

A New Caruncle-bearing *Limnnectes* (Anura: Dicroglossidae) from the Phu Phan Mountain Range in Northeastern Thailand

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ABSTRACT.— We describe a new species of dicroglossid frog in the genus *Limnnectes* from the Phu Phan Mountain Range, Sakon Nakhon Province, northeastern Thailand. Molecular phylogenetic analyses, based on the mitochondrial 16S ribosomal RNA gene, revealed that *Limnnectes sirindhornae* Rujirawan, Yodthong & Aowphol, sp. nov. is closely related to *L. lauhachindai* but has mean uncorrected pairwise genetic divergences of 4.25%. The new species can be distinguished from its congeners by having the combination of SVL 36.7–44.7 mm (n = 8) in adult males, 40.2–45.6 mm (n = 8) in adult females; adult males with hypertrophied heads; adult males with interorbital caruncle weakly developed, low-profiled, U-shaped, without free posterior margin; two enlarged odontoid processes on anterior margin of lower jaw, larger in males than in females; vocal sac opening in males absent; and yellowish coloration in life on ventral surfaces absent.

KEYWORDS: phylogeny, morphology, fanged frog, Indochina, Khorat Plateau

INTRODUCTION

There are currently 102 recognized species of dicroglossid frogs in the genus *Limnnectes* Fitzinger, 1843 (Frost, 2024). The genus is widely distributed from eastern and southern China, south and eastward through Indochina and to the Sunda Islands (Frost, 2024). Adult males of six species in this genus exhibit a swollen or cap-like structure (caruncle; sensu Lambert et al., 2014) on the top of their head: *L. dabanus* (Smith, 1922), *L. gyldenstolpei* (Andersson, 1916), *L. lauhachindai* Aowphol, Rujirawan, Taksintum, Chuaynkern & Stuart, 2015, *L. macrognathus* (Boulenger, 1917), *L. plicatellus* (Stoliczka, 1873) and *L. savan* Phimmachak, Richards, Sivongxay, Seateun, Chuaynkern, Makchai, Som & Stuart, 2019. In Thailand, two caruncle-bearing species were discovered from the Thai-Laos border region in the past decade: *L. lauhachindai* and *L. savan* (Aowphol et al., 2015; Phimmachak et al., 2019). The caruncles of these species are characterized by a low-profile, U-shaped structure with a free posterior edge in *L. lauhachindai*, and a low-profile swelling without a free posterior margin, with the highest point located between the eyes, in *L. savan* (Aowphol et al., 2015; Phimmachak et al., 2019).

During recent herpetological surveys in 2023–2024, a series of *Limnnectes* specimens were collected from the Phu Phan Mountain Range, Sakon Nakhon Province, northeastern Thailand. These specimens generally resembled *L. lauhachindai* from Ubon Ratchathani Province, northeastern Thailand. The taxonomic

status of the Sakon Nakhon specimens was investigated using mitochondrial DNA and morphological analyses. The datasets corroborated differences in the Sakon Nakhon specimens from *L. lauhachindai* and its congeners. Herein, we describe this population as a new species.

MATERIALS AND METHODS

Specimen sampling

Limnnectes specimens were collected by hand from Rai Subdistrict, Phanna Nihom District, Sakon Nakhon Province, northeastern Thailand in October 2023 and January 2024 (Fig. 1). Geographical coordinates and elevation were recorded by Garmin GPS MAP 64s (Garmin Ltd., USA). A Kestrel 400 Weather Meter (Nielsen-Kellerman Co., USA) was used to measure the ambient temperature and humidity. Captured specimens were humanely euthanised using tricaine methanesulfonate (MS-222) (Simmons, 2015). Liver tissues were immediately removed from euthanised individuals, preserved in 95% ethanol and stored at -20 °C for molecular analysis. Euthanised specimens were fixed in 10% formalin and later transferred to 70% ethanol for permanent storage. Specimens and tissues were deposited in the herpetological collection of the Zoological Museum, Kasetsart University, Thailand (ZMKU).

DNA extraction and PCR amplification

We extracted genomic DNA from liver tissue of 13 *Limnnectes* samples from Sakon Nakhon Province

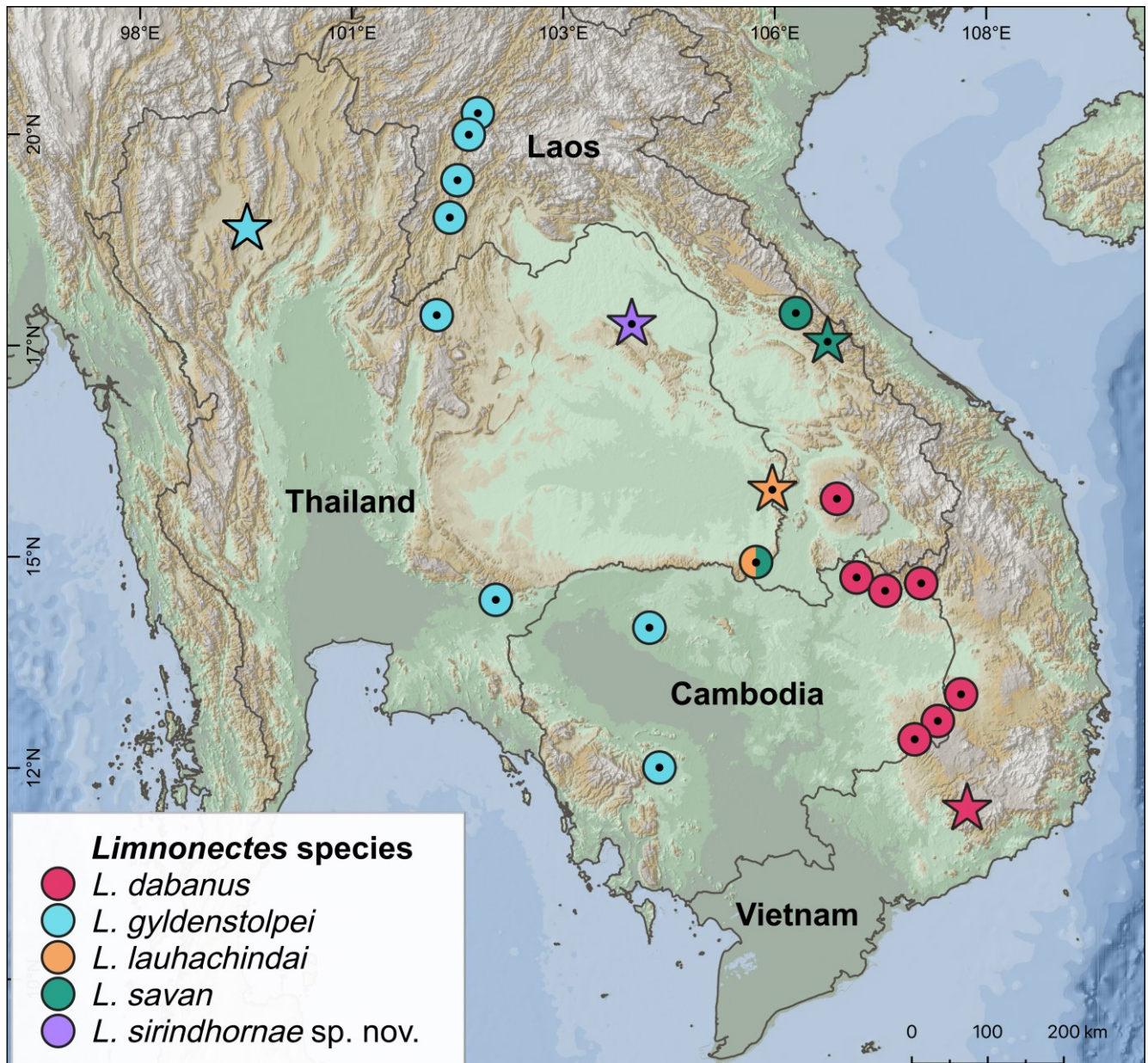


FIGURE 1. Map illustrating localities of *Limnonectes sirindhornae* sp. nov. (purple star) at Rai Subdistrict, Phanna Nikom District, Sakon Nakhon Province, Thailand, and the related species *L. dabanus*, *L. gyldenstolpei*, *L. lauhachindai*, and *L. savan*, used in this study. Key: star = type locality; circle = additional locality; black dot = sample used in molecular analyses.

(Table 1) using the DNeasy (Qiagen, Germany) Blood and Tissue Kit according to manufacturer's protocol. A partial fragment (~520 base pairs) of the mitochondrial 16S ribosomal RNA (*16S*) gene was amplified by the polymerase chain reaction (PCR) using the light strand primer 16Sar (5'-CGCCTGTTTATCAAAAACAT-3') and heavy strand primer 16Sbr (5'-CCGGTCTGAAC TCAGATCACGT-3') (Palumbi, 1996). PCR conditions were as follows: denaturation at 95 °C for 5 min, followed by a second denaturation at 95 °C for 45 s, annealing at 56 °C for 30 s, followed by a cycle extension

at 72 °C for 1 min, for 35 cycles with a final extension at 72 °C for 10 min. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Germany). PCR products were sequenced in both forward and reverse directions using the same amplifying primers at Biobasic Asia Inc. (Singapore) on an ABI 3730XL automatic sequencer (Applied Biosystems, CA, USA). Bidirectional sequences were visually checked and edited in Geneious Prime 2025 (Biomatters, Ltd, Auckland, New Zealand). All new sequences were deposited in GenBank under accession numbers PV939348 to PV939360 (Table 1).

TABLE 1. Samples used in the molecular analyses, including GenBank accession number, voucher number and locality.

Species	GenBank No.	Voucher	Locality	Reference
<i>Fejervarya limnocharis</i>	NC_005055	None	China, Yancheng	Liu et al. (2005)
<i>Quasipaa spinosa</i>	NC_013270	None	China, Zhejiang, Jinhua	Zhou et al. (2009)
<i>Limnonectes bagoensis</i>	MZ578030	HML-030	Myanmar, Bago Yoma	Köhler et al. (2021)
<i>L. bagoensis</i>	MZ578027	SMF 106038	Myanmar, Bago Yoma	Köhler et al. (2021)
<i>L. bagoyoma</i>	MZ578046	SMF 106035	Myanmar, Bago Yoma	Köhler et al. (2021)
<i>L. bagoyoma</i>	MZ578047	SMF 106036	Myanmar, Bago Yoma	Köhler et al. (2021)
<i>L. bannaensis</i>	NC_012837	None	China, Yunnan, Simao	Zhang et al. (2009)
<i>L. coffeatus</i>	KY768794	FMNH 258440	Laos, Champasak, Pakxong	Phimmachak et al. (2018)
<i>L. coffeatus</i>	KY768795	FMNH 258441	Laos, Champasak, Pakxong	Phimmachak et al. (2018)
<i>L. coffeatus</i>	KY768796	NCSM 77785	Laos, Champasak, Pakxong	Phimmachak et al. (2018)
<i>L. dabanus</i>	AF206496	ROM 22081	Vietnam, Dac Lac, Yok Don	Chen et al. (2005)
<i>L. dabanus</i>	GU934329	FMNH 261937	Cambodia, Mondolkiri, Prichrada	Inger and Stuart (2010)
<i>L. dabanus</i>	MK688558	FMNH 258146	Laos, Champasak, Pakxong	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688559	FMNH 258147	Laos, Champasak, Pakxong	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688563	FMNH 261925	Cambodia, Mondolkiri, Pichrada	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688565	FMNH 262747	Cambodia, Mondolkiri, O'Rang	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688566	FMNH 262908	Cambodia, Ratanakiri, Ta Veng	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688567	FMNH 262921	Cambodia, Stung Treng, Siem Pang	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688570	MVZ 258237	Cambodia, Ratanakiri, Veunsai	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688604	FMNH 262744	Cambodia, Stung Treng, Siem Pang	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688606	MVZ 258202	Cambodia, Ratanakiri, Veunsai	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688608	MVZ 258240	Cambodia, Ratanakiri, Veunsai	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688609	MVZ 258240	Cambodia, Ratanakiri, Veunsai	Phimmachak et al. (2019)
<i>L. dabanus</i>	MK688610	MVZ 258247	Cambodia, Ratanakiri, Veunsai	Phimmachak et al. (2019)
<i>L. doriae</i>	MK688574	CAS 229581	Myanmar, Tanintharyi, Kawthaung	Phimmachak et al. (2019)
<i>L. doriae</i>	MK688576	FMNH 268506	Thailand, Surat Thani, Kaeng Krung	Phimmachak et al. (2019)
<i>L. doriae</i>	MW567812	ZMKU AM 01527	Thailand, Tak, Mae Sot	Yodthong et al. (2021)
<i>L. doriae</i>	MW567817	ZMKU AM 01545	Thailand, Tak, Tha Song Yang	Yodthong et al. (2021)
<i>L. fragilis</i>	AY899241	ZNAC 11006	China, Hainan	Zhang et al. (2021)
<i>L. fujianensis</i>	AY974191	DT-FJ002	China, Fujian	Zhang et al. (2021)
<i>L. gyldenstolpei</i>	AF183124	PWRC 002	Thailand, Loei, Phu Luang	Emerson et al. (2000)
<i>L. gyldenstolpei</i>	GU934331	FMNH 266203	Thailand, Sa Kaeo, Mueang Sa Kaeo	Inger and Stuart (2010)
<i>L. gyldenstolpei</i>	MK688580	FMNH 257333	Cambodia, Siem Reap, Bante Sre	Phimmachak et al. (2019)
<i>L. gyldenstolpei</i>	MK688581	NCSM 79297	Laos, Luang Phabang, Luang Phabang	Phimmachak et al. (2019)
<i>L. gyldenstolpei</i>	MK688582	NCSM 79310	Laos, Xaignabouli, Xaignabouli	Phimmachak et al. (2019)
<i>L. gyldenstolpei</i>	MK688583	NCSM 79312	Laos, Xaignabouli, Paklay	Phimmachak et al. (2019)
<i>L. gyldenstolpei</i>	MK688584	NCSM 79549	Cambodia, Kampong Speu, Aural	Phimmachak et al. (2019)
<i>L. gyldenstolpei</i>	MK688585	NCSM 79555	Cambodia, Kampong Speu, Aural	Phimmachak et al. (2019)
<i>L. gyldenstolpei</i>	MK688586	NUOL 00075	Laos, Luang Phabang, Nan	Phimmachak et al. (2019)
<i>L. hascheanus</i>	GU934337	FMNH 270118	Thailand, Nakhon Si Thammarat, Khao Luang	Inger and Stuart (2010)
<i>L. hascheanus</i>	GU934349	LSUHC 6777	Malaysia, Penang	Inger and Stuart (2010)
<i>L. kharsani</i>	AY313693	LSUMZ 81722	Indonesia, Lombok Island	Evans et al. (2003)
<i>L. khasianus</i>	AB981414	KUHE 23158	Thailand, Narathiwat, Bala	Matsui et al. (2014)
<i>L. kohchangae</i>	KY768804	ZMKU AM 01155	Thailand, Trat, Ko Chang	Phimmachak et al. (2018)
<i>L. kohchangae</i>	KY768805	ZMKU AM 01157	Thailand, Trat, Ko Chang	Phimmachak et al. (2018)
<i>L. kohchangae</i>	KY768806	ZMKU AM 01156	Thailand, Trat, Ko Chang	Phimmachak et al. (2018)
<i>L. lauhachindai</i>	KP939072	NCSM 80222	Thailand, Ubon Ratchathani, Sirindhorn	Aowphol et al. (2015)
<i>L. lauhachindai</i>	KP939073	ZMKU AM 01104	Thailand, Ubon Ratchathani, Sirindhorn	Aowphol et al. (2015)
<i>L. lauhachindai</i>	KP939074	ZMKU AM 01105	Thailand, Ubon Ratchathani, Sirindhorn	Aowphol et al. (2015)
<i>L. lauhachindai</i>	KP939075	ZMKU AM 01106	Thailand, Ubon Ratchathani, Sirindhorn	Aowphol et al. (2015)
<i>L. lauhachindai</i>	KP939076	ZMKU AM 01107	Thailand, Ubon Ratchathani, Sirindhorn	Aowphol et al. (2015)
<i>L. lauhachindai</i>	KP939077	ZMKU AM 01109	Thailand, Ubon Ratchathani, Sirindhorn	Aowphol et al. (2015)
<i>L. lauhachindai</i>	KP939078	NCSM 81269	Thailand, Ubon Ratchathani, Sirindhorn	Aowphol et al. (2015)
<i>L. lauhachindai</i>	MK688587	FMNH 266153	Thailand, Ubon Ratchathani, Na Chaluai	Phimmachak et al. (2019)

TABLE 1. continued.

Species	GenBank No.	Voucher	Locality	Reference
<i>L. leporinus</i>	AY313691	A167165	Indonesia, Borneo, Kalimantan Timur	Evans et al. (2003)
<i>L. leytensis</i>	JX911319	USNM222545	Philippines, Leyte Island	Oaks et al. (2013)
<i>L. limborgi</i>	AB981417	KUHE 15614	Malaysia, Janda Baik	Matsui et al. (2014)
<i>L. limborgi</i>	GU934344	FMNH 262817	Cambodia, Mondolkiri, Samling	Inger and Stuart (2010)
<i>L. macrognathus</i>	MK688588	FMNH 268503	Thailand, Surat Thani, Kaeng Krung	Phimmachak et al. (2019)
<i>L. macrognathus</i>	MK688589	FMNH 268505	Thailand, Surat Thani, Kaeng Krung	Phimmachak et al. (2019)
<i>L. malesianus</i>	AY313692	None	Malaysia, Borneo, Sarawak	Evans et al. (2003)
<i>L. microdiscus</i>	AY313688	LSUMZ 81739	Indonesia, Java, Sukabumi	Evans et al. (2003)
<i>L. plicatellus</i>	KJ720981	LSUHC 6710	Malaysia, Pulau Pinang, Empangan Air Hitam	Lambertz et al. (2014)
<i>L. plicatellus</i>	KJ720982	LSUHC 6582	Malaysia, Selangor, Gombak Swamp	Lambertz et al. (2014)
<i>L. plicatellus</i>	KJ720983	LSUHC 4001	Malaysia, Selangor, Kepong	Lambertz et al. (2014)
<i>L. poilani</i>	DQ283378	AMNH A163717	Vietnam, Quang Nam, Tre My	Frost et al. (2006)
<i>L. pseudodoriae</i>	MW567823	ZMKU AM 01565	Thailand, Surat Thani, Ko Pha-ngan Island	Yodthong et al. (2021)
<i>L. pseudodoriae</i>	MW567824	ZMKU AM 01567	Thailand, Surat Thani, Ko Pha-ngan Island	Yodthong et al. (2021)
<i>L. pseudodoriae</i>	MW567825	ZMKU AM 01577	Thailand, Surat Thani, Ko Pha-ngan Island	Yodthong et al. (2021)
<i>L. savan</i>	MK688592	FMNH 255388	Laos, Khammouan, Boualapha	Phimmachak et al. (2019)
<i>L. savan</i>	MK688593	FMNH 255390	Laos, Khammouan, Boualapha	Phimmachak et al. (2019)
<i>L. savan</i>	MK688594	FMNH 266149	Thailand, Ubon Ratchathani, Na Chaluai	Phimmachak et al. (2019)
<i>L. savan</i>	MK688595	FMNH 266156	Thailand, Ubon Ratchathani, Na Chaluai	Phimmachak et al. (2019)
<i>L. savan</i>	MK688596	FMNH 266158	Thailand, Ubon Ratchathani, Buntharik	Phimmachak et al. (2019)
<i>L. savan</i>	MK688597	NCSM 76287	Laos, Savannakhet, Vilabouli	Phimmachak et al. (2019)
<i>L. savan</i>	MK688598	NCSM 76288	Laos, Savannakhet, Vilabouli	Phimmachak et al. (2019)
<i>L. savan</i>	MK688599	NCSM 76289	Laos, Savannakhet, Vilabouli	Phimmachak et al. (2019)
<i>L. savan</i>	MK688600	NCSM 76291	Laos, Savannakhet, Vilabouli	Phimmachak et al. (2019)
<i>L. savan</i>	MK688603	NUOL 00061	Laos, Savannakhet, Vilabouli	Phimmachak et al. (2019)
<i>L. sirindhornae</i> sp. nov.	PV939348	ZMKU AM 01590	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939349	ZMKU AM 01591	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939350	ZMKU AM 01592	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939351	ZMKU AM 01593	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939352	ZMKU AM 01594	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939353	ZMKU AM 01597	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939354	ZMKU AM 01598	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939355	ZMKU AM 01599	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939356	ZMKU AM 01600	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939357	ZMKU AM 01601	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939358	ZMKU AM 01602	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939359	ZMKU AM 01604	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. sirindhornae</i> sp. nov.	PV939360	ZMKU AM 01605	Thailand, Sakon Nakhon, Phanna Nikhom, Rai	This study
<i>L. splendissimus</i>	PQ302572	ITBCZ 8674	Vietnam, Lam Dong, Di Linh	Le et al. (2025)
<i>L. woodworthi</i>	JX911331	RMB 4092	Philippines, Luzon Island	Oaks et al. (2013)

Phylogenetic analyses

Additional homologous *16S* sequences from 81 samples of *Limnonectes* species and the outgroups were downloaded from GenBank, based on previous *Limnonectes* studies (Aowphol et al., 2015; Phimmachak et al., 2018, 2019; Yodthong et al., 2021) (Table 1). *Fejervarya limnocharis* and *Quasipaa spinosa* were selected as outgroups to root the tree following Yodthong et al. (2021). The 13 newly generated *Limnonectes* sequences and GenBank sequences were aligned using the MAFFT v.7 alignment function (Kato and Standley, 2013) with default settings in Geneious Prime 2025 (Biomatters, Ltd, Auckland, New Zealand). The model of sequence evolution that best described the data (GTR+I+G) was determined with PartitionFinder2 (Lanfear et al., 2017) using the Bayesian information criterion (BIC). Bayesian Inference (BI) and Maximum Likelihood (ML) were used to construct phylogenetic trees. The BI analysis was carried out using MrBayes v3.2.7 (Ronquist et al., 2012) on CIPRES Science Gateway V. 3.3 (Miller et al., 2010) with default prior setting. Two independent runs, each with three heated and one cold chain, were performed using Metropolis-coupled Markov Chain Monte Carlo (MCMC). The MCMC chains were run for 10,000,000 generations, with trees sampled every 1,000 generations, and the first 25% of each run was discarded as burn-in. A 50% majority-rule consensus of the sampled trees was constructed to calculate the posterior probabilities of tree nodes. Stationarity was evaluated by ensuring that effective sample sizes (ESS) exceeded 200 for all parameters in Tracer v. 1.7 (Rambaut et al., 2018). The ML analysis was conducted using the IQ-TREE 1.6.12 web server available at “<http://iqtree.cibiv.univie.ac.at>” (Trifinopoulos et al., 2016) with 1,000 bootstrap replicates using the ultrafast bootstrap analysis (Minh et al., 2013; Hoang et al., 2018). Nodal support in BI and ML analyses was quantified using Bayesian posterior probabilities (BPP) and Ultrafast bootstrap support values (UFB), respectively. BPP ≥ 0.95 and UFB values ≥ 95 were considered strongly supported (Huelsenbeck and Ronquist, 2001; Wilcox et al., 2002; Minh et al., 2013). The phylogenetic trees from the BI and ML analyses were visualized and edited using FigTree v. 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). Uncorrected pairwise sequence divergences (*p*-distances) were calculated in MEGA 11 (Tamura et al., 2021) using the pairwise deletion option to remove gaps and missing data from the alignment prior to analysis.

Morphological measurements and analyses

Morphological measurements and their abbreviations followed Aowphol et al. (2015): snout–vent

length (SVL); head length from tip of snout to rear of jaws (HDL); maximum head width at jaw angle (HDW); snout length from tip of snout to anterior margin of eye (SNT); eye diameter (EYE); interorbital distance, measure as minimum distance between eyes on top of head (IOD); horizontal diameter of tympanum (TMP); internarial distance (IND); shank length (SHK); thigh length, from knee to midline of vent (TGH); forearm length, from elbow to base of palmar tubercle (LAL); manus length, from tip of third digit to base of palmar tubercle (HND); pes length, from tip of fourth toe to base of inner metatarsal tubercle (FTL); inner metatarsal tubercle length (IML); and inner metatarsal tubercle width (IMW). Measurements were made with a Mitutoyo digital calipers (Mitutoyo Corp., Japan) to the nearest 0.1 mm and a Nikon SMZ745T Stereomicroscope (Nikon Corp., Japan). Sex and maturity were determined by internal examination of gonads. Terminology for the caruncle (cap or flap-like structure) followed Lambertz et al. (2014).

Specimens of two closely related species were assigned to each group (= lineages, based on their mtDNA below), *Limnoneactes lauhachindai* (holotype and paratypes: males = 20; females = 4) and the Sakon Nakhon population (males = 8; females = 8). Male and female measurements were analyzed separately due to apparent sexual size dimorphism. To remove the effects of allometry, fourteen measurements (except SVL) were corrected for body size variation using allometric growth model in the R package GroupStruct (Chan and Grismer, 2022). The allometric formula is $X_{\text{adj}} = \log_{10}(X) - \beta[\log_{10}(\text{SVL}) - \log_{10}(\text{SVL}_{\text{mean}})]$, where X_{adj} = adjusted value; X = measured value; β = unstandardized regression coefficient for each species and SVL_{mean} = overall average SVL of all each allometry species (Thorpe, 1975; Turan, 1999; Leonart et al., 2000). Morphospacial clustering and positioning between species was analyzed using principal component analysis (PCA) in the FactoMineR and factoextra packages (Lê et al., 2008; Kassambara and Mundt, 2020). A non-parametric permutation-multivariate analysis of variance (PERMANOVA) from vegan package 2.5-3 (Oksanen et al., 2024) was used to determine the significant differences in centroid locations and group clustering between species based on the loadings of the first three dimensions recovered from the PCA. The analysis computed a Euclidean (dis)similarity matrix with 50,000 permutations. For univariate analyses, the dataset was checked for a normal distribution using the Shapiro–Wilk test ($p \geq 0.05$) and equality of variance using the Levene’s test ($p \geq 0.05$). Morphological differences between species were tested by Student’s *t*-test (for normalized and equal variance data), Welch’s *t*-test (for normalized

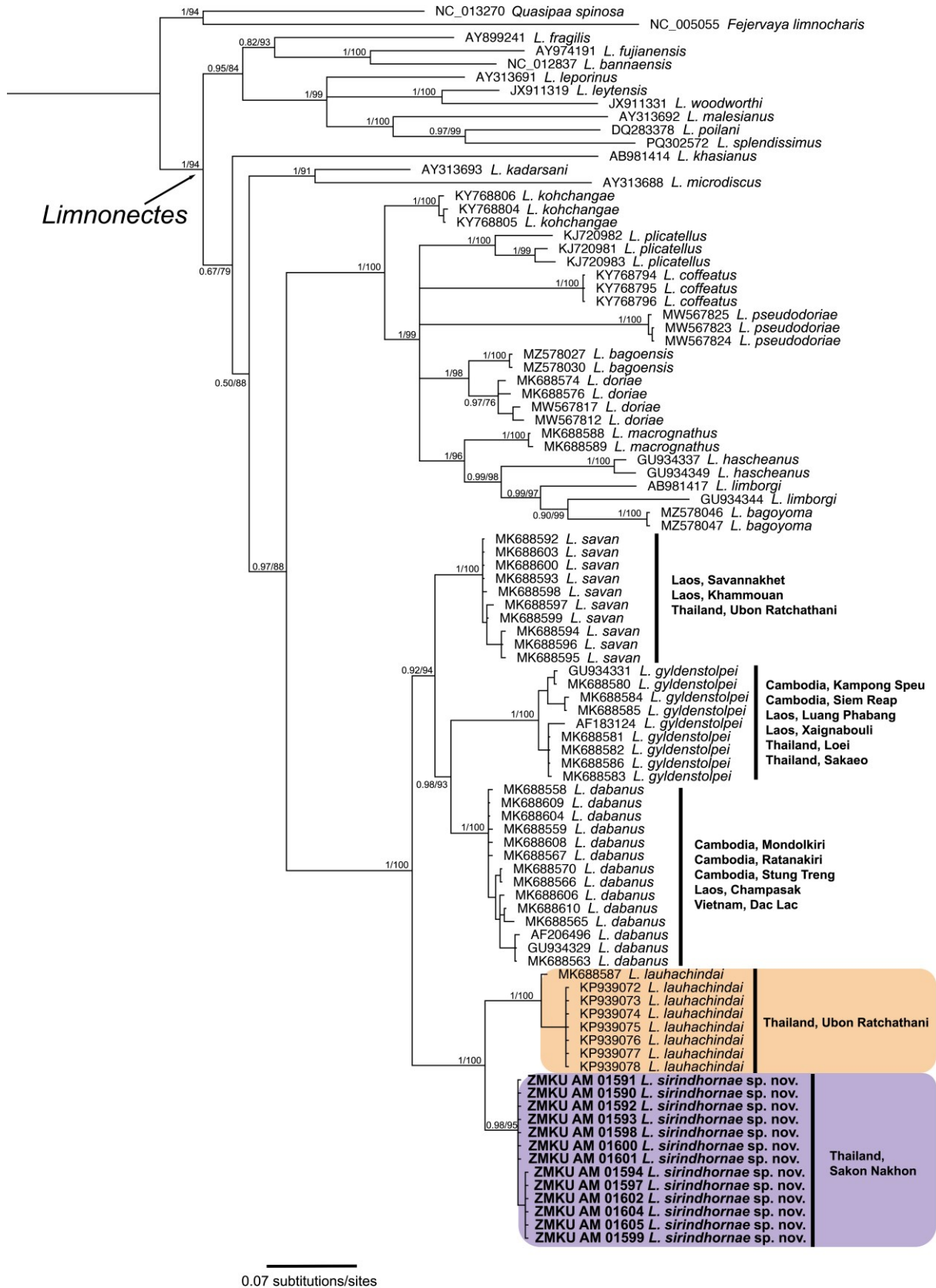


FIGURE 2. Fifty percent majority-rule consensus phylogram resulting from Bayesian analysis of mitochondrial *16S* gene of *Limnionectes* species. Nodal support is indicated by Bayesian posterior probabilities (BPP) and Ultrafast bootstrap (UFB) values. GenBank accession numbers and locality data for sequenced samples are provided in Table 1.

TABLE 2. Mean (min–max) percentage uncorrected pairwise sequence divergences (*p*-distance) in the mitochondrial *16S* gene of *Limnonectes sirindhornae* sp. nov. and related species.

<i>Limnonectes</i> species	n	1.	2.	3.	4.	5.
1. <i>L. dabanus</i>	14	0.65 (0.00–1.36)				
2. <i>L. gyldenstolpei</i>	9	5.92 (5.24–7.19)	0.93 (0.00–2.03)			
3. <i>L. lauhachindai</i>	8	7.50 (7.07–8.52)	8.21 (7.82–9.06)	0.29 (0.00–1.17)		
4. <i>L. savan</i>	10	4.64 (4.23–5.36)	6.15 (5.74–6.81)	7.24 (7.08–7.60)	0.49 (0.00–0.99)	
5. <i>L. sirindhornae</i> sp. nov.	13	5.94 (5.20–6.41)	7.76 (7.51–8.09)	4.25 (3.61–4.45)	6.27 (5.77–6.95)	0.10 (0.00–0.19)

TABLE 3. Factor loadings on the first three principal components (PC) of 15 morphological characters of males and females of *Limnonectes lauhachindai* and *L. sirindhornae* sp. nov. Morphological abbreviations are defined in Materials and methods.

Characters	Male			Female		
	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
SVL	0.9468	-0.0635	-0.0568	0.9567	0.0822	0.0233
HDL	0.8975	0.1214	0.1487	0.9748	-0.1164	-0.0169
HDW	0.9687	0.1314	-0.0366	0.9683	-0.1215	0.0093
SNT	0.9239	0.0377	-0.1487	0.9489	-0.1936	0.1057
EYE	0.2256	0.8101	0.0477	-0.4202	0.8236	0.0099
IOD	0.6527	0.1304	0.5846	0.7883	0.3099	0.3335
TMP	0.8135	0.1878	0.2269	0.9375	0.0945	0.0017
IND	0.6330	0.1039	0.6036	0.8161	0.3400	0.1704
SHK	0.9573	-0.0503	-0.2015	0.9094	0.2026	-0.1131
TGH	0.9443	-0.0682	-0.1874	0.9776	0.1259	0.0562
LAL	0.9329	-0.1563	-0.0536	0.9098	0.2385	-0.1058
HND	0.9312	-0.1514	-0.1883	0.9142	-0.1034	-0.2907
FTL	0.9268	-0.1074	-0.2961	0.9563	0.0005	-0.1816
IML	0.9123	-0.1174	-0.0558	0.8565	-0.2540	-0.3181
IMW	0.1235	-0.7415	0.4445	0.4659	-0.3021	0.7333
Eigenvalue	10.29	1.39	1.21	11.36	1.25	0.94
Percentage of variance	68.57	9.24	8.07	75.72	8.34	6.24
Cumulative percentage of variance	68.57	77.81	85.89	75.72	84.06	90.30

and unequal variance data), and Mann-Whitney *U* test (for the non-normalized data). All statistical analyses were conducted using R program v. 4.3.2 (R Core Team, 2025). Morphological data for comparisons were obtained from their original descriptions and literature on the caruncle-bearing species of *Limnonectes* (Stoliczka, 1873; Andersson, 1916; Boulenger, 1917, 1920; Smith, 1922; Taylor, 1962; Lambert et al., 2014; Rowley et al., 2014; Aowphol et al., 2015; Phimmachak et al., 2019).

RESULTS

The final alignment of *16S* contained 836 characters of *Limnonectes* and the outgroups. The phylogenetic ML and BI analyses recovered largely identical trees topologies (Fig. 2). The 13 samples from Sakon Nakhon Province formed a strongly supported monophyletic lineage (0.98 BPP, 95 UFB) and as a member

of a clade containing *L. dabanus*, *L. gyldenstolpei*, *L. lauhachindai* and *L. savan*. The Sakon Nakhon population was recovered as the sister taxon (1.00 BPP, 100 UFB) to *L. lauhachindai* from Ubon Ratchathani Province. Both lineages, the Sakon Nakhon population and *L. lauhachindai* lineages, formed a clade as the sister taxon (1.00 BPP, 100 UFB) to the clade containing *L. dabanus*, *L. gyldenstolpei* and *L. savan*. Uncorrected pairwise genetic divergence (*p*-distances) within the Sakon Nakhon population was 0.00–0.19%. The Sakon Nakhon population had uncorrected *p*-distances of 3.61–4.45% (mean 4.25%) from its sister species *L. lauhachindai* and ≥ 5.20 % from its related caruncle-bearing species (Table 2).

The PCA analyses of male and female specimens recovered morphological differences between *L. lauhachindai* and the Sakon Nakhon population on a scatter plot of the first two PC axes (PC 1 and PC 2; Fig. 3). PCA of male specimens revealed that the

Sakon Nakhon population was separated from *L. lauhachindai* with partial overlap on a plot of the first two axes (Fig. 3A). The first two axes accounted for 77.81% of cumulative of the total variation (68.57% for PC 1 axis and 9.24% for PC 2 axis; Table 3, Fig. 3). The factor loading of PC 1 was heavily loaded on most characters except EYE and IMW. The factor loading of PC 2 was heavily loaded on EYE and IMW (Table 3). PCA of female specimens showed that the Sakon Nakhon population was distinctly separated from *L. lauhachindai* on a plot of the first two axes (Fig. 3B). The first two axes accounted for 84.06% of cumulative of the total variation (75.72% for PC 1 axis and 8.34% for PC 2 axis; Table 3). The factor loading of PC 1 was heavily loaded on most characters except EYE and IMW. The factor loading of PC 2 was heavily loaded on EYE (Table 3). The results from PERMANOVA of both male and female specimens demonstrated statistically significant morphological differences between the Sakon Nakhon population and *L. lauhachindai* (male: $F = 32.545$, $R^2 = 0.556$, $p < 0.0001$; female: $F = 28.657$, $R^2 = 0.741$, $p = 0.0020$). The t -test (or Mann–Whitney U test) revealed that the Sakon Nakhon population differ significantly from *L. lauhachindai* in most characters ($p < 0.05$) both male and female specimens (except EYE and IMW in males and EYE, IOD, and IML in females; Figs 4, 5).

Systematics

Family Dicroglossidae Anderson, 1871

Genus *Limnonectes* Fitzinger, 1843

Limnonectes sirindhornae Rujirawan, Yodthong & Aowphol, sp. nov.

<http://zoobank.org/urn:lsid:zoobank.org:act:A58980FF-698F-4426-AA35-C364AF1AAAE3>

(Figs 6–9)

Type material.— Holotype: ZMKU AM 01600, adult male, Thailand, Sakon Nakhon Province, Phanna Nikhom District, Rai Subdistrict, 17.256789°N, 103.81097°E, 279 m elev., collected on 11 January 2024 by Anchalee Aowphol, Attapol Rujirawan, Siriporn Yodthong, Akrachai Aksornneam and Pratyaporn Wanchai. Paratypes: ZMKU AM 01597–01599, ZMKU AM 01601–01602 (five adult males), ZMKU AM 01603–01606 (four adult females) and ZMKU AM 01607 (one immature) same data as holotype; ZMKU AM 01590–01591 (two adult males), ZMKU AM 01592–01594, ZMKU AM 01596 (four adult females) and ZMKU AM 01595 (one immature) same data as holotype except collected on 7 October 2023 by Anchalee Aowphol, Attapol Rujirawan, Siriporn Yodthong and Pratyaporn Wanchai.

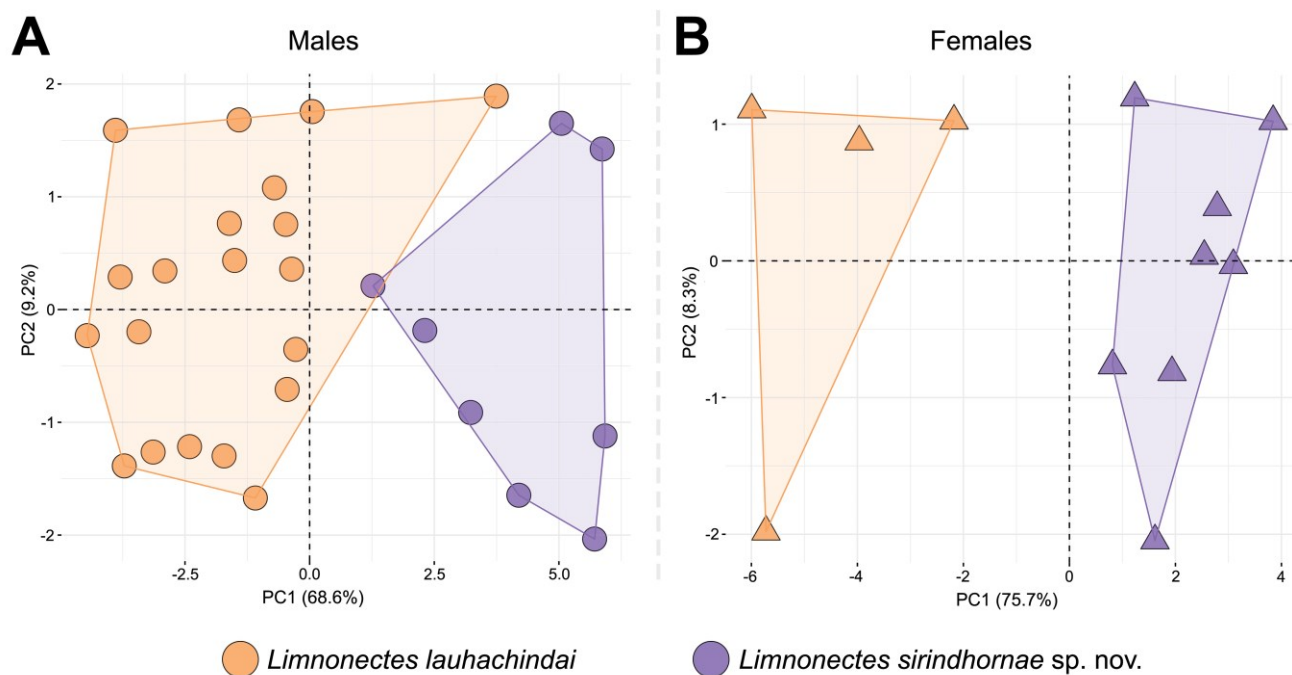


FIGURE 3. Scatter plot of the first and second principal components (PC1 and PC2) from principal component analysis of 15 morphometric measurements of *Limnonectes lauhachindai* and *L. sirindhornae* sp. nov. **A.** males and **B.** females.

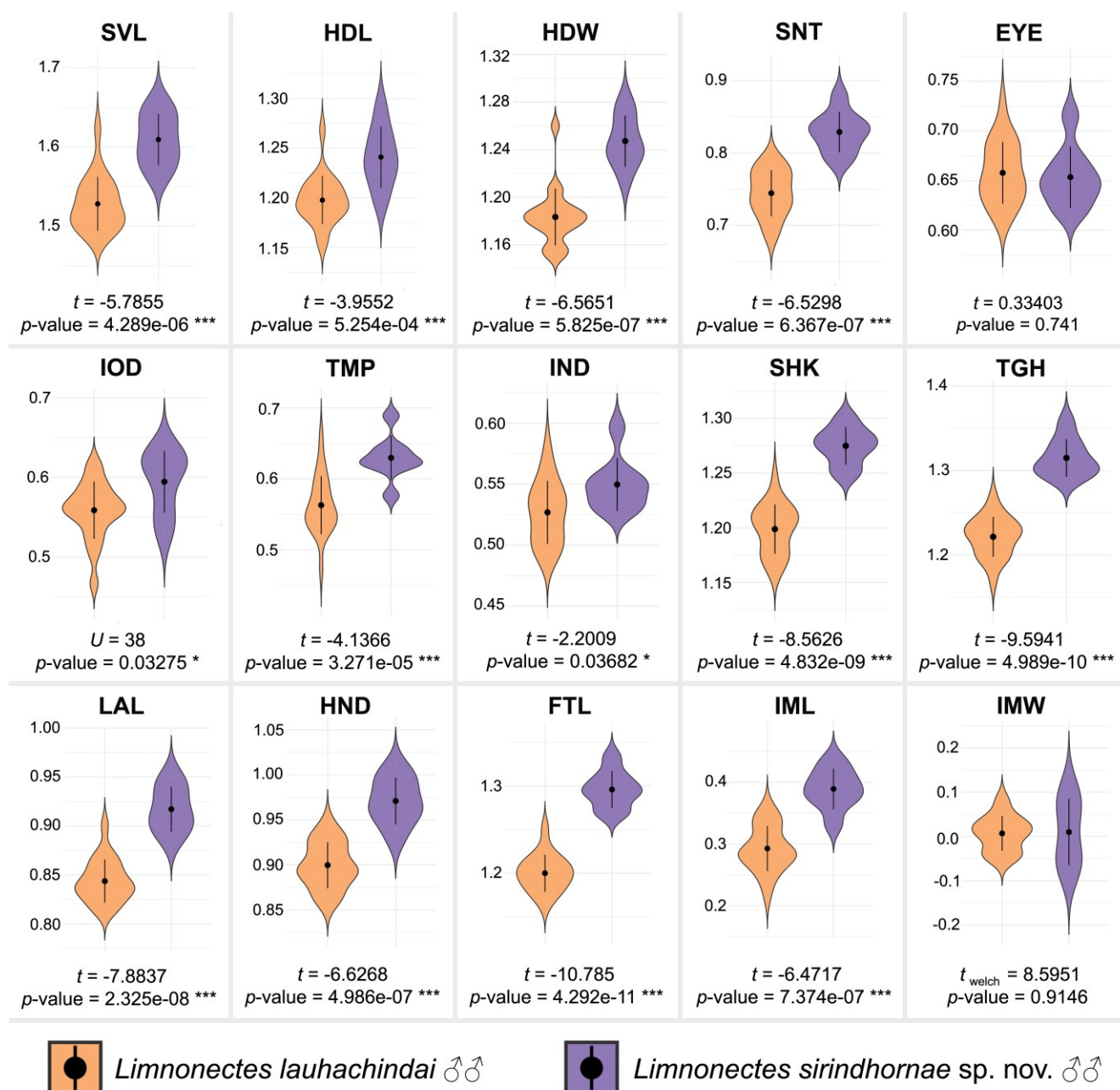


FIGURE 4. Statistical comparisons of males of *Limnonectes lauhachindai* and *L. sirindhornae* sp. nov. tested by t -test (t), welch t -test (t_{welch}), or Mann-Whitney U-test (U). Key: *** = $p < 0.001$, ** = $p < 0.01$, and * = $p < 0.05$.

Etymology.— The specific epithet is a matronym in honor of Her Royal Highness Princess Maha Chakri Sirindhorn, recognizing her significant contributions to the conservation of Thailand's biodiversity and environment.

Diagnosis.— *Limnonectes sirindhornae* sp. nov. is assigned to the genus *Limnonectes* based on its phylogenetic relationship (Fig. 2), the presence of fang-like odontoid processes on the lower jaw (Emerson et al., 2000; Lambertz et al., 2014), and adult males with hypertrophied heads (Lambertz et al., 2014). The new

species can be distinguished from its congeners by having the following combination of characters: SVL 36.7–44.7 mm (40.8 ± 3.0 ; $n = 8$) in adult males, 40.2–45.6 mm (42.7 ± 2.2 ; $n = 8$) in adult females; adult males with hypertrophied heads; adult males with interorbital caruncle weakly developed, low-profiled, U-shaped, without free posterior margin; two enlarged odontoid processes on anterior margin of lower jaw, larger in males than in female; vocal sac opening in males absent; and yellowish coloration in life on ventral surfaces absent.

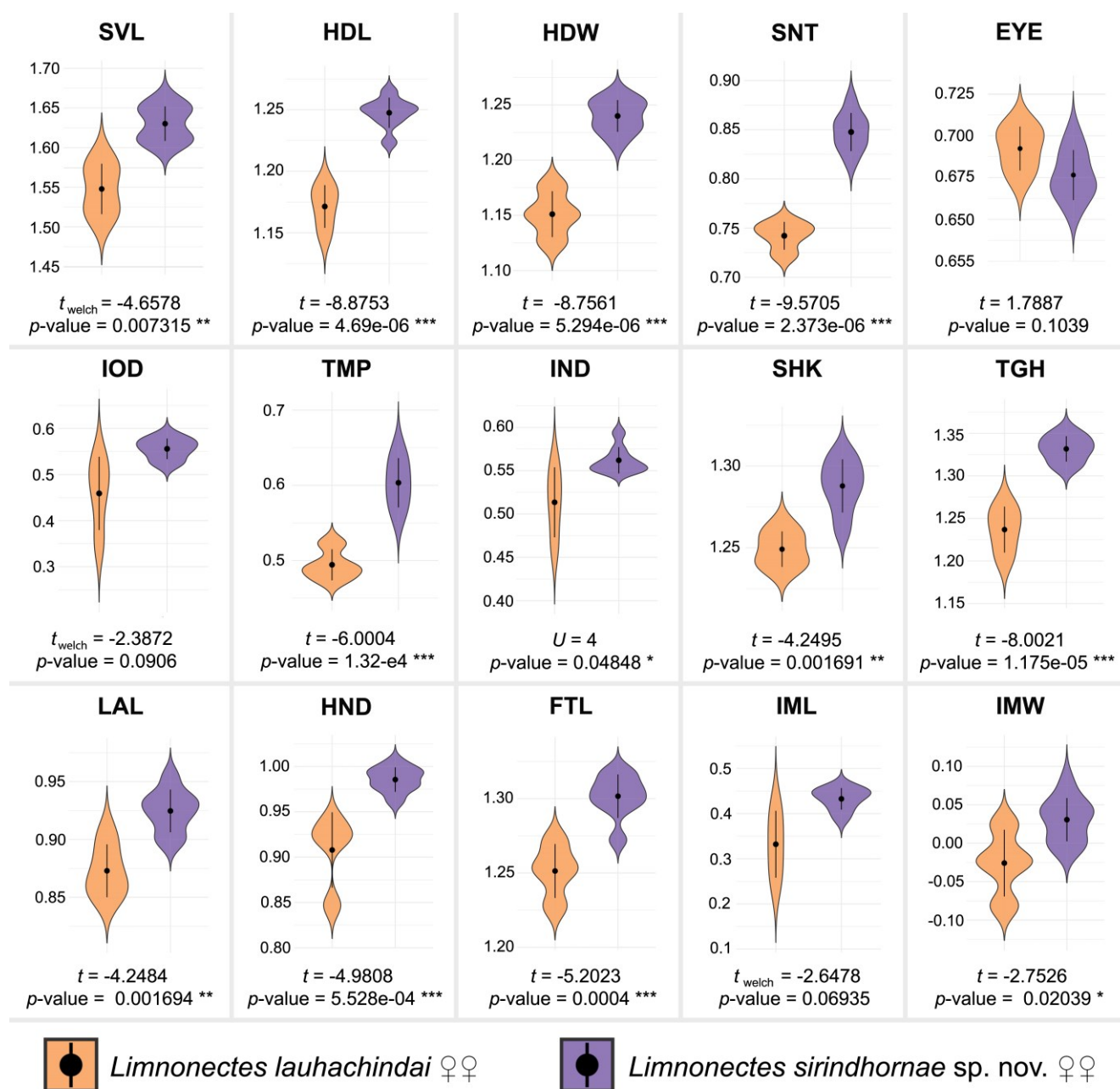


FIGURE 5. Statistical comparisons of females of *Limnonectes lauhachindai* and *L. sirindhornae* sp. nov. tested by t -test (t), welch t -test (t_{welch}), or Mann-Whitney U -test (U). Key: *** = $p < 0.001$, ** = $p < 0.01$, and * = $p < 0.05$.

Description of holotype.— Habitus moderately stocky; body tapering to groin. Head broad and depressed; head length and width subequal (HDL/HDW 0.96). Snout obtusely pointed in dorsal view, round in profile, projecting well beyond lower jaw in profile; nostril dorsolateral, below canthus, much closer to tip of snout than to eye; internarial distance 83% of interorbital distance; canthus rostralis indistinct, rounded, slightly constricted behind nostrils; lores concave, oblique; eye diameter 74% snout length, interorbital distance slightly greater than upper eyelid width; pineal ocellus visible; tympanum round, not elevated from side of

head, annulus weakly visible, tympanum diameter about 81% eye diameter and greater than distance between tympanum and eye; vocal sac openings absent; vomerine teeth on two oblique ridges, closer to choanae than to each other; tongue distinctly notched posteriorly; two enlarged odontoid processes at front of mandible, triangular; median triangular protuberance at mandibular symphysis.

Forelimb moderately robust. Fingers moderately slender, without webbing, with fringe of skin on pre-axial and postaxial sides of all fingers, fringes on Fingers II–III movable; tip of fingers rounded, weakly

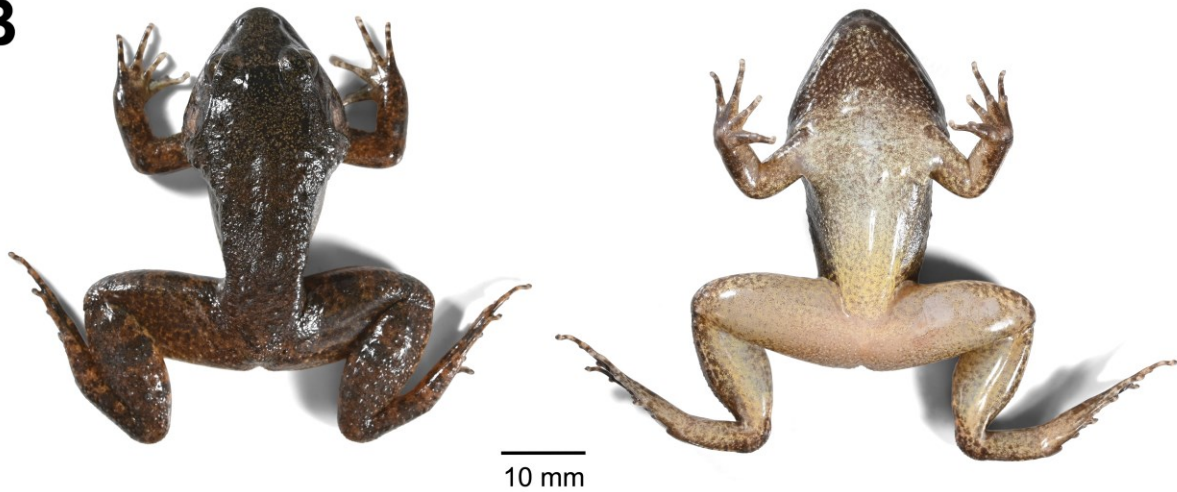
A**B**

FIGURE 6. Adult male holotype of *Limnonectes sirindhornae* sp. nov. (ZMKU AM 01600) **A.** dorsolateral view in life **B.** dorsal and ventral views immediately after euthanasia.

expanded into discs; relative finger lengths $II < IV < I < III$; distinct subarticular tubercles, one on Fingers I–II, two on Fingers III–IV; distinct palmar tubercles, one at base of Finger I, two in contact as base of Fingers II–IV; nuptial pad absent.

Hindlimb robust. Toes moderately slender; tips of toes rounded, expanded into small discs; relative toe lengths $I < II < III < V < IV$; webbing on Toe I to base of disc, on preaxial side of Toe II to level of subarticular tubercle continuing as a fringe to base of disc, on postaxial side of Toe II to base of disc, on preaxial side of Toe III to level of distal subarticular tubercle continuing as a fringe to base of disc, on postaxial side of Toe III to base of disc, on preaxial and postaxial sides of Toe IV to level midway between distal and penultimate subarticular tubercles and continuing as a fringe to base of disc, and on Toe V to midway between distal subarticular tubercle and disc continuing as a fringe to base of disc; moveable flap of

skin on outer margins of Toes I and V; subarticular tubercle, prominent, rounded, one on Toes I–II, two on Toe III and V, three on Toe IV; distinct fold on distal two-third of tarsus; distinct, elongate, oval, inner metatarsal tubercle (IMW/IML 0.36); no outer metatarsal tubercle.

Skin on dorsum and flank shagreened with enlarged, irregular, scattered tubercles, most concentrated near flank and lower back; tubercles tipped with single, whitish spinules on eyelid, flank, lower back near groin, around vent, thigh, and ventral surfaces of tibiotarsus and foot; dense enlarged tubercles, each tipped with numerous whitish spinules, on dorsal surfaces of shank; interorbital caruncle weakly developed, low-profiled, U-shaped, without free posterior margin, extending from level of anterior margin of eye to level midway between posterior margin of eye and tympanum (Fig. 7A); distinct supratympanic fold from

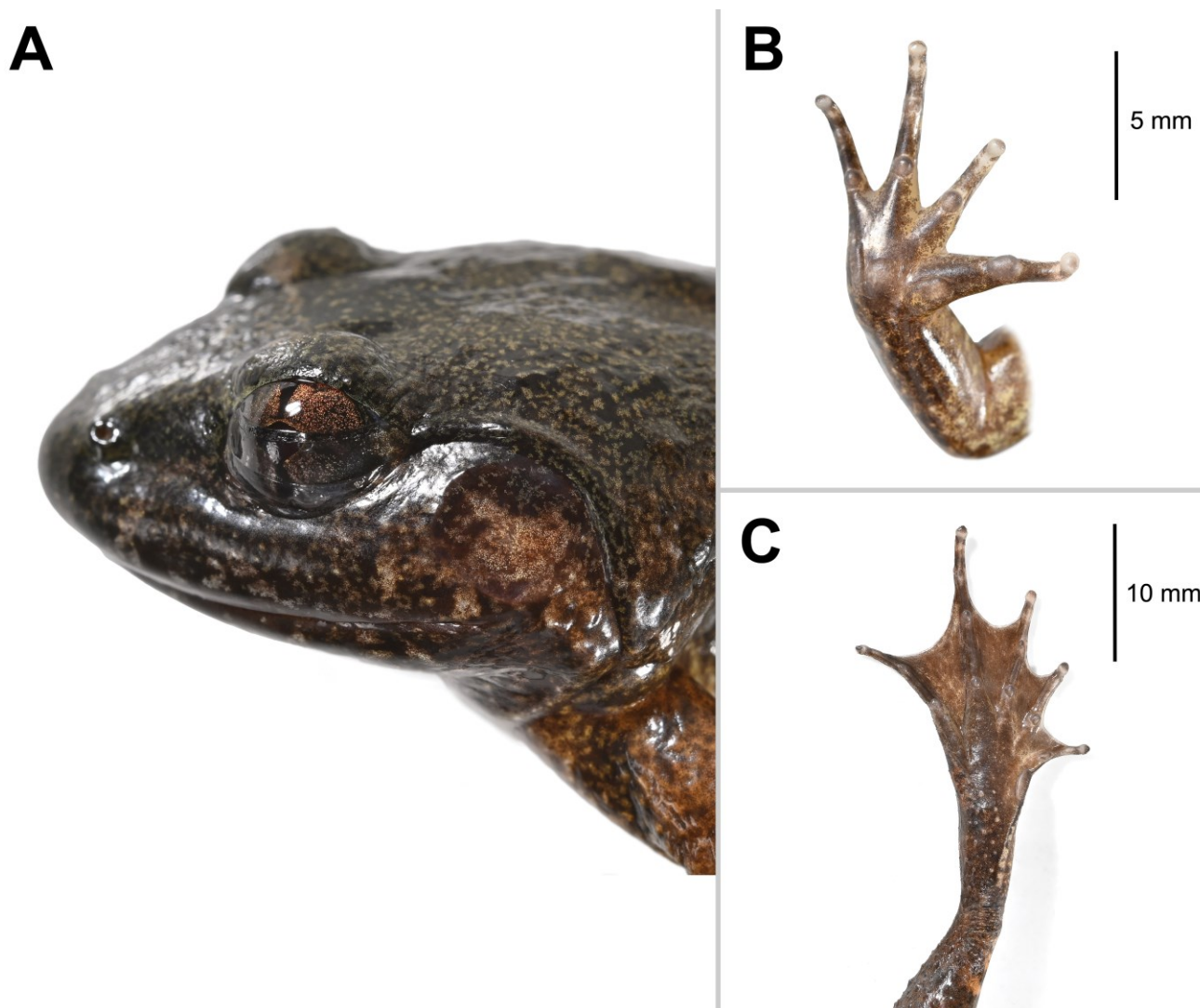


FIGURE 7. Adult male holotype of *Limnonectes sirindhornae* sp. nov. (ZMKU AM 01600) immediately after euthanasia. **A.** lateral view of head. **B.** palmar view of the right hand. **C.** plantar view of the right foot.

posterior corner of eye to axilla; rictal gland absent; dorsolateral fold absent; skin on venter smooth.

Color of holotype in life. Dorsum and upper surface of flank dark brown diffused with irregular dark spots; interorbital region dark with an indistinct light-colored transverse stripe between eyelids; dark streak under canthus from nostril to anterior margin of eye; lips brown with broad black bars; tympanum brown with dark blotched on anterior and dorsoposterior portion. Iris bronze with black vermiform mottling, black vertical and horizontal bars forming shape of a single plus sign (“+”) over eye. Dorsal surfaces of limbs brown with dark cross-bands; posterior surface of thigh brown with dark gray mottling. Venter creamy white with diffused dark spots; chin with dark coloration; ventral surfaces of hands and feet uniformly dark brown.

Color of holotype in preservative. Overall color pattern similar to that in life with some fading. Dorsal

ground color of head, body, flanks, dark brown with indistinct dark spots; limbs brown with indistinct cross-bands. Venter beige with diffused dark spots; chin become dark gray mottling; ventral surfaces of hands and feet dark gray.

Variation.— Females lack interorbital caruncle; have narrower heads than males (Table 4; Fig. 8); have relatively smaller tympana than males (Table 4); have smaller and shorter odontoid processes than males; and contain ova with pigmented poles. Interorbital regions of ZMKU AM 01593, ZMKU AM 01596, ZMKU AM 01601–01602 and ZMKU AM 01607 with distinct light-colored transverse stripe between eyelids. Dorsum coloration varies from dark brown, brown to yellowish-brown with irregular pattern of dark spots (Fig. 9). Three adult females (ZMKU AM 01592, ZMKU AM 01594 and ZMKU AM 01606) have a pale vertebral stripe from tip of snout to vent. Coloration of

TABLE 4. Descriptive measurements (mm) of holotype and paratypes of *Limnonectes sirindhornae* sp. nov. Morphological abbreviations are defined in Materials and Methods.

Characters	Holotype	Male holotype and paratypes n = 8		Female paratypes n = 8	
		Mean $\pm$ SD	Min–Max	Mean $\pm$ SD	Min–Max
SVL	41.0	40.8 $\pm$ 3.0	36.7–44.7	42.7 $\pm$ 2.2	40.2–45.6
HDL	17.5	17.5 $\pm$ 1.8	15.3–20.5	17.7 $\pm$ 0.7	16.4–18.8
HDW	18.2	17.7 $\pm$ 1.4	15.7–19.8	17.4 $\pm$ 0.8	16.1–18.4
SNT	7.0	6.8 $\pm$ 0.6	6.0–7.9	7.1 $\pm$ 0.4	6.4–7.8
EYE	5.2	4.5 $\pm$ 0.4	4.1–5.2	4.8 $\pm$ 0.2	4.5–5
IOD	4.1	4.0 $\pm$ 0.5	3.3–4.4	3.6 $\pm$ 0.2	3.3–3.9
TMP	4.2	4.3 $\pm$ 0.4	3.6–5.0	4.0 $\pm$ 0.4	3.5–4.6
IND	3.4	3.6 $\pm$ 0.2	3.3–4.0	3.7 $\pm$ 0.2	3.5–4.0
SHK	19.5	18.9 $\pm$ 1.2	17.2–20.6	19.4 $\pm$ 1.0	17.9–20.8
TGH	22.8	20.7 $\pm$ 1.3	19.1–22.8	21.5 $\pm$ 0.9	20.2–22.9
LAL	8.6	8.3 $\pm$ 0.6	7.4–9.1	8.4 $\pm$ 0.4	7.8–9.1
HND	9.4	9.4 $\pm$ 0.8	8.1–10.6	9.7 $\pm$ 0.3	9.1–10.2
FTL	20.6	19.8 $\pm$ 1.4	17.9–22.2	20 $\pm$ 0.8	18.5–21.2
IML	2.5	2.5 $\pm$ 0.3	2.0–2.9	2.7 $\pm$ 0.2	2.4–2.9
IMW	0.9	1.0 $\pm$ 0.2	0.8–1.3	1.1 $\pm$ 0.1	1.0–1.2
HDL/HDW	0.96	0.99 $\pm$ 0.04	0.92–1.04	1.05 $\pm$ 0.09	0.99–1.25
EYE/SNT	0.74	0.67 $\pm$ 0.06	0.61–0.75	0.68 $\pm$ 0.05	0.63–0.78
TMP/EYE	0.81	0.95 $\pm$ 0.1	0.80–1.11	0.85 $\pm$ 0.08	0.74–0.98
IND/IOD	0.83	0.91 $\pm$ 0.09	0.82–1.06	1.02 $\pm$ 0.06	0.92–1.09
SHK/TGH	0.86	0.91 $\pm$ 0.04	0.86–0.96	0.90 $\pm$ 0.02	0.88–0.93
IMW/IML	0.36	0.42 $\pm$ 0.07	0.32–0.52	0.40 $\pm$ 0.03	0.34–0.44

ventral surface varies from immaculate creamy white (ZMKU AM 01590–01591, ZMKU AM 01595–01596, ZMKU AM 01601, ZMKU AM 01604, ZMKU AM 01606; three males, three females and one immature), immaculate creamy white and chin with gray mottling (ZMKU AM 01592–01593, ZMKU AM 01597–01598; two males and two females), to creamy white with indistinct gray mottling or diffused gray spots (ZMKU AM 01594, ZMKU AM 01599, ZMKU AM 01602–01603, ZMKU AM 01605, ZMKU AM 01607; two males, three female, and one immature). The interorbital caruncle of the smallest adult male ZMKU AM 01601 (SVL 36.7 mm) is not swollen but the caruncle margin is indistinctly present. Measurements of adults are summarized in Table 4.

Distribution and Natural history.— *Limnonectes sirindhornae* sp. nov. is known only from the type locality in part of the Phu Phan Mountain Range in northeastern Thailand (Figs 1, 10). The type locality of this species is dry evergreen forest at 293 m elevation. The type series was collected in October 2023 at 1800–2000 hrs (air temp = 27.3°C, RH = 91.5%) and January 2024 at 1900–2100 hrs (air temp = 24.9°C, RH = 80.0%). The holotype was found on intermittent stream in a small pool. Most paratypes were found in various microhabitats along the stream including on leaf litter, on and under rocks and boulders, in rock crevices, and in small intermittent pools. ZMKU AM 01602 and

some uncollected individuals were found in rock shelters (or a sandstone cave) in sandstone landforms approximately ~200 m from the stream. Calls and oviposition sites are unknown. Other sympatric frog species found at the type locality included *Fejervarya triora* Stuart, Chuaynkern, Chan-ard & Inger, 2006, *Hylarana mortenseni* (Boulenger, 1903), *Microhyla heymonsi* Vogt, 1911, *Microhyla mukhlesuri* Hasan, Islam, Kuramoto, Kurabayashi & Sumida, 2014, *Micryletta* cf. *immaculata*, *Occidozyga magnapustulosa* (Taylor & Elbel, 1958), *Polypedates megacephalus* Hallowell, 1861, and *Zhangixalus jarujini* (Matsui & Panha, 2006).

Comparisons.— *Limnonectes sirindhornae* sp. nov. is distinguishable from all other species of mainland Southeast Asian *Limnonectes*, except *L. dabanus*, *L. gyldenstolpei*, *L. lauhachindai*, *L. macrognathus*, and *L. plicatellus* and *L. savan*, by having an interorbital caruncle in adult males (sensu Lambert et al., 2014).

Limnonectes sirindhornae sp. nov. can be differentiated from *L. dabanus* by having smaller SVL of 36.7–44.7 mm in males (vs. 48.8–64.4 mm; Phimmachack et al., 2019); interorbital caruncle weakly developed, low-profiled, U-shaped, without free posterior margin (vs. caruncle high-profiled and domed).

Limnonectes sirindhornae sp. nov. can be differentiated from *L. gyldenstolpei* by having smaller SVL of 36.7–44.7 mm in males (vs. 51.1–68.4 mm; Aowphol



FIGURE 8. Dorsal and ventral views of *Limnionectes sirindhornae* sp. nov. in preservative **A.** adult male holotype (ZMKU AM 01600). **B.** adult female paratype (ZMKU AM 01606).

et al., 2015); and interorbital caruncle weakly developed, low-profiled, U-shaped, without free posterior margin (vs. caruncle well-developed, low-profiled, U-shaped with free posterior margin).

Limnionectes sirindhornae sp. nov. is most closely related in mitochondrial DNA to *L. lauhachindai* (Fig. 2) but differs in morphology by having interorbital caruncle weakly developed, low-profiled, U-shaped, without free posterior margin (vs. caruncle well-developed, low-profiled, U-shaped with free posterior margin); vocal sac opening in males absent (vs. present); adult males having statistically larger SVL, HDL, HDL, SNT, IOD, TMP, IND, SHK, TGH, LAL, HND, FTL, and IML ($p < 0.0001$ – 0.0328 ; Fig. 4); and females having statistically larger SVL, HDL, HDL, SNT, TMP, IND, SHK, TGH, LAL, HND, FTL, and IMW ($p < 0.0001$ – 0.0485 ; Fig. 5).

Limnionectes sirindhornae sp. nov. can be differentiated from *L. macrognathus* by having smaller SVL of 36.7–44.7 mm in males (vs. 47–57 mm; Boulenger, 1920); interorbital caruncle weakly developed, low-profiled, U-shaped, without free posterior margin (vs. caruncle lacking U-shape); and ventral surface in life without yellowish coloration (vs. belly and ventral surface of thighs pale yellow).

Limnionectes sirindhornae sp. nov. can be differentiated from *L. plicatellus* by having interorbital caruncle weakly developed, low-profiled, U-shaped, without free posterior margin (vs. caruncle high-profiled and horned); and longitudinal folds on dorsum absent (vs. dorsum with ten to twelve longitudinal folds); and ventral surface in life without yellowish coloration (vs. belly yellow).

Limnonectes sirindhornae sp. nov. can be differentiated from *L. savan* by having interorbital caruncle weakly developed, low-profiled, U-shaped, without free posterior margin (vs. caruncle lacking U-shaped); vocal sac opening in adult males absent (vs. present); relative finger lengths $II < IV < I < III$ (vs. $II < I < IV < III$); relative toe lengths $I < II < III < V < IV$ (vs. $I < II < III = V < IV$ or $I < II < V < III < IV$); and lacking yellowish wash coloration on ventral surface in life (vs. inguinal region and ventral surface of shank with yellowish wash).

DISCUSSION

Our phylogenetic analyses based on a fragment of mitochondrial *16S* gene revealed that *L. sirindhornae* sp. nov. is a member of a clade containing four Indo-chinese caruncle-bearing species, *L. dabamus*, *L. gyldenstolpei*, *L. lauhachindai* and *L. savan*. The new species is most closely related to *L. lauhachindai* from Ubon Ratchathani Province, approximately 300 km to the southeast. However, the new species can be distinguished from its sister species *L. lauhachindai* by having larger body size in both sexes and a weakly

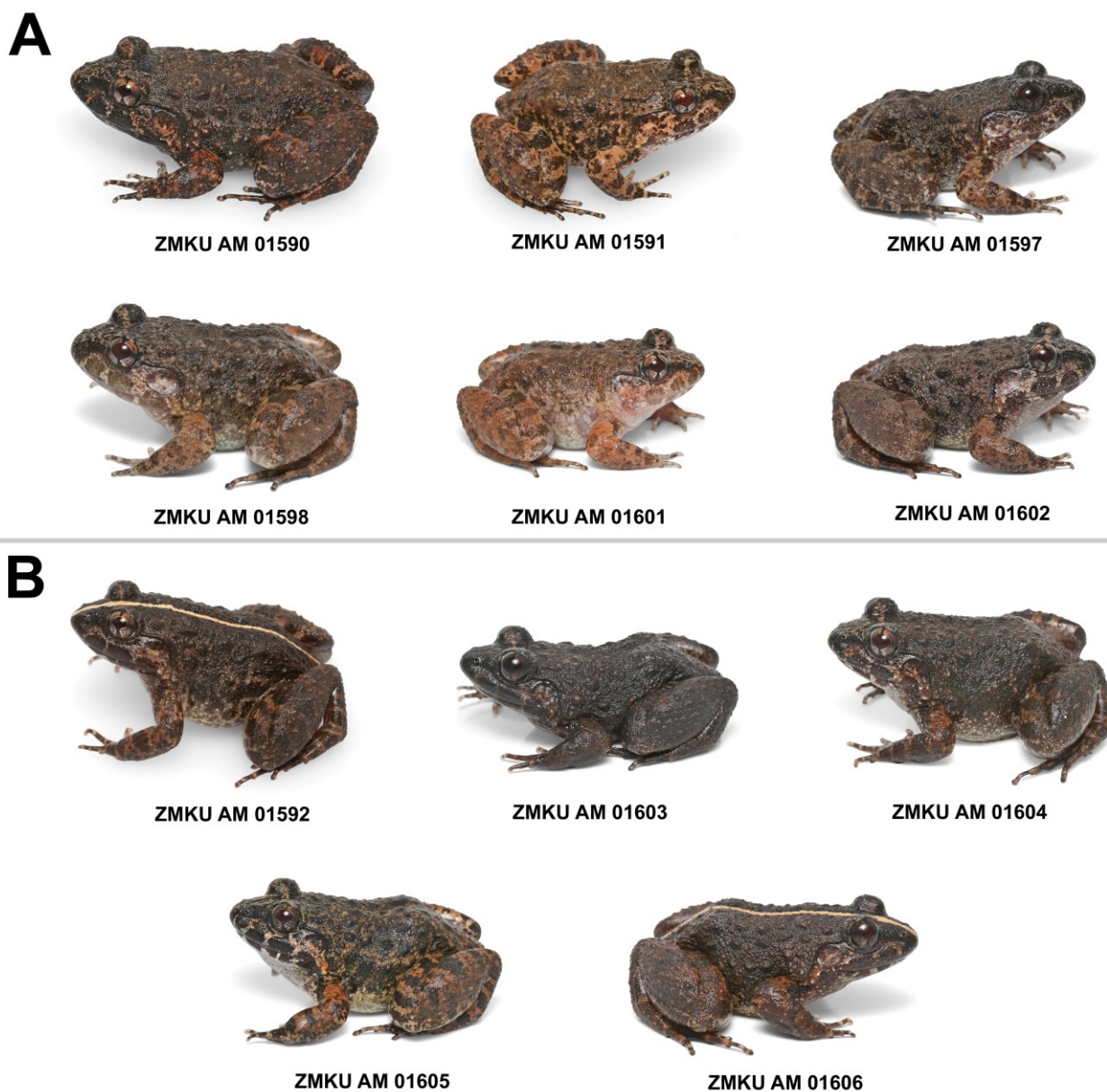


FIGURE 9. Paratypes of *Limnonectes sirindhornae* sp. nov. in life. **A.** adult males **B.** adult females.

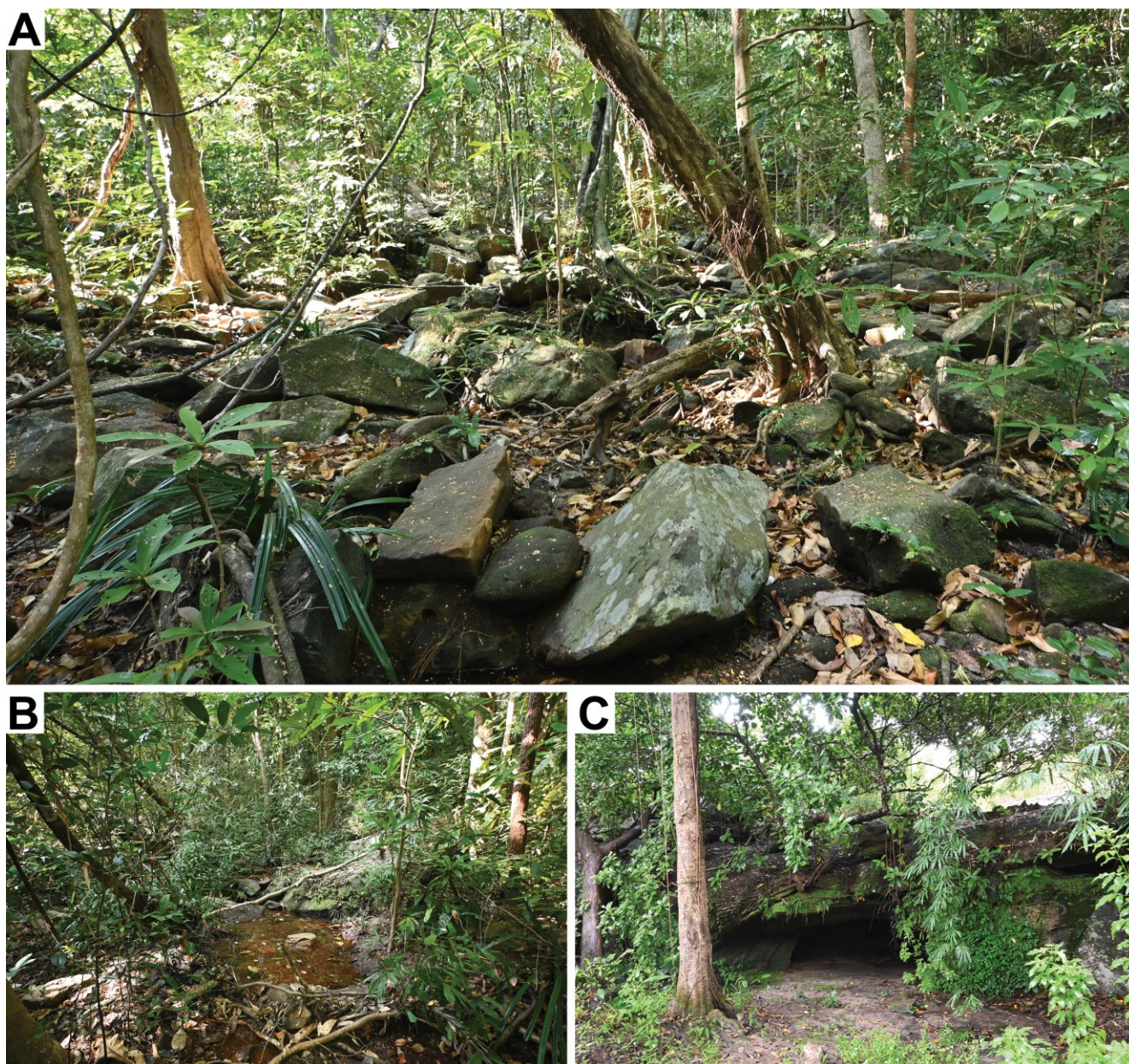


FIGURE 10. Habitat of *Limnionectes sirindhornae* sp. nov. at the type locality in Rai Subdistrict, Phanna Nikhom District, Sakon Nakhon Province, Thailand. **A.** rocky stream and **B.** pool along side of intermittent stream in dry season (January) **C.** sandstone cave in wet season (October).

developed male caruncle. *Limnionectes sirindhornae* sp. nov. is currently known only from the Phu Phan Mountain Range in Phanna Nikhom District, Sakon Nakhon Province in northeastern Thailand. The Phu Phan Range is a long, narrow mountain range located in the central part of the Khorat Plateau, extending into the Savannakhet and Salavan regions of southern Laos (Veeravinantanakul et al., 2018). The description of *L. sirindhornae* sp. nov. brings the total number of species of Thai *Limnionectes* to 21 and caruncle-bearing *Limnionectes* to seven (Aowphol et al., 2015; Phimmachak et al., 2019; Frost 2024). Previously, several range-restricted amphibians and reptiles were discovered from the Phu Phan Range e.g., *Fejervarya*

triora Stuart, Chuaynkern, Chan-ard & Inger, 2006, *Leiolepis glaurung* Wanchai et al., 2024, *Tropidophorus matsuii* Hikida, Orlov, Nabhitabhata & Ota, 2002, and *Zhangixalus jarujini* (Matsui & Panha, 2006) (Hikida et al., 2002; Matsui and Panha, 2006; Stuart et al., 2006; Wanchai et al., 2024). The discovery of the new *Limnionectes* species highlights the importance of herpetofauna diversity and endemism in the Khorat Plateau along the Thai-Laos border. This finding underscores the need for additional field surveys across the Phu Phan Range and other regions of the Khorat Plateau to determine the actual geographic range of *L. sirindhornae* sp. nov. and enhance the herpetofaunal inventory. Furthermore,

investigating the natural history and ecological aspects of this new species are necessary for its effective conservation.

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LITERATURE CITED

- Andersson, L.G. 1916. Zoological results of the Swedish zoological expeditions to Siam 1911–1912 and 1914. 3. Batrachians. Kongliga Svenska Vetenskapsakademiens Handlingar, 55: 13–17.
- Anderson, J. 1871. A list of the reptilian accession to the Indian Museum, Calcutta from 1865 to 1870, with a description of some new species. Journal of the Asiatic Society of Bengal, 40: 12–39.
- Aowphol, A., Rujirawan, A., Taksintum, W., Chuaynkern, Y. and Stuart, B.L. 2015. A new caruncle-bearing *Limnonectes* (Anura: Dicroglossidae) from northeastern Thailand. Zootaxa, 3956: 258–270. <https://doi.org/10.11646/zootaxa.3956.2.6>
- Boulenger, G.A. 1903. On a new frog from Upper Burma and Siam. Annals and Magazine of Natural History, Series 7, 12: 219. <https://doi.org/10.1080/00222930308678844>
- Boulenger, G.A. 1917. Descriptions of new frogs of the genus *Rana*. Annals and Magazine of Natural History, Series 8, 20: 413–418. <https://doi.org/10.1080/00222931709487029>
- Boulenger, G.A. 1920. A monograph of the South Asian, Papuan, Melanesian and Australian frogs of the genus *Rana*. Records of the Indian Museum, 20: 1–226. <https://doi.org/10.5962/bhl.title.12471>
- Chan, K.O. and Grismer, L.L. 2022. GroupStruct: an R package for allometric size correction. Zootaxa, 5124(4): 471–482. <https://doi.org/10.11646/zootaxa.5124.4.4>
- Chen, L., Murphy, R.W., Lathrop, A., Ngo, A., Orlov N.L., Ho, C.T. and Somorjai, I.L.M. 2005. Taxonomic chaos in Asian ranid frogs: an initial phylogenetic resolution. Herpetological Journal, 15: 231–243.
- Emerson, S.B., Inger, R.F. and Iskandar, D. 2000. Molecular systematics and biogeography of the fanged frogs of Southeast Asia. Molecular Phylogenetics and Evolution, 16: 131–142. <https://doi.org/10.1006/mpev.2000.0778>
- Evans, B.J., Brown, R.M., McGuire, J.A., Supriatna, J., Andayani, N., Diesmos, A., Iskandar, D., Melnick D.J. and Cannatella, D.C. 2003. Phylogenetics of fanged frogs: testing biogeographical hypotheses at the interface of the Asian and Australian faunal zones. Systematic Biology, 52: 794–819. <https://doi.org/10.1080/10635150390251063>
- Fitzinger, L.J.F.J. 1843. Systema Reptilium. Fasciculus Primus. Braumüller et Seidel Bibliopolas, Wien, 120 pp.
- Frost, D.R. 2024. Amphibian Species of the World: an Online Reference. Version 6.2. American Museum of Natural History, New York, USA. Available from <https://amphibiansoftheworld.amnh.org/index.php> (accessed 24 July 2025).
- Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. and Wheeler, W.C. 2006. The amphibian tree of life. Bulletin of the American Museum of Natural History, 297: 1–370. [https://doi.org/10.1206/0003-0090\(2006\)297\[0001:TATOL\]2.0.CO;2](https://doi.org/10.1206/0003-0090(2006)297[0001:TATOL]2.0.CO;2)
- Hallowell, E. 1861 [1860]. Report upon the Reptilia of the North Pacific Exploring Expedition, under command of Capt. John Rogers, U.S. N. Proceedings of the Academy of Natural Sciences of Philadelphia, 12: 480–510.
- Hasan, M.K., Islam, M.M., Kuramoto, M., Kurabayashi, A. and Sumida, M. 2014. Description of two new species of *Microhyla* (Anura: Microhylidae) from Bangladesh. Zootaxa, 3755: 401–408. <http://dx.doi.org/10.11646/zootaxa.3755.5.1>
- Hikida, T., Orlov, N.L., Nabhitabhata, J. and Ota, H. 2002. Three new depressed-bodied water skinks of the genus *Tropidophorus* (Lacertilia: Scincidae) from Thailand and Vietnam. Current Herpetology 21 (1): 9–23. <https://doi.org/10.5358/hsj.21.9>
- Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. and Vinh, L.S. 2018. UFBoot2: improving the ultrafast bootstrap approximation. Molecular Biology and Evolution, 35(2): 518–522. <https://doi.org/10.1093/molbev/msx281>
- Huelsenbeck, J.P. and Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, 17(8): 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Inger, R.F. and Stuart, B.L. 2010. Systematics of *Limnonectes* (*Taylorana*) Dubois. Current Herpetology, 29: 51–68. <https://doi.org/10.3105/018.029.0201>
- Kassambara, A. and Mundt, F. 2020. factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7. <https://CRAN.R-project.org/package=factoextra>
- Katoh, K. and Standley, D.M. 2013. MAFFT Multiple Sequence Alignment software version 7: improvements in performance and stability. Molecular Biology and Evolution, 30: 772–780. <https://doi.org/10.1093/molbev/mst010>
- Köhler, G., Zwiters, B., Than, N.L., Gupta, D.K., Janke, A., Pauls, S.U. and Thammachoti, P. 2021. Bioacoustics reveal hidden diversity in frogs: two new species of the Genus *Limnonectes* from Myanmar (Amphibia, Anura, Dicroglossidae). Diversity, 13(9): 399. <https://doi.org/10.3390/d13090399>
- Lambertz, M., Hartmann, T., Walsh, S., Geissler, P. and McLeod, D.S. 2014. Anatomy, histology, and systematic implications of the head ornamentation in the males of four species of *Limnonectes* (Anura: Dicroglossidae). Zoological Journal of the Linnean Society, 172: 117–132. <https://doi.org/10.1111/zoj.12171>
- Lanfear, R., Frandsen, P.B., Wright, A. M., Senfeld, T. and Calcott, B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic Analyses. Molecular biology and evolution, 34(3): 772–773. <https://doi.org/10.1093/molbev/msw260>
- Le, M.V., Nguyen, L.T., Murphy, R.W., Tran, T.D.T., Nguyen, S.N. and Che, J. 2025. A new fanged frog of the genus *Limnonectes* Fitzinger, 1843 (Anura: Dicroglossidae) from Langbian Plateau, southern Vietnam. Zootaxa, 5570 (1): 57–80. <https://doi.org/10.11646/zootaxa.5570.1.2>
- Lê, S., Josse, J. and Husson, F. 2008. FactoMineR: an R package for multivariate analysis. Journal of Statistical Software, 25(1):1–18. <https://doi.org/10.18637/jss.v025.i01>
- Liu, Z.Q., Wang, Y.Q. and Su, B. 2005. The mitochondrial genome organization of the rice frog, *Fejervarya limnocharis* (Amphibia: Anura): a new gene order in the vertebrate mtDNA. Gene, 346: 145–151. <https://doi.org/10.1016/j.gene.2004.10.013>

- Lleonart, J., Salat, J. and Torres, G.J. 2000. Removing allometric effects of body size in morphological analysis. *Journal of Theoretical Biology* 205(1): 85–93. <https://doi.org/10.1006/jtbi.2000.2043>
- Matsui, M. and Panha, S. 2006. A new species of *Rhacophorus* from eastern Thailand (Anura: Rhacophoridae). *Zoological Science*, 23: 477–481. <https://doi.org/10.2108/zsj.23.477>
- Matsui, M., Nishikawa, K. and Eto, K. 2014. A new burrow-utilising fanged frog from Sarawak, East Malaysia (Anura: Dicroglossidae). *Raffles Bulletin of Zoology*, 62: 679–687.
- Miller, M.A., Pfeiffer, W. and Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop (GCE), 1–8. <https://doi.org/10.1109/GCE.2010.5676129>
- Minh, Q., Nguyen, M.A.T. and von Haeseler, A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, 30(5): 1188–1195. <https://doi.org/10.1093/molbev/mst024>
- Oaks, J.R., Sukumaran, J., Esselstyn, J.A., Linkem, C.W., Siler, C.D., Holder, M.T. and Brown, R.M. 2013. Evidence for climate-driven diversification? A caution for interpreting ABC inferences of simultaneous historical events. *Evolution*, 67: 991–1010. <https://doi.org/10.1111/j.1558-5646.2012.01840.x>
- Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S. and Weedon, J. 2024. vegan: community ecology R package version 2.6-10. <https://cran.r-project.org/web/packages/vegan/index.html>
- Palumbi, S.R. 1996. Nucleic acids II: the polymerase chain reaction. In Hillis, D.M., Moritz, C. and Mable, B.K. (Eds). *Molecular Systematics*, Second edition, Sinauer Associates, Inc., Sunderland, p. 205–247.
- Phimmachak, S., Richards, S.J., Sivongxay, N., Saeteun, S., Chuaynkern, Y., Makchai, S., Som, H.E. and Stuart, B.L. 2019. A caruncle-bearing fanged frog (*Limnonectes*, Dicroglossidae) from Laos and Thailand. *ZooKeys*, 846: 133–155. <https://doi.org/10.3897/zookeys.846.33200>
- Phimmachak, S., Sivongxay, N., Seateun, S., Yodthong, S., Rujirawan, A., Neang, T., Aowphol, A. and Stuart, B.L. 2018. A new *Limnonectes* (Anura: Dicroglossidae) from southern Laos. *Zootaxa*, 4375: 325–340. <https://doi.org/10.11646/zootaxa.4375.3.2>
- R Core Team 2025. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G. and Suchard, M.A. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67(5): 901–904. <https://doi.org/10.1093/sysbio/syy032>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61: 1–4. <https://doi.org/10.1093/sysbio/sys029>
- Rowley, J.J.L., Le, D.T.T., Hoang, H.D. and Altig, R. 2014. The breeding behaviour, advertisement call and tadpole of *Limnonectes dabanus* (Anura: Dicroglossidae). *Zootaxa*, 3881: 195–200. <https://doi.org/10.11646/zootaxa.3881.2.8>
- Simmons, J.E. 2015. *Herpetological Collecting and Collections Management*, Society for the Study of Amphibians and Reptiles Herpetological Circular No. 42. Salt Lake City, UT, 191 pp.
- Smith, M.A. 1922. The frogs allied to *Rana doriae*. *Journal of the Natural History Society of Siam*, 4: 215–229.
- Stoliczka, F. 1873. Notes on some species of Malayan Amphibia and Reptilia. *Journal of the Asiatic Society of Bengal*, 42: 111–126.
- Stuart, B.L., Chuaynkern, Y., Chan-ard, T. and Inger, R.F. 2006. Three species of frogs and a new tadpole from eastern Thailand. *Fieldiana Zoology, New Series*, 1543: 1–10.
- Tamura, K., Stecher, G. and Kumar, S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7): 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Taylor, E.H. 1962. The amphibian fauna of Thailand. *The University of Kansas Science Bulletin*, 43: 265–599. <https://doi.org/10.5962/bhl.part.13347>
- Taylor, E.H. and Elbel, R.E. 1958. Contribution to the herpetology of Thailand. *University of Kansas Science Bulletin*, 38: 1033–1189.
- Thorpe, R.S. 1975. Quantitative handling of characters useful in snake systematics with particular reference to intraspecific variation in the Ringed Snake *Natrix natrix* (L.). *Biological Journal of the Linnean Society*, 7(1): 27–43. <https://doi.org/10.1111/j.1095-8312.1975.tb00732.x>
- Trifinopoulos, J., Nguyen, L.T., von Haeseler, A. and Minh, B.Q. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*, 44(W1): W232–W235. <https://doi.org/10.1093/nar/gkw256>
- Turan, C. 1999. A note on the examination of morphometric differentiation among fish populations: The truss system. *Turkish Journal of Zoology*, 23(3): 259–263.
- Veeravanantanakul, A., Kanjanapayont, P., Sangsompom, A., Hasebe, N. and Charusiri, P. 2018. Structure of Phu Phan Range in the Khorat Plateau: Its Apatite Fission Track Ages and Geological Syntheses. *Bulletin of Earth Sciences of Thailand*, 10(1): 8–16.
- Vogt, T. 1911. Beitrag zur Amphibien-fauna der Insel Formosa. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin* 1911: 179–184.
- Wanchai, P., Rujirawan, A., Murdoch, M.L., Aksornneam, A., Promnun, P., Kaatz, A., Gregory, J.J., Nguyen, E., Iderstein, W.V., Quah, E.S.H., Grismer, L.L., Grismer, J.L. and Aowphol, A. 2024. The description of the first rock-dwelling species of butterfly lizard *Leiolepis* Cuvier, 1829 (Squamata, Agamidae) from the Khorat Plateau in northeastern Thailand. *ZooKeys*, 1210: 299–324. <https://doi.org/10.3897/zookeys.1210.127557>
- Wilcox, T.P., Zwickl, D.J., Heath, T.A. and Hillis, D.M. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution*, 25(2): 361–371. [https://doi.org/10.1016/S1055-7903\(02\)00244-0](https://doi.org/10.1016/S1055-7903(02)00244-0)
- Yodthong, S., Rujirawan, A., Stuart, B.L. and Aowphol, A. 2021. A New *Limnonectes* (Anura: Dicroglossidae) from Southern Thailand. *Animals*, 11(2): 566. <https://doi.org/10.3390/ani11020566>
- Zhang, J., Miao, G., Hu, S., Sun, Q., Ding, H., Ji, Z., Guo, P., Yan, S., Wang, C., Kan, X. and Nie, L. 2021. Quantification and evolution of mitochondrial genome rearrangement in Amphibians. *BMC Ecology and Evolution*, 21: 19. <https://doi.org/10.1186/s12862-021-01755-3>
- Zhang, J.F., Nie, L.W., Wang, Y. and Hu, L.L. 2009. The complete mitochondrial genome of the large headed frog, *Limnonectes bannaensis* (Amphibia: Anura), and a novel gene organization in the vertebrate mtDNA. *Gene*, 442: 119–127. <https://doi.org/10.1016/j.gene.2009.04.018>
- Zhou, Y., Zhang, J.Y., Zheng, R.Q., Yu, B.G. and Yang, G. 2009. Complete nucleotide sequence and gene organization of the mitochondrial genome of *Paa spinosa* (Anura: Ranoidae). *Gene*, 447: 86–96. <https://doi.org/10.1016/j.gene.2009.07.009>