**A new species of upland Stream Toad of the genus *Ansonia* Stoliczka, 1870 (Anura: Bufonidae) from northeastern Peninsular Malaysia**

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c) Five plates; 4 Tables; 27 references

d) Amphibia; one new taxon

**Abstract**

A new species of *Ansonia* is described based on genetic and morphological differentiation. *Ansonia* *lumut* **sp. nov.** is most closely related to three other Peninsular Malaysian species, *A. penangensis, A. malayana*, and *A. jeetsukumarani* but differs from these and other congeners by at least 6.9% sequence divergence at the 12S, 16S rRNA and t-RNA-val genes and the following combination of morphological characters: (1) SVL 21.0–23.6 mm in males, 27.7–31.6 mm in females; (2) first finger shorter than second; (3) interorbital and tarsal ridges absent; (4) light interscapular spot absent; (5) presence of large, yellow rictal tubercle; (6) dorsum black with greenish-yellow reticulations; (7) flanks with small yellow spots; (8) fore and hind limbs with yellow cross-bars; and (9) venter light gray with fine, white spotting.

**Key words:** Conservation, Morphology, Phylogeny, Southeast Asia, Systematics

**Introduction**

The Bufonid genus *Ansonia* is currently represented by 26 species and collective range from as far north as northwestern Thailand, southward through Myanmar, the Malay Peninsula, Indonesia, and as far east as Borneo and the Philippines (Frost 2013). Of these, six species occur exclusively in Peninsular Malaysia (*A. endauensis* Grismer, *A. jeetsukumarani* Wood, Grismer, Grismer, Norhayati, Chan & Bauer, *A. latiffi* Wood, Grismer, Grismer, Norhayati, Chan & Bauer, *A. latirostra* Grismer, *A. penangensis* Stoliczka, and *A. tiomanica* Hendrickson). The exception is *A. malayana* Inger, which occurs in Perak, Peninsular Malaysia (type locality), but has also been reported from Peninsular Thailand, south of the Isthmus of Kra (Inger 1960).

Recent expeditions to the poorly studied and highly fragmented Banjaran Timur (=Timur mountain range) in northeastern Peninsular Malaysia have begun to reveal the undiscovered diversity that lies therein (Dring 1979; Grismer 2006; Grismer *et al*. 2013; Wood *et al.* 2008). Our fieldwork to the previously unsurveyed Gunung Tebu (=Tebu Mountain) in the northeastern state of Terengganu uncovered a population of Stream Toad (genus *Ansonia*) that could not be readily assigned to any currently known species. This population closely matches Dring’s (1979) description of *A. malayana* from Gunung Lawit, an adjacent peak on the same mountain range, but is incongruent with topotypes of true *A. malayana* from Bukit Larut, Perak from a different mountain range (Banjaran Bintang) in northwestern Peninsular Malaysia. We adopted a pluristic approach, combining morphology and molecular phylogenetics to evaluate the taxonomic and phylogenetic placement of this population. Our results show that the population from Gunung Tebu represents a genetically distinct lineage that is also morphologically diagnosable. Under the Unified Concept of Species (De Queiroz 2005), we consider this separately evolving lineage as a new species that is morphologically distinct from other species in this genus.

**Materials and Methods**

*Morphology*

The following characters were measured with a Mitutoyo digitmatic caliper to the nearest 0.1 mm on the left side of the body for symmetrical characters: snout–vent length (SVL), from tip of snout to vent; head length (HL), from posterior margin of mandible to tip of snout; head width (HW), measured at the level of the jaw articulation; snout length (SL), from anterior margins of eye to tip of snout; snout width (SW), distance between anterior margins of eyes; internarial distance (IND), measured from medial, inner margins of nostrils; interorbital diameter (IOD), distance between medial margins of palpebrae at its closest point; eye diameter (ED), length between anterior and posterior margins of eye; tympanum diameter (TD), length of the vertical axis; manus length (ML), distance from the proximal edge of the outer metacarpal tubercle to the tip of third finger; crus length (CL), distance from knee inflection to tarsal inflection; pes length (PL), measured from proximal edge of inner metatarsal tubercle to tip of fourth toe. Toe webbing formula follows Savage & Heyer (1997). These characters were compared to additional discrete character states for all congeners listed in Wood *et al.* (2008) and Wilkinson *et al.* (2012).

The holotype was deposited at the Zoological Reference Collection at the Raffles Museum of Biodiversity Research, University of Singapore (ZRC); paratypes were deposited at La Sierra University Herpetological Collection (LSUHC), La Sierra University, Riverside, California, USA.

*Molecular phylogeny*

Genomic DNA was extracted from liver samples stored in 95% ethanol using the animal tissue protocol in the Qiagen DNeasyTM tissue kit (Qiagen. Valencia, California). A 2685 bp fragment of the mitochondrial genes 12S, 16S rRNA and t-RNA-val was amplified using a double stranded polymerase chain reaction (PCR) under the following conditions: 1.0 μl genomic DNA, 1.0 μl of each external primer, 1.0 μl of dinucleotide pairs, 2.0 μl of 5x buffer, 1.0 μl of MgCl 10x buffer, 0.18 μl of Taq polymerase, and 7.5 μl of diH2O. All PCR reactions were carried out on an Eppendorf Mastercycler gradient thermocycler following the thermo profile in Wilkinson *et al.* (2012): initial denaturation at 94°C for 2 min, followed by 35 cycles of a secondary denaturation 94°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 1.5 min, with a final extension at 72°C for 10 min. PCR products were visualized on a 10% agarose gel electrophoresis. PCR products that were the same size as the targeted regions were purified using MANU 30 PCR ultrafiltration (Millipore) plates and the products were re-suspended in diH2O. Purified PCR products were sequenced using an ABI Big-Dye Terminator v3.1 Cycle Sequencing Kit in an ABI GeneAmp PCR 9700 thermal cycler. All cycle sequencing reactions were purified using Sephadex G-50 Fine (GE Healthcare) and analyzed on an ABI 3730xl DNA Analyzer at the Brigham Young University Sequencing center. All primers used in this study for amplification and sequencing are presented in Table 1.

Sequences were edited and aligned in GENEIOUSv5.5.6 (Drummond *et al.* 2011). Initial alignment was constructed using the MUSCLEv3.831 algorithm (Edgar 2004) implemented in GENEIOUSv5.5.6. The alignment was than adjusted by eye in MacClade v4.08 (Maddison & Maddison 2005). Six new sequences of the new species (GenBank Accession numbers KF906318–KF906323) and three new sequences of *Ansonia jeetsukumarani*, LSUHC 11122–24 (KF906324–KF906326) were added to previously published sequences of *Ansonia*+outgroups (Wilkinson *et al.* 2012; Matsui *et al.* 2010). Information for samples used to construct the molecular phylogeny is summarized in Table 2.

Maximum Likelihood (ML) and Bayesian Inference (BI) were used for phylogenetic reconstructions. Models of sequence evolution were based off Matsui *et al.* (2010) and Wilkinson *et al.* (2012). For both the ML and BI analyses, the datasets were partitioned by gene (three partitions). The ML analysis was performed in RAxML HPC 7.5.4 (Stamatakis *et al.* 2008) for 1000 bootstrap pseudoreplicates via the rapid hill-climbing algorithm. The Bayesian analysis was executed in MrBayes v3.2.2 (Ronquist & Huelsenbeck 2003). Two simultaneous runs were performed with eight chains per run, seven hot and one cold for 5,000,000 generations and sampling every 500 generations from the Markov Chain Monte Carlo (MCMC). After 5,000,000 generations the standard deviation of split frequencies was well below 0.01 and chain convergence was assumed. The first 10% of each run was discarded as burn-in and a Maximum Clade Credibility (MCC) tree was computed in TreeAnotator v1.7.5 (Drummond *et al.* 2012). We considered Bayesian posterior probabilities (BPP) above 0.95 and ML bootstrap support values (MLBS) greater than 70 as significantly supported (Huelsenbeck & Ronquist 2001; Wilcox *et al*. 2002). Uncorrected percent sequence divergences were calculated in Mega v5.2.1 (Tamura *et al.* 2011).

**Results**

**Phylogeny and genetic divergences.** Both Bayesian and ML analyses produced trees with largely the same topology with the exception of the placement of *Ansonia penangensis* and the Tebu population*.* Both trees strongly supported *A. penangensis* and the Tebu population as part of a clade containing two other Peninsular Malaysian species, *A. malayana* and *A. jeetsukumarani* (100% BPP, 99% MLBS). In the ML tree, *A. penangensis* was weakly supported to be basal to that clade (65% MLBS), with the Tebu population being sister to *A. malayana* and *A. jeetsukumarani* (53% MLBS) whereas the Bayesian tree recovered the Tebu population as basal to the clade (99% BPP), with *A. penangensis* being sister to *A. malayana* and *A. jeetsukumarani* (70% BPP) (Fig. 1). In terms of uncorrected percent sequence divergence (*p*-distance), the Tebu population is closest to *A. malayana* (6.9%), followed by *A. jeetsukumarani* (7.2%) and *A. penangensis* (7.4%). The *p*-distance between the Tebu population and other Peninsular Malaysian *Ansonia* is 8.2% (*A. endauensis*), 9.1% (*A. latirostra*), 9.2% (*A. tiomanica*), and 9.8% (*A. latiffi*). Other species in geographic proximity include *A. siamensis* Kiew and *A. kraensis* Matsui, Khonsue & Nabhitabhata on the Thai Peninsula, and *A. thinthinae* Wilkinson, Sellas & Vindum in southern Myanmar. From these species, the Tebu population is separated by a distance of 8.1%, 8.5%, and 8.2% respectively. These levels of divergences are within or greater than most species level divergences of this genus (Table 3).

**Systematics.** Results from the phylogenetic analyses and morphological data clearly place the Tebu population within the genus *Ansonia*. Although its species level phylogenetic relationship can not be unambiguously resolved at this point, our results demonstrate that the *Ansonia* from Gunung Tebu represents a separately evolving lineage that can be morphologically diagnosed from all other congeners and is thus described herein as a new species.

**Species description**

*Ansonia lumut* **sp. nov.**

Fig. 2

*Ansonia malayana* Dring 1979:184

**Holotype:** Adult female (ZRC 1.12503) collected on 31 August 2012 from Gunung Tebu, Terengganu, Malaysia (05°36.11’ N 102°36.19’E; 610 m a.s.l) by Shahrul Anuar, Mohd. A. Muin, E. Quah, L. Grismer, B. Beltran, A. Cobos, A. Alonso, C. Thompson, and C. Ogle.

**Paratypes:** Adult male (LSUHC 10899) collected on 31 August 2012 from Gunung Tebu, Terengganu, Malaysia (05°36.11’ N 102°36.19’E; 610 m a.s.l) by Shahrul Anuar, Mohd. A. Muin, E. Quah, L. Grismer, B. Beltran, A. Cobos, A. Alonso, C. Thompson, and C. Ogle. Adult male (LSUHC 11172) and adult females (LSUHC 11211, 11214) collected on 1 July 2013 from the same locality by Chan Kin Onn, Shahrul Anuar, Mohd, A. Muin, A. Sumarli, J. Chan, H. Heinz and L. Grismer.

**Diagnosis:** *Ansonia lumut* **sp. nov.** is assigned to the genus *Ansonia* based on its phylogenetic placement and the following morphological characters: small body size; slender limbs; no parotoid glands; weak subarticular tubercles; and membranous foot webbing (Inger 1960, 1992). *Ansonia lumut* can be differentiated from all other congeners by the following combination of characters: SVL 21–23.6 mm in males, 27.7–31.6 mm in females; first finger shorter than second; absence of interorbital and tarsal ridges; distinct dorsal tubercles and dorsolateral row of enlarged tubercles; finger tips not expanded into discs; slightly less than two phalanges free of web on fifth toe; light interscapular spot and light patch below eye absent; presence of large, yellow rictal tubercle; dorsum black with greenish-yellow reticulations; flanks with small yellow spots; fore and hind limbs with yellow cross-bars; venter light gray with fine, white spotting; males with nuptial pads on first finger.

**Description of holotype:** Adult female, SVL 29.3 mm; head longer than wide (HL/HW=1.12); snout wider than long (SW/SL=1.18), longer than eye diameter (SL/ED=1.46), slightly projecting beyond lower jaw, dorsally convex with a midline depression, truncated with slight median point in dorsal view, truncated and caudoventrally sloping in lateral view; canthus rostralis distinct, lores vertical, slightly concave; nares open laterally, just below canthus, nearly terminal on snout, distance between nares smaller than snout length (IND/SL=0.58), approximately half of snout width (IND/SW=0.49); eyes large, slightly protruding beyond labials in dorsal view, diameter less than snout length (ED/SL=0.68) and interorbital distance (ED/IOD=0.87), pupils circular; interorbital region flat, distance smaller than snout width (IOD/SW=0.67) and snout length (IOD/SL=0.79); tympanum distinct, oval, taller than wide, vertical diameter smaller than eye diameter (TD/ED=0.65); choanae subcircular, separated by distance larger than their diameter; vomerine ridge and teeth absent; tongue narrow, ending in median point, posterior ⅓ free.

Forelimbs and fingers long and slender; finger length from shortest to longest: I<II<IV<III; basal webbing not extending beyond proximal subarticular tubercle; tips rounded, slightly dilated but not forming discs; subarticular tubercles indistinct; inner and outer metacarpal tubercles weak, oval, flat, inner smaller than outer; supernumerary tubercles absent (Fig. 3A).

Hindlimbs and toes long and slender (CL/SVL=0.47), foot shorter than tibia (PL/CL=0.76); toe length from shortest to longest: I<II<III≤V<IV; webbing formula: I ½ – 2 II ½ – 3- III 1 – 3½ IV 3½ – 2- V; tips rounded, slightly dilated but not forming discs; subarticular tubercles indistinct; inner metatarsal tubercle elongate, flat; outer metatarsal tubercle slightly raised, oval, slightly smaller than inner (Fig. 3B).

Upper eyelid, interorbital region, dorsal part of snout and canthus covered with small, flat tubercles bearing brown, keratinized tips; interorbital ridges absent; tubercles absent on lores; single row of small spinules on upper lip and outer margin of upper eyelid; large tubercle at posterior end of upper lip, level with anterior margin of tympanum and a larger tubercle just above rictus, posterior to tympanum; supratympanic fold and parotoid gland absent; slight scapular swelling; back, flanks and dorsal part of limbs with irregularly spaced large and small tubercles bearing brown keratinized spinules, larger tubercles may have more than one spinule; dorsal tubercles largest around nuchal, scapular and dorsolateral region, smallest on dorsal part of limbs (Fig. 3C); entire ventral surface except for manus and pes with fine, evenly spaced spinules that are most dense around rictal and pectoral region (Fig. 3D). Complete measurements of holotype and paratypes are presented in Table 4.

**Coloration in life:** Dorsal base color dark brown to black. Top of head, lores and back with yellow reticulations bearing greenish flecks. Large, yellow tubercle at rictus, followed anteriorly by smaller yellow tubercle at posterior end of upper lip. An even smaller, yellow tubercle present between rictal and posterior upper labial tubercles on left side of head but absent on right side. Three small, yellow patches along upper lip, below eye, lore, and rostrum. Yellow patches on ventral side of mandible. Flanks with small yellow spots. Yellow crossbars on front and hind limbs. Venter light gray with fine, white spotting.

**Coloration in preservative:** Yellow coloration creamy white and dorsal reticulations light gray. Keratinized tips on tubercles brown. Venter uniform creamy white with very fine dark brown stippling that is most dense on gular region, tibia, tarsus, manus, and pes.

**Variation:** All paratypes closely resemble holotype in coloration and pattern. Female paratypes (LSUHC 11211, 11214) have additional yellow tubercle below tympanum between rictal and posterior upper labial tubercle, tubercle on right side larger than one on the left. Male paratypes (LSUHC 10899, 11172) smaller in size, SVL 21.0 mm and 23.6 mm respectively. Vocal slit present on left side of mouth leads into median subgular vocal sac. Small, indistinct nuptial pad of brown asperities on medial surface of first finger between base and distal phalangeal joint. Nuptial pad not visible on LSUHC 10899.

**Comparisons:** Morphological data for species comparisons were obtained from Wood *et al.* (2008), Wilkinson *et al.* (2012), and material examined (see Appendix). *Ansonia lumut* **sp. nov.** is most closely related to three other Peninsular Malaysian species, *A. malayana*, *A. penangensis,* and *A. jeetsukumarani* but can be morphologically distinguished from them by the absence of a light interscapular spot (vs. present in *A. malayana*, *A. penangensis*, and *A. jeetsukumarani*), dorsum with greenish-yellow reticulations (vs. orange hourglass-shaped lines in *A. malayana*, almost uniform black in *A. penangensis* and *A. jeetsukumarani*), large, yellow rictal tubercle (vs. whitish in *A. malayana, A. penangensis*, and *A. jeetsukumarani*), and yellow cross-bars on limbs (vs. orange in *A. malayana, A. penangensis*, and *A. jeetsukumarani*). On the Thai-Malay Peninsula and Indochina, it differs from *A. latiffi* by smaller body size in males (vs. SVL 34.1–38.2 mm) and females (vs. SVL 50.5–50.7 mm), first finger shorter than second (vs. first finger reaching tip of second), absence of tarsal ridge (vs. presence), dorsum blackish with greenish-yellow reticulations (vs. almost uniform brown), presence of large, yellow rictal tubercle (vs. absence), and yellow crossbars on limbs (vs. pale orange); from *A. latirostra* by the absence of interorbital ridges (vs. present), no rictal gland (vs. present), and greenish-yellow reticulations on dorsum (vs. almost uniform black); from *A. tiomanica* by smaller body size in males (vs. SVL 31.2 mm) and females (vs. SVL 38.4 mm), and greenish-yellow reticulations on dorsum (vs. yellow spots); from *A. endauensis* by having blackish dorsum with greenish-yellow reticulations (vs. almost uniform black), and yellow crossbars on limbs (vs. orange); from *A. siamensis* by smaller body size in males (vs. SVL 28.0 mm) and females (vs. SVL 35.0 mm), finger tips not expanded into discs (vs. expanded into distinct discs), distinct dorsal tubercles (vs. reduced or absent), presence of large, yellow rictal tubercle (vs. absence), and slightly less than two phalanges free of web on fifth toe (vs. one phalanx free); from *A. kraensis* by the absence of a light interscapular spot (vs. present), presence of large, yellow rictal tubercle (vs. absence); slightly less than two phalanges free of web on fifth toe (vs. half phalanx free), dorsum with greenish-yellow reticulations (vs. dorsum brown with darker hourglass-shaped pattern), and venter light gray with fine, white spots (vs. dark brown with distinct, white reticulations); from *A. inthanon* Matsui, Nabhitabhata & Panha by larger body size in females (vs. SVL 23.3–25.2 mm), absence of light, interscapular spot (vs. presence), presence of large, yellow tubercle (vs. absence), dorsum blackish with greenish-yellow reticulations (vs. brown with lighter, indistinct markings), presence of dorsolateral row of enlarged tubercles (vs. absence), slightly less than two phalanges free of web on fifth toe (vs. one phalanx free), and venter light gray with fine, white spots (vs. brown with bright yellow blotches); from *A. thinthinae* by the absence of a light patch below eye (vs. presence), absence of light interscapular spot (vs. presence), dorsum black with greenish-yellow reticulations (vs. dark brown, lacking distinct markings), slightly less than two phalanges free of web on fifth toe (vs. half phalanx free), venter light gray with fine, white spots (vs. dark brown with yellow reticulations), and males with nuptial pads on first finger (vs. nuptial pad covering first and second fingers). Differences between the new species and other geographically and phylogenetically distant congeners are summarized in Wood *et al*. (2008: Table 1).

**Distribution and Natural History:** *Ansonia lumut* occurs in hill dipterocarp forest on Gunung Tebu (at 610 m elevation) and Gunung Lawit (at 790–1280 m elevation; Dring 1979) in the northeastern state of Terengganu (Fig. 4). During the day, specimens were found in small rock fissures along a slow moving stream. The stream was approximately 5 m wide under a semi-closed canopy forest and consisted of dark-colored granite rock covered with algae and moss (Fig. 5). At night, toads were found on rocks and small, overhanging vegetation within the stream and along its banks away from strong torrent zones. Female paratype LSUHC 11214 was found to be gravid with unfertilized, unpigmented eggs that were approximately 1.7–1.8 mm in diameter.

**Etymology:** The specific epithet “lumut” is derived from the Malay word for moss, in reference to the new species’ color pattern, which gives it a mossy appearance. Suggested English name: Mossy Stream Toad; Malay name: Kodok lumut

**Discussion**

Although we were unable to examine specimens of *Ansonia malayana* collected by Dring (1979) from Gunung Lawit, Terengganu, his description of the following characters clearly corresponds to *A. lumut* rather than *A. malayana*: “the yellow tubercle at the mouth commissure varies in size from one half to two thirds of the tympanum diameter; fine lichenate vermiculation of green on black covering the dorsal surfaces; and ventrolateral tubercles capped with yellowish pigment”. These characters are diagnostic of *A. lumut* and absent in *A. malayana* (see species comparisons). Furthermore, Gunung Lawit is located in the same mountain range as Gunung Tebu, separated in a straight-line distance of only 20 km, whereas, *A. malayana* has only been confirmed to occur along the Bintang mountain range along the west coast of Peninsular Malaysia, more than 200 km to the west and separated by the extensive Banjaran Titiwangsa. Although *A. lumut* can be readily distinguished from *A. malayana* by color-pattern in life, these two species are morphometrically similar and are hard to differentiate from preserved specimens. Matsui *et al.* (2005) showed that *A. malayana* from Khao Lak, Phang Nga Province in southern Thailand (Grossmann & Tillack 2001) was undoubtedly a misidentification of *A. kraensis*. Additionally, Inger (1960) and Matsui *et al.* (1998) noted distinct differences in color pattern, webbing, and body size between Thai and Malaysian *A. malayana,* As such, reports of *A. malayana* from Thailand should be treated with suspect.

The discovery of *Ansonia lumut* and species from other groups in the recent past (Grismer *et al*. 2013) highlight the understudied nature of northeastern Peninsular Malaysia and the Timur range in particular. Furthermore, our preliminary data indicate that a large amount of biodiversity is yet to be uncovered from this region, which is vital to realizing and understanding the true biodiversity of Peninsular Malaysia and Southeast Asia as a whole.

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**Appendix**

Material examined

*Ansonia jeetsukumarani*. MALAYSIA: Kelantan, Gunung Stong, LSUHC 11122–34; 11140–41.

**Figures and Tables**

Fig. 1. Bayesian phylogeny of *Ansonia* based on 2685 bp fragment of the mitochondrial genes 12S, 16S rRNA and tRNA-val. Nodal support are indicated by Bayesian posterior probabilitiy (BPP) / Maximum Likelihood bootstrap (MLBS). Dashes indicate nodes that were not supported in the ML analysis.

Fig. 2. Live coloration of female paratype LSUHC 11211.

Fig. 3. Holotype of *Ansonia lumut* (ZRC 1.12503). A: Ventral view of right manus; B: Ventral view of right pes; C: Dorsal surface; D: Ventral surface.

Fig. 4. Distribution of *Ansonia* in Peninsular Malaysia. Localities are color coded to corresponding species. Star denotes the type locality of *A. lumut*; polygons denote members in the *A. lumut* clade; circles denote members outside of the *A. lumut* clade.

Fig. 5. Habitat of *Ansonia lumut* at Gunung Tebu, Terengganu.

Table 1. Primers used in this study for PCR amplification and sequencing reactions.

|  |  |  |  |
| --- | --- | --- | --- |
| **Primer** | **Location** | **Primer sequence 5'–3'** | **Primer reference** |
| ThrLm | External | AAARCATKGGTCTTGTAARCC | (Matsui *et al.* 2010) |
| Hedges16H1 | External | CTCCGGTCTGAACTCAGATCACGTAGG | (Hedges & Maxson 1993) |
| 12S-3H | Internal | CAAGTCCTTTGAGTTTTAAGCT | (Matsui *et al.* 2010) |
| 12SL1091 | Internal | AAACTGGGATTAGATACCCCACTAT | (Matsui *et al.* 2010) |
| 12StVal-H | Internal | AAGTAGCTCGCTTAGTTTCGG | (Matsui *et al.* 2010) |
| 12SL2021 | Internal | CCTACCGAGCTTAGTRATAGCTGGTT | (Tominaga *et al*. 2006) |
| 16SH2715 | Internal | AAGCTCCATAGGGTCTTCTCGTC | (Tominaga *et al*. 2006) |
| 16S1M | Internal | CCGACTGTTTACCAAAAACAT | (Fu 2000) |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Table 2. Information for speciems used in this study. Museum accronyms follow that of Matsui *et al*. (2010). | | | | |
| **Voucher #** | **Species** | **Locality** | **GenBank #** | **Source** |
| KUHE 17377 | *Ansonia albomaculata* | East Malaysia, Sarawak, Lanjak Entimau | AB435303 | Matsui *et al.* 2010 |
| KUHE 17499 | *Ansonia albomaculata* | East Malaysia, Sarawak, Lanjak Entimau | AB435304 | Matsui *et al.* 2010 |
| KUHE 17503 | *Ansonia albomaculata* | East Malaysia, Sarawak, Lanjak Entimau | AB435305 | Matsui *et al.* 2010 |
| LSUHC 7726 | *Ansonia endauensis* | West Malaysia, Johor, Endau-Rompin | AB435257 | Matsui *et al.* 2010 |
| BOR 22640 | *Ansonia hanitschi* | East Malaysia, Sabah, Kinabalu, Silau Silau | AB331710 | Matsui *et al.* 2010 |
| BORN 22641 | *Ansonia hanitschi* | East Malaysia, Sabah, Kinabalu, Silau Silau | AB435277 | Matsui *et al.* 2010 |
| KUHE 19050 | *Ansonia inthanon* | Thailand, Doi Inthanon | AB435253 | Matsui *et al.* 2010 |
| LSUHC 11122 | *Ansonia jeetsukumarani* | West Malaysia, Kelantan, Gunung Stong | KF906324 | This study |
| LSUHC 11123 | *Ansonia jeetsukumarani* | West Malaysia, Kelantan, Gunung Stong | KF906325 | This study |
| LSUHC 11124 | *Ansonia jeetsukumarani* | West Malaysia, Kelantan, Gunung Stong | KF906326 | This study |
| LSUHC 8049 | *Ansonia jeetsukumarani* | West Malaysia, Pahang, Fraser‘s Hill | AB435265 | Matsui *et al.* 2010 |
| LSUHC 8050 | *Ansonia jeetsukumarani* | West Malaysia, Pahang, Fraser‘s Hill | AB435266 | Matsui *et al.* 2010 |
| KUHE 23517 | *Ansonia kraensis* | Thailand, Ranong | AB435250 | Matsui *et al.* 2010 |
| KUHE 35184 | *Ansonia kraensis* | Thailand, Ranong | AB435251 | Matsui *et al.* 2010 |
| KUHE 35817 | *Ansonia kraensis* | Thailand, Ranong | AB435252 | Matsui *et al.* 2010 |
| LSUHC 4991 | *Ansonia latiffi* | West Malaysia, Pahang, Sg. Lembing | AB435299 | Matsui *et al.* 2010 |
| LSUHC 4923 | *Ansonia latirostra* | West Malaysia, Pahang, Sg. Lembing | AB435260 | Matsui *et al.* 2010 |
| LSUHC 4924 | *Ansonia latirostra* | West Malaysia, Pahang, Sg. Lembing | AB435261 | Matsui *et al.* 2010 |
| LSUHC 10898 | *Ansonia lumut* sp. nov. | West Malaysia, Terengganu, Gunung Tebu | KF906319 | This study |
| LSUHC 10899 | *Ansonia lumut* sp. nov. | West Malaysia, Terengganu, Gunung Tebu | KF906318 | This study |
| LSUHC 10900 | *Ansonia lumut* sp. nov. | West Malaysia, Terengganu, Gunung Tebu | KF906320 | This study |
| LSUHC 11211 | *Ansonia lumut* sp. nov. | West Malaysia, Terengganu, Gunung Tebu | KF906321 | This study |
| LSUHC11213 | *Ansonia lumut* sp. nov. | West Malaysia, Terengganu, Gunung Tebu | KF906322 | This study |
| LSUHC11214 | *Ansonia lumut* sp. nov. | West Malaysia, Terengganu, Gunung Tebu | KF906323 | This study |
| BORN 22139 | *Ansonia leptopus* | East Malaysia, Sabah, Tawau | AB435297 | Matsui *et al.* 2010 |
| KUHE 15467 | *Ansonia malayana* | West Malaysia, Larut | AB331712 | Matsui *et al.* 2010 |
| KUHE 15472 | *Ansonia malayana* | West Malaysia, Larut | AB435264 | Matsui *et al.* 2010 |
| ACD 3600 | *Ansonia mcgregori* | Philippines, Mindanao | AB435316 | Matsui *et al.* 2010 |
| KUHE 12058 | *Ansonia minuta* | East Malaysia, Sarawak, Kuching | AB435281 | Matsui *et al.* 2010 |
| KUHE 17233 | *Ansonia minuta* | East Malaysia, Sarawak, Kuching | AB435282 | Matsui *et al.* 2010 |
| KUHE 17274 | *Ansonia minuta* | East Malaysia, Sarawak, Kuching | AB435283 | Matsui *et al.* 2010 |
| ACD 2702 | *Ansonia muelleri* | Philippines, Mindanao, Mt. Hamigitan | AB435315 | Matsui *et al.* 2010 |
| KUHEUNL1 | *Ansonia penangensis* | West Malaysia, Penang Is. | AB435262 | Matsui *et al.* 2010 |
| KUHEUNL2 | *Ansonia penangensis* | West Malaysia, Penang Is. | AB435263 | Matsui *et al.* 2010 |
| BORN 12499 | *Ansonia platysoma* | East Malaysia, Sabah, Crocker, Mahua | AB435267 | Matsui *et al.* 2010 |
| BORN 12500 | *Ansonia platysoma* | East Malaysia, Sabah, Crocker, Mahua | AB435268 | Matsui *et al.* 2010 |
| KUHE 23438 | *Ansonia siamensis* | Thailand, Khaochong | AB435255 | Matsui *et al.* 2010 |
| KUHE 23515 | *Ansonia siamensis* | Thailand, Khaochong | AB435256 | Matsui *et al.* 2010 |
| KUHE 35066 | *Ansonia* sp. | Thailand, Kanchanaburi, Pilok | AB435249 | Matsui *et al.* 2010 |
| KUHE 38071 | *Ansonia* sp. | Thailand, Pukhet | AB435254 | Matsui *et al.* 2010 |
| BORN 09247 | *Ansonia spinulifer* | East Malaysia, Sabah, Tawau | AB435284 | Matsui *et al.* 2010 |
| CAS 243857 | *Ansonia thinthinae* | Myanmar, Tanintharyi | JN664248 | Wilkinson *et al*. 2012 |
| CAS 243871 | *Ansonia thinthinae* | Myanmar, Tanintharyi | JN664249 | Wilkinson *et al*. 2012 |
| CAS 243873 | *Ansonia thinthinae* | Myanmar, Tanintharyi | JN664250 | Wilkinson *et al*. 2012 |
| CAS 243947 | *Ansonia thinthinae* | Myanmar, Tanintharyi | JN664251 | Wilkinson *et al*. 2012 |
| CAS 244136 | *Ansonia thinthinae* | Myanmar, Tanintharyi | JN664246 | Wilkinson *et al*. 2012 |
| CAS 244216 | *Ansonia thinthinae* | Myanmar, Tanintharyi | JN664247 | Wilkinson *et al*. 2012 |
| LSUHC 2616 | *Ansonia tiomanica* | West Malaysia, Pahang, Tioman Is. | AB435258 | Matsui *et al.* 2010 |
| LSUHC 4443 | *Ansonia tiomanica* | West Malaysia, Pahang, Tioman Is. | AB435259 | Matsui *et al.* 2010 |
| ZRC 1.11918 | *Ansonia torrentis* | East Malaysia, Sarawak, Gn. Mulu NP | AB435296 | Matsui *et al.* 2010 |
| BOR 08127 | *Leptophryne borbonica* | East Malaysia, Sabah, Crocker, Ulu Kimanis | AB331716 | Matsui *et al.* 2010 |
| BOR 22088 | *Pedostibes hosii* | East Malaysia, Sabah, Tawau | AB331717 | Matsui *et al.* 2010 |
| KUHE 35585 | *Pelophryne signata* | West Malaysia, Genting | AB331720 | Matsui *et al.* 2010 |

Table 3. Uncorrected p-distances calculated for *Ansonia* using MEGA5 (Tamura *et al*. 2011).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | *A. albomaculata* | *A. endauensis* | *A. hanitschi* | *A. inthanon* | *A. jeetsukumarani* | *A. kraensis* | *A. latiffi* | *A. latirostra* | *A. lumut* | *A. leptopus* |
| *A. albomaculata* | – |  |  |  |  |  |  |  |  |  |
| *A. endauensis* | 0.124 | – |  |  |  |  |  |  |  |  |
| *A. hanitschi* | 0.116 | 0.087 | – |  |  |  |  |  |  |  |
| *A. inthanon* | 0.126 | 0.091 | 0.095 | – |  |  |  |  |  |  |
| *A. jeetsukumarani* | 0.107 | 0.088 | 0.090 | 0.093 | – |  |  |  |  |  |
| *A. kraensis* | 0.120 | 0.082 | 0.086 | 0.069 | 0.089 | – |  |  |  |  |
| *A. latiffi* | 0.105 | 0.107 | 0.091 | 0.112 | 0.101 | 0.108 | – |  |  |  |
| *A. latirostra* | 0.117 | 0.098 | 0.088 | 0.090 | 0.082 | 0.092 | 0.101 | – |  |  |
| *A. lumut* | 0.106 | 0.082 | 0.076 | 0.093 | 0.072 | 0.085 | 0.098 | 0.091 | – |  |
| *A. leptopus* | 0.106 | 0.106 | 0.088 | 0.111 | 0.099 | 0.105 | 0.017 | 0.099 | 0.097 | – |
| *A. malayana* | 0.113 | 0.094 | 0.087 | 0.096 | 0.049 | 0.089 | 0.104 | 0.085 | 0.069 | 0.106 |
| *A. mcgregori* | 0.113 | 0.111 | 0.100 | 0.113 | 0.108 | 0.115 | 0.093 | 0.110 | 0.095 | 0.094 |
| *A. minuta* | 0.115 | 0.101 | 0.070 | 0.097 | 0.097 | 0.092 | 0.098 | 0.099 | 0.086 | 0.099 |
| *A. muelleri* | 0.112 | 0.124 | 0.105 | 0.124 | 0.118 | 0.124 | 0.100 | 0.119 | 0.102 | 0.102 |
| *A. penangensis* | 0.120 | 0.088 | 0.084 | 0.099 | 0.081 | 0.092 | 0.106 | 0.090 | 0.074 | 0.103 |
| *A. platysoma* | 0.117 | 0.089 | 0.060 | 0.091 | 0.089 | 0.091 | 0.099 | 0.086 | 0.079 | 0.095 |
| *A. siamensis* | 0.121 | 0.087 | 0.090 | 0.084 | 0.096 | 0.079 | 0.106 | 0.096 | 0.081 | 0.104 |
| *A.* sp\_35066 | 0.118 | 0.079 | 0.083 | 0.065 | 0.091 | 0.053 | 0.105 | 0.087 | 0.078 | 0.102 |
| *A.* sp\_38071 | 0.120 | 0.085 | 0.091 | 0.071 | 0.081 | 0.057 | 0.110 | 0.092 | 0.088 | 0.107 |
| *A. spinulifer* | 0.128 | 0.118 | 0.094 | 0.120 | 0.113 | 0.115 | 0.116 | 0.116 | 0.109 | 0.117 |
| *A. thinthinae* | 0.120 | 0.083 | 0.091 | 0.065 | 0.083 | 0.044 | 0.109 | 0.085 | 0.082 | 0.109 |
| *A. tiomanica* | 0.130 | 0.104 | 0.097 | 0.096 | 0.095 | 0.096 | 0.119 | 0.094 | 0.092 | 0.115 |
| *A. torrentis* | 0.097 | 0.102 | 0.085 | 0.105 | 0.094 | 0.098 | 0.048 | 0.102 | 0.091 | 0.048 |

Table 3 continued

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *A. malayana* | *A. mcgregori* | *A. minuta* | *A. muelleri* | *A. penangensis* | *A. platysoma* | *A. siamensis* | *A.* sp\_35066 | *A.* sp\_38071 | *A. spinulifer* | *A. thinthinae* | *A. tiomanica* | *A. torrentis* |
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|  |  |  |  |  |  |  |  |  |  |  |  |  |
| – |  |  |  |  |  |  |  |  |  |  |  |  |
| 0.115 | – |  |  |  |  |  |  |  |  |  |  |  |
| 0.100 | 0.108 | – |  |  |  |  |  |  |  |  |  |  |
| 0.119 | 0.038 | 0.109 | – |  |  |  |  |  |  |  |  |  |
| 0.082 | 0.107 | 0.096 | 0.112 | – |  |  |  |  |  |  |  |  |
| 0.090 | 0.102 | 0.075 | 0.110 | 0.086 | – |  |  |  |  |  |  |  |
| 0.101 | 0.111 | 0.095 | 0.119 | 0.093 | 0.089 | – |  |  |  |  |  |  |
| 0.092 | 0.109 | 0.091 | 0.117 | 0.093 | 0.091 | 0.070 | – |  |  |  |  |  |
| 0.090 | 0.110 | 0.095 | 0.121 | 0.086 | 0.094 | 0.084 | 0.064 | – |  |  |  |  |
| 0.114 | 0.123 | 0.101 | 0.127 | 0.111 | 0.103 | 0.124 | 0.113 | 0.116 | – |  |  |  |
| 0.091 | 0.109 | 0.095 | 0.124 | 0.091 | 0.089 | 0.076 | 0.057 | 0.065 | 0.110 | – |  |  |
| 0.097 | 0.123 | 0.107 | 0.133 | 0.102 | 0.095 | 0.101 | 0.088 | 0.100 | 0.124 | 0.095 | – |  |
| 0.102 | 0.086 | 0.102 | 0.096 | 0.099 | 0.088 | 0.100 | 0.098 | 0.101 | 0.114 | 0.101 | 0.119 | – |

Table 4. Measurements of the type series summarized by gender. Ranges follow mean ± standard error.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | ZRC 1.12503 Holotype | LSUHC 11214 Paratype | LSUHC 11211 Paratype | Mean ± Std Error Min–Max | LSUHC 11172 Paratype | LSUHC 10899 Paratype | Mean ± Std Error Min–Max |
| sex | f | f | f | Female (n=3) | m | m | Male (n=2) |
| SVL | 29.3 | 27.7 | 31.6 | 29.5 ± 1.1 27.7–31.6 | 23.6 | 21.0 | 22.3 ± 1.3 21.0–23.6 |
| HL | 8.5 | 8.2 | 10.0 | 8.9 ± 0.6 8.2–10.0 | 7.1 | 6.9 | 7.0 ± 0.1 6.9–7.1 |
| HW | 7.6 | 7.4 | 8.5 | 7.8 ± 0.3 7.4–8.5 | 6.6 | 6.3 | 6.5 ± 0.2 6.3–6.6 |
| SL | 3.8 | 3.3 | 4 | 3.7 ± 0.2 3.3–4.0 | 3.3 | 3.1 | 3.2 ± 0.1 3.1–3.3 |
| SW | 4.5 | 4.2 | 4.6 | 4.4 ± 0.1 4.2–4.6 | 3.8 | 3.3 | 3.6 ± 0.3 3.3–3.8 |
| IND | 2.2 | 2.3 | 2.4 | 2.3 ± 0.1 2.2–2.4 | 2 | 1.8 | 1.9 ± 0.1 1.8–2.0 |
| IOD | 3.0 | 3.0 | 3.3 | 3.1 ± 0.1 3.0–3.3 | 2.7 | 2.2 | 2.5 ± 0.3 2.2–2.7 |
| ED | 2.6 | 2.6 | 3.1 | 2.8 ± 0.2 2.6–3.1 | 2.4 | 2.3 | 2.4 ± 0.1 2.3–2.4 |
| TD | 1.7 | 1.6 | 1.8 | 1.7 ± 0.1 1.6–1.8 | 1.6 | 1.0 | 1.3 ± 0.3 1.0–1.6 |
| ML | 7.8 | 7.4 | 8.6 | 7.9 ± 0.4 7.4–8.6 | 6.2 | 5.5 | 5.9 ± 0.4 5.5–6.2 |
| CL | 13.9 | 13.7 | 15.7 | 14.4 ± 0.6 13.7–15.7 | 11.6 | 10.9 | 11.3 ± 0.4 10.9–11.6 |
| PL | 10.5 | 10.5 | 11.3 | 10.8 ± 0.3 10.5–11.3 | 8.4 | 7.7 | 8.1 ± 0.4 7.7–8.4 |