.1%), and first toe length (RITOE 10.0% vs. 9.2–9.9%). The pectoral gland is distinct and very large (vs. indistinct in *L. sinorensis* sp. nov.). Toe fringes are less developed than in *L. sinorensis* sp. nov. In *L. korifi* sp. nov., ventral surfaces dusted with light brown (vs. abdomen creamy white in *L. sinorensis* sp. nov.). From *L. cf. minima*, *L. korifi* sp. nov. differs in smaller body size (SVL 22.7 mm vs. 36.4 mm in female *L. cf. minima*), with a relatively wider head (RHW 37.4% vs. 34.0–36.5%) and internarial distance (RIND 12.3% vs. 9.6–11.2%), and longer forelimb (RFL 73.6% vs. 68.8–71.1%), inner palmer tubercle (RIPTL 6.9% vs. 2.6–5.7%), tibia (RTL 51.1% vs. 46.5–47.0%), foot (RFL 48.5% vs. 40.6–45.6%), and hindlimb (RHLL 163.0% vs. 148.3–159.0%). Pectoral gland and ventral coloration also differ between them (distinct and very large vs. small, and dusty vs. immaculate white, respectively).

### *Leptobranchella sinorensis* sp. nov. (Figs. 3, 4A, B)

### Diagnosis

A medium-sized form, adult males 26.6–27.1 mm in SVL (n=3); tibiotarsal articulation reaching center of eye; pectoral gland indistinct; ventral side of head and body creamy white.

### Etymology

The specific epithet, *sinorensis*, is from the Greek “seenoro,” meaning border, in reference to the type locality, which lies near the Myanmar border of Thailand.

### Holotype

KUHE 19816, an adult male from Huay Sa Kud, Mae Hong Son Province, northern Thailand (19°13' N, 97°57' E, 270 m asl), collected on 9 September 1995 by Masafumi Matsui.

### Paratypes

KUHE 19809 and 19815, two adult males, data same as the holotype.

### Description of holotype (measurements in mm)

SVL 26.6; habitus moderately stocky; head longer (HL 10.3, 38.7%SVL) than broad (HW 9.2, 34.6%SVL); snout round in dorsal view, rounded in profile, projecting beyond lower jaw; eye length (EL 4.5, 16.8%SVL) larger than snout length (SL 4.0, 15.0%SVL); canthus distinct, very slightly constricted; lore oblique, slightly concave; nostril lateral, below canthus, distinctly closer to tip of snout (NEL 1.7, 6.4%SVL) than to eye; internarial distance (IND 2.6, 9.7%SVL), narrower than interorbital distance (IOD 3.1, 11.7%SVL), latter wider than upper eyelid (UEW 2.4, 9.0%SVL); pineal spot indistinct, but present; tympanum visible, diameter (TD 1.5, 5.5%SVL) one-third that of eye, and separated from eye by two-fifths of tympanic diameter (T-EL 0.6, 2.3%SVL); vomerine teeth absent; tongue notched, without papillae; a median, subgular vocal sac in the mid-ventral line; vocal slits posterior to rictus.

Forelimb long (FLL 18.8, 70.7%SVL, LAL 13.5, 50.8%SVL) and slender; fingers slender, unwebbed, finger length formula I<II<IV<III; tips slightly swollen; inner palmar tubercle large (IPTL 1.7, 6.4%SVL), not extending onto first metacarpal and smaller outer palmar tubercle (OPTL 1.0, 3.8%SVL); subarticular tubercles indistinct; nuptial pads absent.

Hindlimb moderately long (HLL 41.2, 154.9%SVL); tibia relatively long (TL 13.1, 49.2%SVL), heels overlapping when limbs are held at right angles to body; tibiotarsal articulation of adpressed limb reaching center of eye; foot (FL 11.5, 43.2%SVL) shorter than tibia; third toe longer than fifth; toe tips less rounded than those of fingers; webbing confined to bases of toes; toes weakly fringed laterally on the second and third toes; subarticular tubercles obscure under base of toes, distally replaced by low keratinous dermal ridges; inner metatarsal tubercle low, length (IMTL 1.4, 5.4%SVL) about half of first toe (ITOEL

2.6, 9.7%SVL); no outer metatarsal tubercle.

Skin nearly smooth, with a few tubercles of varying sizes above; a low, supratympanic ridge from eye to axilla; sides more tuberculate than dorsum; chest and abdomen smooth; pectoral gland inconspicuous, femoral gland distinct on the rear surface of thigh at distal one-third; a pair of small glands ventral to cloaca; a small, round, white gland just above axilla; ventrolateral glandular ridges distinct but regularly interrupted; skin of gular region not modified.

### Color

In life, light brown dorsally on head and body, with dark spots, partly dotted with pink; spots on snout and shoulder region faint, but triangle between eyes clear on upper eyelids; spots on upper part of iliac region faint, merging with brownish ground color, and triangle on lower part of iliac region clear posteriorly; tympanum largely masked black; supratympanic fold brown spotted with red dorsally, and edged with black ventrally; black band below canthus; lips barred with black; blackish spots on flanks large; groin with dark spot not extending onto thigh; limbs marked dorsally with narrow dark brown crossbars, narrow on dorsal surfaces of thigh; elbow and upper arm without bar dorsally; sides of tibia with black spots, not continuous as bars across dorsal surface; posterior side of thigh dark brown; femoral and circum-cloacal glands forming elongated white oval spots; throat purplish; chest and abdomen milky white; ventral surfaces of legs purplish, dotted with white; iris reddish orange in upper half, copper reticulated with black in lower half. In preservative, the dorsal coloration has faded to olive brown. Eye color has faded and lower halves of iris have become indistinguishable.

### Variation

Individuals of the type series are nearly uniform in morphology, but their coloration is variable. In the two paratypes, dorsal dark spots are clearer than in the holotype. In KUHE 19815, the dark spot on snout, triangle between

eyes, and spots on shoulder region are separated, and the spots on upper part of iliac region and triangle on lower part of iliac region are continuous. Blackish spots on flanks are large, and only the upper portion of tympanum is contained within the black mask. In KUHE 19809, the dark spot on snout is faint, and the triangle between eyes and spots on shoulder region continuous. Spots on upper part of iliac region and triangle on lower part of iliac region are present, and blackish spots on flanks are medium-sized, with pinkish tips (Fig. 4).

#### Range

Known only from the type locality, Huay Sa Kud, 270 m asl, Mae Hong Son Province, northern Thailand, near the Thai-Myanmar border.

#### Natural history

In early September, males were found at night among litter on the floor of a forest far from a stream. Associated species observed were *Xenophrys major* (Boulenger, 1908), *Dutaphrynus melanostictus* (Schneider, 1799), *Kaloula pulchra* Gray, 1831, *Fejervarya limnocharis* (Gravenhorst, 1829), *Occidozyga martensii* (Peters, 1867), *Sylvirana nigrovittata* (Blyth, 1856), and *Amolops marmoratus* (Blyth, 1855).

#### Call characteristics

Calls not known.

#### Comparisons

Comparisons with *L. korifi* sp. nov. are described above, under that species. In males, *L. sinorensis* sp. nov. with SVL of 26.6–27.1 mm is smaller than *L. cf. minima* (27.7–28.9 mm), but for the first toe length relative to SVL, the new species is larger than *L. cf. minima* (R1TOE 9.2–9.9% vs. 7.0–8.8% in *L. cf. minima*). In males, *L. sinorensis* sp. nov. has smaller values relative to SVL than *L. pelodytoides* in internarial distance (RIND 9.0–9.7% vs. 10.1–10.5% in *L. pelodytoides*), upper eyelid width (RUEW 8.9–9.9% vs. 10.1–10.5%), snout length (RSL 14.4–15.1% vs.

15.4%), eye to tympanum length (RE-TL 2.3–3.7% vs. 4.0%), tibia length (RTL 46.9–49.4% vs. 49.7–49.8%), foot length (RFL 43.2–46.1% vs. 49.4–50.3%), and hindlimb length (RHLL 154.9–164.8% vs. 165.0–165.6%). The new species (26.6–27.1 mm) is larger than *L. murphyi* (22.0–24.8 mm) in male SVL and had much less well-developed toe fringes than does *L. murphyi* (Fig. 5).

#### DISCUSSION

Doi Inthanon is the highest mountain in Thailand, with the peak at 2,565 m asl, and *L. korifi* sp. nov. was found 265 m below the peak. At lower elevations (1,350–1,650 m asl.), *L. murphyi* was abundant, and *L. fuliginosa* was also collected (Matsui et al., 2017a). Together with *L. melanoleuca*, *L. fuliginosa* occupies a unique position in *Leptobranchella* phylogeny (Matsui et al., 2017a; Chen et al., 2018), basal to Chinese and Vietnamese clades. Thus, Chen et al. (2018) placed it in their clade of Tenasserim Range species, but such a geographic division is not valid. Chen et al. (2021) also recorded *L. minima* on Doi Inthanon, indicating that there are four congeneric species on this mountain. Other than these *Leptobranchella* species, this high mountain harbors other unique anurans, such as *Ansonia inthanon* (Matsui et al., 1998), *Gracixalus seesom* (Matsui et al., 2015), and possibly *G. carinensis* (Matsui et al., 2017b). Future extensive surveys might reveal presence of more interesting species.

Huay Sa Kud, in Mae Hong Son Province, where *L. sinorensis* sp. nov. was obtained, is very close to the type locality of *L. pelodytoides*, Thao, Kayah State of Myanmar (Capocaccia, 1957). Although records of *L. pelodytoides* frequently occur in the surrounding countries, no reliable data have been obtained after the original collection by Leonardo Fea in the late 1880s. We first expected our Thai specimens to represent that species, but close morphological comparison with museum specimens of *L. pelodytoides* revealed slight morphological differences, as shown in

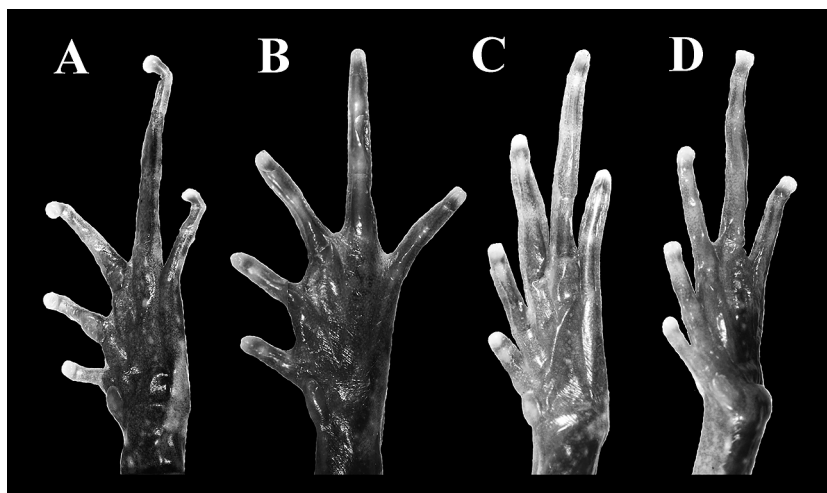


FIG. 5. Ventral view of foot of *Leptobranchella korifi* sp. nov. (A: KUHE 19134, female holotype), *L. sinorensis* sp. nov. (B: KUHE 19816, male holotype), *L. murphyi* (C: KUHE 19221, a male), and *L. cf. minima* (D: KUHE 19200, a male). Not to scale.

our species comparisons. Molecular information on topotypic *L. pelodytoides* is still unavailable. A recent monograph of Myanmar amphibians and reptiles (Zug, 2022: 27) refers to the species, but only with general comments. It is thus desirable in the future to obtain detailed information of *L. pelodytoides* from Myanmar, to clarify its phylogenetic relationships within the genus *Leptobranchella*. Similarly, a male specimen of *L. korifi* sp. nov. and female specimens of *L. sinorensis* sp. nov. have not yet been obtained, and their calls are unknown. Urgent intensive field surveys in the region from northern Thailand to eastern Myanmar must be undertaken immediately, because of ongoing environmental degradation.

From the known phylogenetic relationships, the two new species described herein form a clade with species from Southern China and Vietnam to Indo-Burma and assigned to the former subgenus *Lalos* of the genus *Leptolalax* (Ohler et al., 2011). Such a classification is much more useful and readily understandable than using *Leptobranchella*. We here add some comments about this problem. The small Southeast Asian megophryid frogs, to which

the species treated herein belong, were first known as *Leptobranchium* (Boulenger, 1893), but were later moved to *Megophrys* (Boulenger, 1908). Then they were again placed in *Leptobranchium* as its subgenus *Leptolalax* (Dubois, 1980). It was elevated to a distinct genus *Leptolalax* (Dubois, 1983), which was later split into two subgenera, Bornean *Leptolalax* and continental *Lalos* (Dubois et al., 2010).

A mitochondrial phylogenetic study, however, revealed that *Leptolalax* forms a clade with *Leptobranchella* (Smith, 1925), another genus of more tiny megophryids, mainly from Borneo (Matsui et al., 2017a). Monophyly of *Leptolalax* and *Leptobranchella* with respect to *Leptobranchium* was fully supported, and three monophyletic lineages (Bornean *Leptolalax*, *Leptobranchella*, and continental *Leptolalax*) were recognized in this clade, but their relationships were unresolved. Within the continental clade, the species from the Malay Peninsula formed a sister clade to the clade of the remaining regions, and the species from southern Thailand formed a sister clade to the species from more northern regions. Unfortunately, the study lacked samples from Cambo-

dia and southern Vietnam, which seem to form a distinct clade (Poyarkov et al., 2015; Rowley et al., 2016), because sequences available from GenBank were not suitable for detailed comparisons.

Finally, employing larger samples, including Cambodian and southern Vietnamese ones, Chen et al. (2018) obtained a similar result that *Leptobranchella* is nested within *Leptolalax* and is the sister group of Bornean *Leptolalax*, rendering *Leptolalax* paraphyletic. Chen et al. (2018) recognized six clades (A: Southern China and Indo-Burma, B: Central Highlands, C: Mount Fansipan, D: Tenasserim Range, E: Borneo, and F: Malay Peninsula). For these clades, Chen et al. (2018) adopted a one-genus concept, placing all species into *Leptobranchella*, because they thought such an idea to be the most conservative. However, a four-genera concept, retaining *Leptobranchella*, assigning *Leptolalax* to the Bornean species (Clade E), and erecting new genera for the species from Southern China to the Tenasserim Range (Clades A–D) and for those from Malay Peninsula (Clade F), better reflects phylogenetic relationships.

The one-genus concept of Chen et al. (2018) is now prevalent, but it usually requires notation like “diagnostic characters for species occurring north of the Isthmus of Kra” (Table 4 of Chen et al., 2021). This is highly inconvenient from the view of taxonomic usefulness. Moreover, such an idea strongly contradicts the case in *Megophrys* (sensu lato), including species only slightly more divergent than (about 1.3 times) the species number of *Leptobranchella*, in which molecular clades each of which was originally recognized as a subgenus (Mahony et al., 2017), were later arbitrarily treated as distinct genera (e.g., Deuti et al., 2017; Lyu et al., 2021). In order to solve the generic problem of *Leptobranchella* (sensu lato), confirmation of morphological/ecological synapomorphies for each clade is required.

#### ACKNOWLEDGMENTS

The National Research Council of Thailand and the Royal Forest Department of Thailand kindly permitted MM to conduct the project, and Chulalongkorn University kindly provided all the facilities for conducting the research. SP would like to express gratitude to Chulalongkorn University for providing some research funding. We thank the late J. Nabhitabhata, T. Hikida, H. Ota, and K. Araya for help in the field, and A. Hamidy for laboratory assistance. MM is grateful to C. McCarthy and B. T. Clarke (BM), R. Günther and U. Manthey (ZMB), G. Doria (MSNG), and the late R. F. Inger (FMNH) for allowing him to examine specimens under their care. We thank A. Savitzky and anonymous reviewers for improving an earlier version of the manuscript. This study was supported by grants under The Monbusho International Scientific Research Program (Field Research, Nos. 06041066, 08041144, and 10041166) to MM.

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Accepted: 26 December 2022