SUPPLEMENTARY MATERIALS

Supplementary Methods

Laboratory methods. For molecular phylogenetic analyses, we extracted total genomic DNA from ethanol-preserved femoral muscle tissue using standard phenol-chloroform-proteinase K extraction procedures with consequent isopropanol precipitation, to a final concentration of ~1 mg/mL (protocols followed Hillis et al., 1996 and Sambrook & Russell, 2001). We visualized the isolated total genomic DNA in agarose electrophoresis in the presence of ethidium bromide. We measured the concentration of total DNA in 1 μL using a NanoDrop 2000 (Thermo Scientific, USA), and consequently adjusted the concentration to ca. 100 ng DNA/μL.

We amplified mtDNA fragments covering partial sequences of the 16S rRNA mtDNA gene to obtain a 569 bp length continuous fragment of mtDNA. The 16S rRNA gene is widely applied in biodiversity surveys in amphibians (Vences et al., 2005a, 2005b; Vieites et al., 2009), and has been used in most recent phylogenetic studies on Microhylinae (Matsui et al., 2011; Peloso et al., 2016; Tu et al., 2018). We performed DNA amplification in 20 μL reactions using ca. 50 ng genomic DNA, 10 nmol of each primer, 15 nMol of each dNTP, 50 nMol additional $MgCl_2$, Taq PCR buffer (10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.1 mmol/L $MgCl_2$, and 0.01% gelatin), and 1 unit of Taq DNA polymerase. Primers used in PCR and sequencing included 16sL1 (CTGACCGTGCAAAGGTAGCGTAATCACT) and 16H-1 (CTCCGGTCTGAACTCAGATCACGTAGG) (Hedges, 1994). The PCR conditions included an initial denaturation step of 5 min at 94 °C and 43 cycles of denaturation for 1 min at 94 °C, primer annealing for 1 min with the TouchDown program from 65 °C to 55 °C reducing 1 °C every cycle, extension for 1 min at 72 °C, and final extension step for 5 min at 72 °C.

The PCR products were loaded onto 1.5% agarose gels in the presence of ethidium bromide and visualized via agarose electrophoresis. When distinct bands were produced, we purified the PCR products using 2 μL of a 1:4 dilution of ExoSapIt (Amersham, USA) per 5 μL of PCR product prior to cycle sequencing. The 10 μL sequencing reaction included 2 μL of template, 2.5 μL of sequencing buffer, 0.8 μL of 10 pmol primer, 0.4 μL of BigDye Terminator v3.1 Sequencing Standard (Applied

Biosystems, USA), and 4.2 μL of water. The cycle sequencing used 35 cycles of 10 s at 96 °C, 10 s at 50 °C, and 4 min at 60 °C. We purified the cycle sequencing products by ethanol precipitation. We carried out sequence data collection and visualization on an ABI 3730xl Automated Sequencer (Applied Biosystems, USA). The obtained sequences were deposited in GenBank under accession numbers MT573413–MT573416 (see Supplementary Table S1).

Phylogenetic analyses. To reconstruct the matrilineal genealogy, we used 16S rRNA sequences of the *Micryletta* sp. from Songkhla Province, as well as 16S rRNA sequences of all currently recognized *Micryletta* species and subspecies and several undescribed lineages reported in earlier phylogenetic studies of the genus (Alhadi et al., 2019; Das et al., 2019; Munir et al., 2020; Poyarkov et al., 2018). GenBank accession numbers, museum vouchers, and localities of origin for sequences used in this study are summarized in Supplementary Table S1. We also added a sequence of *Mysticellus franki*, the sister taxon of *Micryletta*, as an outgroup (Garg & Biju, 2019); sequences of *Kaloula pulchra* and *Uperodon systoma* were used to root the tree. In total, we obtained 16S rRNA data from 31 specimens, including four sequences of *Micryletta* sp. from Songkhla, 27 sequences of all other species of *Micryletta* from Thailand, Laos, Vietnam, India, China, and Indonesia, including type specimens of *M. sumatrana* (Sumatra, Indonesia), *M. aishani* (India), and *M. nigromaculata* (Vietnam), topotype specimens of *M. inornata* (Sumatra), *M. erythropoda* (Ma Da, Dong Nai, Vietnam), and *M. steinegeri* (China, Taiwan), and three outgroup sequences of other Microhylinae (*Mysticellus franki*, *Uperodon systoma*, *Kaloula pulchra*) (see Supplementary Table S1).

We initially aligned nucleotide sequences using ClustalX 1.81 (Thompson et al., 1997) with default parameters, and then optimized them manually in BioEdit 7.0.5.2 (Hall, 1999). We used MODELTEST v.3.06 (Posada & Crandall, 1998) to estimate the optimal evolutionary models to be used for dataset analysis. The best-fitting model for the 16S rRNA gene fragment was the GTR+I+G model of DNA evolution, as suggested by the Akaike Information Criterion (AIC). We determined mean uncorrected genetic distances (*p*-distances) between sequences with MEGA 6.0 (Tamura et al., 2013).

We inferred matrilineal genealogy using Bayesian inference (BI) and maximum-likelihood (ML) approaches. We conducted BI in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003); Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses were run with one cold chain and three heated chains for one million generations, with sampling every 100 generations. We performed five independent MCMCMC runs and the initial 2 500 trees were discarded as burn-in. We assessed confidence in tree topology by the frequency of nodal resolution (posterior probability; BI PP) (Huelsenbeck & Ronquist, 2001). We conducted ML analyses with the RAxML web server (http://embnet.vital-it.ch/raxml-bb/, Stamatakis et al., 2008), which was used to search ML trees with the gamma model of rate heterogeneity option. We assessed nodal confidence by non-parametric bootstrapping (ML BS) with 1 000 pseudoreplicates (Felsenstein, 1985).

In both datasets, we regarded tree nodes with ML BS values of 75% or greater and BI PP values over 0.95 to be sufficiently resolved *a priori*. ML BS values between 75% and 50% and BI PP values between 0.95 and 0.90 were regarded as tendencies. Lower values were considered to indicate unresolved nodes (Huelsenbeck & Hillis, 1993).

Morphological description. The morphometrics of adults and character terminology followed Poyarkov et al. (2018): (1) snout-vent length (SVL; tip of snout to cloaca); (2) head length (HL; tip of snout to hind border of jaw angle); (3) snout length (SL; anterior corner of eye to tip of snout); (4) eye length (EL; distance between anterior and posterior corners of eye); (5) nostril-eye length (N-EL; distance between anterior corner of eye and nostril center); (6) head width (HW; maximum width of head on level of mouth angles in ventral view); (7) internarial distance (IND; distance between central points of nostrils); (8) interorbital distance (IOD; shortest distance between medial edges of eyeballs in dorsal view); (9) upper eyelid width (UEW; maximum distance between medial edge of eyeball and lateral edge of upper eyelid); (10) tympanum length (horizontal tympanum diameter (TMP); (11) forelimb length (FLL; length of straightened forelimb to tip of third finger); (12) lower arm and hand length (LAL; distance between elbow and tip of third finger); (13) hand length (HAL; distance between proximal end of outer palmar (metacarpal) tubercle and tip of third finger); (14) first finger length (1FL, distance between tip and distal end of inner palmar tubercle); (15) inner palmar tubercle length (IPTL; maximum distance

between proximal and distal ends of inner palmar tubercle); (16) outer palmar tubercle length (OPTL; maximum diameter of outer palmar tubercle); (17) third finger disk diameter (3FDD); (18) hindlimb length (HLL; length of straightened hindlimb from groin to tip of fourth toe); (19) tibia length (TL; distance between knee and tibiotarsal articulation); (20) foot length (FL; distance between distal end of tibia and tip of fourth toe); (21) inner metatarsal tubercle length (IMTL; maximum length of inner metatarsal tubercle); (22) first toe length (1TOEL), distance between distal end of inner metatarsal tubercle and tip of first toe; (23) fourth toe disk diameter (4TDD). For holotype description, we took the following measurements: (24–26) second to fourth finger lengths (2–3FL-O, 4FL-I; for outer side (O) of second and third, inner side (I) of fourth, distance between tip and junction of neighboring finger); (27–30) second to fifth toe lengths (outer lengths for toes II–IV, inner length for toe V; 2–5TOEL); (31) nostril-snout length (N-SN), distance between middle of nostril and snout tip.

Terminology for describing eye coloration in living individuals was in accordance with Glaw & Vences (1997); subarticular tubercle formulas followed those of Savage (1975). All measurements were taken on the right side of the examined specimen. Sex was determined by gonadal inspection following dissection.

Comparative data on the morphology and taxonomy of *Micryletta* were obtained from previous publications on the genus: including *M. aishani* (Das et al., 2019); *M. erythropoda* (Tarkhnishvili, 1994; Vassilieva et al., 2016); *M. inornata* (Alhadi et al., 2019; Boulenger, 1890; Taylor, 1962), *M. lineata* (Taylor, 1962; Zug & Mulcahy, 2020); *M. nigromaculata* (Poyarkov et al., 2018); *M. steinegeri* (Boulenger, 1909; Das et al., 2019; Fei et al., 2009, 2012); and *M. sumatrana* (Munir et al., 2020).

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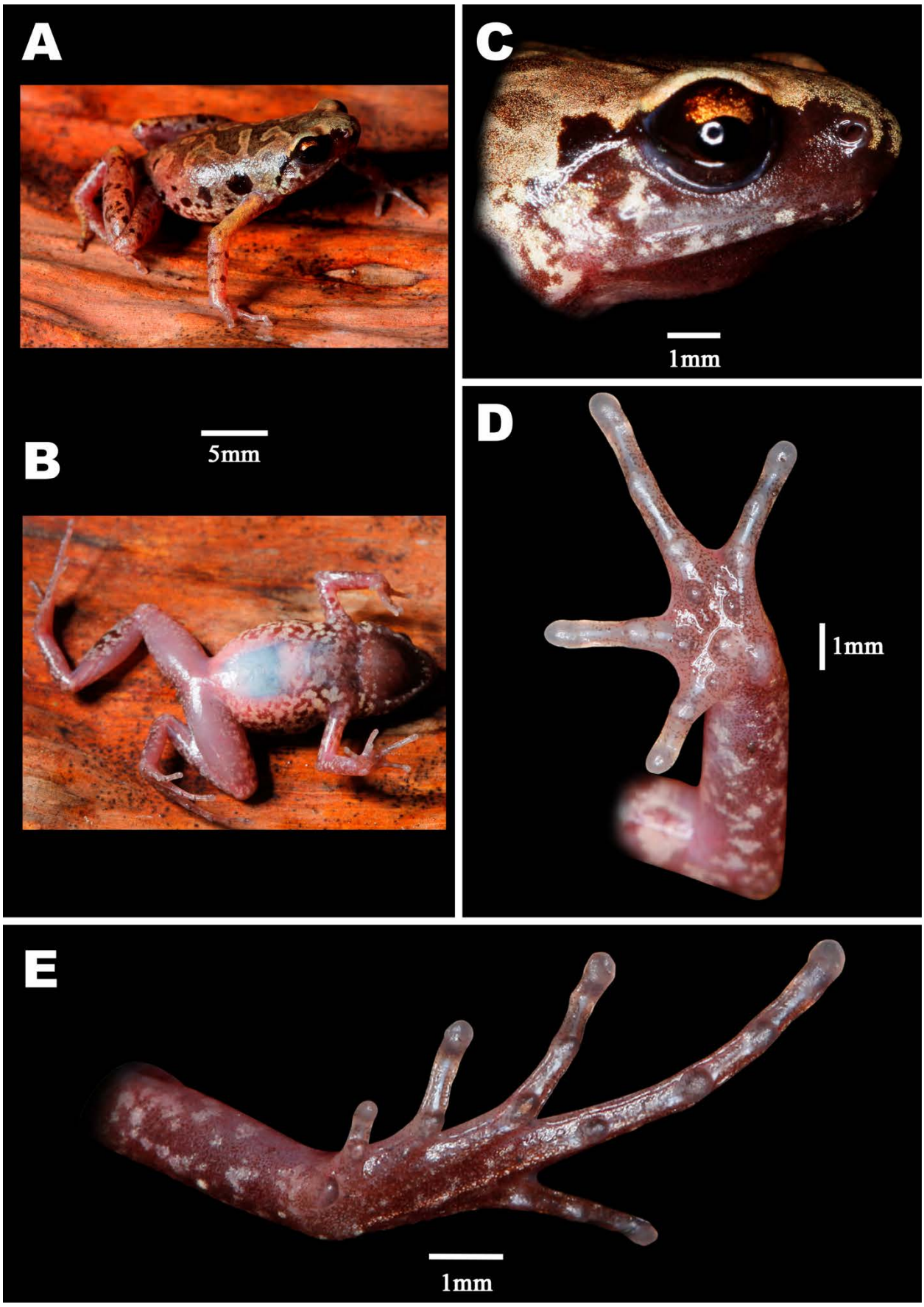
Supplementary Table S1. Localities, voucher information, and GenBank accession numbers for all specimens used in molecular analyses in this study. Locality numbers correspond to those in Figure 1. For references see Supplementary Methods section.

Species	Specimen ID	Locality	GenBank no.	References
M. aishani	SDBDU 3920	India: Assam, Cachar district, Subhong	MK889218	Das et al. (2019)
M. dissimulans **sp. nov.**	AUP01690	Thailand: Songkla Prov., Saba Yoi district	MT573414	*this study*
M. dissimulans **sp. nov.**	AUP01691	Thailand: Songkla Prov., Saba Yoi district	MT573415	*this study*
M. dissimulans **sp. nov.**	AUP01696	Thailand: Songkla Prov., Saba Yoi district	MT573416	*this study*
M. dissimulans **sp. nov.**	AUP01698	Thailand: Songkla Prov., Saba Yoi district	MT573413	*this study*
M. erythropoda	ZMMU A4721-1533	Vietnam: Dong Nai, Ma Da (Vinh Cuu) N.R.	MH756146	Poyarkov et al. (2018)
M. erythropoda	ZMMU A4721-1542	Vietnam: Dong Nai, Ma Da (Vinh Cuu) N.R.	MH756147	Poyarkov et al. (2018)
M. inornata	MZB Amph 23949	Indonesia: Sumatra, Deli Serdang	LC208135	Alhadi et al. (2019)
M. inornata	MZB Amph 23947	Indonesia: Sumatra, Deli Serdang	LC208136	Alhadi et al. (2019)
M. inornata	MZB Amph 23948	Indonesia: Sumatra, Deli Serdang	LC208137	Alhadi et al. (2019)
M. inornata	MZB Amph 27242	Indonesia: Sumatra, Aceh	LC208138	Alhadi et al. (2019)
M. cf. *lineata*	KUHE 23858	Thailand: Ranong	AB634695	Matsui et al. (2011)
M. cf. *lineata*	CAS 247206	Myanmar: Tanintharyi Div., Kawthaung dist.	KM509167	Peloso et al. (2016)
M. nigromaculata	ZMMU A5947	Vietnam: Hai Phong, Cat Ba N.P.	MH756148	Poyarkov et al. (2018)
M. nigromaculata	ZMMU A5937	Vietnam: Hai Phong, Cat Ba N.P.	MH756149	Poyarkov et al. (2018)
M. nigromaculata	ZMMU A5946	Vietnam: Hai Phong, Cat Ba N.P.	MH756151	Poyarkov et al. (2018)
M. nigromaculata	DTU 301	Vietnam: Ninh Binh, Cuc Phuong N.P.	MH756154	Poyarkov et al. (2018)
M. steinegeri	KUHE 35937	China: Taiwan: Yunlin	AB634696	Matsui et al. (2011)
M. cf. *steinegeri*	KUHE 20497	Thailand: Phrae, Mae Yom	AB598341	Matsui et al. (2011)
M. cf. *steinegeri*	K 3246	Laos: Luangprabang Prov., Ban Sop Chuna	KC180027	de Sá et al. (2012)
M. cf. *steinegeri*	TZ9892	Vietnam: Ha Tinh, Ke Go	AF285206	*unpublished*

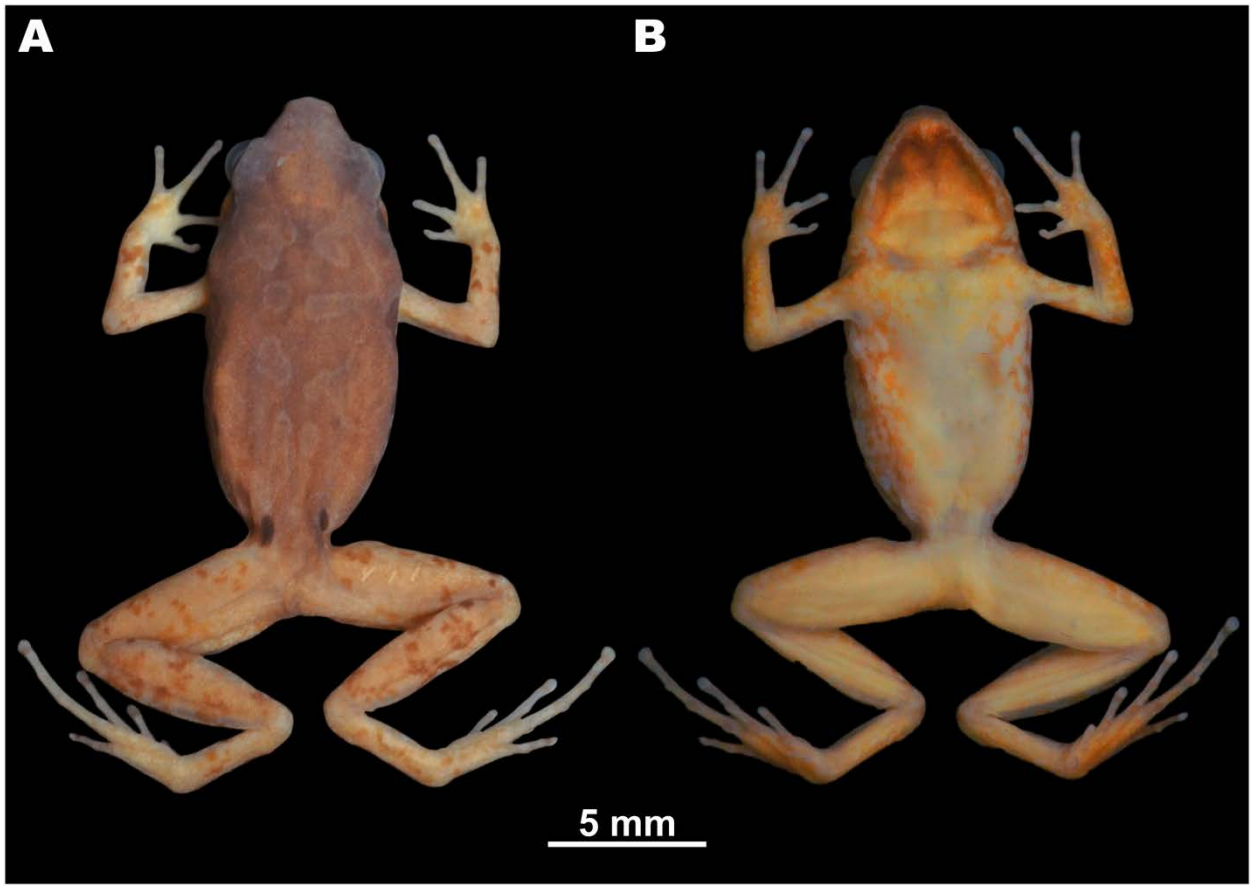
M. cf. *steinegeri*	ZMMU NAP-3580	Vietnam: Hai Phong, Cat Ba N.P.	MH879845	Poyarkov et al. (2018)
M. cf. *steinegeri*	K3068	Thailand: Chiang Mai, Doi Chiang Dao	KR827953	Grosjean et al. (2015)
M. cf. *steinegeri*	KUHE 35133	Laos	AB611968	Kurabayashi et al. (2011)
M. cf. *steinegeri*	FMNH 255121	Laos: Boualapha, Khammouan Prov.	KC822494	Blackburn et al. (2013)
M. cf. *steinegeri*	DTU 310	Vietnam: Ninh Binh, Cuc Phuong N.P.	MH879840	Poyarkov et al. (2018)
M. sumatrana	MZB Amph 30594	Indonesia, Sumatra Selatan, Musi Banyuasin	MN727065	Munir et al. (2020)
Micryletta sp. 1	FMNH 255123	Laos: Thaphabat, Bolikhamxay	KC822493	Blackburn et al. (2013)
Outgroup				
Mysticellus franki	ZSI/WGRC/V/A/967	India: Kerala, Wayand	MK285340	Garg and Biju (2019)
Uperodon systoma	SDBDU 2005.4723	India: Tamil Nadu: Kunnapattu	MG557949	Garg et al. (2019)
Kaloula pulchra	NMNS 3208	China	KC822614	Blackburn et al. (2013)

Supplementary Table S2. Uncorrected *p*-distance (percentage) 16S rRNA sequences of *Micryletta* species included in phylogenetic analyses (below diagonal), average intraspecific genetic *p*-distances (on diagonal), and standard error estimates (above diagonal).

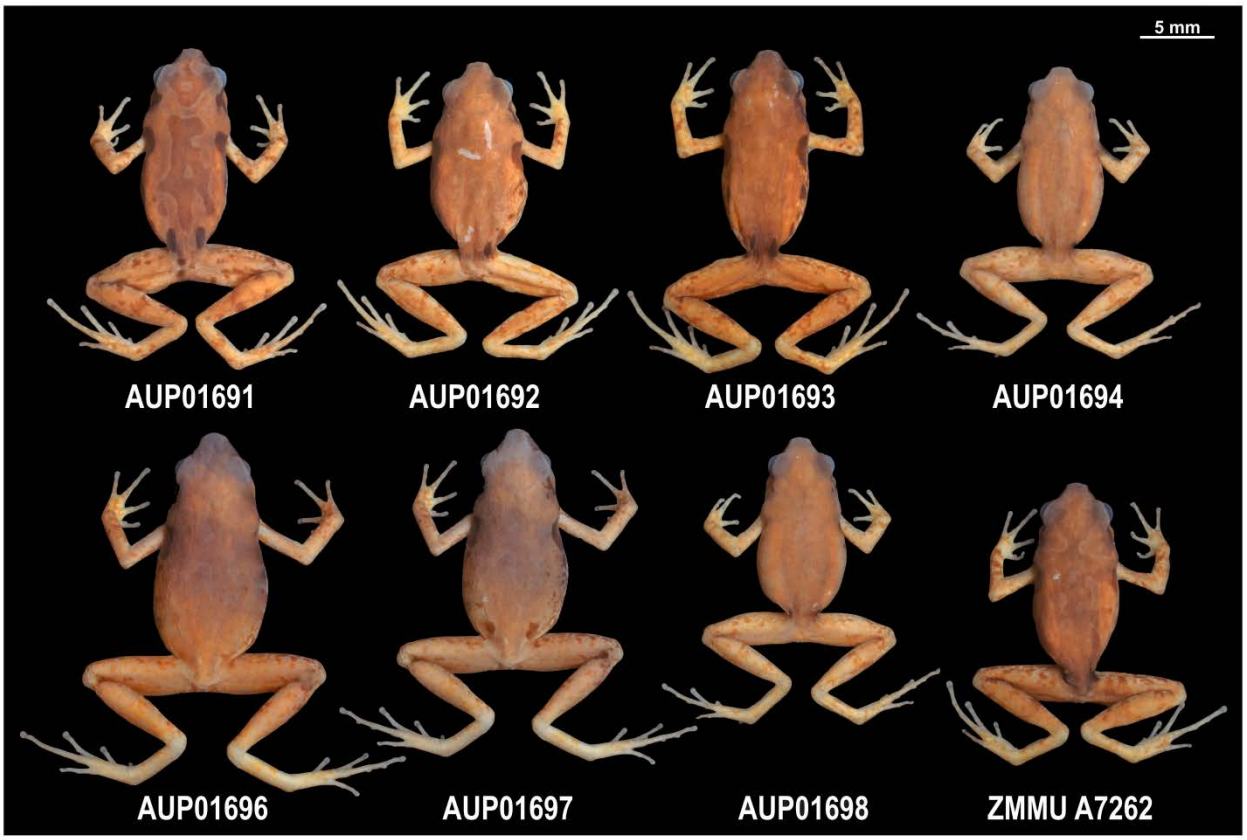
	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*M.* cf. *steinegeri* A	**0.3**	0.6	0.7	0.7	0.6	0.9	0.9	0.7	1.1	1.0	1.2	1.1	0.9	1.3
2	*M.* cf. *steinegeri* B	2.4	**1.8**	0.8	0.8	0.7	1.0	1.0	0.8	1.2	1.1	1.4	1.2	1.0	1.4
3	*M.* cf. *steinegeri* C	2.9	4.1	**0.4**	0.7	0.6	0.9	0.9	0.7	1.0	1.1	1.4	1.1	1.0	1.3
4	*M. steinegeri* s. str. D	2.1	3.5	2.8	-	0.5	0.9	1.0	0.8	1.0	1.0	1.3	1.1	1.0	1.4
5	*M.* cf. *steinegeri* E	1.9	3.1	2.2	1.2	-	0.8	0.9	0.7	1.0	1.0	1.2	1.1	0.9	1.3
6	*M.* cf. *lineata*	4.4	5.3	4.6	4.2	3.4	**0.0**	0.7	0.8	1.1	1.0	1.4	1.1	1.0	1.3
7	*M. erythropoda*	5.6	6.5	5.8	5.6	4.8	2.6	**0.0**	0.9	1.0	1.2	1.6	1.2	1.1	1.3
8	*M. aishani*	3.4	4.5	3.2	3.6	3.0	3.4	4.8	-	0.9	0.9	1.1	1.0	0.9	1.4
9	*M. inornata*	5.9	6.9	5.0	5.0	4.8	6.1	7.1	4.7	**0.6**	1.1	1.5	1.2	1.1	1.3
10	*M. nigromaculata*	5.9	7.0	5.9	5.3	5.2	5.6	7.7	4.7	6.0	**0.7**	0.9	1.0	0.9	1.4
11	*Micryletta* sp. 1	5.7	6.5	7.0	6.3	4.9	5.5	7.4	4.3	6.5	2.8	-	1.8	1.3	2.0
12	*M. sumatrana*	6.5	8.0	7.5	6.0	6.5	7.5	9.3	6.0	7.9	5.9	9.1	-	1.0	1.4
13	*M. dissimulans* **sp. nov.**	**5.1**	**6.6**	**5.8**	**4.8**	**4.8**	**6.0**	**7.4**	**4.4**	**5.5**	**5.0**	**5.5**	**5.2**	**0.0**	1.3
14	*Mysticellus franki*	8.9	10.1	8.8	8.8	8.5	8.8	9.8	9.0	9.5	9.6	12.1	10.8	9.0	-



Supplementary Figure S1. Holotype of *Micryletta dissimulans* **sp. nov.** (AUP01690), adult male, in life. (A) General dorsolateral view; (B) ventral view; (C) lateral view of head; (D) volar view of left hand; (E) plantar view of right foot. Photos by P. Pawangkhanant.

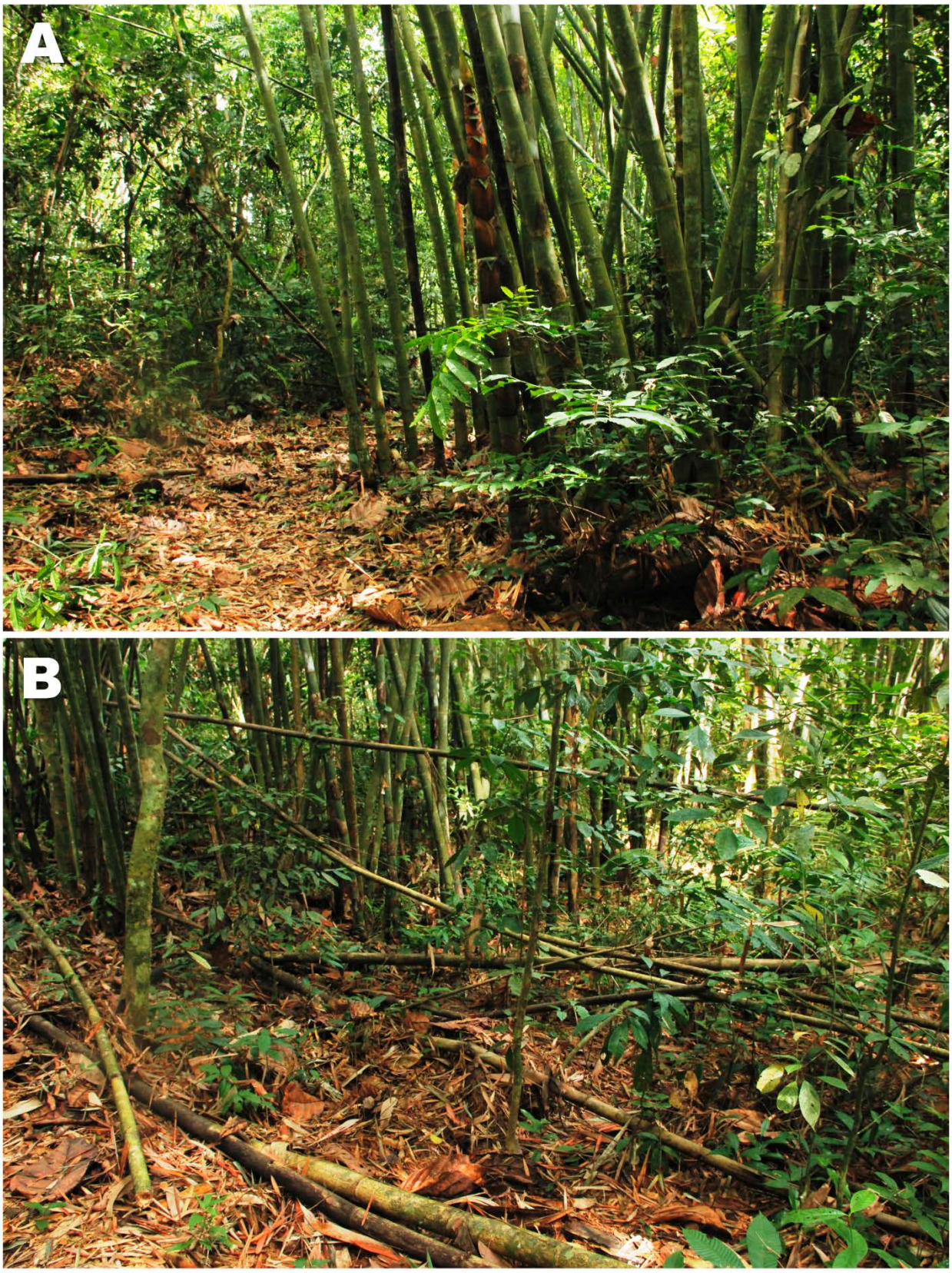


Supplementary Figure S2. Holotype of *Micryletta dissimulans* **sp. nov.** (AUP01690), adult male in preservative. (A) Dorsal view; (B) ventral view. Scale bar equals 5 mm. Photos by C. Suwannapoom.



Supplementary Figure S3. Paratypes of *Micryletta dissimulans* **sp. nov.** in preservative (dorsal view). Scale bar equals 5 mm. Photos by C. Suwannapoom.

Supplementary Figure S4. Paratype of *Micryletta dissimulans* **sp. nov.** (AUP01696), gravid female, in life. (A) General dorsolateral view; (B) ventral view. Photos by P. Pawangkhanant.



Supplementary Figure S5. Habitat of *Micryletta dissimulans* **sp. nov.** at the type locality in Saba Yoi District, Songkhla Province, Southern Thailand. (A), (B) Secondary lowland bamboo forest showing microhabitat of the new species. Photos by P. Pawangkhanant.