Zoosyst. Evol. 100 (4) 2024, 1361–1373 | DOI 10.3897/zse.100.129398

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Taxonomic revision of the genus *Micryletta* (Amphibia, Microhylidae), with description of a new species from Thailand

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Academic editor: Umilaela Arifin ♦ **Received** 11 June 2024 ♦ **Accepted** 31 August 2024 ♦ **Published** 10 October 2024

Abstract

The genus *Micryletta* is widely distributed in South China and Southeast Asia. Although significant progress has been made in the diversity and taxonomy of this genus over the past few years, the distribution range and taxonomy of some species still remain controversial, especially in *M. inornata* sensu lato. Consequently, limitations at national borders have resulted in a lack of comparative research on species from different countries. To resolve the classification dispute, assess species diversity, and determine the distribution range of *Micryletta*, a series of specimens were collected from the Yunnan Province of China and Thailand during herpetological surveys from 2009 to 2020. Subsequent analyses based on morphological and molecular data revealed a distinct and previously unknown lineage from western Thailand, which we formally describe as a new species. Furthermore, our study confirms that *M.* '*inornata*', previously known from Mengla, Yunnan, was actually *M. menglienica*, and further extends its distribution range to Thailand and Laos. In addition, our findings extend the latitudinal distribution of *M. inornata* and *M. subaraji* northward into southern Thailand. Notably, this study brings the total number of known species in the genus *Micryletta* from 13 to 14, with the count rising from three to six species in Thailand and from one to three in Laos. Our study further confirms that species diversity within the genus *Micryletta* is underestimated and emphasizes the important role that international collaborations play in taxonomy. Intensifying field surveys in other regions (e.g., Myanmar, Vietnam, and Guangxi of China) will be extremely necessary in the future to clarify any taxonomic questions and reevaluate the distribution range of these species.

Key Words

Distribution range, diversity, *Micryletta*, *M. inornata* sensu lato, new species

* These authors contributed equally to this work.

Introduction

South China and Southeast Asia span the Himalaya, Indo-Burma, and Sundaland biodiversity hotspots and host a high diversity of species (Myers et al. 2000). The region's complex evolutionary histories and highly diverse habitats, primarily attributed to the wide variation in landforms, climate, and latitude, are deemed to support the high species diversity (Janssens et al. 2016; Huang et al. 2023). Over the past few years, research on *Sylvirana* (Sheridan and Stuart 2018), *Leptobrachella* (Chen et al. 2021), *Megophrys* (Chen et al. 2017), and *Amolops* (Wang et al. 2020) suggests the presence of cryptic amphibian species diversity within this region. Additionally, some new genera and new species have been discovered in this area (e.g., Wu et al. 2021; Yodthong et al. 2021; Suwannapoom et al. 2022; Matsui et al. 2023). Despite being home to an extensively rich amphibian diversity, southern China and Southeast Asia are also threatened by human activity and global warming (Hughes 2018), which increases the risk of extinction prior to the discovery of new species.

The genus *Micryletta*, originally described by Dubois (1987) to accommodate two previously known species, *M. inornata* (Boulenger, 1890) and *M. steinegeri* (Boulenger, 1909), now includes 13 recognized species (Frost 2024). Among these, nine new species have been described in the last five years (e.g., Das et al. 2019; Liu et al. 2021a; Miller et al. 2021; Poyarkov et al. 2021; Yang and Poyarkov 2021; Sankar et al. 2022). Members of the genus *Micryletta* are widely distributed from southern China to northern Indonesia and eastern India (Frost 2024), with most species only known from their type localities (e.g., Poyarkov et al. 2018, 2021; Munir et al. 2020; Suwannapoom et al. 2020; Yang and Poyarkov 2021; Sankar et al. 2022). National boundaries have posed a challenge to the documentation of species distribution ranges, resulting in paucity of comparative research between different countries.

Moreover, species classification within *Micryletta* has been faced with a long-standing controversy, especially *M. inornata* sensu lato (Liu et al. 2021a). Its morphological similarities with other species of *Micryletta* led *M. inornata* to be historically considered a widespread species that occurred in Sumatra throughout the Malay Peninsula and Indochina. However, a recent study by Alhadi et al. (2019) restricted the distribution of *M. inornata* to the island of Sumatra, resulting in numerous unnamed lineages eventually described as new species by other authors (Das et al. 2019; Munir et al. 2020; Suwannapoom et al. 2020; Liu et al. 2021a; Poyarkov et al. 2021; Yang and Poyarkov 2021). However, *M. inornata* sensu lato is still paraphyletic with a disjunct distribution as it contains undescribed paraphyletic lineages. Among these, lineages from Kanchanaburi in Thailand (Miller et al. 2021), Phongsaly in Laos (Das et al. 2019), and Yunnan and Guangxi in China (Liu et al. 2021a; Yeung et al. 2023) remain uncertain.

To resolve the classification dispute, assess species diversity, and determine the distribution range of *Micryletta*, we sort out a number of specimens from fieldwork collections in southern China and Southeast Asia from 2009 to 2020. Subsequent genetic analyses and morphological comparisons revealed that populations from western Thailand represent a distinct evolutionary lineage that could not be classified into any known taxa; therefore, we identify it as a new species. In addition, we further clarify and update the distribution ranges for *M. menglienica* and *M. subaraji*.

Materials and methods

Sampling

A total of 17 samples were collected from China and Thailand in different seasons (Fig. 1). After euthanasia, liver tissues were stored in 95% ethanol. All specimens were fixed in 10% formalin, then transferred to 75% ethanol, and finally deposited at Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ). Research protocols were approved by the Ethics Committee of the Kunming Institute of Zoology, Chinese Academy of Science (IACUC no.: IACUC-OE-2021-07-001).

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from the liver or muscle tissues using the standard phenol-chloroform extraction protocol (Sambrook et al. 1989). A fragment of the mitochondrial 16S ribosomal RNA gene (16S rRNA) was amplified and sequenced for all samples using primers 16S rRNA-F (CGCCTGTTTAYCAAAAACAT) and 16S rRNA-R (CCGGTYTGAACTCAGATCAYGT) (Kocher et al. 1989). The reaction volume of PCR amplifications was 25 µl, and the reaction procedure was as follows: initial denaturing step at 95 °C for 4 min, 35 cycles of denaturation at 94 °C for 40 s, annealing at 55 °C for 40 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The amplified PCR product was purified using a Qiagen PCR purification kit (Watson Biotechnologies, Shanghai, China) and then sequenced in both directions using an ABI 3100 automated sequencer. Sequences generated in this study were assembled and edited using AutoSeqMan (Sun 2018). Sequences were aligned and manually optimized in MEGA 6.0 (Tamura et al. 2013). All the new sequences were submitted to the GenBank. Homologous sequences of the genus *Micryletta* and those of the outgroups *Mysticellus franki*, *Kaloula pulchra*, and *Uperodon systom* were downloaded from the GenBank (Table 1).

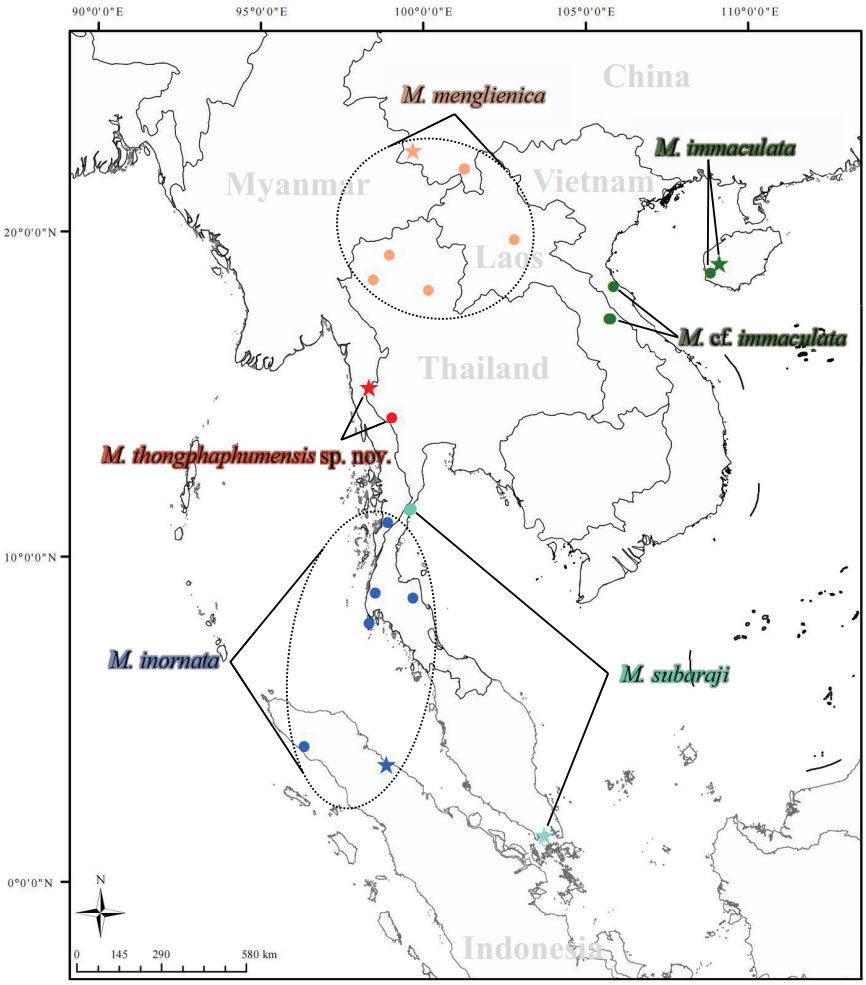

Figure 1. Map showing the distribution of *M. thongphaphumensis* sp. nov., *M. menglienica*, *M. immaculata*, *M. subaraji*, and *M. inornata*. The pentagram represents the type locality of these species. The same color represents the same species.

Molecular analysis

Bayesian inference (BI) and maximum likelihood (ML) methods were used to analyze phylogenetic relationships based on mitochondrial 16S rRNA. The BI analysis and ML analysis were conducted using the MrBayes tool on XSEDE and RAxML-HPC BlackBox in CIPRES Web (Miller et al. 2010). For BI analysis, we ran a jModelTest with Bayesian information criteria on the alignment, resulting in the best-fitting nucleotide substitution model of TIM2ef+I+G (Darriba et al. 2012). The Monte Carlo Markov chain length was run for 10,000,000 generations and sampled every 1,000 generations. The first 30% of the sampled trees were discarded as burn-in after the standard deviation of split frequencies of the two runs was less than a value of 0.01. The remaining trees were used to create a 50% majority rule consensus tree and to estimate Bayesian posterior probabilities. For the ML analysis, we used the proportion of invariable sites estimated from the data and 1,000 bootstrap pseudo replicates. We also computed pairwise comparisons of uncorrected sequence divergence (*p*-distance) for the partial sequences of the 16S rRNA using MEGA 6.0 (Tamura et al. 2013).

Morphology

Only adult specimens were examined for morphometric studies. The sex and maturity of the specimens were determined by identifying secondary sexual characteristics (such as vocal sacs in males and eggs in gravid females) or through gonadal examination using a small lateral or ventral incision. Measurements were taken to the nearest 0.1 mm with digital calipers. The morphometrics and character terminology follow Fei et al. (2009): snout-vent length (**SVL**); head length (**HL**); head width (**HW**); snout length (**SL**); internasal space (**INS**); interorbital space (**IOS**); width of upper eyelid (**UEW**); diameter of eye (**ED**); diameter of tympanum (**TD**); distance from anterior border of tympanum to posterior orbital border (**TYE**); distance from the center of the nostril to the tip of the snout (**SN**); distance from the front of the eye to the center of the nostril (**EN**); internal front of eyes, the shortest distance between the anterior orbital borders of the eyes (**IFE**); internal back of eyes, the shortest distance between the posterior orbital borders of the eyes (**IBE**); forearm length, measured from the elbow to the wrist (**FAL**); hand length (**HAL**); diameter of lower arm (**LAD**); thigh length, from the cloaca to the knee (**THL**); tibia length/shank length,

Table 1. Localities, voucher information, and Genbank accession numbers for all specimens used in molecular analyses of this study. The "*" indicates that the sequences are derived from the holotype or paratype. The "#" indicates that the sequences are derived from the type locality.

ID	Species	Voucher ID	Locality	GenBank No.	References
Ingroup					
1	*M. aishani*#	SDBDU 3920	Subhong, Cachar district, Assam, India	MK889218	Das et al. 2019
2	*M. aishani*	CAS 231509	Kachin State, Myanmar	MW035603	Miller et al. 2021
3	*M. dissimulans**	AUP 01690	Saba Yoi District, Songkhla, Thailand	MT573414	Suwannapoom et al. 2020
4	*M. dissimulans**	AUP 01691	Saba Yoi District, Songkhla, Thailand	MT573415	Suwannapoom et al. 2020
5	*M. dissimulans**	AUP 01696	Saba Yoi District, Songkhla, Thailand	MT573416	Suwannapoom et al. 2020
6	*M. erythropoda*#	ZMMUA4721-1542	Ma Da, Dong Nai, Vietnam	MH756147	Poyarkov et al. 2018
7	*M. erythropoda*#	ZMMUA4721-1533	Ma Da, Dong Nai, Vietnam	MH756146	Poyarkov et al. 2018
8	*M. hekouensis**	KIZ 20210510	Nanxi, Hekou, Yunnan, China	MZ536627	Liu et al. 2021a
9	*M. hekouensis**	KIZ 20210511	Nanxi, Hekou, Yunnan, China	MZ536628	Liu et al. 2021a
10	*M. immaculata**	KFBG 14271	Exianling Nature Reserve, Hainan, China	MW376737	Yang and Poyarkov 2021
11	*M. immaculata**	KFBG 14270	Exianling Nature Reserve, Hainan, China	MW376736	Yang and Poyarkov 2021
12	*M.* cf. *immaculata*	TZ 98110	Chin Xai, Ha Tinh, Vietnam	AF285207	Ziegler 2000
13	*M.* cf. *immaculata*	FMNH 255121(1)	Boualapha, Khammouan, Laos	KC822494	Blackburn et al. 2013
14	*M.* cf. *immaculata*	FMNH 255121(2)	Boualapha, Khammouan, Laos	KC179997	De Sá et al. 2012
15	*M. inornata*	USNM 587625	Lenya National Park, Tanintharyi, Myanmar	MT609033	Miller et al. 2021
16	*M. inornata*	MZB 27242	Suka Makmue, Aceh, Sumatra, Indonesia	LC208138	Alhadi et al. 2017
17	*M. inornata*	KIZ 031299	Kanom District, Phuket, Thailand	PQ208536	This study
18	*M. inornata*	KIZ 025629	Nopphitam, Nakhon Si Thammarat, Thailand	PQ208534	This study
19	*M. inornata*	KIZ 031264	Klong Sok District, Suratthani, Thailand	PQ208535	This study
20	*M. inornata*#	MZB 23948	Deli Serdang, Sumatra, Indonesia	LC208137	Alhadi et al. 2017
21	*M. inornata*#	MZB 23947	Deli Serdang, Sumatra, Indonesia	LC208136	Alhadi et al. 2017
22	*M. inornata*#	MZB 23949	Deli Serdang, Sumatra, Indonesia	LC208135	Alhadi et al. 2017
23	*M. lineata*	CAS 247206	Kawthaung, Tanintharyi, Myanmar	KM509167	Peloso et al. 2016
24	*M. lineata*	KUHE 23858	Ranong, Thailand	AB634695	Matsui et al. 2011b
25	*M. lineata*	USNM 587624	Lenya, Ma Noe Lone, Tanintharyi, Myanmar	MT609052	Miller et al. 2021
26	*M. lineata*	USNM 587911	Nint Tenku, Tanintharyi, Myanmar	MT609036	Miller et al. 2021
27	*M. lineata*	CAS 247200	Tanintharyi, Myanmar	MW042901	Miller et al. 2021
28	*M. melanops*#	ZMMU NAP-01381-5	Bidoup Nui Ba National Park, Lam Dong, Vietnam	MZ474685	Poyarkov et al. 2021
29	*M. melanops**	ZMMU A7583	Bidoup Nui Ba National Park, Lam Dong, Vietnam	MZ474684	Poyarkov et al. 2021
30	*M. menglienica*	K 3246	Ban Sop Chuna, Luangprabang, Laos	KC180027	De Sá et al. 2012
31	*M. menglienica*	K 3068	Doi Chiang Dao, Chiang Mai, Thailand	KR827953	Grosjean et al. 2015
32	*M. menglienica*	KUHE 20497	Mae Yom, Phrae, Thailand	AB598341	Matsui 2011a
33	*M. menglienica*	-	Thailand	AF215375	Vences 1999
34	*M. menglienica*	KFBGF14654	Menglun, Mengla, Yunnan, China	OR053963	Yeung et al. 2023
35	*M. menglienica*	KFBGF14653	Menglun, Mengla, Yunnan, China	OR053962	Yeung et al. 2023
36	*M. menglienica*	KIZ 038313	Menglun, Mengla, Yunan, China	PQ208545	This study
37	*M. menglienica*	KIZ 01554	Menglun, Mengla, Yunan, China	PQ208543	This study
38	*M. menglienica*	KIZ 030759	Chomtong, Ban Laung, Chiang Mai, Thailand	PQ208544	This study
39	*M. menglienica*#	KIZ 20210712	Jingxin, Menglian, Yunnan, China	OK335187	Liu et al. 2021b
40	*M. menglienica*#	KIZ 20210711	Jingxin, Menglian, Yunnan, China	OK335186	Liu et al. 2021b
41	*M. nigromaculata*	DTU 301	Cuc Phuong National Park, Ninh Binh, Vietnam	MH756154	Poyarkov et al. 2018
42	*M. nigromaculata**	ZMMUA5934	Cat Ba National Park, Ninh Binh, Vietnam	MH756150	Poyarkov et al. 2018
43	*M. nigromaculata**	ZMMUA5940	Cat Ba National Park, Ninh Binh, Vietnam	MH756152	Poyarkov et al. 2018
44	*M. nigromaculata**	ZMMUA5942	Cat Ba National Park, Ninh Binh, Vietnam	MH756153	Poyarkov et al. 2018
45	*M. steinegeri*	-	Tainan, Taiwan, China	MW376735	Yang and Poyarkov 2021
46	*M. steinegeri*	ZMMUA5336-3	Kaohsiung, Taiwan, China	MW376734	Yang and Poyarkov 2021
47	*M. subaraji*	KIZ 049448	Ang Thong, Prachuap Khiri Khan, Thailand	PQ208547	This study
48	*M. subaraji*	KIZ 018074	Ang Thong, Prachuap Khiri Khan, Thailand	PQ208546	This study
49	*M. subaraji**	ZRC1.13389	Kranji Marshes, Singapore	ON026066	Sankar et al. 2022
50	*M. subaraji**	ZRC1.13370	Kranji Marshes, Singapore	ON026065	Sankar et al. 2022
51	*M. subaraji**	ZRC1.13369	Kranji Marshes, Singapore	ON026064	Sankar et al. 2022
52	*M. subaraji**	ZRC1.13323	Kranji Marshes, Singapore	ON026063	Sankar et al. 2022
53	*M. sumatrana**	MZB. Amph 30594	Hutan Harapan, Musi Banyuasin, Indonesia	MN727065	Munir et al. 2020
54	*M. thongphaphumensis* sp. nov.	KUHE 35133	Kanchanaburi, Thailand	AB611968	Kurabayashi et al. 2011
55	*M. thongphaphumensis* sp. nov.	KIZ 016771	Sai Yok Noi, Kanchanaburi, Thailand	PQ208541	This study
56	*M. thongphaphumensis* sp. nov.	KIZ 016690	Sai Yok Noi, Kanchanaburi, Thailand	PQ208537	This study
57	*M. thongphaphumensis* sp. nov.	KIZ 016768	Sai Yok Noi, Kanchanaburi, Thailand	PQ208539	This study
58	*M. thongphaphumensis* sp. nov.	KIZ 016763	Sai Yok Noi, Kanchanaburi, Thailand	PQ208538	This study
59	*M. thongphaphumensis* sp. nov.	KIZ 016769	Sai Yok Noi, Kanchanaburi, Thailand	PQ208540	This study
60	*M. thongphaphumensis* sp. nov.	KIZ 024670	Thong Pha Phum, Kanchanaburi, Thailand	PQ208542	This study
Outgroup					
61	*Mysticellus franki**	ZSI/WGRC/V/A/967	Suganthagiri, Wayanad district, Kerala state, India	MK285340	Garg and Biju 2019
62	*Uperodon systoma*	SDBDU 2005.4723	Kunnapattu, Tamil Nadu, India	MG557949	Garg et al. 2018
63	*Kaloula pulchra*	NMNS 3208	China	KC822614	Blackburn et al. 2013

from the knee to the ankle (**TL**); foot length (**FL**); length of inner metatarsal tubercle (**IMT**); first finger length (**FIL**); second finger length (**FIIL**); third finger length (**FIIIL**); fourth finger length (**FIVL**); first toe length (**TIL**); second toe length (**TIIL**); third toe length (**TIIIL**); fourth toe length (**TIVL**); fifth toe length (**TVL**).

Results

The BI and ML analyses obtained similar topologies with relatively high nodal support values for most terminal nodes, differing mainly at nodes identified as weakly supported or collapsed (Fig. 2). Our phylogenetic result shows that the monophyly of the genus *Micryletta* was strongly supported (BPP = 1.00; BS = 89; Fig. 2). In addition, phylogenetic analyses showed that our newly collected *Micryletta* specimens were recovered as four major clades (Fig. 2).

The first clade: seven samples (four samples from northern Thailand, two samples from Xishuangbanna, Yunnan, China, and one sample from Laos) clustered with the specimens (including the topotypic specimens) of *M. menglienica* with weak support (Fig. 2, clade I). The genetic distance of this clade was found to be very small, ranging from 0.0 to 1.0% (Suppl. material 1). Therefore, we considered them to be conspecific with *M. menglienica*.

The second clade: the population of *M.* cf. *inornata* from Kanchanaburi in Thailand, represented a distinct phylogenetic lineage with strong support (BPP = 1.00; BS = 100, clade II), which differed notably from topotypic specimens of *M. inornata* sensu stricto from Indonesia (Fig. 2). The interspecific genetic distance between the Kanchanaburi population and the other species of the genus *Micryletta* ranged from 2.8% (*M. hekouensis*) to 12.8% (*M. sumatrana*) (Table 2). It is comparable to the divergences among the nearest neighbor genetic distances of this group,

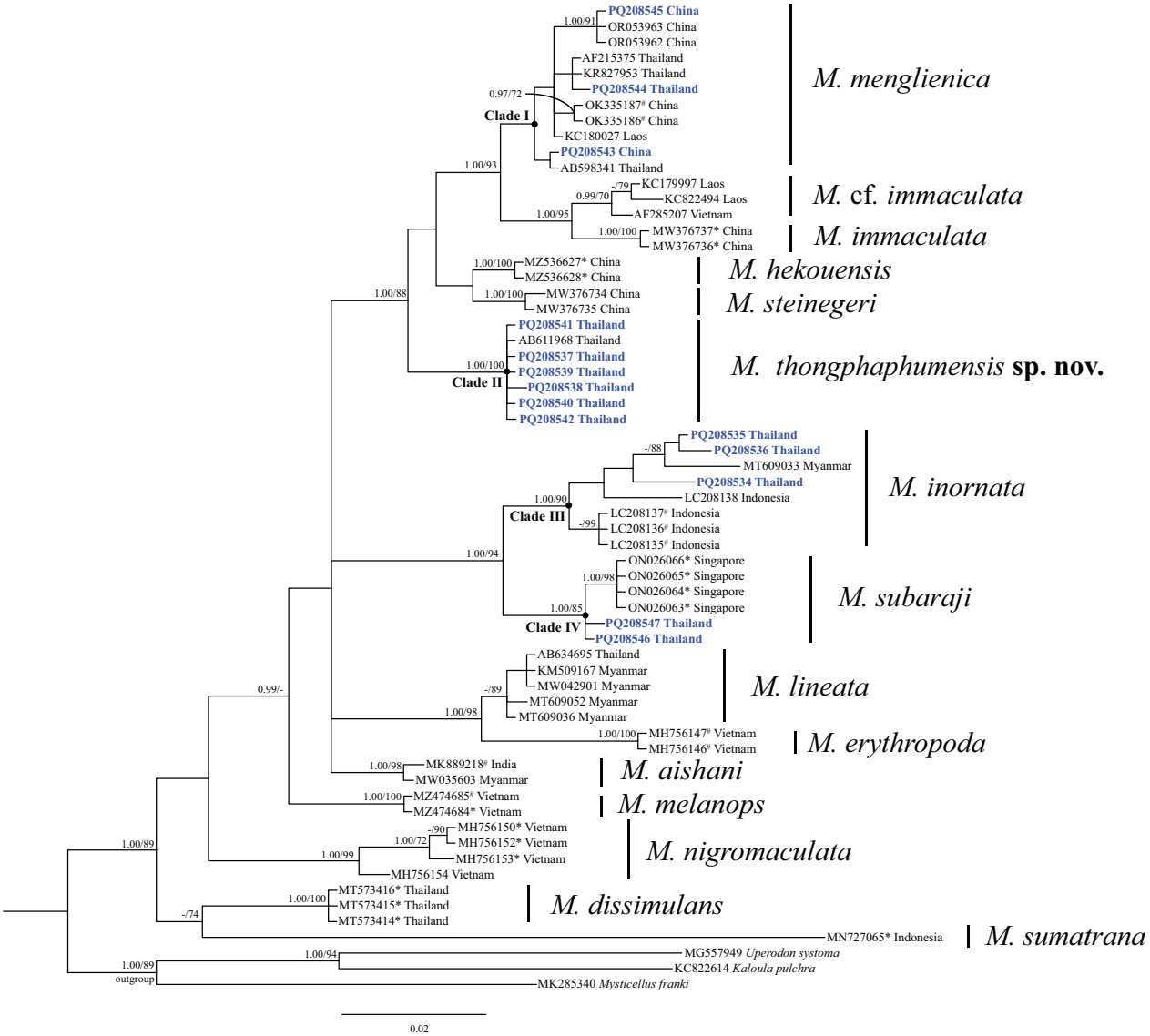

Figure 2. Phylogram of *Micryletta* based on mitochondrial 16S rRNA gene. Bayesian posterior probabilities (BPP) from BI analyses and bootstrap supports (BS) from ML analyses are listed beside the nodes. The symbol '-' represents a value below 0.95/70. '*' denotes the holotype or paratype. '#' denotes the specimen from the type locality. Blue bold text indicates newly generated data.

Table 2. Mean uncorrected *p*-distances (%) of 16S sequences among *Micryletta* species (below the diagonal) and standard error estimates (above the diagonal). The ingroup mean uncorrected *p*-distances are shown on the diagonal in bold.

ID	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*M. aishani*	**0.2**	0.8	0.9	0.9	0.8	0.9	1.0	0.8	0.7	0.8	0.8	1.0	0.8	0.9	1.4
2	*M.* cf. *immaculata*	3.9	**0.5**	1.1	1.0	0.7	0.5	1.1	0.8	0.9	1.0	0.6	1.1	0.7	1.1	1.5
3	*M. dissimulans*	4.4	6.2	**0.0**	1.2	1.0	1.1	1.0	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.4
4	*M. erythropoda*	4.7	5.9	7.4	**0.0**	0.9	1.0	1.1	1.0	0.7	1.0	1.0	1.1	1.0	1.0	1.6
5	*M. hekouensis*	3.2	2.7	5.0	5.0	**0.0**	0.8	1.0	0.7	0.8	0.9	0.6	1.1	0.5	1.0	1.5
6	*M. immaculata*	4.8	1.7	6.4	6.8	3.1	**0.0**	1.2	0.8	1.0	1.0	0.6	1.2	0.7	1.1	1.5
7	*M. inornata*	5.2	7.1	5.9	7.6	5.7	7.4	**1.6**	1.0	1.0	1.0	1.1	1.1	1.0	0.6	1.5
8	*M. thongphaphumensis* sp. nov.	3.5	4.0	6.0	5.7	2.8	4.4	6.1	**1.3**	0.8	0.9	0.7	1.1	0.7	1.0	1.6
9	*M. lineata*	3.4	4.4	5.9	2.7	3.6	5.3	6.3	4.2	**0.2**	0.9	0.9	1.0	0.8	1.0	1.5
10	*M. melanops*	3.1	5.0	5.0	5.4	3.7	5.4	5.6	4.2	4.2	**0.0**	0.9	1.0	0.9	0.9	1.5
11	*M. menglienica*	3.5	2.1	5.3	6.0	2.3	2.4	6.6	3.4	4.6	4.4	**0.5**	1.0	0.6	1.0	1.5
12	*M. nigromaculata*	5.0	7.1	5.6	7.9	6.2	7.4	6.8	6.6	5.9	5.1	6.4	**0.9**	1.0	1.1	1.2
13	*M. steinegeri*	3.5	3.1	4.8	5.7	1.3	3.0	5.6	3.1	4.3	4.4	2.2	5.8	**0.2**	1.0	1.4
14	*M. subaraji*	4.6	6.5	5.8	6.7	5.4	7.0	3.0	5.8	5.4	5.0	6.1	6.3	5.4	**0.3**	1.6
15	*M. sumatrana*	10.8	12.3	9.9	14.0	11.4	12.5	12.9	12.8	12.2	11.2	11.7	9.6	10.8	13.3	-

which varied from 1.3% (*M. steinegeri* and *M. hekouensis*) to 14.0% (*M. sumatrana* and *M. erythropoda*) for 16S rRNA (Table 2). Morphologically, the Kanchanaburi population of *M.* cf. *inornata* differs in a number of taxonomically important diagnostic characters from other congeners, including *M. inornata* from Indonesia. Thus, both molecular and morphological analyses clearly indicate that this population represents a separately evolving lineage and an undescribed species, which we describe below.

The third clade: three samples from Surat Thani, Phuket, and Nakhon Si Thammarat in Thailand clustered with *M. inornata* sensu stricto from Indonesia and Myanmar (BPP = 1.00; BS = 90; Fig. 2, clade III). The mean intraspecific genetic distance of *M. inornata* sensu stricto is 1.6% (Table 2), ranging from 0.0–2.8% (Suppl. material 1).

The fourth clade: two specimens from Prachuap Khiri Khan in Thailand were grouped with type specimens of *M. subaraji* with strongly supported (BPP = 1.00; BS = 85; Fig. 2, clade IV). The uncorrected *p*-distance between the newly-discovered populations from Prachuap Khiri Khan Province and the topotypic *M. subaraji* (Kranji Marshes, Singapore) was very small, ranging from 0.0–0.6% (Suppl. material 1), indicating that they represent the same taxon.

Taxonomic account

Micryletta thongphaphumensis Cao, Suwannapoom, Kilunda, Wu & Che, sp. nov.

https://zoobank.org/6BE93CBC-4833-46E9-A9B2-176804292322

Figs 3–5

Micryletta inornata., Kurabayashi et al. 2011.

Etymology. The specific name is a Latinized toponymic adjective in neuter gender derived from "Thong Pha Phum" in reference to the type locality Thong Pha Phum District in Kanchanaburi Province, Thailand.

Suggested common name. We propose "Thong Pha Phum Paddy Frog" as the common English name.

Type material. ***Holotype*** (Figs 3, 4) • KIZ 024670, adult male from Thong Pha Phum District in Kanchanaburi Province, Thailand (15°11'52.72"N, 98°19'29.71"E; 242 m a.s.l.), collected by Chatmongkon Suwannapoom, Jing Che, Fang Yan, and Wei Gao on 5 August 2013.

Paratypes (Fig. 5) • KIZ 016763, KIZ 016768, KIZ 016771, and KIZ 016690, four adult females from Sai Yok Noi Waterfall in Kanchanaburi Province, Thailand (14°14'19"N, 99°03'30"E; 182 m a.s.l.), collected by Zhi-Yong Yuan and Chatmongkon Suwannapoom on 4 August 2014. KIZ 016769 adult male, with the same collection information as females.

Referred specimens. One adult female KIZ 016762 and two adult males, KIZ 016764 and KIZ 016770, from the same location as paratypes.

Diagnosis. *Micryletta thongphaphumensis* sp. nov. is assigned to the genus *Micryletta* based on the following combination of morphological traits: small body size; absence of vomerine teeth; tympanum small and externally visible; subarticular tubercles on fingers and toes prominent; three well-developed metacarpal tubercles; absence of webbing between fingers and toes (Dubois 1987; Fei et al. 2009). The new species differs from its congeners by a combination of the following characters: medium-sized within the genus (SVL 21.3–25.6 mm in males, n = 5; 24.4–29.7 mm in females, n = 4; Table 3); snout truncate in dorsal view; tympanum distinct; supratympanic fold absent; a black streak extending from tip of the snout to crotch; upper lip white; tibiotarsal articulation adpressed limb reaching level of tympanum; lack of webbing between fingers and toes; relative finger lengths: I < II < IV < III; relative toe lengths: I < II < V < III < IV; outer metatarsal tubercle absent; ventral skin of body and limbs smooth; brown marbling patterns on dorsal limbs; dorsal orange-brown with black spots (Fig. 4).

Description of the holotype (Fig. 4, Table 3). Adult male, body size small (SVL = 25.6 mm), elongated oval-shaped; head wider than long (HW/HL = 1.2); canthus rostralis distinct, rounded; loreal region slightly concave; top of head relatively flat; snout slightly projecting beyond lower jaw, truncate in dorsal view, and bluntly

Figure 3. Adult male holotype (KIZ 024670) of *Micryletta thongphaphumensis* sp. nov. in life. **A.** Dorsal view; **B.** Lateral view of left side; **C.** Lateral view of right side. Photos by Jing Che.

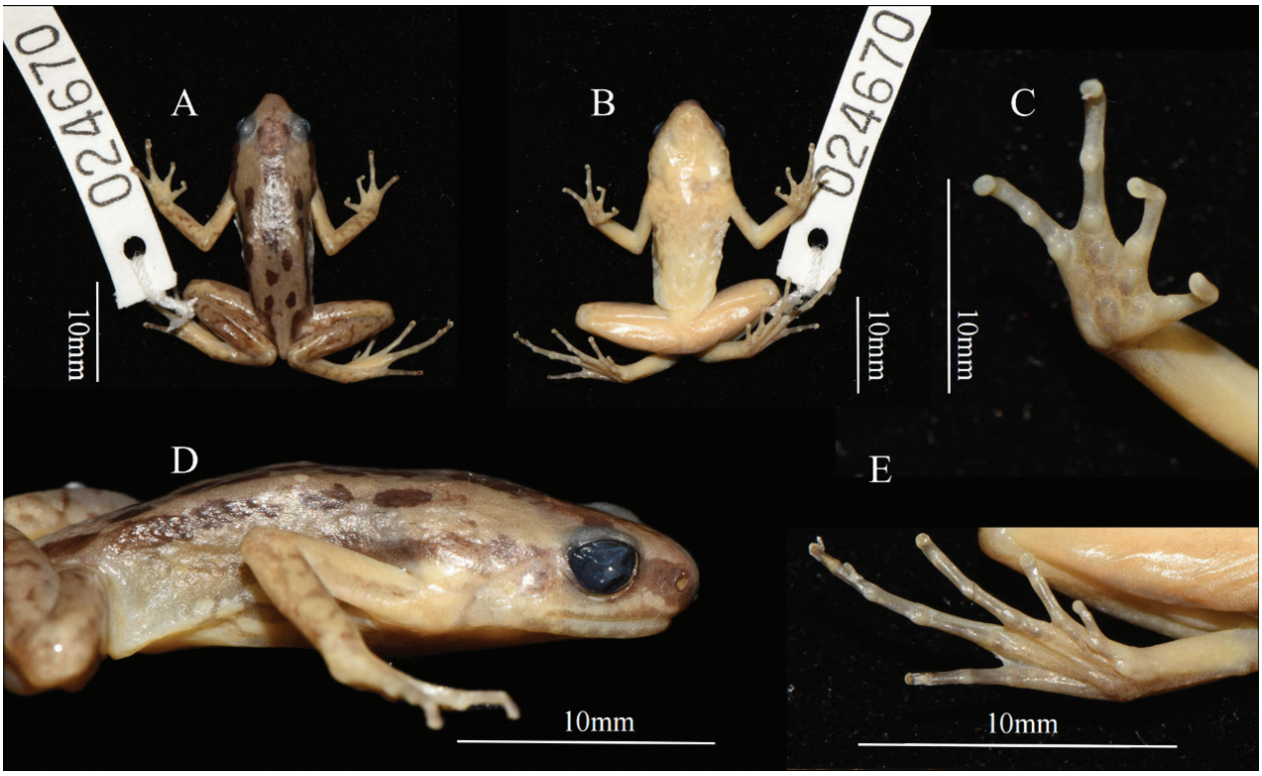

Figure 4. Adult male holotype (KIZ 024670) of *Micryletta thongphaphumensis* sp. nov. in preservative. **A.** Dorsal view; **B.** Ventral view; **C.** Volar view of right hand; **D.** Lateral view of head; **E.** Plantar view of right foot. Photos by Yu-Yang Cao.

rounded in profile; snout length longer than diameter of eye (SL/ED = 1.3); nostril round, closer to tip of snout than to eye (SN/EN = 0.6); internarial distance greater than upper eyelid width (INS/UEW = 1.6), and shorter than interorbital distance (INS/IOS = 0.5); upper eyelid width roughly equal one-third of interorbital distance (UEW/IOS = 0.3) and half of diameter of eye (UEW/ED = 0.5); eyes medium in size (ED/HL = 0.4); vomerine teeth absent; opening of vocal sac long cleft; tongue oval, with no notch at posterior tip; tympanum distinct, small (TD = 0.9 mm) and rounded; diameter of tympanum shorter than diameter of eye (TD/ED = 0.4); interorbital distance between anterior margins of eyes closer than that of posterior margins (IFE/IBE = 0.6); supratympanic fold absent.

Forelimbs slender and long, hand slightly longer than forearm length (HAL/FAL = 1.1); relative finger lengths: I < II < IV < III (FIL = 2.5 mm, FIIL = 3.4 mm, FIIIL = 6.1 mm, FIVL = 4.6 mm); tips of fingers round and not expanded to disks; subarticular tubercle on fingers rounded and prominent, subarticular tubercle formula: 1, 1, 2, 2; nuptial pad absent; webbing between fingers absent; three well-developed metacarpal tubercles at the base of outer three fingers, inner one rounded and smallest, outer metacarpal tubercle elongated, medial metacarpal tubercle large, oval and prominent.

Hindlimbs slender, tibia length shorter than half of snout-vent length (TL/SVL = 0.4), tibia length slightly shorter than foot length (TL/FL = 0.9); heels slightly overlapped when thighs are positioned at right angles to the body;

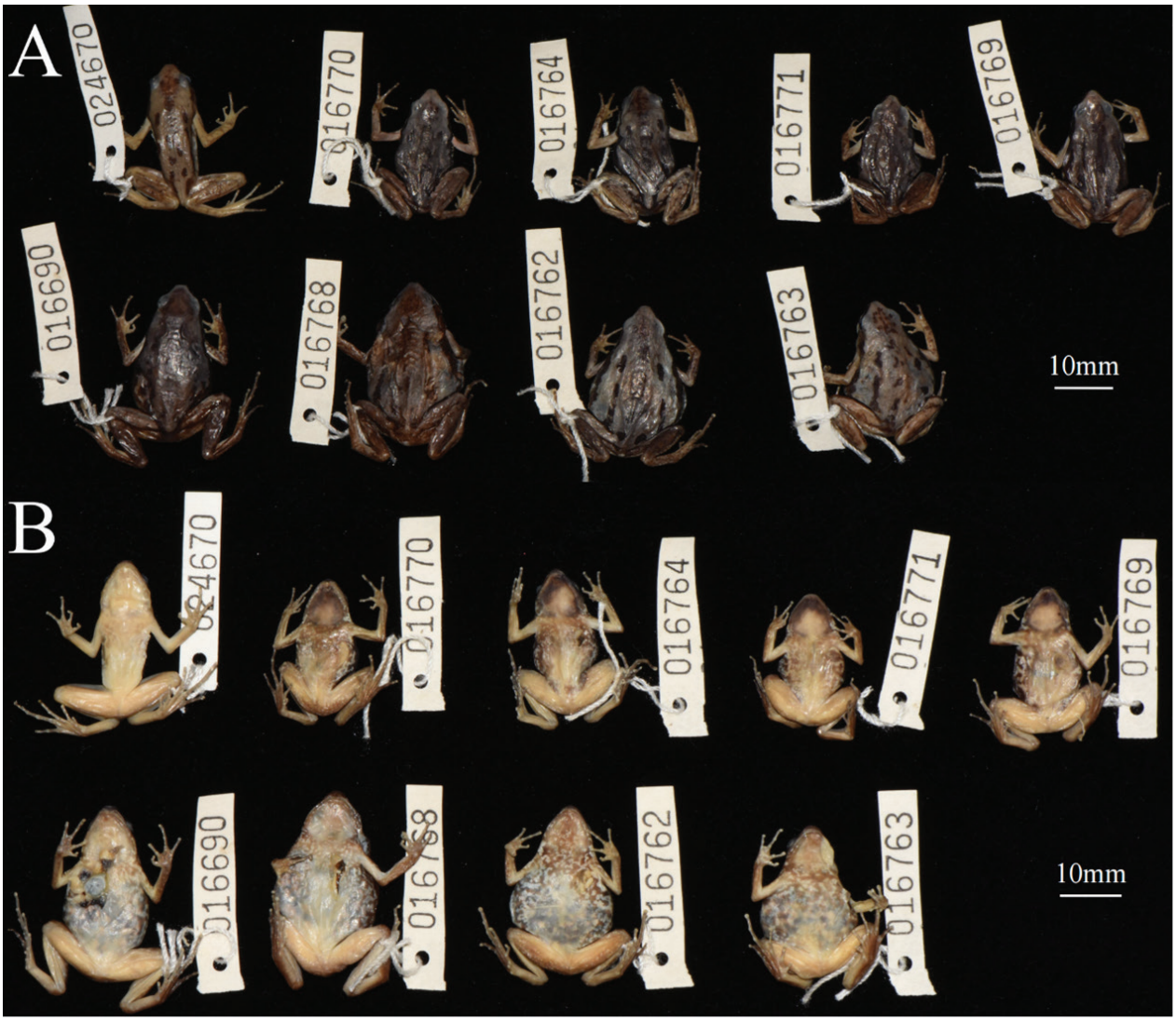

Figure 5. Type series and referred specimens of *Micryletta thongphaphumensis* sp. nov. from Kanchanaburi province in preservative **A.** Dorsal view; **B.** Ventral view. Photos by Yu-Yang Cao.

tibiotarsal articulation adpressed limb reaching level of tympanum; tips of toes round and not expanded to disks; subarticular tubercles on toes rounded and prominent, formula: 1, 1, 2, 3, 2; relative toe lengths: I < II < V < III < IV (TIL = 2.4 mm, TIIL = 4.7 mm, TIIIL = 8.3 mm, TIVL = 11.2 mm, TVL = 6.6 mm); three small supernumerary tubercles at base of toes II–IV, smaller than proximal subarticular tubercles; inner metatarsal tubercle oval and distinct (IMT = 0.9 mm); outer metatarsal tubercle absent; webbing between toes absent.

Dorsum skin of body and limbs smooth without small tubercles; dorsolateral fold absent; ventral surface of throat, belly, forelimbs, and hindlimbs smooth; upper eyelid lacking tubercles; dorsal skin of body sparsely granular in life; vent area smooth.

Color of holotype in life (Fig. 3). Dorsum skin of body orange-brown with some scattered blackish black spots elongate; lateral sides of dorsum with brownish black stripes from snout to groin; subtle longitudinal median line present on dorsum; upper lip white, extending up to the anterior forelimb; lower lip puce with white mottling along the margins; dorsal surface of forearms orange-brown without brown marbling patterns; dorsal surface of hindlimbs orange-brown without dark transverse bands, brown marbling patterns clearly, extending to dorsal of fifth toe; finger and toes I–IV gray-brown with white mottling; groin, anterior and posterior parts of thigh, and lateral surfaces of shank and tarsus brown with a few fuzzy white spots; irregular white and brown spots on chest and lateral belly; iris bicolored, with upper third bronze and lower two-thirds dark brown.

Color of holotype in preservative (Fig. 4). Colors considerably faded; dorsum skin of body grey-brown with blackish black spots; the pattern generally remains unchanged; subtle longitudinal median line fuzzy on dorsum; upper lip, belly, and white spots turned to light grey.

Variation and sexual dimorphism (Fig. 5). The body sizes of males are smaller than females generally, although there is overlap in the ranges of values (male SVL 21.3–25.6 mm, female SVL 24.4–29.7 mm); translucent skin on belly through which the large bicolored white and black eggs are visible; dark-brown spots markings variable in density from few to many and most somewhat elongate rather than round; the shapes of spots varied; subtle longitudinal median line clear or fuzzy on dorsum.

Table 3. Measurements (in mm) of *Micryletta thongphaphumensis* sp. nov. from Thailand. Abbreviations are defined in Methods.

	KIZ 024670	KIZ 016769	KIZ 016771	KIZ 016763	KIZ 016768	KIZ 016690	KIZ 016770	KIZ 016764	KIZ 016762
	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Referred specimen	Referred specimen	Referred specimen
Gender	Male	Male	Female	Female	Female	Female	Male	Male	Female
SVL	25.6	23.5	21.3	24.4	29.7	28.1	22.3	23.7	27.3
HL	6.6	6.9	6.6	6.6	6.8	6.5	6.4	6.7	6.9
HW	8.1	7.2	7.5	7.7	8.9	8.0	7.4	8.1	8.2
HW/HL	1.2	1.0	1.1	1.2	1.3	1.2	1.2	1.2	1.2
SL	3.3	3.4	3.0	3.4	3.2	2.9	3.1	3.2	3.3
INS	2.1	2.1	1.6	2.2	2.1	2.1	2.1	2.0	2.2
IOS	4.5	3.5	3.4	3.8	3.9	3.7	3.8	3.6	3.8
INS/IOS	0.5	0.6	0.5	0.6	0.5	0.6	0.6	0.6	0.6
UEW	1.3	1.4	1.1	1.2	1.1	1.4	1.2	1.2	1.2
INS/UEW	1.6	1.5	1.5	1.8	1.9	1.5	1.8	1.7	1.8
UEW/IOS	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3
ED	2.5	2.0	2.0	2.5	2.1	2.6	2.4	2.0	2.7
UEW/ED	0.5	0.7	0.6	0.5	0.5	0.5	0.5	0.6	0.4
ED/HL	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.3	0.4
SL/ED	1.3	1.7	1.5	1.4	1.5	1.1	1.3	1.6	1.2
TD	0.9	1.2	1.2	1.4	1.3	1.5	1.1	1.0	1.2
TD/ED	0.4	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.4
TYE	0.9	0.6	0.4	0.6	0.6	0.6	0.6	0.6	0.7
SN	1.1	0.9	1.0	1.0	1.1	0.7	1.0	1.1	1.2
EN	2.0	1.9	2.1	2.1	1.9	2.2	1.8	2.1	2.3
SN/EN	0.6	0.5	0.5	0.5	0.6	0.3	0.6	0.5	0.5
IFE	4.0	4.2	4.0	4.1	4.8	4.7	3.8	4.2	4.3
IBE	6.6	6.6	6.1	6.8	7.3	6.6	6.1	6.4	6.8
IFE/IBE	0.6	0.6	0.7	0.6	0.7	0.7	0.6	0.7	0.6
FAL	6.7	6.1	5.7	6.5	6.4	6.6	6.3	6.1	6.5
HAL	7.2	6.2	6.0	6.2	7.4	7.2	6.4	6.0	7.0
HAL/FAL	1.1	1.0	1.0	1.0	1.1	1.1	1.0	1.0	1.1
LAD	1.4	1.3	1.6	1.4	1.9	1.6	1.4	1.7	1.7
THL	11.0	10.1	9.7	11.9	12.4	12.0	11.0	11.0	11.9
TL	11.4	10.7	10.0	11.0	12.5	11.9	11.3	11.1	11.5
TL/SVL	0.4	0.5	0.5	0.5	0.4	0.4	0.5	0.5	0.4
FL	12.9	11.9	10.7	11.7	14.0	13.1	11.9	12.8	12.0
TL/FL	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	1.0
IMT	0.9	0.6	0.5	0.6	0.9	0.8	0.8	0.7	0.9
FIL	2.5	2.2	2.0	2.5	2.6	2.1	2.1	2.0	2.3
FIIL	3.4	2.9	2.5	3.3	3.6	3.9	3.0	3.2	2.9
FIIIL	6.1	5.0	4.8	5.7	5.9	5.9	5.3	5.4	5.5
FIVL	4.6	4.0	2.8	3.6	4.5	4.0	3.4	3.9	3.9
TIL	2.4	2.6	1.7	2.0	2.1	2.4	2.0	2.3	2.1
TIIL	4.7	4.3	4.0	4.3	4.8	4.8	4.3	4.3	4.5
TIIIL	8.3	7.6	6.8	7.1	8.1	8.1	6.8	7.1	7.2
TIVL	11.2	10.2	9.7	9.9	11.3	11.2	10.1	10.2	11.1
TVL	6.6	6.2	5.4	5.6	6.4	6.3	5.5	5.5	6.2

Distribution and ecology. *Micryletta thongphaphumensis* sp. nov. is currently known from the Thong Pha Phum District and Sai Yok Noi in Kanchanaburi Province, Thailand (Fig. 1). Considering that the region borders Myanmar, it is speculated that this species may also be present in Myanmar. Eggs were visible in the females we collected, thus indicating that August is the breeding season for this species. Other frog species were observed at the same location, including *Microhyla mukhlesuri*, *M. heymonsi*, *Hylarana nigrovittata*, *Occidozyga martensii*, *Limnonectes jarujini*, and *Fejervarya limnocharis*.

Morphological comparison. We compared *Micryletta thongphaphumensis* sp. nov. to all other recognized species of the genus *Micryletta* (Tarkhnishvili 1994; Poyarkov et al. 2018, 2021; Alhadi et al. 2019; Das et al. 2019; Munir et al. 2020; Suwannapoom et al. 2020; Liu et al. 2021a, b; Miller et al. 2021; Yang and Poyarkov 2021; Sankar et al. 2022).

Micryletta thongphaphumensis sp. nov. differs from *M. hekouensis* by its supratympanic fold absent (vs. supratympanic fold distinct); dorsum of upper arms and dorsum of body orange-brown with brown marbling patterns in life (vs. dorsum of upper arms golden, dorsum of hindlimbs solid black with brownish black stripes); tibiotarsal articulation adpressed limb reaching level of tympanum (vs. reaching front of eye); dermal ridges absent (vs. dermal ridges present under 2nd to 4th toes but indistinct).

Micryletta thongphaphumensis sp. nov. differs from *M. steinegeri* by snout length slightly longer than diameter of eye (vs. snout length shorter than diameter of eye); webbing between fingers and toes absent (vs. rudimentary webbing between toes); second finger shorter than fourth finger, relative finger lengths: I < II < IV < III (vs. second finger longer than fourth finger, relative finger lengths: I < IV < II < III); supratympanic fold absent (vs. distinct).

Micryletta thongphaphumensis sp. nov. differs from *M. immaculata* by dorsum orange-brown in life (vs. dorsum bronze brown to reddish brown); black stripes extending from tip of snout to crotch and dark brown spots on dorsum obvious (vs. dark brown spots and stripes on dorsum and flank absent); white stripes on upper lips present (vs. irregular white spots along upper lips present); supratympanic fold absent (vs. distinct and lower margin of supratympanic fold black); webbing between toes absent (vs. webbing between toes basal and poorly developed).

Micryletta thongphaphumensis sp. nov. differs from *M. menglienica* by supratympanic fold absent (vs. distinct); dermal ridges absent (vs. dermal ridges present under 2nd to 4th toes but indistinct); dorsum of forelimbs and body orange-brown in life (vs. dorsum of forelimbs light yellow, dorsum of hindlimbs the same color as dorsum of body); ventral side of head and chest brown (vs. ventral side of head and chest greyish brown or purple grey); tibiotarsal articulation adpressed limb reaching level of tympanum (vs. reaching eye or between eye and tympanum).

Micryletta thongphaphumensis sp. nov. differs from *M. aishani* by white stripes on upper lips (vs. ash-grey mottling along the margins of the upper lips); dorsum orange-brown with many large or small scattered blackish black spots in life (vs. dorsum reddish-brown with few scattered blackish-brown spots on posterior parts of the back and near the groin); tibiotarsal articulation adpressed limb reaching level of tympanum (vs. reaching up to the level of armpit); lateral sides of dorsum with brownish black stripes from snout to groin (vs. blackish-brown streak extending from snout to lower abdomen); fifth toe longer than second toe, relative toes lengths: I < II < V < III < IV (vs. fifth toe shorter than second toe, relative toe lengths: I < V < II < III < IV).

Micryletta thongphaphumensis sp. nov. differs from *M. sumatrana* by tibiotarsal articulation adpressed limb reaching level of tympanum (vs. tibiotarsal articulation reaching front of eye); upper lip white (vs. cream spots between lip and axilla); supratympanic fold absent (vs. supratympanic fold thick, rounded, glandular, curving from posterior corner of the eye to shoulder); brown marbling patterns on tibia and tarsal (vs. complete and incomplete dark brown cross bands on tibia and tarsus); dorsum orange-brown with large or small scattered blackish black spots (vs. dorsal coloration of body golden brown with some irregular dark spots and dark thin vertebrae line).

Micryletta thongphaphumensis sp. nov. differs from *M. erythropoda* by the outer metatarsal tubercle absent (vs. presence of an outer metatarsal tubercle); orange-brown dorsum in life (vs. brick-reddish dorsum); webbing between toes absent (vs. toes with rudimentary web); second finger shorter than fourth (vs. second finger equal to fourth).

Micryletta thongphaphumensis sp. nov. differs from *M. inornata* by tibiotarsal articulation adpressed limb reaching level of tympanum (vs. reaching the eye); dorsum orange-brown with large or small scattered blackish spots (vs. dorsum brownish-grey with a silver tinge and irregular blackish-brown blotches of variable size); lateral sides of dorsum with continuous brownish black stripes from snout to groin (vs. presence of a discontinuous lateral blackish-brown streak from the tip of the snout to near the groin); throat brown with irregular fuzzy white spots (vs. throat light reddish-grey without prominent spots); dorsal of limbs with black marbling patterns (vs. dorsal of limbs with irregular golden yellow spots).

Micryletta thongphaphumensis sp. nov. differs from *M. dissimulans* by hand wider than long (vs. head longer than wide); snout length longer than diameter of eye (vs. diameter of eye equal to snout length); dorsal orange-brown with black spots (vs. dorsal shagreened with irregular-shaped brown blotches edged in beige, no black spots on dorsum); without large black spot behind eye (vs. large black spot behind eye); lateral sides of dorsum with brownish black stripes from snout to groin (vs. two large black blotches in axillary and inguinal areas on each side); white patches on lips (vs. absent).

Micryletta thongphaphumensis sp. nov. differs from *M. subaraji* by tympanum distinct (vs. hidden); dorsum orange-brown with black spots in life (vs. greyish brown); upper eyelid width equals one-third of interorbital distance UEW/IOS = 0.3 (vs. UEW/IOS = 0.7); internarial distance equals half of interorbital distance INS/IOS = 0.5 (vs. INS/IOS = 0.8).

Micryletta thongphaphumensis sp. nov. differs from *M. menlanops* by tibiotarsal articulation adpressed limb reaching level of tympanum (vs. reaching eye); supratympanic fold absent (vs. present); webbing between fingers and toes absent (vs. toe webbing rudimentary between toes II–III and III–IV); dorsum orange-brown with large black spots in life (vs. dorsal pale dark brown with small reddish speckles); dorsal surfaces of limbs orange-brown with brown marbling patterns (vs. dark brown with orange-red speckles); fifth toe longer than second toe, relative toes lengths: I < II < V < III < IV (vs. fifth toe shorter than second toe, relative toe lengths: I < V < II < III < IV); white patches on lips (vs. absent); iris bicoloured, with upper third bronze and lower two-thirds brownish black (vs. iris uniform black).

Micryletta thongphaphumensis sp. nov. differs from *M. nigromaculata* by tibiotarsal articulation adpressed limb reaching level of tympanum (vs. reaching eye); supratympanic fold absent (vs. present); dorsum light brown to orange-brown with large black spots (vs. dorsum with dark-brown irregular hourglass-shaped pattern edged with orange); white patches on lips (vs. absent); lateral sides of dorsum with brownish black stripes from snout to groin (vs. a large black blotch in inguinal area on each side).

Micryletta thongphaphumensis sp. nov. differs from *M. lineata* by second finger longer than fourth finger, relative finger lengths: I < II < IV < III (vs. second finger equal to fourth finger, relative finger lengths: I < IV = II < III); fifth toe longer than third toe, relative toes lengths: I < II < V < III < IV (vs. fifth toe equal to third toe, relative toes lengths: I < II < V = III < IV); ventral side of body smooth (vs. chest and abdomen with large flat abutting tubercles); tympanum diameter about one-third that diameter of eye (vs. about two-thirds).

Discussion

In this study, we explored the diversity, distribution, and classification of *Micryletta* by integrating our long-term fieldwork and published data. Our study supported the monophyly of *Micryletta*, consistent with previous studies by Sankar et al. (2022), Liu et al. (2021a), and Munir et al. (2020). Our study further clarified the classification controversy and distribution range of *M. inornata* sensu lato as follows:

The distribution range of *M. menglienica*

Micryletta menglienica was reported as *Kalophrynus menglienicus*, according to the original publication by Yang and Su (1980). Liu et al. (2021b) transferred the species to *Micryletta* based on both 16S rRNA and morphological analysis. Previously, this species was known only from the type locality (Menglian, Yunnan in China) and northern Vietnam (Frost 2024). In addition, the taxonomic status of *M.* '*inornata*' recorded in Mengla of Yunnan and Guangxi, China, was also controversial (Liu et al. 2021a; Yeung et al. 2023). Among these, Yeung et al. (2023) indicated that *M.* '*inornata*' from Mengla, Yunnan, was actually *M. menglienica*. Our study agrees with the findings by Yeung et al. (2023) and further extends the distribution range to Thailand and Laos for the first time. Therefore, *M. menglienica* is currently found in Yunnan province, China, Laos, Vietnam, and Thailand, with a potential occurrence in Myanmar. However, the support value of the *M. menglienica* lineage was low, possibly due to limited loci. Moreover, the *M.* cf. *inornata* from Guangxi, China, remains unknown. Future studies should employ multiple nuclear markers using phylogenomic approaches combined with extensive sampling to resolve this question.

The distribution range of *M. inornata*

Micryletta inornata is currently confirmed to be distributed in Sumatra, Indonesia, and southern Myanmar, but distribution records from the uplands of Thailand, Cambodia, China, and Vietnam require further verification (Frost 2024). Our study confirms the distribution of this species in Thailand for the first time. In addition, our study confirms that *M.* cf. *inornata* from Kanchanaburi, Thailand, is a new species based on molecular and morphological evidence, herein described as *M. thongphaphumensis* sp. nov. However, our study did not confirm the existence of this species in other areas (e.g., Yaephy, Tanintharyi in Myanmar) with questionable distribution. Therefore, future collaborative research among herpetologists from different countries is necessary to employ more extensive sampling across the range of *M. inornata* and further clarify its distribution.

The distribution range of *M. immaculata*

Micryletta immaculata was reported by Yang and Poyarkov (2021) and is only known from Hainan Island, China. Our study shows that populations from central Laos and central Vietnam formed a monophyletic lineage, which was the sister taxon to the type specimens of *M. immaculata*. The uncorrected genetic *p*-distances between the two clades are 1.7%, which is higher than the minimum interspecific genetic distance (*M. steinegeri* and *M. hekouensis*, Table 2). However, due to the absence of specimens from central Laos and central Vietnam, we could not determine whether this lineage represents an undescribed species or *M. immaculata*. We agree with Sankar et al. (2022) and consider the lineage from Vietnam and Laos as *M.* cf. *immaculata* provisionally. Although the Gulf of Tonkin acts as a geographical barrier isolating northern Vietnam and Hainan Island, China, they occur in the same zoogeographic region. Both regions still share some species, such as *Odorrana nasuta*, *Theloderma corticale*, *Rhacophorus rhodopus*, and *Rohanixalus hansenae* (Frost 2024). We recommend strengthening fieldwork surveys in the Indochina Peninsula paired with the integration of population genomics and acoustic data in order to clarify the taxonomic status of the lineage from Laos and Vietnam.

The distribution range of *M. subaraji*

Micryletta subaraji was reported by Sankar et al. (2022) and is only known from Kranji Marshes in Singapore. Our study indicates that specimens collected from Thap Sakae and Prachuap Khiri Khan in Thailand are *M. subaraji*. This is the first recorded instance of *M. subaraji* in Thailand, thus extending its latitudinal distribution northward for more than 1000 km to southern Thailand. Given that Malaysia is located between Thailand and Singapore, we presume that this species might also be present there. Future studies should conduct thorough investigations of *Micryletta* species in Malaysia to confirm the distribution of *M. subaraji*.

Acknowledgments

This work was supported by the National Key R&D Program of China (2022YFC2602500), the Second Tibetan Plateau Scientific Expedition and Research (STEP) program (Grant No. 2019QZKK0501), Science and Technology Basic Resources Investigation Program of China (2021FY100200); National Natural Science Foundation of China (NSFC 32100371); Major Science and Technique Programs in Yunnan Province (202102AA310055), the Key R & D program of Yunnan Province (202103AC100003, 202303AH310055), Yunnan Applied Basic Research Projects (No. 202301AT070312, 202301AT070431), Yunnan Revitalization Talent Support Program Yunling Scholar Project; China's Biodiversity Observation Network (Sino-BON), the Animal Branch

of the Germplasm Bank of Wild Species, CAS (Large Research Infrastructure Funding); the Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, University of Phayao and the Thailand Science Research and Innovation Fund and the University of Phayao (Unit of Excellence 2025 on Aquatic animals biodiversity assessment (Phase I)). Specimen collection protocols were approved by the Institutional Ethical Committee of Animal Experimentation of the University of Phayao (certificate number UP-AE59-01-04-0022 issued to Chatmongkon Suwannapoom) and the Institute of Animal for Scientific Purposes Development Thailand (No. U1-01205-2558). We thank Jie-Qiong Jin, Wen-Jie Dong, Chen-Qi Lu, Fang Yan, Zhi-Yong Yuan, Ke Jiang, Jin-Min Chen, Parinya Pawangkhant, and Zhong-Xiong Fu for helping with collecting samples in the field.

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Supplementary material 1

Uncorrected *p*-distance (percentage) 16S rRNA sequences of individuals included in phylogenetic analyses and standard error estimates

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Data type: xlsx

Link: https://doi.org/10.3897/zse.100.129398.suppl1