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A new species of *Ansonia* (Anura: Bufonidae) from northern Tanintharyi Division, Myanmar

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Abstract

A new species of *Ansonia* is described from northern Tanintharyi Division, Myanmar. The new species is the first record of *Ansonia* from Myanmar. It is phylogenetically most closely related to *A. kraensis* at the Isthmus of Kra, Thailand, but can be distinguished from *A. kraensis* and all other species of *Ansonia* from Thailand and the Malay Peninsula by a combination of several morphological characters and dorsal and ventral color patterns.

Key words: *Ansonia thinthinae*, *A. kraensis*, stream toad, molecular phylogenetic analysis

Introduction

As part of an ongoing survey of the reptiles and amphibians of Myanmar, members of the Nature and Wildlife Conservation Division (NWCD) of the Myanmar Forest Department, National Museum of Natural History, Smithsonian Institution (USNM), and California Academy of Sciences (CAS) surveyed the Tanintharyi Nature Reserve (TNR) in March 2008 and again in September and October 2009. The TNR is an approximately 1,700 km^2 reserve ca. 70 km north of Dawei in the Tanintharyi Division of Myanmar. The TNR includes part of the Thein Ze Kalein Aung forest (857 km^2) and Lu Wine forest (843 km^2). The area is predominantly semi-evergreen dipterocarpacean rain forest (Thein 2009), and was established as a sanctuary for the Asian elephant and possibly the tiger (Lynam *et al.* 2006).

Approximately 22 species of amphibians and 41 species of reptiles were found during the two surveys, including several unidentified species of frogs. Amongst these, a species of *Ansonia* Stoliczka was determined to be new and is the first record of *Ansonia* in Myanmar.

Material and methods

Specimens were collected by hand, euthanized, tissue samples removed, and then fixed in 10% buffered formalin before preserving in 70% ethanol. The tissue samples were stored in 95% ethanol. Latitude and longitude of specimen localities were recorded with a Garmin 12 GPS, datum WGS84. Specimens are housed in the Department of Herpetology, CAS and the Division of Amphibians and Reptiles, USNM. Additional specimens, although not used in this paper, are housed in the Myanmar Biodiversity Museum. The preserved specimens were examined, sexed (using body size and presence or absence of vocal sacs, nuptial pads, and eggs), and measured. Comparisons were made with available specimens (see material examined; museum acronyms follow Sabaj Pérez [2010]) and published descriptions of currently recognized (Frost 2011) and phylogenetically closely related (Matsui *et al.* 2010) or geographically proximate species of *Ansonia* from Thailand and the Malay Peninsula (Inger 1960; Kiew 1985; Matsui *et al.* 1998, 2005; Grismer 2006a,b; Wood *et al.* 2008; Quah *et al*. 2011).

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Measurements (Table 1) on adult specimens were taken using digital calipers to the nearest 0.1 mm as follows: snout-vent length (SVL, from tip of snout to vent); head length (HL, from tip of snout to hind border of angle of jaw); 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); head width (HW, width of head at its widest point); snout width (SW, width of snout at anterior corner of eyes); 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); tympanum diameter (TD; horizontal diameter of tympanum); tympanum to eye distance (T-ED, from anterior edge of tympanum to posterior edge of eye); forelimb length (FLL, 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 thenar 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); and inner metatarsal tubercle length (IMTL, greatest length of tubercle).

TABLE 1. Measurements of the type series of *Ansonia thinthinae*. Mean (in mm) followed by standard deviation and range (in parenthesis). See text for abbreviations.

	Male (N = 23)	Female (N = 1)
SVL	25.0 ± 1.4 (22.1–28.1)	31.8
HL	7.3 ± 0.4 (6.7–7.9)	8.6
HW	7.4 ± 0.3 (6.9–8.1)	8.4
SW	3.8 ± 0.2 (3.4–4.2)	4.2
SL	3.2 ± 0.2 (2.9–3.6)	3.7
DNE	1.9 ± 0.1 (1.6–2.1)	2.2
IND	2.4 ± 0.1 (2.0–2.6)	2.8
IOD	2.9 ± 0.1 (2.5–3.2)	3.3
ED	2.7 ± 0.2 (2.3–3.1)	2.9
UEW	2.1 ± 0.2 (1.6–2.4)	2.3
VTD	1.5 ± 0.2 (1.1–1.8)	1.8
HTD	1.2 ± 0.2 (0.9–1.5)	1.4
T-ED	0.5 ± 0.1 (0.3–0.6)	0.8
FLL	13.3 ± 0.7 (11.8–14.2)	16.8
HAL	7.0 ± 0.4 (6.1–7.7)	8.3
1FL	1.7 ± 0.2 (1.5–2.2)	2.0
THL	12.2 ± 0.8 (10.8–13.6)	14.9
TIL	12.1 ± 0.6 (11.0–13.4)	14.8
FL	10.0 ± 0.6 (8.7–11.0)	12.2
1TL	1.7 ± 0.2 (1.5–2.0)	2.1
IMTL	1.2 ± 0.2 (0.9–1.6)	1.8
OMTL	0.7 ± 0.1 (0.5–0.9)	1.3

Measurements on tadpole specimen CAS 244137 were taken following Inger (1985) and Rao *et al*. (2006) using a binocular 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 distance, eye to tip of snout, internarial distance, width of oral disc, tail length, maximum tail depth, tail muscular depth, forelimb length, hand length, thigh length, tibia length, and foot length. Staging followed Gosner (1960), oral apparatus terminology followed Altig and McDiarmid (1999), and labial tooth row formula followed Altig *et al*. (1998).

Genomic DNA was extracted from approximately 25mg of tissue from four adult male specimens (CAS 243857, 243873, 243947, 244216), one adult female specimen (CAS 243871), and one tadpole specimen (CAS

244136) using the Qiagen DNeasy tissue kit (Qiagen, Valencia, California) as per the manufacturer's instructions. DNA quality was then checked by running 5ul of the extraction out on a 1% agarose gel.

A 2685bp fragment of the mitochondrial 12S and 16S rRNA genes was amplified from the extracted genomic DNA via PCR using primers ThrLm (Matsui *et al.* 2010) and Hedges16H1 (Hedges & Maxson 1993). Amplification reactions (25 µl) contained 50 ng DNA, 1 X PCR buffer (20 mM Tris-HCl, 50 mM KCl), 1.5 mM $MgCl_2$, 0.4 µM each primer, 200 µM each dNTP, and 1.25 U of Taq. The thermal cycler profile consisted of initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72ºC for 1.5 min, with a final extension at 72°C for 10 min. PCR products were then visualized on a 1% agarose gel and purified using ExoSAP-IT (USB, Cleveland, Ohio) as per the manufacturer's instructions. Cycle-sequencing was then carried out using the original, external primers as well as six other internal primers: 12S-3H, 12SL1091, 12StVal-H (Matsui *et al.* 2010), 12SL2021, 16SH2715 (Tominaga *et al*. 2006), and 16S1M (Fu 2000). Cycle sequence reactions were then purified using a standard ethanol precipitation protocol and run on an ABI 3130*xl* Genetic Analyzer.

The sequences were inserted into a data matrix of already aligned sequences of species of *Ansonia* from Matsui *et al.* (2010) and inspected by eye to maximize positional homology. The six new sequences were deposited in GenBank (JN664246–JN664251).

Uncorrected pairwise genetic distances (*p*-distance) were computed in PAUP* 4.0b10 (Swofford 2002) to estimate how genetically divergent these specimens are from one another and from currently known species of *Ansonia*. A maximum parsimony (MP) analysis using PAUP* 4.0b10 and a Bayesian analysis using MrBayes version 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) were performed on this data matrix of 36 sequences of 22 species of *Ansonia*, five of the six sequences of specimens from Myanmar (two sequences from CAS 243873 and CAS 243947 were identical so only the sequence from CAS 243873 was used), and one outgroup species, *Pedostibes hosii* (Boulenger) (Matsui *et al.* 2010). Each nucleotide was treated as an unordered character with four alternative states, and gaps were considered missing data in all analyses.

In the MP analysis, data were treated with equal weight (Kjer 1995; Allard & Carpenter 1996; Cibois *et al.* 1999). We performed 100 heuristic searches with the starting trees obtained by random stepwise addition, followed by tree-bisection-reconnection (TBR) branch-swapping. We also performed a nonparametric bootstrap analysis (Felsenstein 1985) with 1000 replicates, each executed as a heuristic search as above, to evaluate support for relationships, as implemented in PAUP* 4.0b10.

In the Bayesian analysis, the general-time-reversal model of base substitution (Nei & Kumar 2000) was selected based on the results of Matsui *et al.* (2010). For the tree-partition analysis, the Monte Carlo Markov Chains were simulated for 50,000,000 generations and sampled every 1000 generations. Four chains were run and 10,000 initial trees were discarded (burn-in).

Results

The Bayesian and MP analyses resulted in similar trees that were also similar to the tree presented in Matsui *et al.* (2010). The Bayesian tree is presented in Figure 1. In both trees, a clade of species from Thailand and the specimens from Myanmar is strongly supported by both Bayesian (1.00 PP) and MP (86%) analyses. Within this clade, the specimens from Myanmar form a strongly supported clade (1.00 PP, 100%) that is most closely related with strong support (1.00 PP, 86%) to a clade of specimens representing *A. kraensis* Matsui, Khonsue, and Nabhitabhata. *Ansonia inthanon* Matsu, Nabhitabhata, and Panha, *Ansonia* sp. 1, and *Ansonia* sp. 2 were outside of this clade with weaker support.

The *p*-distance between the specimens from Myanmar and *A. kraensis* is 5%, and between the specimens from Myanmar and *Ansonia* sp. 1 is 6.5%, even though *Ansonia* sp. 1 is geographically the closest species to the specimens from Myanmar, possibly as close as eight km to the east in Thailand, on the opposite side of the Bilauktaung Mountain Range, whereas, *A. kraensis* is over 500 km to the south at the Isthmus of Kra. These *p*-distances are within the range (2.1%–13.2%) between different species of *Ansonia* by Matsui *et al*. (2010), and similar to *p*-distances between the species pairs *A. jeetsukumarani* Wood, Grismer, Ahmed, and Senawi / *A. malayana* Inger (5.5%), *A. torrentis* Dring / *A. latiffi* Wood, Grismer, Ahmad, and Senawi (5.6%), *A. torrentis* / *A. leptopus* (Günther) (5.7%), and *A. muelleri* (Boulenger) / *A. mcgregori* (Taylor) (4.5%), and greater than *p*-distances between the species pairs *A. torrentis* / *A. longidigita* Inger (2.5%), and *A. latiffi* / *A. leptopus* (2.1%).

The results of the phylogenetic and morphological analyses clearly places the specimens from Myanmar as members of the genus *Ansonia* but these specimens cannot be assigned to any described species and are described herein as new.

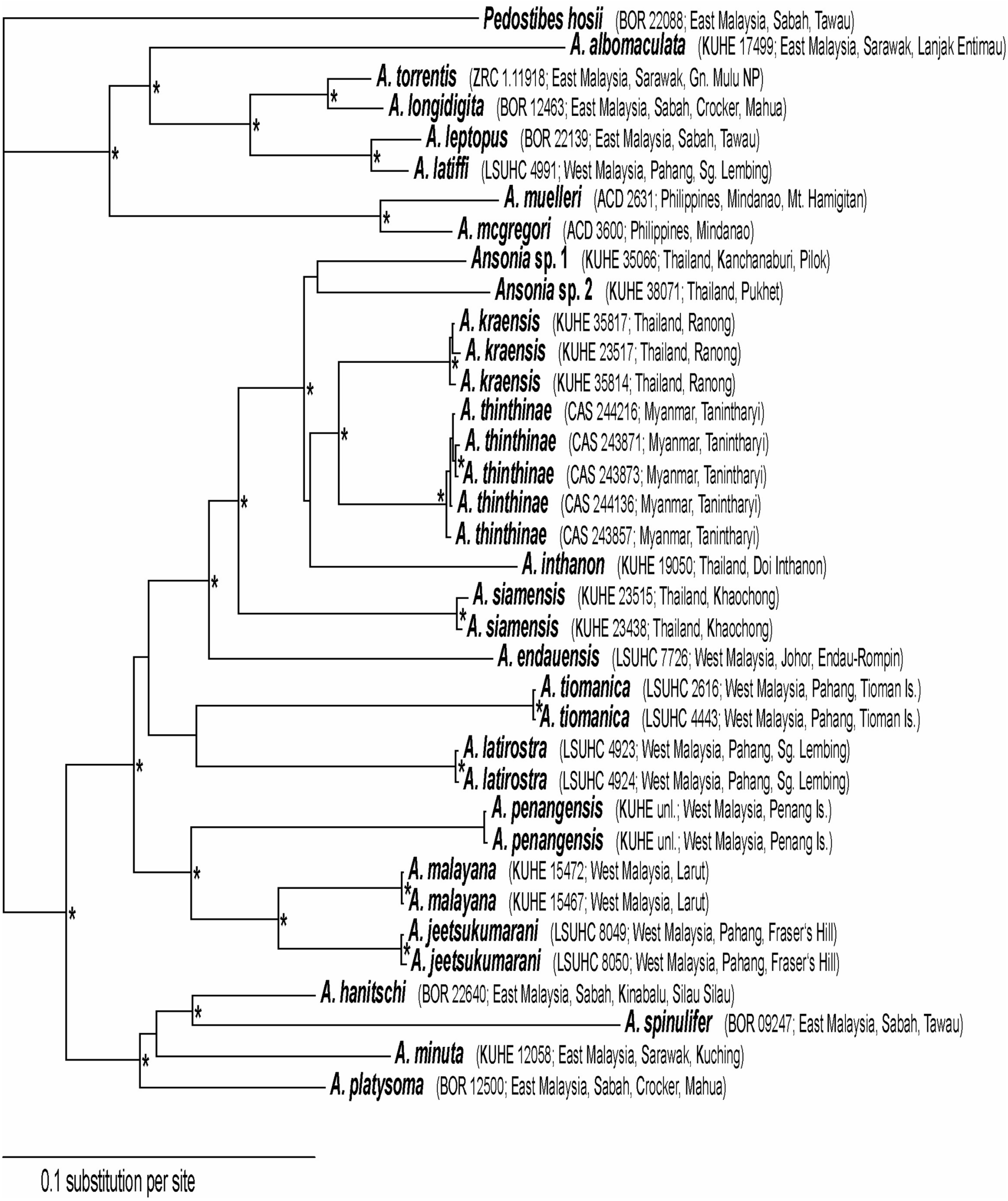

FIGURE 1. Bayesian phylogram (50% majority rule) obtained from a combined analysis of 12S and 16S genes from species of *Ansonia* and *Pedostibes* from Matsui *et al.* (2010) and new species from Myanmar (voucher specimen number and collection locality are in parentheses following species name). Asterisks at nodes indicate significant (≥0.95) posterior probabilities for those nodes.

Species description

Ansonia thinthinae sp. nov.

Thin Thin's Stream Toad

Holotype. CAS 243857 (Figures 2 and 3), an adult male, collected from a stream at 14° 41' 4.6" N, 98° 19' 59.3" E, Tanintharyi Nature Reserve, Yebyu Township, Dawei District, Tanintharyi Division, Myanmar, ca. 409 m, on 21 March 2009 by K. S. Lwin, Y. M. Win, Z. H. Aung, and J. V. Vindum.

Paratypes. CAS 243858–243861, 243869, USNM 558323, 558324, seven adult males, collected at same locality as holotype; CAS 243868, an adult male, collected from a stream at 14° 41' 11.7" N, 98° 20' 15.3" E, Tanintharyi Nature Reserve, Yebyu Township, Dawei District, Tanintharyi Division, Myanmar, 441 m, on 21 March 2009 by K. S. Lwin, Y. M. Win, Z. H. Aung, and J. V. Vindum; CAS 243871–243873, USNM 558325, three adult males and one adult female, collected from a stream at 14° 41' 12.6" N, 98° 20' 7.9" E, Tanintharyi Nature Reserve, Yebyu Township, Dawei District, Tanintharyi Division, Myanmar, ca. 436 m, on 21 March 2009 by K. S. Lwin, Y. M. Win, Z. H. Aung, and J. V. Vindum; CAS 243943–243947, USNM 558326–558329, nine adult males, collected from a stream at 14° 41' 12.6" N, 98° 20' 7.9" E, Tanintharyi Nature Reserve, Yebyu Township, Dawei District, Tanintharyi Division, Myanmar, ca. 436 m, on 26 March 2009 by S. L. Oo, Z. H. Aung, M. Hlaing, and J. V. Vindum; CAS 244216, an adult male, collected from the Me Kyauklonegyi Stream at 14° 41' 35.8" N, 98° 17' 24.9" E, Tanintharyi Nature Reserve, Yebyu Township, Dawei District, Tanintharyi Division, Myanmar, ca. 161 m, on 13 October 2009 by M. Hlaing, Z. H. Aung, S. L. Oo, K. S. Lwin, Y. M. Win, J. V. Vindum, and J. A. Wilkinson; CAS 244136, 244137, two tadpoles collected from the Me Kyauklonegyi Stream at 14° 41' 35.1" N, 98° 17' 32.6" E, Tanintharyi Nature Reserve, Yebyu Township, Dawei District, Tanintharyi Division, Myanmar, ca. 155 m, on 13 October 2009 by M. Hlaing, Z. H. Aung, S. L. Oo, K. S. Lwin, Y. M. Win, J. V. Vindum, and J. A. Wilkinson; CAS 244217, an adult male, collected from a stream at 14° 41' 08.7" N, 98° 20' 12.3" E, Tanintharyi Nature Reserve, Yebyu Township, Dawei District, Tanintharyi Division, Myanmar, ca. 434 m, on 22 October 2009 by M. Hlaing, Z. H. Aung, S. L. Oo, K. S. Lwin, Y. M. Win, J. V. Vindum, and J. A. Wilkinson.

Diagnosis. *Ansonia thinthinae* is considered a member of the genus *Ansonia* based on small body size, presence of slender limbs, weak subarticular tubercles, and membranous foot webbing, but absence of parotoid glands in adults, and presence of cup-like ventral oral disc, wide areas peripheral to tooth rows for both lips, expanded post-dental portion of lower lip with continuous papillae along margin, upper jaw sheath divided into two separate, keratinized parts, markedly flattened body, and eyes set relatively back on body in larvae (Inger 1960, 1992). *Ansonia thinthinae* can be distinguished from other congeners that are geographically (Thailand and Malay Peninsula) or phylogenetically (clade A1 of Matsui *et al.* [2010]) close by a combination of the following characters: male SVL of 22.1–29.0 mm, female SVL of 31.8 mm; head wider than long in males; light patch below eye; several yellow tubercles at corner of jaw; one vocal slit; spotting on gular region; dorsum tuberculated; first finger much shorter than second; first and second fingers with nuptial pads in males; finger tips rounded without forming disks; webbing reaching disks on third and fifth toes; iris yellow in life; dorsum brown with yellow interscapular spot; venter with yellow spotting fusing into larger patterning on brown background.

Description of holotype. Snout-vent length 25.6 mm; head slightly wider than body, and slightly wider (7.6 mm) than long (7.5 mm); snout (3.2 mm) longer than eye (2.8 mm), wide (4.2 mm; SW/SVL 16%), square (SW/HW 55%), constricted in front of eyes, dorsally concave, sloping anteroventrally between eyes and nostrils, truncate, projecting beyond lower jaw, sloping posteroventrally to mandibular symphysis when viewed laterally, with slight midline point in dorsal profile that appears as distinct vertical ridge on anterior margin when viewed from front; canthus rostralis distinct, slightly curved; lores straight, vertical; nostrils closer to tip of snout than eyes (DNE 1.9 mm; DNE/SL 59%), at level of symphysis of lower jaw, directed laterally, oval, angled anterodorsally; internarial distance (2.6 mm) narrower than interorbital distance (3.2 mm), latter wider than upper eyelid (2.2 mm); cranial crests absent; parotids absent; margin of eyelid with row of small tubercles making it appear sharp; tympanum distinct, oval, taller (1.6 mm) than wide (1.3 mm), horizontal diameter 46% of horizontal diameter of eye, separated from eye by 38% of tympanum diameter (0.5 mm).

Longitudinal vocal slit on left side of mouth into median subgular vocal sac; vomerine ridge absent; choanae slightly oval, interior to lingual shelf when viewed from below; tongue narrow, ending in median point; posterior ⅔ free.

FIGURE 2. Dorsal (A) and ventral (B) views of the holotype of *Ansonia thinthinae* (CAS 243857).

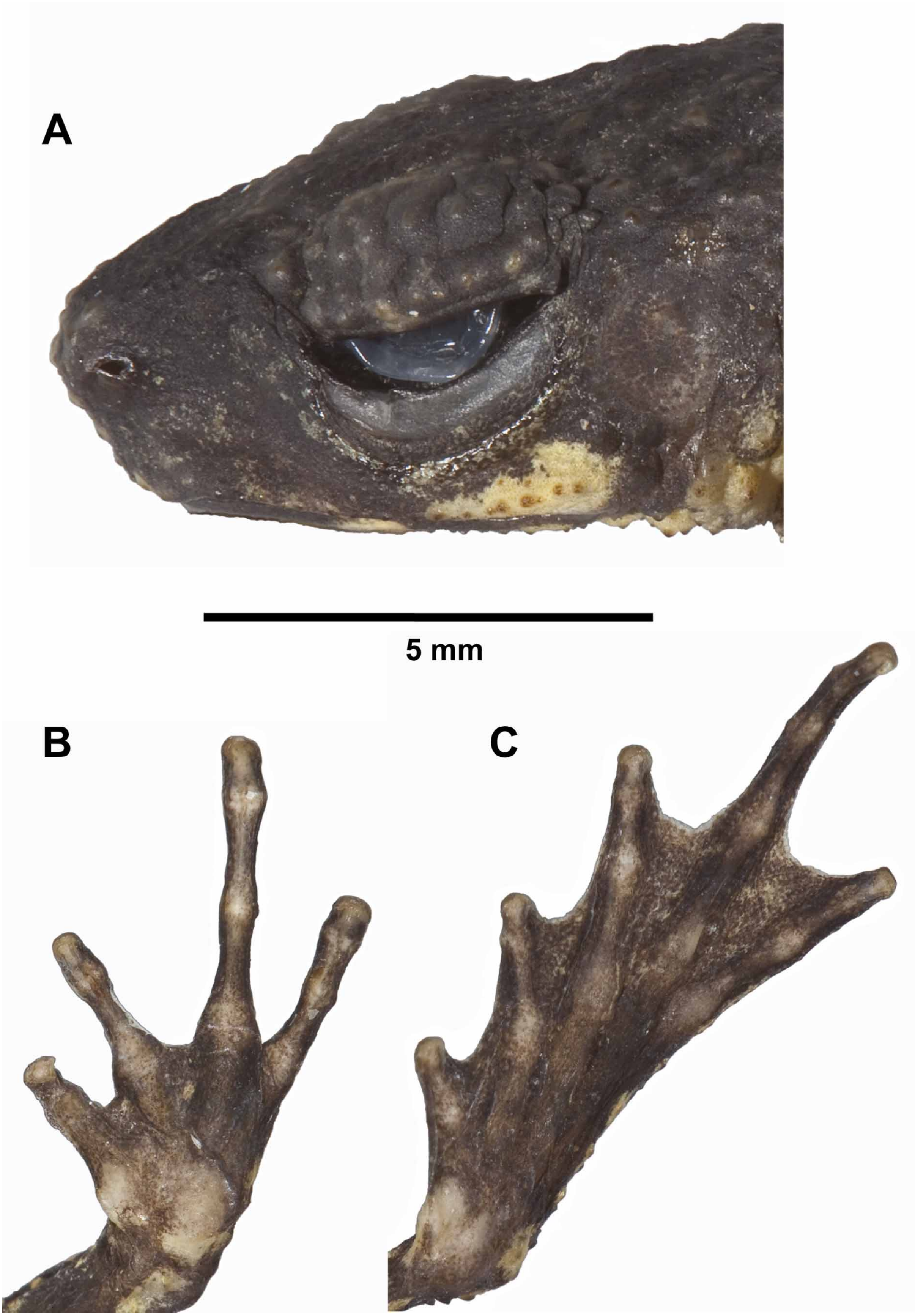

FIGURE 3. Lateral view of the head (A), palmar view of the left hand (B), and planter view of the left foot (C) of the holotype of *Ansonia thinthinae* (CAS 243857).

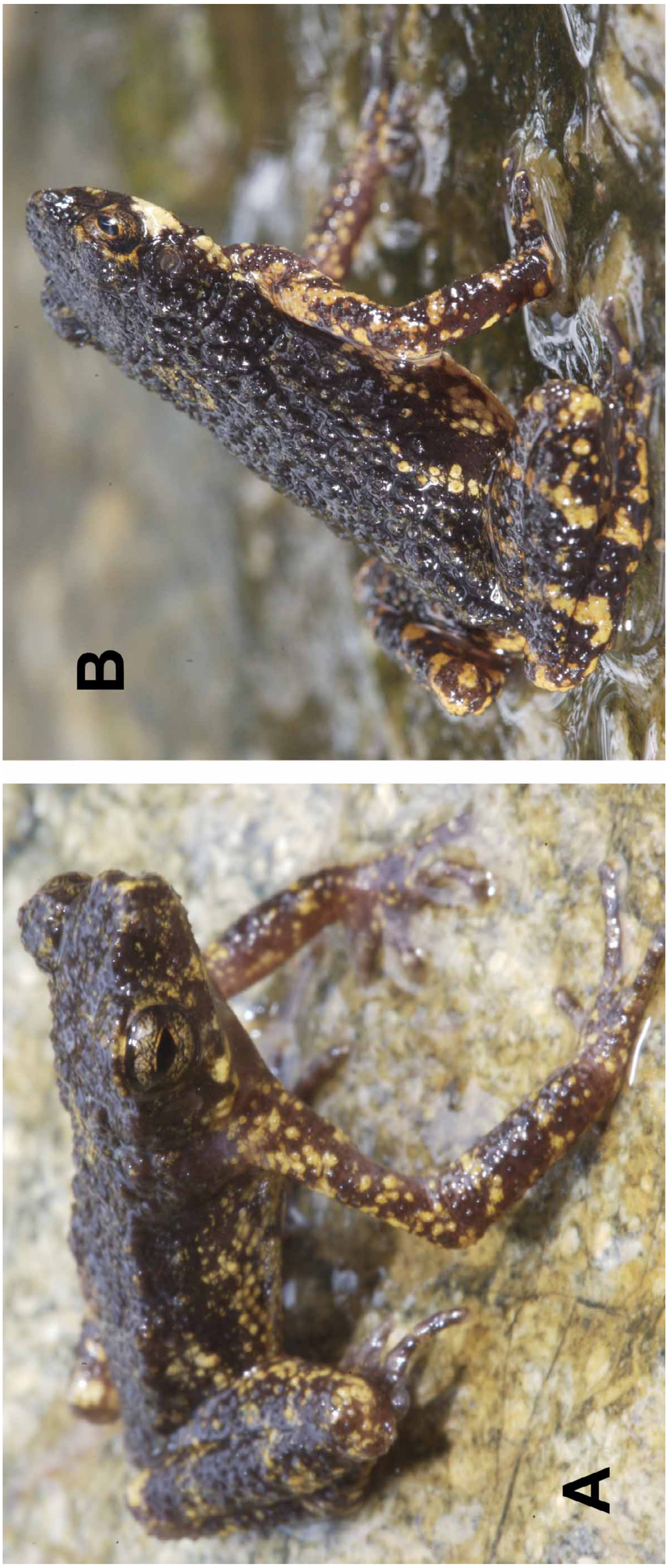

FIGURE 4. Photograph in life of (A) the holotype (CAS 243857) and (B) a paratype (CAS 244216) of *Ansonia thinthinae*.

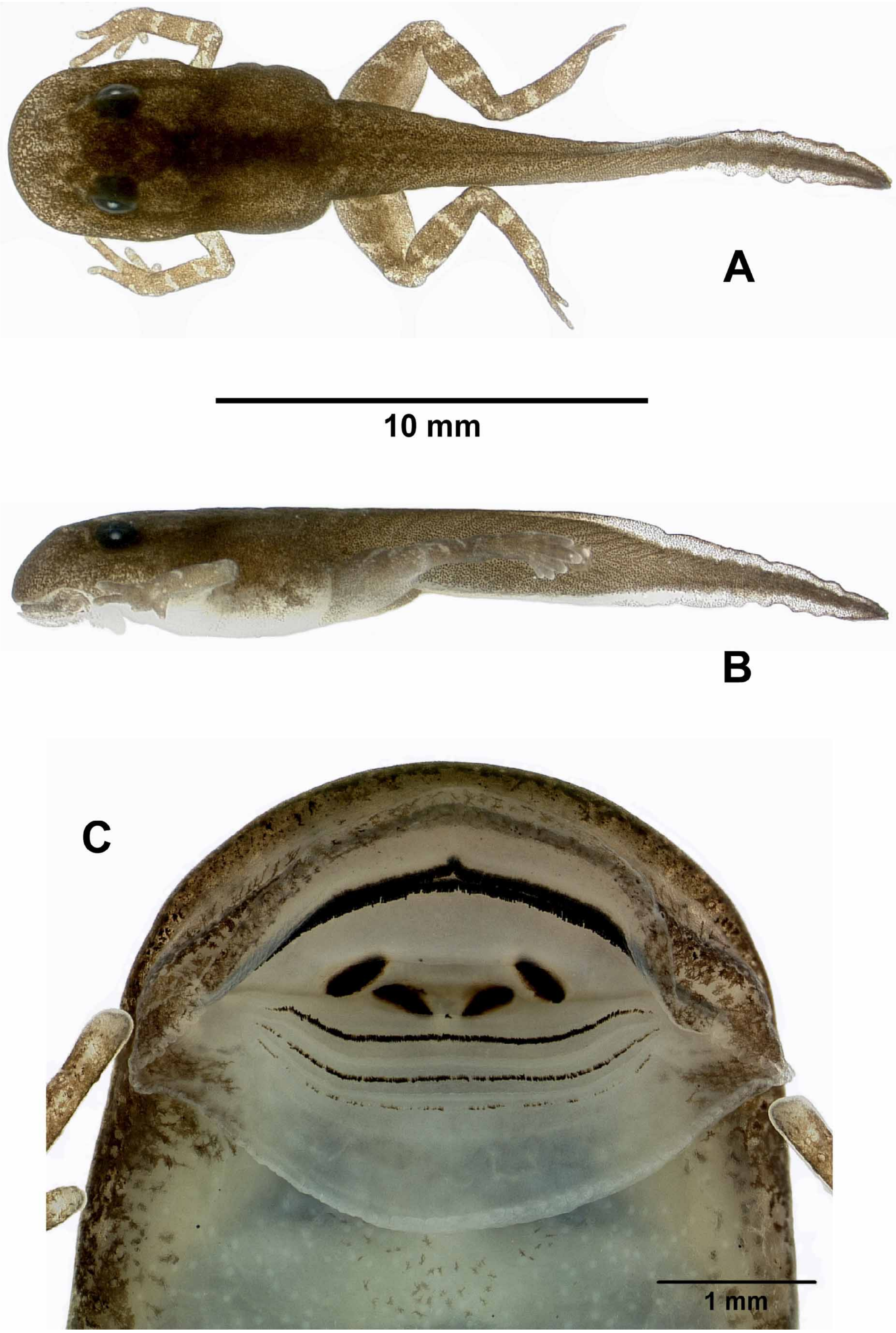

FIGURE 5. Dorsal (A), lateral (B), and mouth (C) views of the tadpole of *Ansonia thinthinae* (CAS 244137).

Forelimbs long (13.8 mm) and slender; hand length (7.2 mm) more than half of forelimb length; fingers long, slender, webbing reaching base of most proximal subarticular tubercle of each finger, slight fringes from distal subarticular tubercles to tips; tips rounded, not forming disks wider than phalanges; relative finger length 1<2<4<3, first finger (2.0 mm) much shorter than second, reaching just beyond subarticular tubercle of second when adpressed; broad round palmar tubercle; very small, oval thenar tubercle; subarticular tubercles weak, subarticular tubercle formula I 1, II 1, III 2, IV 1; nuptial pad of coarse dark asperities broadly distributed on medial to medial dorsal surface of first finger from base of finger to proximal end of distal phalange and restricted to base and lower half of dorsal ridge of second finger.

Thigh (12.6 mm) slightly longer than tibia (12.0 mm), heels barely touching when flexed limbs are held at right angles to body; tibiotarsal articulation of adpressed limb reaching anterior corner of eye; foot (9.7 mm) much shorter than tibia; toes long and slender, tips round, slightly swollen into small disks; toes with weak subarticular tubercles, subarticular tubercle formula I 1 II 1 III 2 IV 3 V 2; first toe 1.7 mm in length; fifth toe slightly longer than third; toes strongly webbed, webbing forming broad sheet, continuing as slight fringe to base of disk on fourth toe; webbing formula: I ½–1 II ½–2 III ½–3^- IV 2¾–½ V following Myers and Duellman (1982) as modified by Savage and Heyer (1997); inner metatarsal tubercle flat and oval (length 1.1 mm); outer metatarsal tubercle round, prominent, smaller in diameter (0.7 mm) than inner; no tarsal fold.

Ridges absent on forehead, interorbital, or parietal region; large tubercle anterior to tuberculated eyelid; single row of dark brown asperities along upper lip from tip of snout to mid-eye (left side) or posterior of eye (right side) where they are raised and surrounding area becomes light cream color (light patch below eye); another line of asperities along anterior edge of tympanum; group of four tubercles on left side and five on right side, ending in keratinized darker spinules, from posterior of jaw to anterior to insertion of forelimb; underside of mandible with single row of dark brown conical keratinized tubercles along edge becoming slightly scattered anteriorly at chin and posteriorly at corners of jaw; dorsum very tuberculate with mixture of large and small conical tubercles, some terminating in darker keratinized spinules, larger ones with more than one spinule; concentration of larger tubercles above tympanum and in scapular region forming loose dorsolateral rows posterior to scapular region extending to insertion of hindlimb; side of body granular but not tuberculate; abdominal area coarsely granulate, with evenly spaced keratinized pointed tubercles, becoming much more finely granular on pectoral and gular region; dorsal and lateral surfaces of limbs with scattering of small conical tubercles, most of which terminating in keratinized points, ventral side of limbs with fewer tubercles.

Coloration in preservative. Dorsum dark brown, with light yellow diamond shaped interscapular spot; light cream-colored flecking on medial edges of eyelids, around edges of eyes, and on lores; light cream-colored patch below eye; vertical narrow light cream-colored line from symphysis of lower jaw to tip of snout; group of tubercles on side of head below and posterior to tympanum to anterior of insertion of arm light cream-colored; limbs brown dorsally with narrow, indistinct light yellow crossbars; skin above joints of phalanges lighter (Figure 2A); yellow spots along edge of underside of mandible; gular region brown with cream-colored spotting extending to chest; abdominal region with large yellow spots, some fused into elongate patterns, on brown background, becoming cream-colored on ventral aspect of thigh; anterior and posterior of thighs and flanks with yellow spotting on brown (Figure 2B).

Coloration in life (based on digital image). Patterning is same as in preservative, light cream-color and yellow on dorsum is bright yellow. Iris dull yellow with dark brown thin reticulations, concentrated along horizontal midline across pupil to appear as dark brown bar (Figure 4A).

Variation. Male paratypes closely resemble holotype, with snout-vent length ranging from 22.1 mm to 28.1 mm; and with varying degrees of keratinized asperities on mandibles. Vocal slit on left side of mouth as with holotype in 13 male paratypes (CAS 243858–243860, 243869, 243873, 243947, 243952, 243953, USNM 558324, 558325, 558327–558328) while on right side of mouth in 10 male paratypes (CAS 243861, 243868, 243872, 243943–243946, USNM 558323, 558326, 558329); light crossbars on limbs orange in life (Figure 4B) in some individuals (CAS 244216, 244217), other individuals (CAS 243868, 243869) with white patterning on brown venter in preservative. Nuptial spines present on first and second finger of all male paratypes. The single female (CAS 243871) larger than males (31.8 mm), with narrower and shorter head in relation to snout-vent length; single row of asperities on mandible less developed and lacking brown caps of keratin.

Tadpole. Description based on CAS 244137 at Gosner (1960) stage 42. Total length 24.0 mm, head-body length 10.0 mm, head-body depth 3.9 mm, maximum head-body width 4.5 mm, diameter of eyeball 1.1 mm, interorbital distance 1.4 mm, eye to tip of snout 2.6 mm, internarial distance 1.5 mm, width of oral disc 4.3 mm, tail length 14.0 mm, maximum tail depth 2.4 mm, tail muscular depth 2.4 mm, forelimb length 4.1 mm; hand length 2.4 mm, thigh length 3.8 mm, tibia length 3.3 mm, foot length 2.6 mm. Body broadly oval in dorsal view, maximum width posterior to eyes (Figure 5A); dorso-ventrally depressed (Figure 5B); snout broadly rounded in dorsal view; eyes dorsal, oriented laterally; nares closer to eyes than to tip of snout, directed antero-laterally. Oral disc ventral, 96% of head-body width, not emarginate, both labia expanded; anterior labium separated from tip of snout by groove; marginal papillae in a single row across posterior labium but only on lateral corners of anterior labium, submarginal papillae not observed; black, serrated jaw sheaths, upper divided with gap 145% length of single sheath; lower divided with gap 50% length of single sheath; labial tooth row formula 2/3, anterior rows longer than posterior rows, well separated from jaw sheaths (Figure 5C); tail musculature tapering posteriorly to pointed tail tip; tail deepest midway; dorsal and ventral fins approximately equal in depth.

Tadpole color in preservative: Dorsum of body brown due to densely scattered brown chromatophores; venter transparent with very few chromatophores medially, and more chromatophores laterally; muscular tail same color as body, few chromatophores on more transparent dorsal and ventral fins, becoming more concentrated along edges; dorsal aspect of fore and hind limbs with white transverse barring.

Etymology. The specific epithet is given in honor of the late Daw [Ms.] Thin Thin, who was a dedicated member of the Myanmar Herpetological Survey Team and contributed greatly to the understanding of the diversity and natural history of amphibians and reptiles in Myanmar.

Comparisons. *Ansonia thinthinae* can be distinguished from other species of *Ansonia* from Thailand and the Malay Peninsula that are not geographically or phylogenetically close as follows: from *A. malayana* by larger body size in both male (mean SVL 25.0 mm in *A. thinthinae*, mean SVL 22.1 mm in *A. malayana*) and female (SVL 31.8 mm in single specimen of *A. thinthinae*, SVL less than 30.0 mm in *A. malayana*), several small yellow tubercles at corner of jaw (single yellow tubercle at corner of jaw in *A. malayana*), two fingers with nuptial pads in males (only first finger with nuptial pad in males of *A. malayana*), venter with large yellow spots and patterning on brown background (small yellow spots on dusky background in *A. malayana*), and no interrupted arc anterior to sacrum (interrupted light dorso-lateral arc in *A. malayana*); from *A. penangensis* Stoliczka by smaller female body size (female SVL 37.2 mm in *A. penangensis*), tarsal ridge absent (present in some individuals of *A. penangensis*), first finger much smaller than second (first finger up to but not reaching base of disk of second finger when adpressed in *A. penangensis*), 0.5 phalange free of webbing on fifth toe (1.5 phalanges free in *A. penangensis*), yellow iris (red iris in *A. penangensis*), and venter with large yellow spotting some fusing to elongate patterning on brown background (venter with small white spotting on grey background in *A. penangensis*); from *A. jeetsukumarani* by larger body size in both male (SVL up to 20 mm in *A. jeetsukumarani*) and female (SVL less than 25.4 mm in *A. jeetsukumarani*), two fingers with nuptial pads in males (only first finger with nuptial pad in *A. jeetsukumarani*), 0.5 phalange free of webbing on fifth toe (1.5–2.0 phalanges free in *A. jeetsukumarani*), several yellow tubercles at corner of jaw (one or two white tubercles at corner of jaw in *A. jeetsukumarani*), and yellow iris (reddish/orange in *A. jeetsukumarani*); from *A. latiffi* by smaller body size in both male (SVL range 34.1–38.2 mm in *A. latiffi*) and female (SVL range 50.5–50.7 in *A. latiffi*), first finger much shorter than second (first finger reaching tip of second in *A. latiffi*), two fingers with nuptial pads in males (occasionally on two fingers in *A. latiffi*), no tarsal ridge (present in *A. latiffi*), yellow iris (reddish-gold in *A. latiffi*), and light patch below eye (absent in *A. latiffi*); from *A. latirostra* Grismer by several yellow tubercles at corner of jaw (one to three large yellow tubercles present at corner of jaw in *A. latirostra*), no interorbital tubercular ridges (present in *A. latirostra*), no rictal gland (large rictal gland in *A. latirostra*), two fingers with nuptial pads in males (nuptial pad only on first finger in *A. latirostra*), 0.5 phalange free of webbing on fifth toe (1.0–2.0 phalanges free in *A. latirostra*), light spot between scapulae always present (absent in some individuals of *A. latirostra*), and venter with large yellow spotting on brown background (venter with widely spaced, small light spotting on raised bumps on darker grey background in *A. latirostra*); from *A. endauensis* Grismer by larger body size in both male (SVL to 17.4 mm in *A. endauensis*) and female (SVL to 28.5 mm in *A. endauensis*), two fingers with nuptial pads in males (nuptial pads absent in *A. endauensis*), 0.5 phalange free of webbing on fifth toe (2.0 phalanges free in *A. endauensis*), yellow iris (red iris in *A. endauensis*), one vocal slit on left or right side of mouth (two vocal slits on both sides of mouth in *A. endauensis*), spotting on gular region (spotting on gular region absent in *A. endauensis*), interscapular spot present (absent or present in *A. endauensis*), and venter with large yellow spotting on brown background (venter grey with no patterning in *A. endauensis*); from *A. tiomanica* Hendrickson by smaller body size in both male (SVL 31.2 mm in *A. tiomanica*) and female (SVL 38.4 mm in *A. tiomanica*), two fingers with nuptial pads in males (only first finger with nuptial pad in *A. tiomanica*), 0.5 phalange free of webbing on fifth toe (2.0 phalanges free in *A. tiomanica*), yellow spotting on lower jaw (absent in *A. tiomanica*), light patch below eye (absent in *A. tiomanica*), and light spot between scapulae (absent in *A. tioman-*

ica); and from *A. leptopus* by smaller body size in both male (SVL 30–40 mm in *A. leptopus*) and female (SVL 45–65 mm in *A. leptopus*), first finger much smaller than second (first finger reaching disk of second in *A. leptopus*), two fingers with nuptial pads in males (only first finger with nuptial pad in *A. leptopus*), interscapular spot present (absent in *A. leptopus*), and 0.5 phalange free of webbing on fifth toe (2.0–2.5 phalanges free in *A. leptopus*).

FIGURE 6. Type locality of *Ansonia thinthinae* **sp. nov.** (indicated by star) in Tanintharyi Division, Myanmar.

Ansonia thinthinae is phylogenetically and geographically close to *A. kraensis*, *A. inthanon*, and *A. siamensis* Kiew. These three species are known from neighboring Thailand both north (*A. inthanon*) and south (*A. kraensis*, *A. siamensis*) of the type locality of *A. thinthinae*. *Ansonia thinthinae* also shares two molecular synapomorphies with these three species (bases C and T at loci 72 and 312, respectively; database available at http://dx.doi.org/10.5061/dryad.mj0rs0dt). *Ansonia thinthinae* can be distinguished from *A. inthanon* by a larger female body size (female SVL 23.3–25.2 in *A. inthanon*), two fingers with nuptial pads in males (only first finger with nuptial pad in *A. inthanon*), light patch below eye (absent in *A. inthanon*), and venter with large yellow spotting some fusing to elongate patterning on brown background (venter with small yellow spots on brown background in *A. inthanon*); and from *A. siamensis* by smaller female body size (female SVL 35 mm in *A. siamensis*), tips of fingers not expanded into disks (tips of fingers expanded into disks in *A. siamensis*), two fingers with nuptial pads in males (first finger with nuptial pad in *A. siamensis*), presence of dorsal tubercles (dorsal tubercles much reduced or absent in *A. siamensis*), and absence of greenish-yellow irregular markings on dorsum (present in *A. siamensis*).

Ansonia thinthinae is morphologically very similar to its phylogenetic sister species, *A. kraensis*. In addition, it shares two unique molecular synapomorphies with *A. kraensis* (bases T and A at loci 1220 and 2132, respectively). However, it can be distinguished from *A. kraensis* by a larger body size in both male (mean SVL 21.3 mm in *A. kraensis*) and female (mean SVL 25.3 mm in *A. kraensis*), head wider than long in males (longer than wide in males of *A. kraensis*), two fingers with nuptial pads in males (one or two fingers with nuptial pads in *A. kraensis*), dorsum brown with no pattern except for interscapular spot (dorsum brown with darker Y shaped pattern beginning at rear of eyes, merging before interscapular spot, surrounding spot, then, as inverse Y, diverging onto sides of sacrum in *A. kraensis*), and venter with yellow spotting on brown background (silvery white mottling with brown in *A. kraensis*). Additionally, six molecular characters are not only absent from specimens of *A. kraensis* but are unique to all specimens of *A. thinthinae* among all species of *Ansonia* in the molecular phylogenetic analysis (bases T, T, C, G, T, and T at loci 99, 130, 305, 1155, 1379, and 1380, respectively). Reciprocally, specimens of *A. kraensis* contain six molecular characters that are not only absent in *A. thinthinae* but are unique to *A. kraensis* among all species of *Ansonia* in the molecular phylogenetic analysis (bases T, C, C, A, G, and C at loci 392, 1617, 1744, 1755, 2089, and 2366, respectively).

Distribution and natural history. *Ansonia thinthinae* is only known from the stream of the type locality and approximately 4.7 km to the west along Me Kyauklonegyi Stream (Figure 6), but is expected in other streams that flow westward from the Bilauktaung Mountain Range. Adult specimens were collected between 10:00 and 14:00 h under stones along the edge of a second-order stream approximately 3 m wide at an altitude of 400–450 m and at 24:00 h among stones at the edge of the approximately 10 m wide and 0.6 m deep Me Kyauklonegyi Stream at an altitude of 158 m. The tadpoles were collected from the upper surface of a boulder midstream of the Me Kyauklonegyi Stream where they were attached by their oral discs. The boulder was submerged under a sheet of approximately 3 mm of flowing water.

Discussion

Ansonia thinthinae, with a male body size of 22–29 mm and a female body size of 31 mm, seems to follow a trend of phylogenetically related, small-sized species of *Ansonia* from the Malay Peninsula and Thailand (most species of clade A1 of Matsui *et al.* [2010]). What is surprising is the phylogenetically closer relationship of *A. thinthinae* with *A. kraensis* than with *Ansonia* sp. 1 of Matsui *et al.* (2010), even though *Ansonia* sp. 1 was found just on the eastern side of the Bilauktaung Mountain Range that separates Thailand from Myanmar. This result indicates that the mountain range may be a barrier to dispersal even though it is only approximately 770 to 1000 m high nearest the type locality, and that *A. thinthinae* may be closely tied to the Dawei (Tavoy) River watershed west of this mountain range. This also conforms to the observations of limited ranges in other species of this genus on the Malay Peninsula (Wood *et al.* 2008; Matsui *et al.* 2010). Additional surveys to both the north and south of the localities on both the western and eastern sides of the Bilauktaung mountains will help to better delimit the range of *A. thinthinae* and determine if the range extends into other watersheds, such as the Tenasserim River watershed to the south, and if it intersects with neighboring species, such as *A. kraensis*, *A. inthanon*, and *Ansonia* sp. 1, or if there are intervening populations that may be species yet unknown to science.

Material examined

Ansonia endauensis, ZRC 1.11557–58 [Paratypes], Malaysia; *A. jeetsukumarani*, ZRC 1.12415–16, Malaysia; *A. kraensis*, CAS 73718, ZRC 1.8454–55, 1.8457–58, Thailand; *A. latiffi*, FMNH 173326 [Paratype], ZRC 1.12419 [Paratype], Malaysia; *A. latirostra*, ZRC 1.11560–63 [Paratypes], Malaysia; *A. malayana*, CAS-SU 3238, Malaysia; *A. mcgregori*, CAS 61839 [Holotype], 61845–51 [Paratypes], 89808–09, Philippine Islands; *A. muelleri*, CAS 89810–11; Philippine Islands; *A. siamensis*, FMNH 216106–08, Thailand; *A. thinthinae*, see holotype and paratypes.

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