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FIRST DESCRIPTIONS OF THE ADULT MALE AND TADPOLES OF *Leptobrachella korifi* MATSUI, PANHA & ETO, 2023 (AMPHIBIA: MEGOPHRYIDAE)

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Abstract

We provide the first identification of male specimens of *Leptobrachella korifi* from its type locality in Doi Inthanon, Thailand, along with a description of its tadpole, using DNA barcoding identification. This account is based on the discovery of male and tadpole specimens of *L. korifi* from Inthanon National Park. Less than 0.1% of the 12S rRNA, tRNA-Val, and 16S rRNA gene sequences from the tadpoles deviate from those of the holotype of *L. korifi* in GenBank and the adult, thereby clarifying the identification. Adults were typically seen at night calling while resting on the dry litter of bushes surrounding streams and waterfalls, where tadpoles of this species were collected. The characters of adult males of this species are: small-sized (SVL 22.8±1.85 mm); dorsal skin smooth; venter dusted with a fine brown network; ventrolateral glands completed; distinct axillary gland, parabrachial gland, and femoral gland; toe webbing with basal and lateral fringes narrow. Tadpole's coloration in life is beige with brown blotches on the body, tail, and fin. The Keratodont Row Formula is 1:3+3/2+2:1.

Keywords: mtDNA phylogeny, morphology, larva, Ang Kha Luang, Megophryidae, Thailand

Introduction

The genus *Leptobrachella* is a diverse group within the family Megophryidae and is widely distributed across Southeast Asia, from northeastern India to the Malay Archipelago. *Leptobrachella* currently contains 106 recognized species (Frost 2025). Members of the genus are notable for their small size and often

cryptic coloration adapted to streamside environments (Ohler *et al.* 2011). Their tadpoles also have unique eel-like bodies, an adaptation to living in gravel beds in stream ecosystems. Currently, thirteen species of *Leptobrachella* are recognized in Thailand (Frost 2025): *L. bourreti* (Dubois, 1983), *L. eos* (Ohler, Wollenberg, Grosjean *et al.*, 2011, *L. fuliginosa* (Matsui,

2006), *L. heteropus* (Boulenger, 1900), *L. korifi* Matsui, Panha & Eto, 2023, *L. melanoleuca* (Matsui, 2006), *L. minima* (Taylor, 1962), *L. murphyi* Chen, Suwannapoom, Wu *et al.*, 2021, *L. pelodytoides* (Boulenger, 1983), *L. sinorensis* Matsui, Panha & Eto, 2023, *L. sola* (Matsui, 2006), *L. ventripunctata* (Fei, Ye & Li, 1990), and *L. zhangyapingi* (Jiang, Yan, Suwannapoom *et al.*, 2013). These species are part of the diverse genus found primarily in mountainous forested areas, often near streams, where they inhabit leaf litter and rocky substrates.

Leptobranchella korifi was described in 2023 based on a single specimen (holotype: KUHE 19134, an adult female) collected near the summit of Doi Inthanon (18°35' N, 98°29' E, 2,300 m a.s.l.), Chom Thong, Chiang Mai Province, Thailand (Matsui *et al.* 2023). The species is currently known only from its type locality. *Leptobranchella korifi* is distinguished from its congeners by a unique combination of morphological features, including its small size and dorsal coloration. Molecular analyses also indicate genetic divergence from other species of *Leptobranchella korifi*, which exemplifies high-altitude endemism in Thailand's montane habitats, the preservation of which are crucial to preserving regional biodiversity (Chen *et al.* 2021). Given its restricted distribution, the habitat of *L. korifi* is vulnerable to threats like deforestation and climate change. However, information on male specimens and tadpoles of *L. korifi* has been entirely lacking. In this study, we provide the first description of male specimens and tadpoles of *L. korifi*.

Tadpoles and adults can be matched thanks to recent developments in molecular techniques, which enable an accurate description of their tadpoles (Grosjean & Inthara 2016, Chunskul *et al.* 2021, Thongproh *et al.* 2023). Morphological and molecular identification of *Leptobranchella* tadpoles has produced accurate descriptions of 22 species (Smith 1917, Inger 1985, Malkmus *et al.* 1999, Grismer *et al.* 2004, Ohler *et al.* 2011, Oberhummer *et al.* 2014, Nguyen *et al.* 2018, 2020, Hou *et al.* 2018, Lyu *et al.* 2020, Le *et al.* 2021, Shi *et al.* 2021, Vassilieva 2021, Haas *et al.* 2022, Duong *et al.* 2023, Rongchapho *et al.* 2024) from 104 species. This indicates that tadpole descriptions are still far from complete for *Leptobranchella*. DNA barcoding and matching tadpoles with adults are essential because tadpoles often lack distinct morphological traits that allow for reliable species-level identification. Without molecular

confirmation of larval-adult associations, there is a risk of misidentification, which can mislead taxonomic decisions and compromise studies in ecology and conservation biology (Haas *et al.* 2022).

In this study, we examined specimens previously assigned to *L. bourreti* and *L. korifi*. New material of *L. korifi* was added to this study, based on molecular analyses and a morphological comparison. Furthermore, this description of *Leptobranchella* tadpoles accounts for less than 25% of the total number of species that have been identified so far, many of which have not been identified using modern genetic techniques. Therefore, the present study aims to describe the morphology of *L. korifi* tadpoles identified through molecular comparisons.

Material and methods

Sampling. We conducted a field survey in Doi Inthanon National Park, Chiang Mai Province, Northern Thailand (Fig. 1).

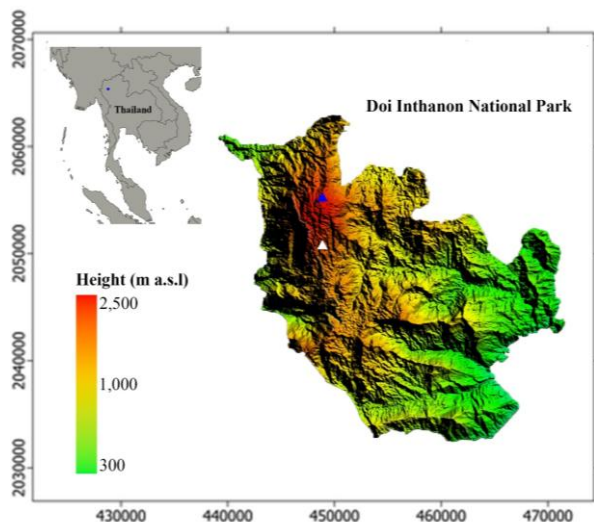


Figure 1. Elevation map of Doi Inthanon National Park, Chiang Mai Province, northern Thailand, showing the collection sites of *L. korifi* in Ang Kha Luang (blue triangle near the mountain peak) and the Kiew Mae Pan nature trail (white triangle).

The adults were euthanized using 1,1,1-Trichloro-2-methyl-2-propanol hemihydrate, and the tadpoles using 70% ethyl alcohol dissolved in water. For molecular analyses, pieces of liver and tail muscle from tadpoles were taken before the fixed specimens were preserved in absolute ethanol. Specimens were fixed in 10% formaldehyde (overnight), and tadpoles fixed in an equal ratio of 10% formaldehyde: 70% ethyl alcohol (1:1) (Inthara *et al.* 2009, Chuaynkern *et al.* 2019, 2023, Chunskul *et al.* 2021, Thongproh

et al. 2023). The research was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, based on the Ethics of Animal Experimentation of the National Research Council of Thailand (reference no. 660201.2.11/310[57]). Specimens were deposited at the Khon Kaen Vertebrate Collection (KKUC), Khon Kaen University, Khon Kaen Province, northeastern Thailand.

Molecular data and phylogenetic analyses:

Total genomic DNA was extracted from tissue samples using the GF-1 Tissue DNA Extraction Kit (Vivantis, Inc., Malaysia) according to the manufacturer's recommendations. ~2,000 base pairs of the 12S rRNA, tRNA-Val, 16S rRNA gene of the mitochondrial genome were amplified and sequenced using the two primers pair sequence of Rongchapho *et al.* (2024) (5'-3') Leptobranchella_12SF (forward) 5'-AAGCCACA CCCCCACGGGTA-3', and Leptobranchella_12SR (reverse) 5'-TTGCCACAGAGACGGGT TAACTC-3', and 16S Primer with sequence (5'-3') Leptobranchella_16SF (forward) 5'-CAGTCT TTAGTATGTGCGACAG-3', and Leptobranchella_16SR (reverse) 5'-CCACAGGGTCTT CTCGTCTTATG-3'. The PCR amplification as described by Dever (2017), using the same primers and modified PCR conditions as follows: 1 cycle initial denaturation at 94°C for 30s, 30 cycles of denaturation at 94°C for 40s, annealing at 62°C for 40s, extension at 68°C for 1 min/kb, and 1 cycle final extension at 68°C for 10 mins. The PCR products were purified and sequenced by U2Bio (Bangkok, Thailand). The DNA sequences were aligned using Seaview version 4. Phylogenies were constructed under Maximum Parsimony (MP) and Bayesian inference (BI). A heuristic search for the most parsimonious tree was performed using PAUP* 4.0a 169 (Swofford 2022) with 1,000 replicates; the TBR (tree bisection-reconnection) branch swapping option was used, and gaps were treated as missing data. The Bayesian analysis parameter model was estimated from the dataset using MrModelTest 2.2 (Nylander 2004). The best-fit nucleotide substitution models for 12S rRNA, tRNA-Val, 16S rRNA genes based on the Akaike information criterion was GTR+I+G (-lnLs=29882.118; p-inv=0.204809; gamma shape=0.628285), respectively. BI was performed using MrBayes 3.2.7 (Ronquist *et al.* 2012). Markov chains were run for 10 million generations; trees were sampled every 1,000 generations, with the first 25% samples discarded as burn-in, resulting in a potential scale reduction

factor of < 0.005. A 50% majority-rule consensus of the sampled trees was constructed to calculate the posterior probabilities of tree nodes. Uncorrected pairwise sequence divergences (p-distance) were calculated in MEGA X (Kumar *et al.* 2018).

Morphological study: Measurements were taken with digital calipers to the nearest 0.1 mm (Mitutoyo Corp, Kawasaki, Japan). Abbreviations used for measurements: SVL=snout-vent length; HW= maximal head width; HL=head length (from the back of the mandible to the tip of snout); MN=distance from the back of the mandible to the nostril; MBE=distance from the back of the mandible to posterior corner of eye; IFE=distance between anterior corners of eyes; IBE=distance between the back of the eyes; FLL=forelimb length (from elbow to base of the outer tubercle); HAL=hand length (from base of outer palmar tubercle to the tip of the toe); TFL=third finger length (from base of first subarticular tubercle); TL=tibia length; FOL=foot length (from base of inner metatarsal tubercle to the tip of the toe); FTL=fourth toe length (from the base of the first subarticular tubercle to the tip of the toe); IN=internarial distance; EN=distance from the front of the eye to the nostril; EL=eye length (from the anterior of eye to posterior of eye); TYD=greatest tympanum diameter; TYE=distance from tympanum to the back of the eye; IUE=minimum distance between upper eyelids; UEW=maximum width of inter upper eyelid; WTF=webbing between third and fourth toe (from the base of the first subarticular tubercle); WFF=webbing between fourth and fifth toe (from the base of the first subarticular tubercle); WI=webbing between third and fourth toe when folded along fourth toe (from the base of the first subarticular tubercle); WII=webbing between fourth and fifth toe when folded along fourth toe (from the base of the first subarticular tubercle); IMT=length of inner metatarsal tubercle; ITL=inner toe length. Webbing; MTTF=distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between third and fourth toe; TFTF=distance from the maximum incurvation of the web between third and fourth toe to the tip of the fourth toe; MTFF=distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between fourth and fifth toe; FFTF=distance from the maximum incurvation of the web between fourth and fifth toe to the tip of fourth toe; FL=femur length

(from vent to knee); SL=distance from front of the eye to tip of the snout; NS=distance from nostril to tip of the snout; TW=width of tibia; TFOl= third of distance from base of tarsus to

tip of toe IV; fd1-fd4=width of pads of finger 1 to 4; fw1-fw4=width of fingers 1 to 4; td1-td5=width of pads of the toes 1 to 5 and tw1-tw5=width of toes 1 to 5.

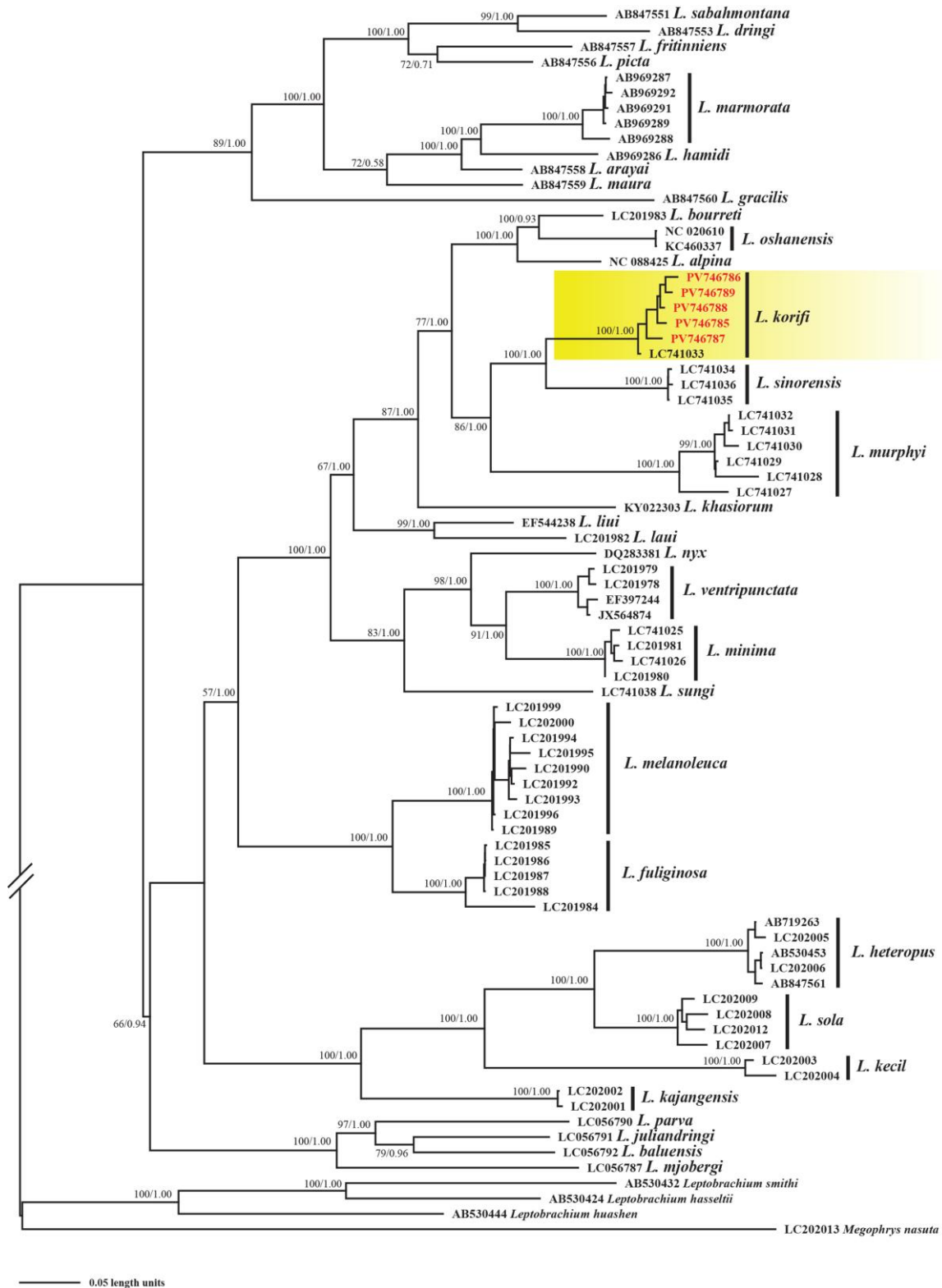


Figure 2. Bayesian Inference and Maximum Parsimony phylogenetic trees of *L. korifi* and other species inferred from 12S rRNA, tVal RNA, 16S rRNA mtDNA gene fragments. Numbers above or below branches represent bootstrap supports for MP inference and Bayesian posterior probability (MP-BS/BPP). Bayesian posterior probabilities (BPP > 0.95), and bootstrap support values (BS > 70%).

Morphological characteristics were examined on preserved tadpoles. Measurements of the head, body, and tail base were recorded to the nearest 0.1 mm using digital calipers and a Nikon C-LEDS stereo microscope with an ocular scale. Developmental stages were determined following Gosner (1960), and terminology for external morphology follows Grosjean (2005). Measurements include BH=body height; BL=body length; BW=maximum body width; ED=maximum eye diameter; LF=maximum lower fin height; MTH=maximum tail height; ND=nostril diameter; NN=internarial distance; NP=naro-pupillar distance; PP=interpupillar distance; RN=rostronarial distance; SD=spiracle diameter; SU=distance from tip of snout-insertion of upper tail fin, TAL=tail length; TL=total length; TMH=maximum tail muscle height; TMW=tail muscle width at the end of body; UF=maximum upper fin height; AL=anterior labium; PL=posterior labium; and ODW=oral disc width. Tadpole photographs were taken using a digital camera and improved using Adobe® Photoshop version 25.11.0 and photographs of tadpole were taken using Nikon D7500 with 40.0 mm f/2.8 and Nikon SMZ745T stereomicroscope with NIS-Elements D Package Software.

The lateral lines identified in this study include the canthoinfraorbital line (CIL), supraorbital line (SOL), infraorbital line (IOL), lateral line (LL), dorsal line (DL), dorsolateral line (DLL), angular line (AL), medial line (ML), ventrolateral line (VLL), and ventral line (VL). The SOL, IOL, DL, AL, ML, and VL are based on Quinzo *et al.* (2006), while the SOL and VLL follow Rongchapho *et al.* (2024). The LL and DLL are newly named in this study.

Results

Phylogenetic analysis and genetic divergence.

Bayesian inference (BI) and maximum parsimony (MP) analyses resulted in identical topologies with no differences in the branching pattern among taxa (Fig. 2). However, support values for some nodes varied slightly between the two methods. Obtained mtDNA fragments consisted of 2,184 bp. The matrilineal genealogy of *Leptobranchella* based on 12S rRNA, tVal RNA, 16S rRNA genes contained 68 individuals with ~2,000 bp (Sup. Table 1). The most effective parsimony-informative substitution model was chosen. Two different optimality criteria yielded identical relationships in the phylogenetic analyses employed. As shown in

the BI tree, only MP-BS $\geq 70\%$ and BPP $\geq 95\%$ are considered as significant support. Matrilineal genealogy was discovered by phylogenetic analysis of the 12S rRNA, tVal RNA, 16S rRNA sequences (Fig. 2). The pairwise uncorrected p-distances support this placement, revealing a value of 0.00 (12S rRNA) - 0.02 (16S rRNA) between simple of *L. korifi* this study, which signifies genetic identity, and values of 0.00 (12S rRNA) - 0.01 (16S rRNA) when compared to the holotype of *L. korifi* sequences, indicating low intraspecific variation (Sup. Table 2).

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(Figs. 3–7; Sup. Table 3)

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Holotype. KUHE 19134 (by original designation) from near the summit of Doi Inthanon, Chom Thong, Chiang Mai Province, Thailand (18°35' N, 98°29' E, 2,300 m a.s.l.).

Materials examined. PR 03323–24 (two adult males), collected by Y. Chuaynkern, P. Rongchapho, N. Paitafong, P. Tongsuk, and S. Soonthong on 24 September 2023 from Ang Kha Luang (18°35'16.2"N, 98°28'53.7"E, 2,513 m a.s.l.), Doi Inthanon National Park, Chiang Mai Province, northern Thailand; PR 03501–02 (two adult males), PR 03504 (one adult male), collected by P. Rongchapho and N. Khagjitmathee between 22 and 23 November 2023 from Kiew Mae Pan Nature Trail (18°33'29.6"N, 98°28'52.6"E, 2,234 m a.s.l.), Doi Inthanon National Park, Chiang Mai Province, northern Thailand; THNHM 09480 (one adult male), THNHM 09482–85 (four adult males), collected by T. Chan-ard and S. Makchai on 19 November 2005, from Huay Mae Rake, Doi Inthanon National Park, Chiang Mai Province, northern Thailand.

Extended diagnosis. (1) Size small, 22.76±1.85 mm in males (20.2–26.5 mm; $n=10$), 26.27±0.41 mm in females (27.8–28.8 mm; $n=3$); (2) tibiotarsal articulation reaching anterior corner of eye when hindlimb adpressed along body; (3) tympanum distinct, supratympanic fold present and almost dark tympanic mask; (4) dorsal skin smooth; (5) ventral side dusted with fine brown network; (6) dorsal surface with distinct markings: a reverse-triangle marking between eyes, a butterfly on the shoulder regions, and triangle marking on lower part of iliac

region; (7) ventrolateral glands completed; (8) body glands distinct: a large axillary gland, a suprabrachal gland, a large femoral gland and postcloacal glands alongside of vent (9) flank with distinct black small spots; (10) fingers without webbing and fringes; (11) toe webbing at bases and lateral fringes narrow; (12) iris bicolored, usually typically yellowish orange with black reticular in upper half, white with black reticular in lower half; (13) vocal sac present, internal, paired.

Description of the adult male. Based on PR 03323. (A) *Size and general aspect.* (1) Size small (SVL 21.5 mm), body rather stout.

(B) *Head.* (2) Head longer than wide (HL 7.7 mm; HW 6.6 mm; MN 6.7 mm; MFE 5.2 mm; MBE 2.8 mm), flat above. (3) Snout rounded in dorsal view, slightly projecting beyond lower jaw, its length longer than horizontal diameter of eye (SL 3.0 mm, EL 2.5 mm). (4) Canthus rostralis distinct, loreal region obtuse in cross-section, concave. (5) Interorbital space flat, broader than upper eyelid (IUE 2.8 mm; UEW 2.0 mm) and internarial distance (IN 2.1 mm); distance between anterior margins of orbits 1.8 times greater than distance between posterior margins of orbits (IFE 3.6 mm; IBE 6.3 mm). (6) Nostril round with flap of skin laterally, closer to tip of snout than to eye (NS 1.3 mm; EN 1.7 mm). (7) Pupil oval, horizontal. (8) Tympanum (TYD 1.2 mm) distinct, rounded, 48% of tympanum diameter; tympanum-eye distance (TYE 0.9 mm) 22% of tympanum diameter. (11) Tongue large, cordate, emarginated, bearing no median lingual process; lower jaw without tooth-like projections.

(C) *Forelimbs.* (12) Arm short and thin; forearm shorter than hand (FLL 4.8 mm; HAL 5.6 mm). (13) Fingers I, II, and IV short and thin, finger III long and thin (TFL 2.7 mm). (14) Relative finger lengths: $I < II < IV < III$. (15) Tips of fingers blunt, enlarged, without grooves, narrower than finger width (fd1 0.3 mm, fw1 0.2 mm; fd2 0.3 mm, fw2 0.3 mm; fd3 0.3 mm, fw3 0.3 mm; fd4 0.3 mm, fw4 0.2 mm). (16) Dermal fringe on inner and outer sides of fingers absent; fingers without webbing. (17) Subarticular tubercles absent. (18) Prepollex distinct, rounded; two distinct palmar tubercles, inner larger than outer; no supernumerary tubercle.

(D) *Hindlimbs.* (19) Hind limbs long, heels overlapping when limbs are folded at right angles to body; tibia 6.4 times longer than wide (TL 10.3 mm; TW 1.6 mm), slightly shorter than thigh (FL 10.5 mm) and shorter than the distance

from base of internal metatarsal tubercle to tip of toe IV (FOL 11.4 mm). (20) Toes long and thin, toe IV 3.3 times distance from base of tarsus to tip of toe IV (FTL 4.6 mm; TFOF 15.2 mm). (21) Relative toe lengths: $I < II < V < III < IV$. (22) Tips of all toes rounded, slightly enlarged; disc absent on toes I-V, without grooves, narrower than toe width (td1 0.2 mm, tw1 0.3 mm; td2 0.2 mm, tw2 0.3 mm; td3 0.3 mm, tw3 0.2 mm; td4 0.2 mm, tw4 0.2 mm; td5 0.3 mm, tw5 0.2 mm). (23) Webbing rudimentary, formula: $I2-2\frac{1}{2}II2-3\frac{1}{2}III3-4IV4-3\frac{1}{2}V$ (WTF 1.2 mm, WFF 0.9 mm; WI 1.2 mm, WII 0.9 mm; MTTF 3.8 mm, MTFF 3.3 mm, TFTF 5.9 mm, FFTF 5.9 mm). (24) Toe V without dermal fringe. (25) Subarticular tubercle absent. (26) Inner metatarsal tubercle distinct, oblong, elongate, its length 0.38 times in length of toe I (IMT 0.5 mm; ITL 1.3 mm). (27) Tarsal folds absent. (28) Outer metatarsal tubercle absent; supernumerary tubercles and tarsal tubercles absent.

(E) *Skin.* (29) Skin on head, body, and dorsal surface of limbs smooth. (30) Dorso-lateral folds absent; supratympanic fold present and prominent, from behind the eye to the shoulders. (31) Dorsal surface of limbs and tarsi smooth; thigh and shank with glandular warts. (32) Throat, chest, and belly smooth. (33) Macroglans present: lateroventral gland present, formed as a completed continuous series of glandular fold; femoral gland present, very large; axillary glands present, vary large; suprabrachial gland present, small; femoral gland present, very large.

(F) *Coloration.* In preservation, (34) Dorsal and lateral surface of head and body dark brown, dorsum pattern indistinct; loreal and tympanic region black; tympanum entirely black. (35) Dorsal surface of forelimb light brown; forearm, thigh, shank and foot dark brown with black cross bars; posterior part of thigh brown, with finely black spots and black crossbars. (36) Ventral surface head, body and limbs creamy white with marbling; throat margin marbled; chest and belly creamy white with dark brown spots; under thigh dark brown with creamy white spots; webbing dark brown; macroglans creamy white.

In life, (37) Dorsal and lateral surface of head and body olive brown, back without spots, a darker brown triangle pattern present behind eyes, a butterfly marking on shoulder, a triangle marking on lower iliac region, loreal and tympanic regions black; tympanum entirely black. (35) Dorsal surface of forelimb yellowish

creamy brown; forearm, thigh, shank, and foot brown with black crossbars. (36) Ventral surface of head, body, limbs, chin, and throat pinkish with marbling; chest and belly yellow-brown with dark brown spots; thigh brown with small yellow-brown speckles; webbing black; macroglands creamy white; iris bicolored, upper half orange with black reticulations, lower half silver-white with black reticulations.

(G) Male secondary characters. (37) Nuptial pads absent. (38) Inner openings of vocal sacs present, slit-like, located laterally and posteriorly in the floor of the mouth.

Variation. Adult males are smaller than females (SVL 22.8 ± 1.85 mm versus 28.2 ± 0.42 mm). Adult males have a vocal sac, while females have a stocky body and eggs in the abdomen. Dorsal coloration and dark pattern vary in quality among them, but all are gray or brown, with variations in the number of spots. The female holotype smaller than the newly collected females for this study (22.7 mm versus 28.3 ± 0.41 mm); the head of the holotype is smaller than those in this study (HW 8.5 mm versus 9.7 ± 0.14 mm, HL 9.2 mm versus 9.4 ± 0.61 mm) (Matsui *et al.* 2023).

Tadpole description. *Materials examined of tadpoles.* PR 03451.1–5 (5 tadpoles, stages 26–34), collected by Y. Chuaynkern, P. Rongchapho, N. Paitafong, P. Tongasuk, and S. Soonthong on 24 September 2023 from Ang Kha Luang ($18^{\circ}35'16.2''\text{N}$, $98^{\circ}28'53.7''\text{E}$, 2,513 m a.s.l.), Doi Inthanon National Park, Chiang Mai Province, northern Thailand; PR 03503.1–2 (2 tadpoles, stages 27–35), collected by P. Rongchapho and N. Khagjitmathee between 23 November 2023 from Kiew Mae Pan Nature Trail ($18^{\circ}33'29.6''\text{N}$, $98^{\circ}28'52.6''\text{E}$, 2,234 m a.s.l.), Doi Inthanon National Park, Chiang Mai Province, northern Thailand.

Diagnosis. (1) Body elongates, size medium 13.0 ± 1.60 (BL 11.1–15.4 mm; $n=7$, stage 26–35); (2) pineal ocelli absent; (3) nares round with opening and rimmed with four small lobes; (4) spiracle sinistral; (5) oral disc positioned ventrally, directed anteroventrally; (6) Keratodont Row Formula (KRF) $1:(3+3)/(2+2):1$.

Description. The description of the tadpole is based on a voucher specimen PR 03451.1 (stage 34; BL 15.4 mm). The tail description, which is missing in the DNA voucher, is based on specimen PR 03503.1 (stage 35L BL 15.2 mm). All measurements (in mm) and counts are provided (Sup. Table 4). In dorsal view (Fig. 5A), body oval elongate, snout rounded, two

sizable lateral lymphatic sacs from spiracle to end of body. In lateral view (Fig. 5B), body oval, slightly depressed, BW 157.14% of BH, snout oval. Eyes slightly small, ED 5.84% of BL, bulging and not visible in ventral view (Fig. 5D), positioned dorsolaterally, directed anterolaterally. Pineal ocelli absent. Nare moderately sized, rounded with an oval opening, rimmed with four small lobes (Fig. 5F), positioned dorsolateral, directed anterolaterally, closer to snout than to pupils, RN 92.86% of NP; NN 21.21% of PP. Spiracle sinistral, flattened tubular, small (Fig. 5G), positioned laterally, and situated at dorsolaterally, SD 3.25% of BL; opening at closed against body wall, caudal myotomes and hind limb insertion, oriented posterodorsally. Tail musculature strong; TMH 80.85% of BH and 54.29% of MTH, TMW 45.59% of BW, gradually tapering, reaching tail tip. Tail fins moderately sized; UF 28.57% of MTH, upper fin extending onto body, SU 93.74% of BL, slightly convex; lower fin extending onto body, convex; MTH 148.94% of BH, tail tip acute angle. Cloacal tube large, approximately conical, medial, entirely attached to lower fin, opening medial, posteriorly directed.

Oral disc (Fig. 5E). Oral disc moderately sized, positioned ventrally and directed anteroventrally, not emarginated laterally, ODW 22.08% of BL and 38.64% of BW, Marginal papillae of lower labium as of upper labium, cone, decreasing in size towards central, then increasing, absent submarginal papillae. The KRF of $1:(3+3)/(2+2):1$, $A3 > A2 > A4 > A1$, A1 very short, A2 shortly interrupted in the middle, A3 and A4 separated by the upper beak; $P1 > P2 > P3$; A3 short, half of P1 interrupted in the middle, P1 and P2 separated by the upper beak.

There are nine pairs of lateral lines (Fig. 6), all lines visible in dorsal and lateral views. Canthoinfraorbital line paired, 18/18 openings, extending forward from the anterior nostril and curving above it to the infraorbital. Supraorbital line paired, 6/7 openings, running from anterior to posterior margins of eye. Infraorbital line paired, 22/22 openings, running from snout to angular line. Dorsal line paired, 30/31 openings, running from posterior eye along the dorsum of body until tip of the tail, attached to the upper fins, but this tadpole has an incomplete tail on the right side. Dorsolateral line paired, 57/59 openings, running from posterior eye along the dorsum of body until tip of the tail, insert

Plate 20



Figure 3. An adult male of *Leptobrachella korifi* in preservative (PR 03323): (A) dorsal, (B) ventral, (C) lateral (right side) views of the body, left side of the (D) hand and (E) foot.

Plate 21

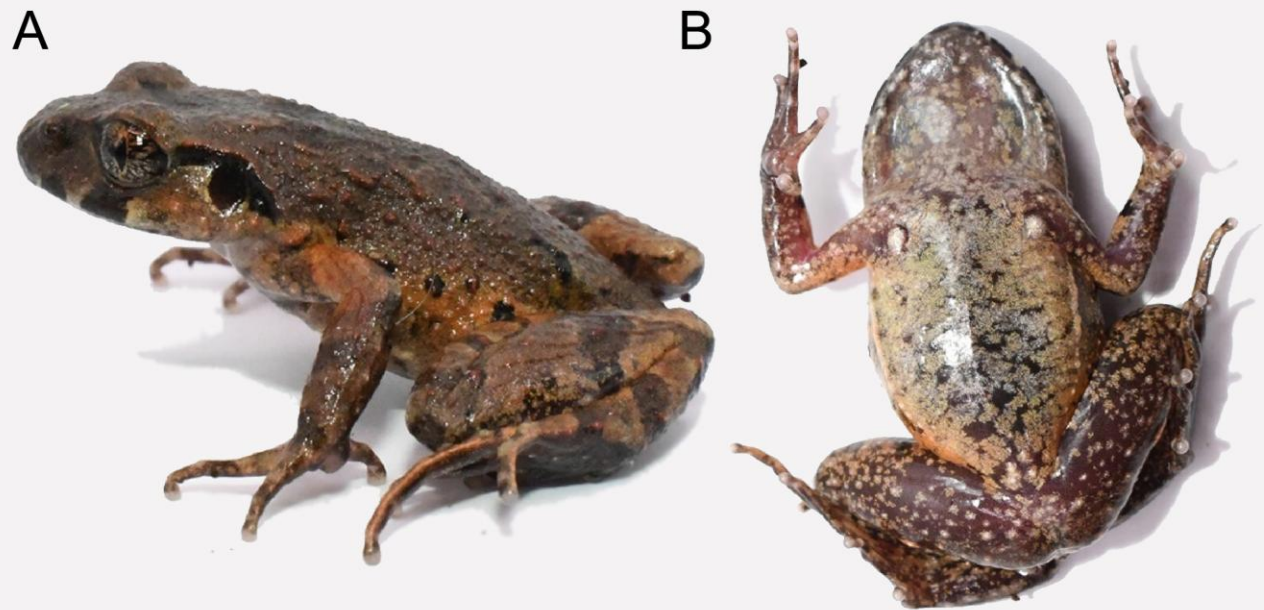


Figure 4. An adult male of *Leptobrachella korifi* in life (PR 03501): (A) dorsolateral and (B) ventral views

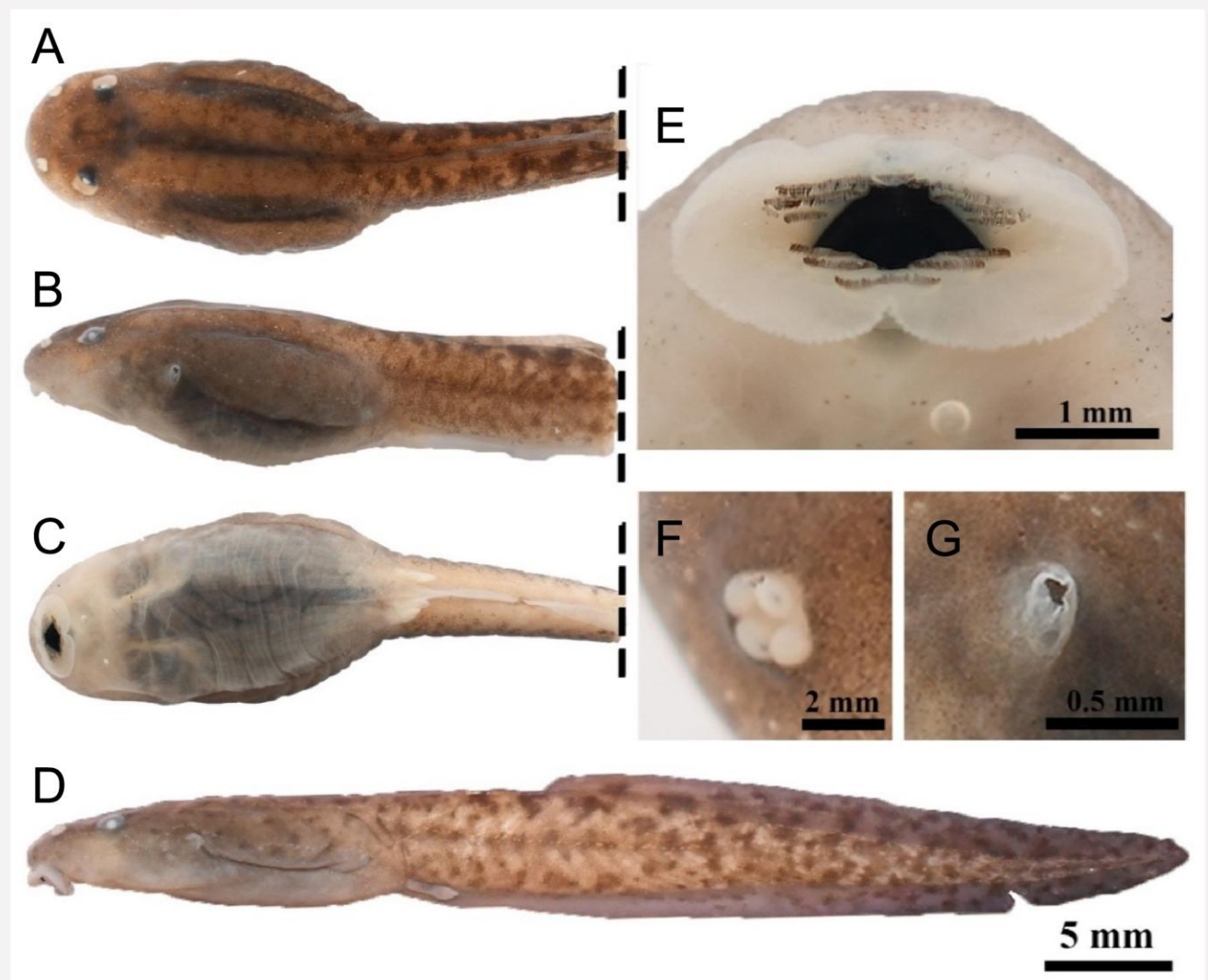


Figure 5. The tadpole of *Leptobrachella korifi* in preservatives (PR 03451.1; stage 34): (A) dorsal, (B) lateral, (C) ventral views of the anterior body; (PR 03504.1, stage 35): (D) lateral view of full body, (E) oral disc, (F) nasal, and (G) spiracle

medium of tail muscle, but this tadpole has an incomplete tail on the right side. Angular line single, 18/18 openings, running from the posterior eye downward across the lateral. Medial line paired, 31/31 openings, running from anterior and upward spiracle (left side) to posterior end of body, on lateral lymphatic sacs. Ventral line paired, 45/45 openings, running forward from the posterior end of the body along the belly to the chest, under lateral lymphatic sacs.

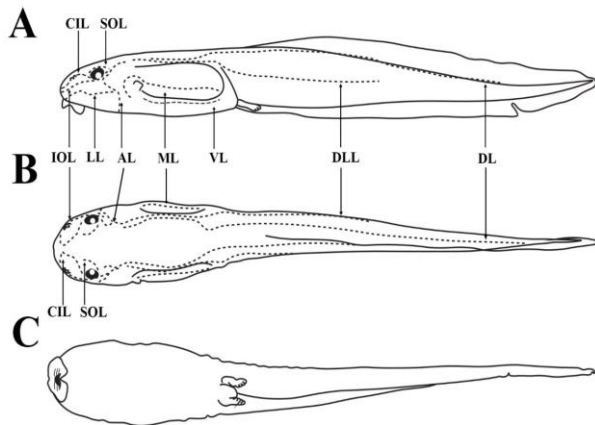


Figure 6. Lateral line system of *Leptobranchella korifi* tadpole: (A) lateral, (B) dorsal, and (C) ventral views.

Coloration. In preservative (Figs. 5A-C), dorsal and lateral surfaces of body dark brown with light brown spots, throat brown with light brown spots, chest dark gray. Tail musculature gray with light brown spots. Fins creamy transparent with less light brown spots.

In life (Fig. 7), dorsal and lateral surfaces of body brown with creamy spots, throat brown with creamy spots, chest gray. Tail musculature gray with creamy spots. Fins creamy transparent with less creamy spots.

Variation. In all, seven tadpoles from two localities were employed in this investigation. The four developmental stages were used to measure the variance among these tadpoles, and they were distributed as follows: 26 (12.38 ± 0.78), 27 (11.2), 34 (15.4), and 35 (15.2) of BL (mm). The KRF of all stages is 1:3+3/2+2:1. The differentiation of lateral lines was unaffected by tadpole development. Each lateral line's number of apertures differed across specimens and between the left and right sides of the same lateral line. The canthoinfraorbital line had 12–18 openings, the supraorbital line contained 5–9 openings, the infraorbital line contained 20–22 openings, the angular line had 15–19 openings, the medial line had 19–33

openings, the ventral line had 20–33 openings, the dorsal line had 30–87 openings, and the dorsolateral line had 56–59 openings. Sup. Table 3 contains measurements, lateral line values, KRF, and meristic counts for every stage that is accessible.

Distribution. Doi Inthanon, Chaing Mai, Thailand (Fig. 1).

Natural history. Specimens of *L. korifi* were collected near the summit of Doi Inthanon, Doi Inthanon National Park, Chiang Mai Province, northern Thailand, in the Thong Chai Range, at elevations ranging from 2,200 to 2,513 m a.s.l. Adult males were observed sitting on litter and leaves, calling at night, near streams and waterfalls along mountain trails in the hill evergreen forest (Fig. 8). Specimens were collected between August and November, with calls audible along the streams during this period.

Discussion

The record of *Leptobranchella bouretti* in the Ang Kha region of Doi Inthanon was mentioned by Chan-ard (2003) without precise evidence. Therefore, *L. bouretti* sensu Chan-ard (2003) may be *L. korifi* or maybe both species occur sympatrically in the region. Matsui *et al.* (2023) subsequently described *L. korifi* based on the holotype from this locality. In this study, we compared the newly collected females with the holotype of *L. korifi* and found a the difference in body size: SVL 22.7 ($n=1$) mm versus 26.27 ± 0.41 mm (27.8–28.8 mm) (Matsui *et al.* 2023). Both species, *Leptobranchella korifi* and *L. murphyi*, are found sympatrically in the Doi Inthanon region, however, *L. korifi* has been recorded at elevations of 2,000–2,513 m a.s.l. (red zone in Fig. 1), while *L. murphyi* has been found at 500–1,200 m a.s.l. (green-yellow zone in Fig. 1), which no overlap in altitudinal range (Chen *et al.* 2021). Males of *Leptobranchella korifi* are smaller in size (SVL 20.2–26.5 mm) compared to those of *L. murphyi* (23.2–24.9 mm). Additionally, *L. korifi* has large and conspicuous pectoral glands, whereas those of *L. murphyi* are small and indistinct. The lateral fringes of the toes are less developed in *L. murphyi*, and the ventral surface is light brown dust, in contrast to the creamy white abdomen of *L. murphyi* (Chen *et al.* 2021).

Currently, comparing the tadpoles of different *Leptobranchella* species is challenging due to the often-vague quality of published descriptions. However, several distinctive features set the

Plate 22



Figure 7. A tadpole of *Leptobranchella korifi* in life (PR 03504.1): (A) lateral and (B) ventral views of the body



Figure 8. The habitat of *Leptobranchella korifi* were collected in Doi Inthanon National Park: (A) Kiew Mae Pan nature trail and (B) Ang Kha Luang

tadpoles of *L. korifi* apart from all other known *Leptobrachella* tadpoles (Sup. Table 4). The lateral line system in *L. korifi* tadpoles, observed from stages 26 to 35, displays significant variability both between individuals and between the left and right sides of the same tadpole. The number of openings varies among different lateral lines, but the ventral line consistently has 18 openings across all tadpoles.

The lateral line system in *L. korifi* tadpoles is notably more complex than that of *L. melanoleuca*. While *L. melanoleuca* possesses six lateral lines, including the canthoinfraorbital line (CIL), supraorbital line (SOL), dorsal Line (DL), angular Line (AL), medial line (ML), and ventrolateral line (VLL), *L. korifi* exhibits nine lateral lines. The infraorbital line (IOL), lateral line (LL), dorsolateral line (DLL), and ventral line (VL) are observed in *L. korifi*, while only ventrolateral line (VLL) is found in *L. melanoleuca*. This distinction highlights the greater complexity of the lateral line system in *L. korifi*, which may reflect differences in their ecological adaptations or sensory needs. Variation in the number of dorsal and dorsolateral lines, independent of the tadpoles' developmental stage. These structures likely serve critical sensory functions throughout development (Quinzio & Fabrezi 2006, Quinzio *et al.* 2014). However, the exact timing of lateral line formation and disappearance remains unknown due to insufficient data, underscoring the need for further research to elucidate their developmental timeline and functional significance.

Our analysis highlights an underestimation of potential biodiversity and a lack of understanding of amphibian taxonomy in Thailand. Many species are challenging to identify due to their wide-ranging movements away from breeding sites or their hibernation during the non-breeding season, making tadpoles crucial for improving estimates of anuran diversity (McDiarmid & Altig 1999). Tadpole descriptions are therefore essential for understanding the ecological needs and natural history of different anuran species (Le *et al.* 2023). However, there is limited knowledge about aspects such as actual distribution in Doi Inthanon, species reproduction, high-level molecular studies, and their specific location. Further research is needed to address these gaps.

Author contributions

All the authors contributed equally.

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Research permits

Permission issued by the Department of National Park, Wildlife & Plant Conservation (No. 0907.4/1313) to conduct research in the protected area, and specimen collections.

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Supplemental data

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