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# Hidden tribe: A new species of Stream Toad of the genus *Ansonia* Stoliczka, 1870 (Anura: Bufonidae) from the poorly explored mountainous borderlands of western Thailand

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*http://zoobank.org/82F0310B-38FF-417B-A2B6-8D8F67AD069D*

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**Academic editor** Raffael Ernst | **Received** 26 August 2021 | **Accepted** 25 October 2021 | **Published** 3 December 2021

**Citation:** Suwannapoom C, Grismer LL, Pawangkhanant P, Naiduangchan M, Yushchenko PV, Arkhipov DV, Wilkinson JA, Poyarkov NA (2021) Hidden tribe: A new species of Stream Toad of the genus Ansonia Stoliczka, 1870 (Anura: Bufonidae) from the poorly explored mountainous borderlands of western Thailand. Vertebrate Zoology 71 763–779. https://doi.org/10.3897/vz.71.e73529

## Abstract

The integrated results of morphological and molecular phylogenetic analyses confirmed the new species status of a recently discovered population of *Ansonia* from Suan Phueng District, Ratchaburi Province, Thailand. *Ansonia karen* **sp. nov.** is separated from all other species of *Ansonia* by a unique combination of mensural, discrete morphological, and color pattern characteristics and is the sister species of *A. thinthinae* from Tanintharyi Division, Myanmar. This discovery fills a geographic hiatus of 350 km between it and *A. kraensis* from Ranong Province, Thailand. *Ansonia karen* **sp. nov.** is the newest member of a long list of range-restricted endemics having been recently discovered in the northern Tenasserim Mountain region of western Thailand and continues to underscore the unexplored nature of this region and its need for conservation.

## Key words

*Ansonia karen* sp. nov., Molecular phylogenetics, Ratchaburi Province, Southeast Asia, Tenasserim Mountains, Thai-Malay Peninsula, toads

## Introduction

Stream toads of the genus *Ansonia* Stoliczka, 1870 comprise a distinctive clade of small anurans with relatively flat bodies and heads, and long thin limbs with slender bulbous digits that are adaptations for their scansorial, lotic life style. Species of *Ansonia* are generally restricted to rocky fast-flowing streams along the mountainous and hilly border regions of southeastern Myanmar and western and southern Thailand, southward through the Thai-Malay Peninsula to Sumatra, Borneo, and the Philippines (Grismer et al. 2016; Quah et al. 2019).

The general life history of their tadpoles—adhering themselves to the surfaces of rocks beneath fast-flowing water or in the spray zones of cascades—restricts the distribution of *Ansonia* to riverine habitats. As such, range-restricted endemism is characteristic of many species in this genus and those with widespread distributions from multiple localities are likely to be species complexes (e.g. Sanguilla et al. 2011; Grismer et al. 2016; Grismer et al. in prep.).

This is the case for a Thai-Burmese clade of nine species of *Ansonia* distributed from eastern Myanmar to western and southern Thailand where each is known from only its type locality or another locality close by with confluent riverine systems (Fig. 1). We report here a new population from western Thailand found in the hilly region of Suan Phueng District in Ratchaburi Province, that fills in a notable 350 km hiatus between *A. thinthinae* Wilkinson, Sellas & Vindum from the Tanintharyi Nature Reserve, Tanintharyi Division, Myanmar (Wilkinson et al. 2012) and *A. kraensis* Matsui, Khonsue & Nabhitabhata from the Punyaban Waterfall, Ranong Province, Thailand (Matsui et al. 2005). A molecular analysis using 12S and 16S ribosomal RNA genes recovered this new population as the sister species of *A. thinthinae*, and univariate and multivariate analyses of mensural characters and comparisons of discrete morphological and color pattern differences among species within the Thai-Burmese clade, clearly differentiate it from all other members. Therefore, based on genetic and morphological data, we describe this new population as a new species.

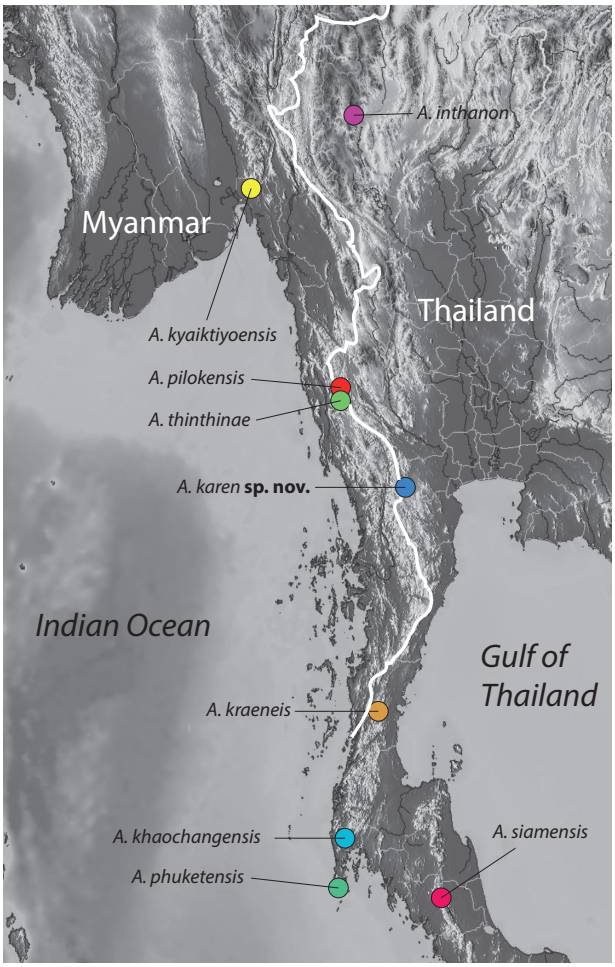


**Figure 1.** Distribution of the species of the Thai-Burmese clade of *Ansonia*.

## Materials and methods

### Sampling

Specimens were collected in Suan Phueng District, Ratchaburi Province, Thailand by Parinya Pawangkhanant, Platon V. Yushchenko, Mali Naiduangchan, Chatmongkon Suwannapoom, and Nikolay A. Poyarkov during several field surveys from 2016 to 2019. The location of the surveyed locality and the distribution of the Thai-Burmese clade of *Ansonia* are shown in Figure 1. Geographic coordinates and elevation were obtained using a Garmin GPSMAP 60CSx and recorded in WGS 84 datum. Specimens were collected by hand, euthanized with 20% benzocaine solution, femoral muscle or liver tissue samples removed, and then fixed in 10% buffered formalin before preserving in 70% ethanol. The tissue samples were stored in 95% ethanol. Specimens and tissues were subsequently deposited in the herpetological collections of the School of Agriculture and Natural Resources, University of Phayao (AUP, Phayao, Thailand) and of the Zoological Museum of Moscow University (ZMMU, Moscow, Russia).

Specimen collection and animal use protocols were approved by the Institutional Ethical Committee of Animal Experimentation of the University of Phayao, Phayao, Thailand (certificate number UP-AE61-01-04-022, issued to Chatmongkon Suwannapoom) and were strictly complacent with the ethical conditions of the Thailand Animal Welfare Act. Field work, including collection of animals in the field and specimen exportation, was authorized by the Institute of Animals for Scientific Purpose Development (IAD), Bangkok, Thailand (permit numbers U1-01205-2558 and UP-AE59-01-04-0022, issued to Chatmongkon Suwannapoom).

**Table 1.** Raw mensural data from the type series of *Ansonia karen* **sp. nov.** Abbreviations are listed in the Materials and methods; an asterisk (*) marks the holotype specimen.

| **males** | **SVL** | **HL** | **HW** | **SW** | **SL** | **DNE** | **IND** | **IOD** | **ED** | **UEW** | **VTD** | **TD** | **T-ED** | **FAL** | **HAL** | **1FL** | **THL** | **TIL** | **FL** | **1TL** | **1MTL** | **OMTL** | **3FDW** | **HLL** | **FLL** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ZMMU A-7605* | 24.9 | 8.3 | 7.5 | 3.8 | 2.9 | 1.9 | 1.8 | 2.5 | 2.7 | 1.9 | 1.4 | 1.1 | 0.3 | 13.1 | 7.4 | 1.3 | 11.4 | 11.8 | 9.9 | 1.8 | 1.8 | 1.3 | 0.7 | 35.6 | 17.3 |
| ZMMU A-7606 | 25.6 | 8.4 | 7.7 | 3.9 | 2.7 | 1.5 | 1.9 | 2.6 | 2.7 | 1.8 | 1.3 | 0.9 | 0.4 | 13.1 | 6.6 | 1.3 | 10.2 | 11.4 | 9.8 | 1.5 | 1.6 | 1.2 | 0.5 | 34.9 | 16.8 |
| ZMMU A-7607 | 23.9 | 6.9 | 7.2 | 3.7 | 2.9 | 1.8 | 1.7 | 2.9 | 2.8 | 2.1 | 1.4 | 1.3 | 0.0 | 12.5 | 6.6 | 1.6 | 10.3 | 11.1 | 8.9 | 1.4 | 1.0 | 0.8 | 0.4 | 33.9 | 16.2 |
| ZMMU A-7608 | 23.2 | 7.6 | 7.8 | 4.4 | 3.0 | 1.7 | 1.8 | 2.5 | 3.3 | 2.5 | 1.5 | 1.2 | 0.4 | 12.7 | 6.7 | 1.4 | 10.0 | 10.8 | 8.9 | 1.5 | 1.6 | 1.2 | 0.7 | 33.1 | 16.7 |
| ZMMU A-7609 | 23.5 | 7.4 | 7.7 | 4.4 | 3.2 | 2.2 | 2.0 | 2.9 | 2.8 | 2.1 | 1.4 | 1.4 | 0.6 | 12.6 | 6.6 | 1.4 | 11.7 | 11.2 | 9.4 | 2.0 | 1.6 | 1.0 | 0.6 | 37.7 | 19.5 |
| ZMMU A-7610 | 23.7 | 7.4 | 7.6 | 4.1 | 3.2 | 2.1 | 2.1 | 2.8 | 3.0 | 2.0 | 1.2 | 1.6 | 0.3 | 12.6 | 6.4 | 1.4 | 10.4 | 11.4 | 9.6 | 1.7 | 1.4 | 1.2 | 0.6 | 35.9 | 19.1 |
| ZMMU A-7611 | 24.7 | 7.4 | 7.7 | 4.3 | 3.5 | 2.0 | 2.2 | 2.9 | 3.2 | 2.1 | 1.4 | 1.4 | 0.4 | 12.6 | 6.7 | 1.3 | 10.8 | 12.0 | 9.8 | 1.7 | 1.7 | 1.2 | 0.6 | 36.8 | 18.1 |
| ZMMU A-7612 | 24.8 | 7.5 | 7.4 | 4.6 | 3.3 | 2.0 | 2.4 | 2.9 | 3.1 | 2.3 | 1.3 | 1.3 | 0.3 | 12.8 | 6.8 | 1.3 | 11.7 | 11.8 | 10.2 | 1.8 | 1.7 | 1.0 | 0.5 | 37.8 | 18.0 |
| ZMMU A-7613 | 25.4 | 7.9 | 10.0 | 4.5 | 3.7 | 2.2 | 2.3 | 2.9 | 3.1 | 2.3 | 1.1 | 1.4 | 0.7 | 13.3 | 7.0 | 1.4 | 11.2 | 11.8 | 9.9 | 1.7 | 1.6 | 0.9 | 0.6 | 35.8 | 19.8 |
| AUP-00661 | 24.4 | 8.0 | 7.5 | 3.8 | 3.1 | 1.5 | 1.9 | 2.4 | 2.6 | 1.9 | 1.4 | 1.0 | 0.3 | 13.2 | 6.5 | 1.3 | 11.4 | 12.1 | 9.8 | 1.8 | 1.8 | 1.2 | 0.5 | 34.8 | 17.4 |
| AUP-00662 | 24.6 | 8.1 | 7.7 | 3.7 | 2.9 | 1.6 | 1.9 | 2.4 | 2.6 | 1.8 | 1.3 | 1.0 | 0.3 | 13.1 | 6.5 | 1.3 | 11.1 | 12.0 | 9.7 | 1.7 | 1.8 | 1.2 | 0.5 | 34.2 | 17.4 |
| **females** | **SVL** | **HL** | **HW** | **SW** | **SL** | **DNE** | **IND** | **IOD** | **ED** | **UEW** | **VTD** | **TD** | **T-ED** | **FAL** | **HAL** | **1FL** | **THL** | **TIL** | **FL** | **1TL** | **1MTL** | **OMTL** | **3FDW** | **HLL** | **FLL** |
| AUP-00663 | 28.9 | 9.2 | 8.7 | 4.4 | 3.5 | 2.3 | 2.2 | 2.8 | 2.9 | 2.4 | 1.5 | 1.4 | 0.4 | 14.3 | 8.0 | 2.1 | 13.4 | 14.1 | 11.6 | 1.9 | 2.4 | 1.4 | 0.9 | 43.6 | 21.8 |
| AUP-00664 | 29.2 | 9.1 | 8.2 | 4.5 | 3.6 | 2.1 | 2.5 | 2.8 | 2.9 | 2.4 | 1,51 | 1.4 | 0.5 | 14.4 | 7.5 | 2.1 | 14.2 | 13.7 | 11.5 | 1.9 | 2.4 | 1.3 | 0.9 | 44.7 | 21.2 |
| ZMMU A-7614 | 26.2 | 8.3 | 7.9 | 4.0 | 3.3 | 2.0 | 2.1 | 3.0 | 3.2 | 2.4 | 1.4 | 1.4 | 0.7 | 13.7 | 7.3 | 1.3 | 12.0 | 12.2 | 10.2 | 1.7 | 2.1 | 1.4 | 0.7 | 39.2 | 19.1 |
| ZMMU A-7615 | 27.9 | 8.5 | 8.3 | 4.7 | 3.1 | 2.1 | 2.1 | 3.2 | 3.2 | 2.8 | 1.6 | 1.4 | 0.5 | 14.3 | 7.1 | 2.9 | 12.7 | 13.0 | 11.6 | 1.9 | 2.0 | 1.3 | 0.8 | 43.5 | 21.8 |
| AUP-00665 | 28.8 | 8.9 | 8.1 | 4.2 | 3.5 | 2.3 | 2.1 | 2.6 | 2.8 | 2.3 | 1.5 | 1.3 | 0.4 | 14.0 | 7.0 | 2.1 | 13.1 | 13.1 | 11.5 | 1.8 | 2.4 | 1.4 | 0.9 | 44.1 | 20.5 |

## Morphological data and analyses

Observations on color pattern were based on the examination of specimens in life as well as digital images of living and euthanized specimens prior to preservation. Measurements were recorded with a Mitutoyo dial caliper under a Nikon SMZ 1500 dissecting microscope to the nearest 0.01 mm. Measurements of adult specimens generally following Wilkinson et al. (2012) were: snout-vent length (SVL, from tip of snout to vent); head length (HL, from tip of snout to hind border of angle of jaw); head width (HW, width of head at its widest point); snout width (SW, width of snout at anterior corner of eyes); snout length (SL, from anterior border of eye to tip of snout); distance from nostril to eye (DNE, from center of nostril to anterior border of eye); internarial distance (IND, distance between center of nares); interorbital distance (IOD, minimum distance between upper eyelids); eye diameter (ED, horizontal diameter of eye); upper eyelid width (UEW, greatest transverse width of eyelid); vertical tympanum diameter (VTD; vertical diameter of tympanum); horizontal tympanum diameter (HTD; horizontal diameter of tympanum); tympanum to eye distance (T-ED, from anterior edge of tympanum to posterior edge of eye); forearm length (FAL, from elbow to tip of third finger); hand length (HAL, from proximal edge of palmar tubercle to tip of third finger); first finger length (1FL, from distal end of inner metacarpal tubercle to tip of first finger); thigh length (THL, from vent to knee); tibia length (TIL, from knee to ankle); foot length (FL, from proximal end of outer metatarsal tubercle to tip of fourth toe); first toe length (1TL, from distal end of inner metatarsal tubercle to tip of first toe); inner metatarsal tubercle length (IMTL, greatest length of tubercle); outer metatarsal tubercle length (OMTL, greatest length of tubercle); third finger disk width (3FDW, maximal width of terminal disk on third finger); hindlimb length (HLL, length of straightened hindlimb from groin to tip of fourth toe); and forelimb length (FLL, length of straightened forelimb from axilla to tip of third finger) (Table 1).

Measurements on a single tadpole specimen AUP-02091 followed Inger (1985) and Wilkinson et al. (2012), and were taken under a Nikon SMZ 1500 dissecting microscope with a micrometer as follows: total length, head-body length (tip of snout to insertion of tail), head-body depth, maximum head-body width, diameter of eyeball, interorbital dis-

tance, eye to tip of snout, internarial distance, width of oral disc, tail length, maximum tail depth, tail muscular depth, thigh length, tibia length, and foot length. Tadpole staging followed Gosner (1960), oral apparatus terminology followed Altig and McDiarmid (1999), and labial tooth row formula followed Altig et al. (1998).

The morphospatial clustering among sampled individuals from a selected subset of species and characters for which there was full coverage for each species were visualized using principal component analysis (PCA) from the ADEGENET package in R (Jombart et al. 2010). Only males were used in the analysis because only one female of *Ansonia thinthinae* was available. Characters analyzed were HL, HW, SL, ED, HTD, IND, IOD, FL, and TIL. To remove potential effects of allometry, size was normalized using the following equation: $X_{adj}=\log(X)-\beta[\log(SVL)-\log(SVL_{mean})]$, where $X_{adj}$=adjusted value; X=measured value; β=unstandardized regression coefficient for each population; and $SVL_{mean}$=overall average SVL of all populations (Thorpe 1975, 1983; Turan 1999; Lleonart et al. 2000, accessible in the R package *GroupStruct* (available at https://github.com/chankinonn/GroupStruct). The morphometrics of each species were normalized separately and then concatenated so as not to conflate intra- with interspecific variation (Reist 1986). All data were scaled to their standard deviation to insure they were analyzed on the basis of correlation and not covariance. For corroboration, a discriminant analysis of principal components (DAPC) was performed on the same data set. DAPC relies on scaled data calculated from its own internal PCA as a prior step to ensure that variables analyzed are not correlated and number fewer than the sample size. Dimension reduction of the DAPC prior to plotting, is accomplished by retaining the first set of PCs that account for 90–99% of the variation in the data set (Jombart and Collins 2015) as determined from a scree plot generated as part of the analysis.

Two-sample *t*-tests of the all the scaled mensural characters were used to search for statistically significant mean morphometric differences between the new Thai population and its sister species, *A. thinthinae* (see below). Characters were subjected to an F-test to test for homogeneity of variances. Those with unequal variances were subjected to a Welch's *t*-test and those with equal variances were subjected to a Student *t*-test. All statistical analyses were performed in R [v3.4.3]. Raw and adjusted data are presented in Table S1.

## Laboratory methods

For the molecular phylogenetic analyses, we extracted the total genomic DNA from ethanol-preserved femoral muscle tissue of six specimens of the new Thai population using standard phenol-chloroform-proteinase K extraction procedures with consequent isopropanol precipitation, for a final concentration of about 1 mg/ml (protocols followed Hillis et al. (1996) and Sambrook and David (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 NanoDrop 2000 (Thermo Scientific), and consequently adjusted to ca. 100 ng DNA/µL.

We amplified mtDNA fragments covering partial 16S rRNA gene sequences to obtain a 560 bp-length continuous fragment per specimen. The 16S rRNA gene has widely been applied in biodiversity surveys in amphibians (Vences et al. 2005a, 2005b; Vieites et al. 2009), and has been used in the most of the recent phylogenetic studies on *Ansonia* (e.g. Grismer et al. 2016; Matsui et al. 2007; Matsui et al. 2010; Wilkinson et al. 2012). 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 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.1 mM $MgCl_2$ and 0.01% gelatine) and 1 unit of Taq DNA polymerase. Primers used in the PCR and sequencing include 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 to 55°C reducing 1°C every cycle, and extension for 1 min at 72°C, and a final extension step for 5 min at 72°C.

PCR products were loaded onto 1.5% agarose gels in the presence of ethidium bromide and visualized in agarose electrophoresis. When distinct bands were produced, we purified the PCR products using 2 µl of a 1:4 dilution of ExoSapIt (Amersham) per 5 µl of PCR product prior to cycle sequencing. A 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 version 3.1 Sequencing Standard (Applied Biosystems) and 4.2 µl of water. The cyclesequencing used 35 cycles of 10 sec at 96°C, 10 sec at 50°C and 4 min at 60°C. We purified the cyclesequencing products by ethanol precipitation. We carried out sequence data collection and visualization on an ABI 3730xl Automated Sequencer (Applied Biosystems).

## Genetic data and phylogenetic analyses

Ingroup samples consisted of 128 individuals representing 32 nominal species and included three of the six samples from the new Thai population. Outgroups used to root the tree were *Rentapia hosii* (Boulenger), *Pelophryne brevipes* (Peters), *P. misera* (Mocquard), and *P. signata* (Boulenger) based in part on the phylogenetic results of Chan et al. (2014), Grismer et al. (2016), Matsui et al. (2007, 2010), and Wilkinson et al. (2012). Sequence data generated for the six individuals from the new Thai population are deposited in GenBank under the accession numbers MZ823491–MZ823496. Sequences for all other individuals were downloaded from GenBank and are listed in Quah et al. (2019: Table 2).

Maximum Likelihood (ML) and Bayesian Inference (BI) were used to estimate phylogenetic trees. Best-fit

models of evolution were determined in IQ-TREE (Nguyen et al. 2015) using the Bayesian information criterion (BIC) implemented in ModelFinder (Kalyaanamoorthy et al. 2017). TIM2+F+I+G4 was the best-fit model of evolution for both 12S and 16S. The ML analysis was performed using the IQ-TREE webserver (Trifinopoulos et al. 2016) with 1000 bootstrap pseudoreplicates using the ultrafast bootstrap (UFB) analysis (Minh et al. 2013; Hoang et al. 2018). The BI analysis was performed on CIPRES Science Gateway (Miller et al. 2010) using MrBayes v3.2.4 (Ronquist et al. 2012). Two independent runs were performed using Metropolis-coupled Markov Chain Monte Carlo (MCMCMC), each with four chains: three hot and one cold. The MCMCMC chains were run for 60,000,000 generations with the cold chain sampled every 6000 generations and the first 25% of each run being discarded as burn-in. The posterior distribution of trees from each run was summarized using the sumt function in MrBayes v3.2.4 (Ronquist et al. 2012).

Stationarity was checked with Tracer v1.6 (Rambaut et al. 2014) to be sure the effective sample sizes (ESS) for all parameters were greater than 200. We considered Bayesian posterior probabilities (BPP) of 0.95 and above and ultrafast bootstrap support values (UFB) of 95 and above as strong nodal support (Huelsenbeck et al. 2001; Minh et al. 2013). Uncorrected pairwise sequence divergences (p-distance) were calculated in MEGA v6.06 (Tamura et al. 2013) using the complete deletion option which removes gaps and missing data from the alignment prior to analysis.

# Results

## Phylogenetic data

The ML and BI analyses recovered nearly identical trees (Fig. 2). Both analyses recovered a strongly supported (UFB 100/BPP 1.00) Thai-Burmese clade of *Ansonia* composed of *A. siamensis* Kiew*, A. khaochangensis* Grismer, Wood, Aowopol, Cota, Grismer, Murdoch, Aguilar, and Grismer*, A. pilokensis* Matsui, Khouse, and Panha*, A. phuketensis* Matsui, Khonsue, and Panha*, A. kyaiktiyoensis* Quah, Grismer, Wood, Myint Kyaw Thura, Oaks, and Aung Lin*, A. inthanon* Matsui, Nabhitabhata, Panha*, A. kraensis, A. thinthinae,* and the new population from Suan Phueng District, Ratchaburi Province, Thailand. The Suan Phueng population was recovered as the strongly supported (100/1.00) sister species of *A. thinthinae* in both analyses with an uncorrected pairwise sequence divergence between them for the 16S rRNA gene of 4.1%. The only difference between the ML and BI analyses was the placement of *A. pilokensis* as a poorly supported (UFB 79) sister species of *A. phuketensis* in the ML analysis, whereas, in the BI analysis, these two species formed an unsupported polytomy with the remaining species in the Thai-Burmese clade. Their sister species relationship was poorly supported in both analyses (84/0.54) in Quah et al. (2019). All other relationships were in complete concordance with Quah et al. (2019).

**Table 2.** Calculated t and p values from the t-tests.

| | t-value | **p-value** | **test** |
|---|---|---|---|
| HLL | –2.9397 | 0.02401 | Welch t-test |
| SL | –5.0071 | 0.00187 | Welch t-test |
| EL | –7.3776 | 0.00022 | Welch t-test |
| HTD | –3.9494 | 0.00363 | Welch t-test |
| HW | –8.1595 | 7.84E-05 | Welch t-test |
| IND | –3.244 | 0.003133 | Student t-test |
| IOD | –3.9681 | 0.00656 | Welch t-test |
| TIL | –10.845 | 1.49E-06 | Welch t-test |
| FLL | –7.8502 | 3.65E-05 | Welch t-test |

## Mensural data

The new Thai population from Suan Phueng and its sister species *Ansonia thinthinae* have statistically different head, body, and limb proportions (Tables 2, 3) with the former having a significantly shorter and wider head (HL and HW, respectively); shorter snout (SL); smaller eyes and tympana (ED and HTD, respectively); smaller internarial and interorbital diameters (IND and IOD, respectively); and shorter hind limbs (TIL and FL, respectively). The two populations are widely separated in the PCA (Fig. 2) where principal component (PC) 1 accounts for 63.8% of the variation in the dataset and loads most heavily for head width (HW), snout length (SL), limb length (FLL and HLL), and eye diameter (ED). PC 2 accounts for an additional 10.4% of the variation and loads most heavily for internarial distance (IND) (Table 4). The DAPC corroborates the PCA in that each population is completely separated from one another along the first discriminant function that accounts for 99.4% of the data set (Fig. 2). Based on all the above data, the new Thai population from Suan Phueng District is described below as a new species.

# Taxonomy

## *Ansonia karen* sp. nov.

Suggested Common Name: Karen Stream Toad

Figures 3–7

http://zoobank.org/151C982A-A85C-4AB5-B969-51BCFE4-5C701

**Holotype.** ZMMU A-7605 (field number NAP-06631), an adult male collected on 8 November 2016 at a forest stream within the montane evergreen forest of Khao

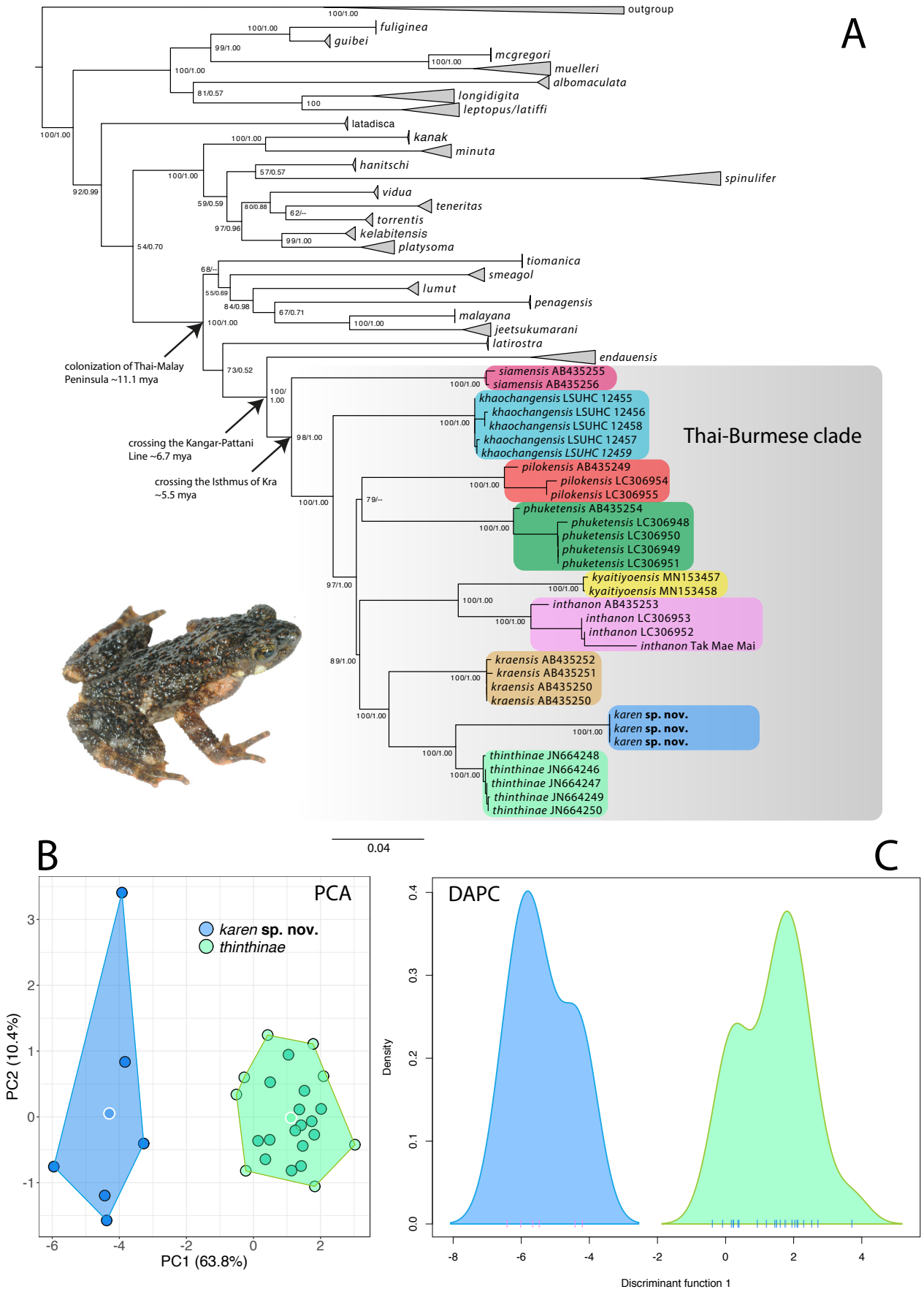


**Figure 2.** A. Majority-rule consensus tree from 1000 ML bootstrap pseudoreplicates of *Ansonia*. Phylogeny is based on 2467 bp of concatenated 12S and 16S ribosomal DNA with UFB and BPP support values, respectively, listed at the nodes. Scale bar denotes substitutions site. B. PCA of *Ansonia karen* **sp. nov.** and *A. thinthinae* based on the adjusted mensural characters. C. DAPC of *Ansonia karen* **sp. nov.** and *A. thinthinae* based on the adjusted mensural characters. Photo showing an adult male of *Ansonia karen* **sp. nov.** in life by N. A. Poyarkov.

**Table 3.** Summary statistics of the adjusted mensural characters used in the statistical analyses of *Ansonia karen* **sp. nov.** and *A. thinthinae.* Abbreviations are listed in the Material and methods.

| ***A. karen* sp. nov. (n=6)** | **HL** | **HW** | **SL** | **EL** | **TD** | **IND** | **IOD** | **TIL** | **FL** |
|---|---|---|---|---|---|---|---|---|---|
| mean | 1.943 | 1.853 | 1.052 | 0.762 | 0.224 | 0.756 | 0.960 | 2.346 | 2.164 |
| ± sd | 0.034 | 0.041 | 0.055 | 0.072 | 0.081 | 0.104 | 0.063 | 0.028 | 0.038 |
| range | 1.907–1.987 | 1.804–1.929 | 0.967–1.112 | 0.672–0.850 | 0.105–0.310 | 0.585–0.851 | 0.847–1.031 | 2.312–2.384 | 2.113–2.212 |
| | 1.987 | 1.929 | 1.112 | 0.850 | 0.310 | 0.851 | 1.031 | 2.384 | 2.212 |
| ***A. thinthinae* (n=24)** | | | | | | | | | |
| mean | 1.983 | 1.995 | 1.167 | 0.986 | 0.369 | 0.853 | 1.066 | 2.486 | 2.298 |
| ± sd | 0.029 | 0.053 | 0.049 | 0.063 | 0.093 | 0.052 | 0.047 | 0.050 | 0.049 |
| range | 1.907–2.030 | 1.804–2.068 | 1.041–1.263 | 0.809–1.108 | 0.198–0.569 | 0.723–0.946 | 0.957–1.161 | 2.312–2.557 | 2.173–2.367 |

**Table 4.** Summary statistics and principal component analysis scores for the mensural characters of *Ansonia karen* **sp. nov.** and *A. thinthinae*. Abbreviations are listed in the Materials and methods.

| | **PC1** | **PC2** | **PC3** | **PC4** | **PC5** | **PC6** | **PC7** | **PC8** | **PC9** |
|---|---|---|---|---|---|---|---|---|---|
| Standard deviation | 2.39726 | 0.96600 | 0.89174 | 0.83157 | 0.57186 | 0.47365 | 0.37752 | 0.32949 | 0.17559 |
| Proportion of variance | 0.63854 | 0.10368 | 0.08836 | 0.07683 | 0.03634 | 0.02493 | 0.01584 | 0.01206 | 0.00343 |
| Cumulative proportion | 0.63854 | 0.74222 | 0.83058 | 0.90741 | 0.94375 | 0.96868 | 0.98451 | 0.99657 | 1 |
| Eigenvalue | 5.74685 | 0.93316 | 0.79520 | 0.69150 | 0.32702 | 0.22434 | 0.14252 | 0.10856 | 0.03083 |
| HIL | 0.27098 | 0.54668 | –0.48265 | 0.10878 | –0.53108 | 0.16594 | –0.09269 | 0.22918 | –0.11062 |
| SL | 0.33941 | –0.03475 | –0.41842 | –0.11058 | 0.72617 | 0.08739 | –0.11690 | 0.33045 | –0.19611 |
| ED | 0.34608 | –0.14423 | 0.01457 | 0.50058 | –0.03803 | –0.62922 | –0.45158 | –0.05594 | 0.06492 |
| HTD | 0.29530 | 0.23246 | 0.58207 | –0.45619 | –0.08608 | –0.26407 | –0.05060 | 0.35025 | –0.33087 |
| HW | 0.38077 | 0.19814 | 0.02006 | 0.16603 | 0.12146 | –0.24659 | 0.79279 | –0.04052 | 0.28647 |
| IND | 0.24973 | –0.73749 | –0.24304 | –0.17910 | –0.39792 | –0.03709 | 0.25438 | 0.12371 | –0.25072 |
| IOD | 0.34435 | 0.06183 | –0.18036 | –0.58885 | –0.02297 | –0.06361 | –0.20669 | –0.60330 | 0.29507 |
| TIL | 0.37290 | 0.01362 | 0.26001 | 0.31988 | 0.05980 | 0.42742 | 0.02970 | –0.48613 | –0.51707 |
| FL | 0.37339 | –0.19475 | 0.30554 | 0.09243 | –0.06762 | 0.50026 | –0.18555 | 0.30861 | 0.57988 |

Laem Mt., Suan Phueng District, Ratchaburi Province, Thailand (N 13.54732, E 099.20394; 715 m a.s.l. in elevation), by P. Pawangkhanant, C. Suwannapoom and N. A. Poyarkov.

**Paratypes (n=15).** ZMMU A-7606 (field number NAP-06630), an adult male with same collection information as holotype; ZMMU A-7607 (no field number) and ZMMU A-7608 (field number AUP-00349), two adult males collected on 15 June 2018 at same locality as holotype by P. Pawangkhanant, C. Suwannapoom and M. Naiduangchan; ZMMU A-7609–A-7614 (field numbers NAP-10193–NAP-10198), five adult males and an adult female collected on 18 June 2019 at a forest stream within the montane evergreen forest on the northern slope of Khao Laem Mt., Suan Phueng District, Ratchaburi Province, Thailand (N 13.54581, E 099.20368; 758 m a.s.l. in elevation), by P. Yushchenko and M. Naiduangchan; AUP-00661–00665, two adult males and three adult females collected on 15 June 2019 at same locality as holotype by P. Pawangkhanant and M. Naiduangchan; and ZMMU A-7615 (field number NAP-09901), an adult female collected on 4 October 2019 at same locality as holotype by P. Pawangkhanant.

**Diagnosis.** *Ansonia karen* **sp. nov.** is recognized as a member of the genus *Ansonia* based on the results of the molecular phylogenetic analyses that recover it as the sister species of *A. thinthinae* (Fig. 2) as well as by a combination of the following morphological characters: small body size (maximum SVL 25.6 mm in males and 29.2 mm in females); long slender limbs bearing long slender digits with bulbous tips; absence of parotoid glands; weak subarticular tubercles; and membranous foot webbing (Inger 1960, 1966, 1992; Wilkinson et al. 2012; Chan et al. 2014; Grismer et al. 2016; Quah et al. 2019). It can be differentiated from all congeners by the following combination of characters: maximum SVL in males 25.4 mm and females 29.2 mm; snout projecting beyond lower jaw; tympanum visible; no interorbital or tarsal ridges; first finger shorter than second; finger tips bulbous, toe tips slightly dilated forming weak discs; approximately 2.5 phalanges free of web on fourth toe and 0.5 phalanges free of web on fifth toe; yellow rictal tubercles at angle of jaw; distinct, red-tipped, spiny tubercles on dorsum and flanks; abdomen coarsely granular; no oblique flaps of skin bordering vent; wide, light-colored patch below eye; light-colored, generally diamond-shaped interscapular spot; large, discrete, yellow, submandibular spots; no

**Figure 3.** Holotype of *Ansonia karen* **sp. nov.** (ZMMU A-7605) from Suan Phueng District, Ratchaburi Province, western Thailand. Photo by N. A. Poyarkov.

light-colored streaks on canthus rostralis; dorsum black, lacking an X-shaped marking surrounding interscapular spot; no dark-colored markings on rump; no dark dorsolateral stripe; iris yellow-gold; fore- and hind limbs bearing irregularly shaped, light-colored crossbars; venter and undersides of limbs dull-yellow bearing thick, grey-brown reticulations; palmar surfaces of hands and thenar surfaces of feet reddish-orange in life.

**Description of holotype.** Adult male, SVL 24.9 mm (Figs. 3, 4); head longer than wide (HL/HW=1.10); snout shorter than wide (SL/SW=0.76), projecting beyond lower jaw, strongly tuberculate, truncate in dorsal view (Fig. 3D), truncate and sloping in lateral view (Fig. 3C); canthus rostralis distinct, lores vertical, flat; nares open laterally just below canthus, located much closer to end of snout than to eye (Fig. 3C); distance between nares smaller than snout length (IND/SL=0.62) and snout width (IND/SW=0.47); eyes large, slightly protruding beyond upper jaws in dorsal view (Fig. 3D), diameter nearly same as snout length (ED/SL=0.94) and interorbital distance (ED/IOD=1.07); pupils horizontal; interorbital region flat, strongly tuberculate, distance smaller than snout width (IOD/SW=0.67) and snout length (IOD/SL=0.88); tympanum distinct, suboval, taller than wide (Fig. 3C), horizontal axis less than eye diameter (HTD/ED=0.53); choanae subcircular, separated by a distance larger than their diameter; vomerine ridge and teeth absent; tongue narrow, ending in median point, posterior one-half free.

Forelimbs and fingers long and slender (HAL/SVL=0.30; FLL/SVL=0.69); finger length from shortest to longest: I<II<IV<III; basal webbing not extending beyond proximal subarticular tubercle (Fig. 3E); fingertips bulbous, slightly dilated but not forming discs; subarticular tubercles indistinct; inner and outer metacarpal tubercles distinct, oval, slightly raised, inner smaller than outer; supernumerary tubercles absent (Fig. 3E). Hind limbs and toes long and slender (FL/SVL=0.40; HLL/SVL 1.43), toe length from shortest to longest: I<II<V<III<IV; webbing formula: I 0.5–0.5 II 0.5–1 III 0.5–3 IV 2.5–0.5 V; toe tips bulbous, slightly dilated forming weak discs (Fig. 3F); subarticular tubercles indistinct; inner metatarsal tubercle small, oval, slightly raised; outer metatarsal tubercle raised, rounded, somewhat smaller than inner (OMTL/IMTL=0.72). Upper eyelid, interorbital region, dorsal part of snout and canthus covered with numerous small and larger tubercles; no interorbital ridges; small, randomly arranged tubercles on lores; single row of small spinules on upper lip and outer margin of upper eyelid (Fig. 3C); four rictal tubercles; no supratympanic folds or parotoid glands; dorsum, flanks, and dorsal surfaces of limbs bearing irregularly spaced, large and small tubercles most of which have brown keratinized spinules, some larger tubercles have more than one spinule; concentration of larger tubercles above tympanum and in scapular region forming an indistinct dorsolateral row extending to insertion of hind limbs; series of brown conical, keratinized tubercles along edges of underside of mandible, absent in gular region (Fig. 3B); abdomen coarsely granular; all ventral surfaces except for manus and pes covered with coarse, evenly spaced, rounded glandular tubercles.

**Coloration in life (Figs. 3, 4).** Top of head black (Fig. 3D); dorsum and flanks black, punctuated with widely spaced, red-tipped tubercles (Fig. 4); dull-yellow, elon-

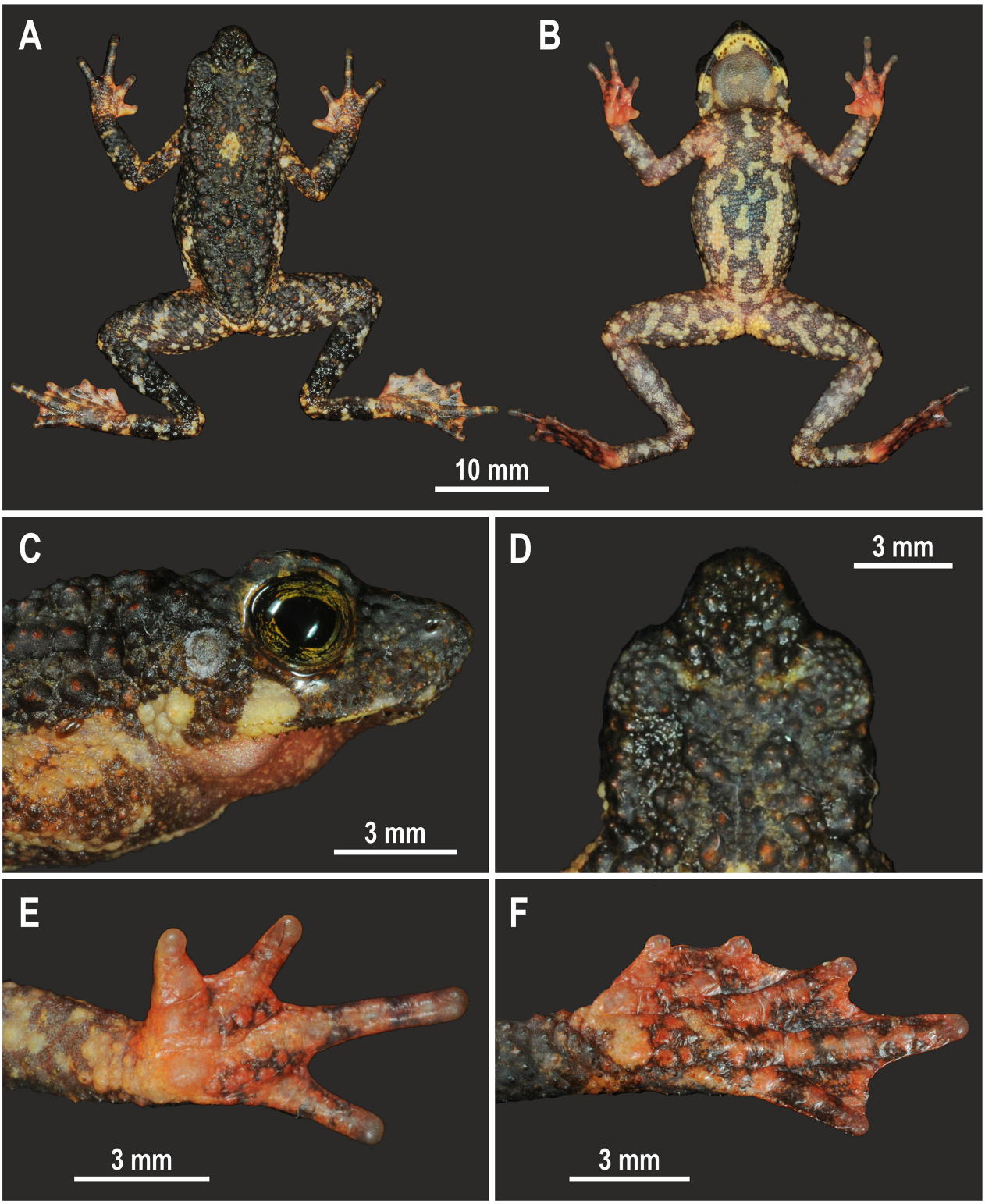


**Figure 4.** Holotype of *Ansonia karen* **sp. nov.** (ZMMU A-7605) in life from Suan Phueng District, Ratchaburi Province, western Thailand. A. Dorsal view. B. Ventral view. C. Right lateral view of head. D. Dorsal view of head. E. Ventral view of left hand. F. Ventral view of left foot. Photos by N. A. Poyarkov.

gate, diamond-shaped insterscapular spot (Fig. 3A); large, yellowish suborbital patch and rictal tubercles (Figs. 3C, 4); forelimbs bearing irregularly shaped, light-colored beige-grey bands, most prominent on brachia and extending anteriorly onto shoulder; dorsomedial surfaces of shoulder and digits I and II orangish; gray, irregularly shaped bands on hind limbs; yellow patch on ankles; large, yellow, widely spaced, submandibular blotches confluent with blotch on lower sections of upper lip (Fig. 3B); gular region light greyish-brown, unicolor; wide, dark-brown, paired, longitudinally oriented pectoral markings grading posteriorly into a wide, black, abdominal reticulum confluent with dark reticulum on ventral surfaces of hind limbs (Fig. 3B); wide yellow patch surrounding vent extending onto ventral surface of thighs (Fig. 3A); and bottoms of hands and feet reddish-orange (Fig. 3E–F).

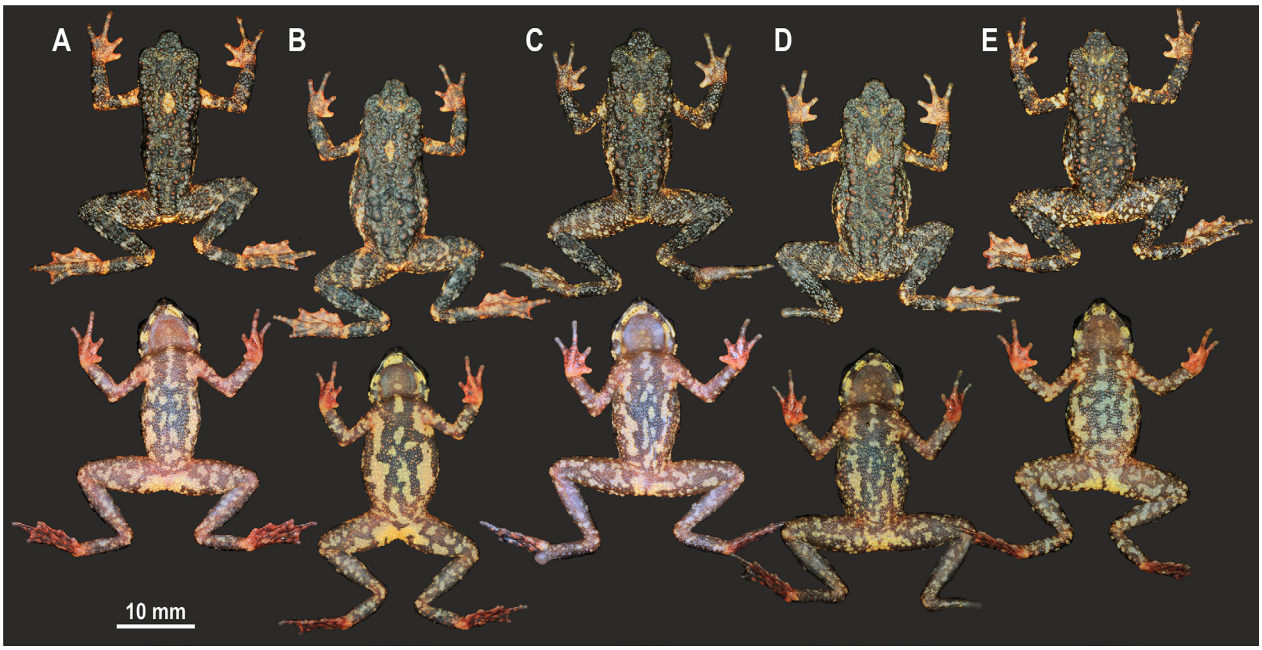


**Figure 5.** Male paratypes of *Ansonia karen* **sp. nov.** in dorsal (upper row) and ventral (lower row) views from Suan Phueng District, Ratchaburi Province, western Thailand. A. ZMMU A-7607. B. ZMMU A-7606. C. ZMMU A-7608. D. AUP-00661. E. AUP-00662. Photos by N. A. Poyarkov.

**Coloration in preservative.** After five years of storage in ethanol, the warm reddish, yellowish and orange tints have significantly faded, the specimen looks dark greyish-brown; however all major features of coloration pattern are still well-discernable.

**Variation.** Raw and adjusted mensural data of the type series are presented in Tables 1 and 1s, respectively. Males have smaller body sizes than females, and their SVL values do not overlap (male SVL = 23.2–25.6 mm, average 24.4±0.8 mm; vs. female SVL = 26.2–29.2 mm, average 28.2±1.2 mm). The members of the type series generally agree in coloration with that of the holotype (see Fig. 5). Males ZMMU A-7607 (Fig. 5A) and ZMMU A-7608 (Fig. 5C) have generally lighter pinkish-grey coloration of ventral surfaces. The shape of ventral reticulum varies from having dense small yellowish and blackish spots (as in male AUP-00662, Fig. 5E) to larger intermittent longitudinal black blotches with yellowish veins between them (as in males ZMMU A-7607 and ZMMU A-7608, Fig. 5A, C). Males AUP-00661 and AUP-00662 originally had damaged and partially regenerated left hind limbs (Fig. 5D–E). Other morphological features showed no significant variation among the type series.

**Larval morphology.** Description based on AUP-02091 at Gosner (1960) stage 38. Total length 16.9 mm, head-body length 6.2 mm, head-body depth 2.4 mm, maximum head-body width 3.3 mm, diameter of eyeball 0.9 mm, interorbital distance 1.4 mm, eye to tip of snout 1.7 mm, internarial distance 1.1 mm, width of oral disc 3.0 mm, tail length 10.8 mm, maximum tail depth 2.4 mm, tail muscular depth 1.7 mm, thigh length 1.0 mm, tibia length 1.2 mm, foot length 1.7 mm. Body distinctly flattened dorsoventrally (Fig. 6A), broadly oval-shaped in dorsal and ventral views with maximum width posterior to eyes (Fig. 6B); snout broadly rounded in dorsal view (Fig. 6B); eyes with dorsolateral orientation; nostrils located closer to eyes than to tip of snout, with anterolateral orientation. Oral disc ventral, forming a sucker, comprising ca. 93% of head-body width, not emarginate, both oral labia expanded (Fig. 6D); anterior labium slightly smaller than posterior labium, separated from tip of snout by deep groove; marginal papillae in single row across posterior labium and not discernable on anterior labium, submarginal papillae in two rows on posterior labium; black, serrated jaw sheaths, upper divided with gap of ca. same length as single sheath, lower continuous; labial tooth (keratodont) row formula 2/3, all rows continued, well separated from jaw sheaths, anterior rows medially curved, slightly longer than posterior rows (Fig. 6D); tail musculature well-developed, tapering posteriorly to pointed tail tip; tail deepest in anterior one third of length; dorsal and ventral fins approximately equal in depth.

**Tadpole coloration.** In life (Fig. 6) dorsal surfaces of body and tail dark violet-brown with numerous, golden and bronze-colored specks scattered along tail, getting denser on body anteriorly and laterally and around eyes (Fig. 6B). Laterally dark violet-gray with bright golden or metal specks on tail and body flanks; limbs dorsally with bronze specks and transverse dark bands (Fig. 6B). Ventrally semi-translucent lavender-gray lacking golden specks (Fig. 6C). After three years in preservative, dorsal surfaces of body turned grayish-brown, with densely well-discernable scattered brown chromatophores; ventral surfaces with very few chromatophores medially getting denser laterally; dorsal and ventral tail fins transparent with few chromotaphores.

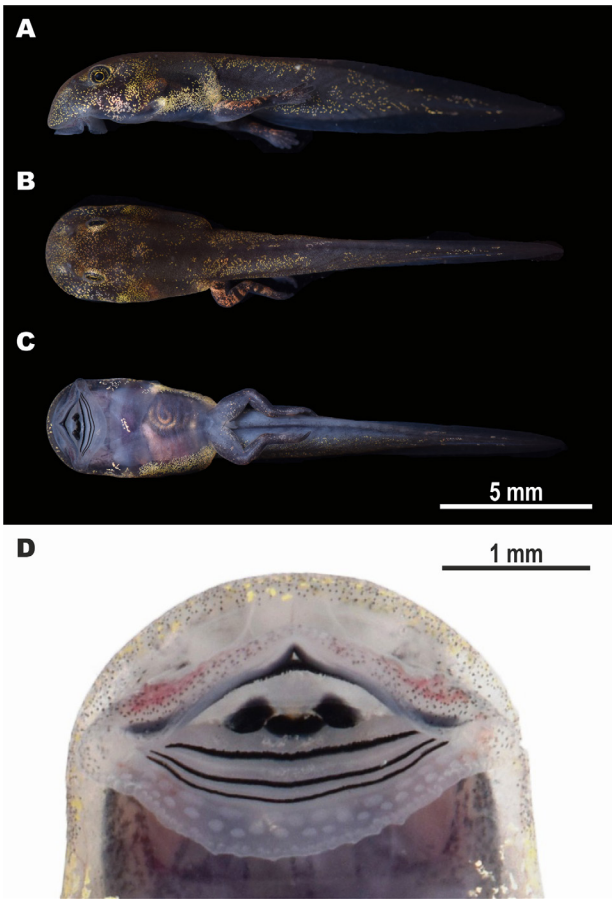


**Figure 6.** The tadpole of *Ansonia karen* **sp. nov.** (AUP-02091) from Suan Phueng District, Ratchaburi Province, western Thailand in life. A. Lateral view. B. Dorsal view. C. Ventral view. D. Close-up of the oral disc. Photos by M. Naiduangchan.

**Distribution.** *Ansonia karen* **sp. nov.** is currently known only from the type locality and nearby locality in same forest stream in the environs of Khao Laem Mountain, in Suan Phueng District of Ratchaburi Province in western Thailand, less than 2.0 km from the international Thai–Myanmar border (Fig. 1). The new species likely inhabits the middle portion of the Northern Tenasserim Mountain range (between the Kanchanaburi and Prachuap Khiri Khan provinces), and is expected to occur in adjacent montane areas in the western part of Phetchaburi Province of Thailand and Thanintharyi Division of Myanmar.

**Natural history.** The new species inhabits a polydominant montane tropical evergreen forest on Khao Laem Mountain at elevations from ca. 700 to 750 m a.s.l., where the adult specimens were observed at night perched on leaves or stones (Fig. 7B–C) along an approximately 1–3 m wide, slow-flowing mountain stream (Fig. 7A), or beneath stones along the stream's edge. The multi-species polydominant tropical forest at the type locality has dense vegetation with tangles of giant bamboo *Dendrocalamus asper* (Schult.) Backer. Males were calling during our field observations from June to November throughout 2017–2019. The tadpoles of the new species were recorded in the same stream and were usually concentrated in pools under small waterfalls, hiding under gravel on the stream bottom, or sitting on the vertical surfaces of large submerged boulders to which they were attached by their oral discs (Fig. 7D).

The species of amphibians and reptiles recorded in sympatry with the new species at the type locality include: *Leptobrachium tenasserimense* Pawangkhanant, Poyarkov, Duong, Naiduangchan & Suwannapoom, *L. smithi* Matsui, *Xenophrys* cf. *major* (Boulenger), *Leptobrachella melanoleuca* (Matsui), *L. fuliginosa* (Matsui), *Amolops panhai* Matsui & Nabhitabhata, *Alcalus tasanae* (Smith), *Limnonectes jarujini* Matsui, Panha, Khonsue & Kuraishi, *L. doriae* (Boulenger), *L. macrognathus* (Boulenger), *M. berdmorei* (Blyth), *Acanthosaura crucigera* Boulenger, *Pseudoxenodon macrops* (Blyth), *Trimeresurus popeiorum* Smith, and *Rhabdophis chrysargos* (Schlegel).

**Etymology.** The specific name "*karen*" is given as a noun in apposition and refers to the name of the Karen people. Originally inhabiting wide areas in southern and southeastern Myanmar, many Karen have migrated to Thailand, having settled mostly on the Thailand–Myanmar border, including the Suan Phueng District, the type locality of the new species, due to the political turmoil during the end of XX – beginning of XXI centuries. We received significant help and assistance from the local Karen community in Suan Phueng during our field surveys and want to thank them for their permanent support. NAP also thanks Karen Sarkisian for his support and encouragement.

**Comparisons.** *Ansonia karen* **sp. nov.** is most closely related to *A. thinthinae* but differs from it by being smaller, more squat and having statistically significant differences in head and limb proportions (see above and Table 2). It differs in coloration and pattern from *A. thinthinae* in having (as opposed to lacking) red-tipped tubercles on the dorsum and flanks; lacking, as opposed to having, gular spotting; having irregularly shaped gray crossbars on the hind limbs as opposed to having regularly shaped, thin, yellowish crossbars; and the bottoms of the hands and feet being reddish-orange as opposed to black. Differences between, and among, other species in the Thai-Burmese clade are summarized in Table 5.

## Discussion

The genus *Ansonia* was hypothesized to have evolved and diversified in Borneo before independently dispersing to the Philippines, Sumatra, and twice onto the Thai-Malay Peninsula (Grismer et al. 2016). The first colonization of the Thai-Malay Peninsula ~11.1 million years ago (mya), ultimately resulted in the evolution of a clade that currently contains at least 17 species, including the new species *Ansonia karen* **sp. nov.** Diversification of this clade at its point of origin in Peninsular Malaysia was followed

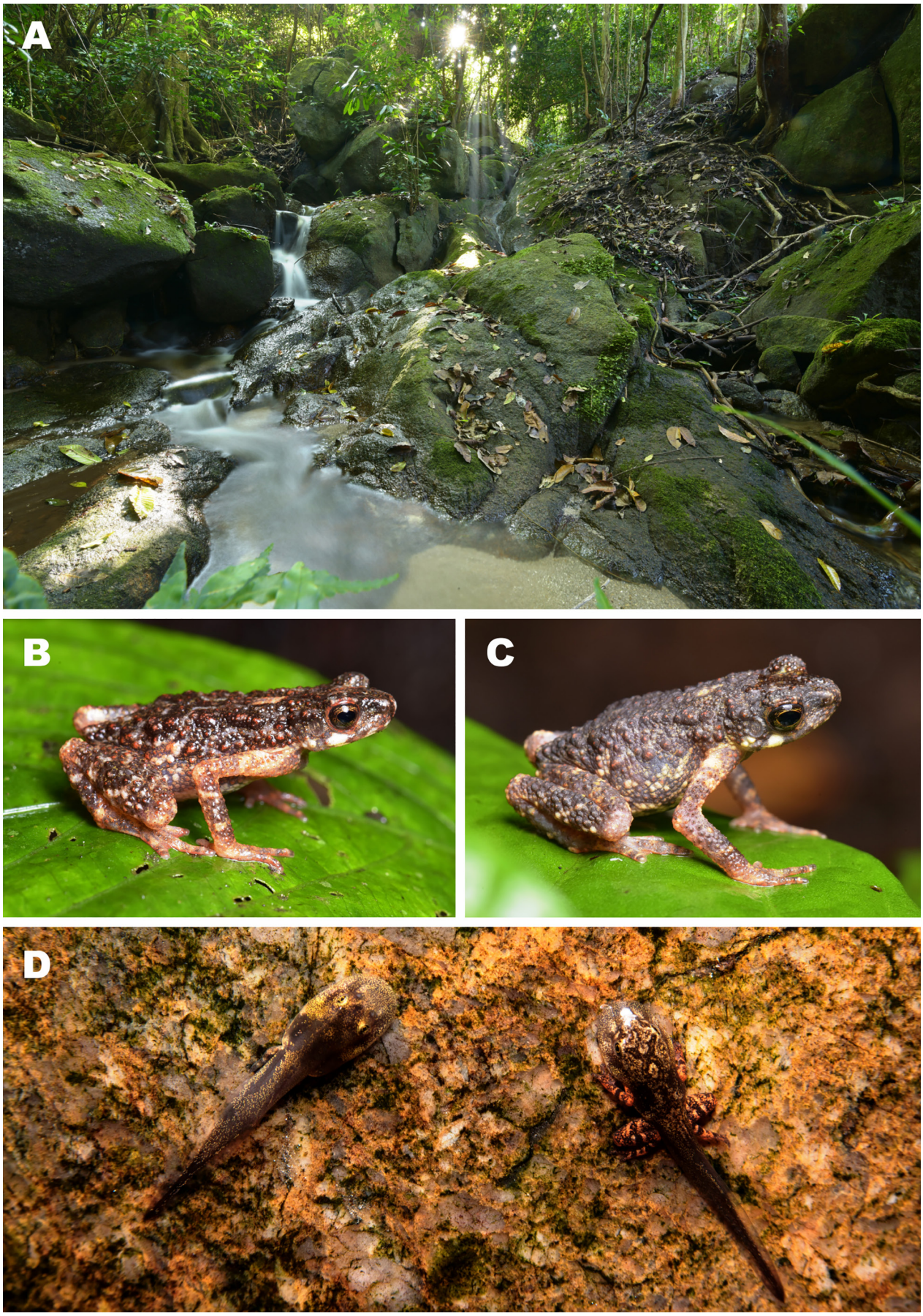


**Figure 7.** Natural history of *Ansonia karen* **sp. nov.** A. Breeding habitat of the new species *Ansonia karen* **sp. nov.** in Suan Phueng District, Ratchaburi Province, western Thailand. B. Female of the new species *in situ* (not collected). C. Male of the new species *in situ* (not collected). D. Tadpole at Gosner (1960) stage 38 (AUP-02091, left), and a metamorph at Gosner (1960) stage 44 (AUP-02092, right) *in situ*. Photos by M. Naiduangchan.

**Table 5.** Morphological and color pattern characters of the species of the Thai-Burmese clade of *Ansonia*. Bold character states are those that discretely separate *A. karen* **sp. nov**. from some or all the other species of the clade.

| **Species** | *karen* **sp. nov** | *inthanon* | *khaochangensis* | *kraensis* | *kyaiktiyoensis* | *pilokensis* | *phukentensis* | *siamensis* | *thinthinae* |
|---|---|---|---|---|---|---|---|---|---|
| SVL (Female) | 26.2–29.2 | **23.3–25.2** | **34–35.3** | 24.0–27.9 | **24** | **24.7–25.4** | **28.1–30.5** | **32.2–34.6** | **31.8** |
| Maximum SVL (Male) | 23.2–25.4 | **22.9–23.3** | **31.9–32** | **19.9–22.3** | 24 | 19.9–23.9 | 23.1–25.4 | **25.5–27.9** | 22.1–28.1 |
| Snout projecting beyond lower jaw (1) or not (0) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Tympanum visible (1) or not (0) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 or 1 | 1 |
| Light coloured rictal tubercle(s) at the corner of the jaw present (1) or not (0) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Interorbital tubercle ridges present (1) or not (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Opening of vocal sac on right (1) or left (0) | 1 | 1 | 1 | 0 | / | 0 | 1 | 0 or 1 | 0 or 1 |
| Finger tips rounded or forming small discs (1) or expanded and spatulate (0) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Toe tips rounded or forming small discs (1) or expanded and spatulate (0) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1st finger reaching the disc of the 2nd (1) or not (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| No. of fingers with nuptial pads | 1 | 1 | 0 | 1 | 0 | 2 | 2 | 1 | 1 + 2 |
| No. of free phalanges of V toe | 0.5 | 0.5–1 | 2 | 0.5 | 1.5 | 0.5 | 0.5 | 1 | 0.5 |
| No. of free phalanges of IV toe | 2.5 | 2.75 | 3–3.5 | 0.5–2.0 | 3 | 2.75–3 | 3 | 2 | 2.75–3 |
| No. of free phalanges of III toe | 0.5 | 0.5–2.66 | 2 | 0.5–2.33 | 1.5–3 | 0.5–2 | 1 | 1 | 0.5–2 |
| No. of free phalanges of II toe | 0.5 | 0.5–2 | 1–1.5 | 0.5–2.0 | 0.5–1.5 | 0.5–1 | 0.5 | 1 | 0.5–1 |
| No. of free phalanges of I toe | 0.5 | 0.5–1 | 0.5 | 0.5–1.0 | 0.5 | 0.5 | 0.5 | 1 | 0.5 |
| Tarsal ridge present (1) or not (0) | 0 | 0 | **1** | 0 | 0 | 0 | 0 | 0 | 0 |
| Inner metatarsal tubercle present (1) or not (0) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Outer metatarsal tubercle present (1) or not (0) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Submandibular tubercles in males present (1) or not (0) | 1 | 1 | **0** | 1 | 1 | 1 | 1 | 0 | 1 |
| Dorsal tubercles present (1) or not (0) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| Dorsolateral row of enlarged tubercles present (1) or not (0) | 1 | **0** | **0** | **0** | **0** | 1 | **0** | **0** | 1 |
| Rows of tubercles on back (1) or not (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oblique flap of skin on each side of vent (1) or not (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | / |
| Abdomen coarsely granular (1), finely granular (2), or tuberculate (0) | 1 | 1 | 1 | 1 | 1 | 1 | **2** | **2** | 1 |
| Color or iris | Yellowish–gold | gold | **black** | Gold | Yellowish–gold | Gold | Gold | / | Yellow |
| Gular spotting present (1) or not (0) | 0 | **1** | 0 | 0 | 0 | **1** | 0 | **1** | **1** |
| Wide, light patch below eye (1) or not (0) | 1 | **0** | **0** | 0–1 | **0** | 1 | 1 | **0** | 1 |
| White postorbital patch present (1) or not (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Light spot between the scapulae present (1) or not (0) | 1 | 1 | **0** | 1 | 1 | 1 | 1 | **0** | 1 |
| Light crossbar on hind limbs present (1) or not (0) | 1, irregular in shape | 1 | **0** | 1 | 1 | 1 (faint) | 1 | 1 | 1 |
| Vertebral stripe present (1) or not (0) | 0 | 0 | 0 | 0 | 0 | **1** | 0–1 | 0 | 0 |
| Discrete white or bright yellow spots along the underside of lower jaw large (2), small (1) or absent (0) | 2 | 2 | **0** | **1** | 2 | 2 | 2 | 2 | 2 |

by a northward expansion across the Kangar-Pattani Line between Thailand and Peninsular Malaysia at approximately 6.7 mya, giving rise to the Thai-Burmese clade and further diversification into at least eight species after crossing the Isthmus of Kra farther north at approximately 5.5 mya (Grismer et al. 2016).

*Ansonia karen* **sp. nov.** is the newest member of the Thai-Burmese species which is confined to the rugged mountainous regions west of the Chao Praya Basin of Thailand. The close geographic proximity of some of its non-sister species (e.g. *A. pilokensis*, *A. khaochangensis*, and *A. phuketensis*) and the discordance between the phylogenetic relationships and geographic distribution of the other *Ansonia* species, is indicative of the complicated biogeographic nature concerning the origin of these range-restricted endemics. The discovery of *Ansonia karen* **sp. nov.** in this section of the Tenasserim Mountains is more of an expectation than a surprise in that it fills a notable hiatus of 350 km between *A. thinthinae* from the Tanintharyi Nature Reserve, Tanintharyi Division, Myanmar and *A. kraensis* from the Punyaban Waterfall, Ranong Province, Thailand (see Fig. 1; Wilkinson et al. 2012).

The northern Tenasserim Mountain region is notable for the recent discoveries of endemic amphibians and reptiles (Matsui 2006; Sumontha et al. 2012, 2017; Wilkinson et al. 2012; Connette et al. 2017; Grismer et al. 2016, 2020a, 2020b, 2020c; Matsui et al. 2018; Pawangkhanant et al. 2018; Suwannapoom et al. 2018; Lee et al. 2019; Chomdej et al. 2020, 2021; Poyarkov et al., 2020). The mountains of northern Tenasserim are recognized as one of multiple local centers of amphibian diversity and endemism in Indochina (Poyarkov et al. 2021). Phylogenetic studies further indicate that these mountains have significantly influenced the cladogenetic structure and faunal exchange between mainland Indochina and Sundaland throughout the Cenozoic (see Chen et al. 2017, 2018; Grismer et al. 2018; Poyarkov et al. 2018; Suwannapoom et al. 2018; Gorin et al. 2020; Al-Razi et al., 2021). Additional field surveys and subsequent integrative taxonomic analyses are necessary to continue expanding our knowledge of this region's exceptional herpetofaunal diversity and endemism in order to effectively put into place science-based conservation management programs.

## Acknowledgements

We would like to thank the Laboratory Animal Research Center, University of Phayao and The Institute of Animal for Scientific Purposes Development (IAD), Thailand for permission to do field work. We also want to thank the Rabbit in the Moon foundation for help during the field work, and especially to Charnchai Bindusen and Juthamas Wangaryattawanich for organizing and supporting our fieldwork; and to Krarok Wohde, Torn Wohde, Jo Wohde, Maiday Ta-Au for assistance in the field; we thank Kanokwan Yimyoo for constant support and Pattarawhich Dawwrueng, Thiti Ruengsuwan, Kawin Jiaranaisakul, Akkrachai Aksornneam for help during the field work. NAP also thanks Karen Sarkisian for his support and encouragement; NAP is grateful to Dr. Valentina F. Orlova and Roman A. Nazarov (ZMMU) for help during the work in collection under their care; NAP thanks the members of MSU HerpLab including Vladislav A. Gorin, Evgeniya N. Solovyeva, Andrei M. Bragin, Sabira S. Idiatullina, Alexey Trofimets, and Anna S. Dubrovskaya for support and assistance. We express our sincere gratitude to two anonymous reviewers for their useful suggestions on an earlier version of the manuscript. Fieldwork, specimen collection, morphological examination, molecular phylogenetic analyses and data analyses for this paper were conducted with the financial support of the Russian Science Foundation (RSF grant No. 19-14-00050 to Nikolay A. Poyarkov); specimen collection and data analysis were also partially supported by the grants of the Unit of Excellence 2022 on Biodiversity and Natural Resources Management, University of Phayao (No. FF65-UoE003, specimen collection) and Thailand Research Fund (DBG6180001; specimen collection, data analysis) to Chatmongkon Suwannapoom.

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# Supplementary material 1

## Table S1

**Authors:** Suwannapoom C, Grismer LL, Pawangkhanant P, Naiduangchan M, Yushchenko PV, Arkhipov DV, Wilkinson JA, Poyarkov NA (2021)

**Data type:** .docx

**Explanation note:** Adjusted and raw mensural data from males of the type series of Ansonia karen sp. nov. and A. thinthinae used in the statistical analyses. Abbreviations are in the Materials and methods.



**Link:** https://doi.org/10.3897/vz.71.e73529.suppl1