

## Dressed in black. A New *Ansonia* Stoliczka, 1870 (Lissamphibia: Anura: Bufonidae) from Gunung Murud, Sarawak, East Malaysia (Borneo)

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### Abstract

A new species of stream toad of the genus *Ansonia* is described from Gunung Murud, Pulong Tau National Park, of northern Sarawak, Malaysia, Borneo. *Ansonia vidua*, sp. nov., is morphologically distinguished from its Bornean congeners by the following combination of characters: medium size (SVL of adult females 33.5–34.4 mm); body uniformly black-brown in life; absence of a visible pattern on dorsum or limbs; presence of two low interorbital ridges; shagreened skin on dorsum, sides and upper surfaces of the limbs with numerous homogeneously small, rounded warts; first finger shorter than second; reduced webbing between the toes and an absence of a sharp tarsal ridge. Uncorrected genetic distances between related taxa of > 4.3% in 16S rRNA gene support its status as a hitherto undescribed species.

**Key words:** Amphibia, *Ansonia vidua* sp. nov., Pulong Tau National Park, systematics

### Introduction

The bufonid genus *Ansonia* Stoliczka 1870 comprises 26 nominal species known from Myanmar to Sundaland and the Phillipines (Frost 2013). Larval synapomorphies, including a large ventral oral disc and a dorsoventrally flattened body, are associated with a rheophilic lifestyle in streams with a moderate to strong current (Inger 1966, 1992; Haas *et al.* 2009; Haas & Das 2008; Matsui 2005; Matsui *et al.* 2010).

With a total of 12 species described and additional undescribed species recognised, the island of Borneo is a centre of diversity for the genus *Ansonia* (Matsui *et al.* 2010). Recent studies using molecular markers reveal that the Bornean species of *Ansonia* do not form a monophyletic group (Matsui *et al.* 2010, 2012). Instead, the genus is divided into two major clades: the first occurs on Borneo, some of the islands of the Philippines and the Malay Peninsula; the second clade is known from Myanmar, Thailand and Sundaland, including the Malay Peninsula and Borneo (Matsui *et al.* 2010). This latter clade includes a monophyletic Bornean group, consisting of *Ansonia hanitschi* Inger, 1960, *A. spinulifer* (Mocquard, 1890), *A. minuta* Inger, 1960 and *A. platysoma* Inger, 1960, as well as two genetically distinct lineages from the Kelabit Highlands in Sarawak and from the Crocker Range in Sabah that probably represent species that have not been formally described (Matsui *et al.* 2010). While a few species, such as *A. leptopus* (Günther, 1872), *A. longidigita* Inger, 1960 and *A. spinulifer*, are widespread on Borneo, the majority of *Ansonia* are distributed in small and isolated areas in mountain ranges or even on a single mountain range (Inger & Stuebing 2005). *Ansonia fuliginea* (Mocquard, 1890), for instance, is a micro-endemic species of Gunung Kinabalu, Sabah. *A. latidisca* Inger, 1966 is only known from Gunung Penrisen, Sarawak and the adjacent Gunung Damus, near Sambas, Kalimantan (Inger & Stuebing 2005, Pui *et al.* 2011). Relatively recently, *A. echinata* Inger & Stuebing, 2009 was described from Bukit Kana, a low, isolated hill near the coast of Sarawak (Inger & Stuebing 2009). The number of species of *Ansonia* will likely grow in the future as sampling of new localities continues.

In this study, we describe a new montane species of *Ansonia* from the summit ridge of Gunung Murud, which at 2,423 m asl is the highest mountain in the state of Sarawak, Malaysia (north-western Borneo). Although only two specimens were collected, the new species is clearly distinguishable from its congeners by external morphology, colouration, and genetic data. The discovery of a new species of toad from this remote area in the Pulong Tau National Park contributes to our knowledge of the highly diverse amphibian fauna of the mountain ranges of northern Sarawak.

## Materials and methods

Both specimens of the type series were photographed alive (Canon EOS 5D Mark II, Canon 100 mm macro lens, various flashes) and euthanized in ca. 2% aqueous Chlorobutanol solution (1,1,1-trichloro-2-methyl-2-propanol). Tissue samples were taken from femoral muscles and stored in RNALater buffer solution (Ambion/Applied Biosystems). Voucher specimens were fixed in 4% neutrally buffered formalin for about three weeks, then washed in tap water and subsequently transferred to ethanol solution for long-term storage. The concentration of ethanol was increased stepwise from 30% to 50% to 75% to avoid shrinkage. The sexes of the type specimens were determined by dissection. The following measurements were taken from the preserved specimens using a MicroScribe® M (Revware Systemy, San Jose, CA, USA): snout-vent length (SVL, from tip of snout to vent); body width (BW, greatest width of body); axilla to groin distance (AGD, distance between posterior edge of forelimb insertion and anterior edge of hind limb insertion); head length (HL, distance between angle of jaws and tip of snout); head width at the anterior of the eye (HWAE, width of the head taken directly anterior of the eye); head width at the angle of jaws (HWAJ, distance between angle of jaws); head depth (HD, greatest depth of head, taken posterior of eye); snout length (SL, distance from anterior margin of eye to tip of snout); nostril-snout distance (NS, distance between centre of nostril and tip of snout); eye to nostril distance (EN, distance between anteriormost point of eye and posterior rim of nostril); internarial distance (IN, distance between outer rims of nostrils); eye diameter (ED, horizontal diameter of exposed portion of eyeball); upper eyelid width (UE, greatest transverse width of upper eyelid); interorbital distance (IO, smallest distance between upper eyelids); tympanum-eye distance (TED, distance between anterior rim of tympanum and posterior edge of eye); horizontal tympanum diameter (HTYD); vertical tympanum diameter (VTYD); tibia length (TBL, distance between anterior surface of knee and posterior surface of heel with both tibia and tarsus flexed); thigh length (THL, from cloaca to most distal apex of knee); hand length (HND, distance from base of palmar tubercle to tip of third finger); and foot length (FOT, distance from base of inner metatarsal tubercle to tip of fourth toe). All measurements were repeated three times and the mean value calculated. Fingers and toes were measured using the software ImageAccess 10.4 (Imagic Bildverarbeitung AG, CH-Glattbrugg) from digital images taken with a calibrated Leica DFC420 camera on a Leica MZ16 with motorfocus: Fingers were measured as follows: FII, from base to tip of first finger; FIII, from base to tip of second finger; FIV, from base to tip of third finger; FV, from base to tip of fourth finger. Toes were measured as follows: TI, from base of foot to tip of longest toe; TII, from base of foot to tip of second toe; TIII, from base of foot to tip of third toe; TIV, from base of foot to tip of fourth toe; and TV, from base of foot to tip of fifth toe. Total genomic DNA was extracted from macerated tissue samples using Wizard SV Genomic DNA Purification System (Promega, Switzerland), according to the manufacturer's protocols. The following primers were used for PCR amplification of partial sequences of the mitochondrial 16S rRNA gene: 16SC (forward) 5'-GTRGGCCTAAAAGCAGCCAC-3', 16SD (reverse) 5'-CTCCGGTCTGAACTCAGATCACGTAG-3'. A 25 µl PCR reaction volume was used containing 1 µl DNA, 1 µl of each primer (20 pmol/µl (20µM), 1.5 µl MgCl<sub>2</sub>, magnesium chloride, 12.5 µl GoTaq Hot Start Green Master Mix (Promega) and 8 µl ddH<sub>2</sub>O: subscript 2 (Promega). The cycling conditions for amplification were: denaturation at 94°C for 2 min; 35 cycles at 94°C for 0:30 min, 48°C for 0:30 min, and 72°C for 1:00 min; then one final extension cycle at 72°C for 5:00 min, stop at 4°C. All PCRs were performed on a Techne TC-512 thermo-cycler. PCR products were excised from agarose gels and cleaned using the Wizard® SV Gel and PCR Clean-UP System (Promega). Sequencing was performed in both directions by LGC Genomics (Berlin, Germany) with the same primers that had been used for amplification. Sequence preparation, editing and management were performed using Geneious Pro 6.1.5 (Biomatters, [www.geneious.com](http://www.geneious.com)).

We compared our data with sequences from GenBank (Table 1). The sequences were aligned using the MAFFT algorithm (Katoh *et al.* 2002) for maximized sequence similarity (Morrison 2009). We used the MAFFT

plugin 1.3.3 in Geneious Pro in E-INS-i mode and with standard parameters. We selected GTR + G + I as the best fitting model of sequence evolution using the PAUP plugin 1.2.6 and the implemented Modeltest 3.7 in Geneious Pro. Maximum Likelihood analysis (ML) was performed with the RaxML plugin 1.0 in Geneious Pro using 1000 bootstrap replicates. Bayesian Inference (BI) was performed using the MrBayes plugin 2.0.9 in Geneious Pro using 10 million generations with sampling every 500 generations from one cold chain and three heated chains with a default temperature of 0.2. We qualitatively checked if the chains reached stationarity after this period using the same plugin and then discarded the first 20% of sampled trees as burn-in. Uncorrected genetic p-distances were also calculated in Geneious Pro. Institutional abbreviations follow Sabaj Pérez (2012), except we retain ZRC for the Raffles Museum of Biodiversity Research, National University of Singapore following local usage (the abbreviation used in Sabaj Pérez 2012, is USDZ\*). Material examined for comparison is listed in Appendix 1.

**TABLE 1.** GenBank accession numbers and authors of the sequences used in this study.

Species	GenBank Accession Nos.	Author
<i>Ansonia vidua</i> sp. nov.	KJ488546, holotype, NMBE 1061645	this study
<i>Ansonia vidua</i> sp. nov.	KJ488547, paratype, NMBE 1066153	this study
<i>Ansonia</i> sp. 1	AB435249	Matsui <i>et al.</i> 2010
<i>Ansonia kraensis</i>	AB435250–AB435252	Matsui <i>et al.</i> 2010
<i>Ansonia inthanon</i>	AB435253	Matsui <i>et al.</i> 2010
<i>Ansonia</i> sp. 2	AB435254	Matsui <i>et al.</i> 2010
<i>Ansonia siamensis</i>	AB435255, AB435256	Matsui <i>et al.</i> 2010
<i>Ansonia endauensis</i>	AB435257	Matsui <i>et al.</i> 2010
<i>Ansonia tiomanica</i>	AB435258, AB435259	Matsui <i>et al.</i> 2010
<i>Ansonia latirostra</i>	AB435260, AB435261	Matsui <i>et al.</i> 2010
<i>Ansonia penangensis</i>	AB435262, AB435263	Matsui <i>et al.</i> 2010
<i>Ansonia malayana</i>	AB331712, AB435264	Matsui <i>et al.</i> 2010
<i>Ansonia jeetskumarani</i>	AB435265, AB435266	Matsui <i>et al.</i> 2010
<i>Ansonia platysoma</i>	AB435267–AB435272	Matsui <i>et al.</i> 2010
<i>Ansonia</i> sp. 3	AB435273–AB435276	Matsui <i>et al.</i> 2010
<i>Ansonia hanitschi</i>	AB331710, AB435277, AB435278	Matsui <i>et al.</i> 2010
<i>Ansonia hanitschi</i>	FJ882794	van Boekelaer <i>et al.</i> 2009
<i>Ansonia hanitschi</i>	EF433427, EF433428	Haas & Das 2008
<i>Ansonia spinulifer</i>	AB435284–AB435292	Matsui <i>et al.</i> 2010
<i>Ansonia</i> sp. 4	AB435279, AB435280	Matsui <i>et al.</i> 2010
<i>Ansonia minuta</i>	AB435281–AB435283	Matsui <i>et al.</i> 2010
<i>Ansonia minuta</i>	GQ281543–GQ281545	Haas <i>et al.</i> 2009
<i>Ansonia longidigita</i>	AB331711, AB435293–AB435295	Matsui <i>et al.</i> 2010
<i>Ansonia longidigita</i>	DQ283341	Frost <i>et al.</i> 2006
<i>Ansonia torrentis</i>	AB435296	Matsui <i>et al.</i> 2010
<i>Ansonia leptopus</i>	AB435297, AB435298	Matsui <i>et al.</i> 2010
<i>Ansonia latifffi</i>	AB435299, AB435300	Matsui <i>et al.</i> 2010
<i>Ansonia</i> sp. 5	AB435301, AB435302	Matsui <i>et al.</i> 2010
<i>Ansonia albomaculata</i>	AB435303–AB435305	Matsui <i>et al.</i> 2010
<i>Ansonia guibei</i>	AB435306, AB435307	Matsui <i>et al.</i> 2010
<i>Ansonia fuliginea</i>	AB331709, AB435308	Matsui <i>et al.</i> 2010
<i>Ansonia muelleri</i>	AB435309–AB435315	Matsui <i>et al.</i> 2010
<i>Ansonia mcgregori</i>	AB435316, AB435317	Matsui <i>et al.</i> 2010
<i>Pelophlyne brevipes</i>	AB331720	Matsui <i>et al.</i> 2010

## Results

The final alignment of 77 sequences has a length of 875 bp. The intraspecific sequence variation between the holotype and the paratype of the new species is 0.2%. Following the phylogenetic hypothesis (Fig. 1) the new species of *Ansonia* is the sister taxon to a clade consisting of *A. platysoma* and an undescribed species from Bario, Kelabit Highlands, Sarawak, Malaysia, referred to as *A. sp.* 3 (Matsui *et al.* 2010). These three species together are part of a moderately supported monophyletic group of Bornean species, including *A. hanitschi*, *A. minuta*, *A. spinulifer* and a further undescribed species from the Crocker Range, Sabah, Malaysia, referred to as *A. sp.* 4 (Matsui *et al.* 2010). This Bornean clade was also found by Matsui *et al.* (2010), but with a different branching pattern of the basal branches.

Within this Bornean clade the new species has an uncorrected genetic p-distance of 4.3–5.0% to *A. sp.* 3, 5.5–6.2% to *A. platysoma*, 5.8–6.6% to *A. hanitschi*, 9.5% to *A. sp.* 4, 7.3–8.3% to *A. minuta*, and 10.4–11.2% to *A. spinulifer*. In comparison, the p-distance between pairs of described and accepted species of Bornean *Ansonia* is 2.7% in *A. guibei* and *A. fuliginea*, 3.2–3.6% in *A. longidigita* and *A. torrentis* (but see discussion below), and 5.9–6.9% in *A. leptopus* and *A. longidigita*. We interpret the genetic data of the new species, therefore, to be a reliable indication of its evolutionary distinctiveness relative to all examined congeners following the Phylogenetic Species Concept (Cracraft 1992; Nixon & Wheeler 1990), the Evolutionary Species Concept (Wiley 1978), and the Unified Species Concept (de Queiroz 2007).

The species from Thailand and Peninsular Malaysia *A. inthanon*, *A. jeetskumarani*, *A. kraensis*, *A. latirostra*, *A. malayana*, *A. penangensis*, *A. siamensis*, *A. tiomanica* and two undescribed species *A. sp.* 1 and *A. sp.* 2 form a second clade (Fig. 1). A third clade comprises *A. albomaculata*, *A. fuliginea*, *A. guibei*, *A. latifffi*, *A. leptopus*, *A. longidigita*, *A. mcgregori*, *A. muelleri*, *A. torrentis* and the undescribed species *A. sp.* 5 from Borneo, Philippines, Sumatra, and Southeast Asia (Fig. 1). These results correspond to previously published studies (Matsui *et al.* 2010, 2012). In contrast to Matsui *et al.* (2012), however, the phylogenetic relationships of the Bornean species *A. latidisca* to the two clades mentioned above are unresolved (Fig. 1).

### *Ansonia vidua*, sp. nov.

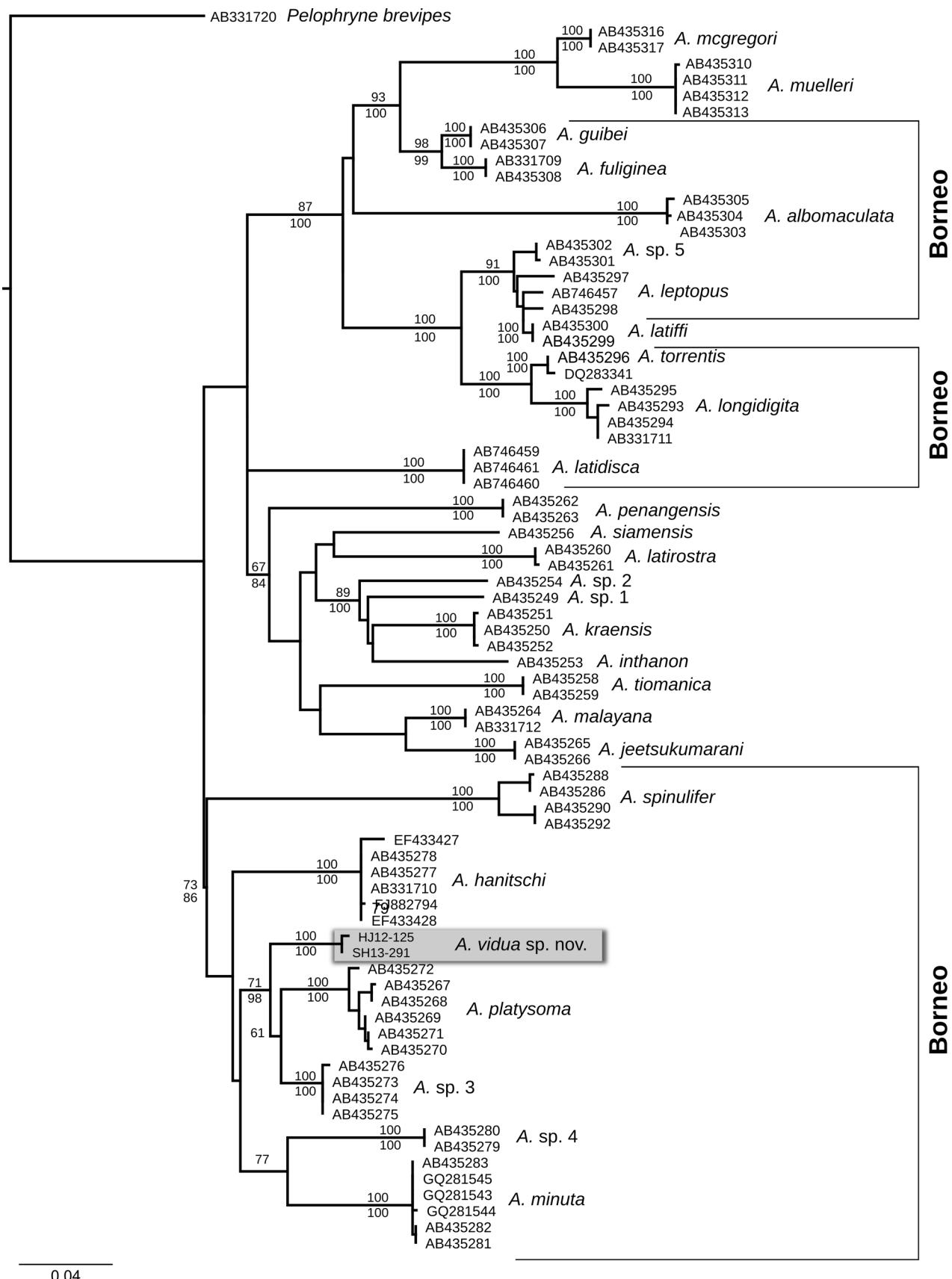
**Holotype:** NMBE 1061645, field number HJ12–125 (Figs. 2–5), GenBank accession number KJ488546, from the summit trail between Church Camp and the summit of Gunung Murud, near the intersection with Sungai Murud, Gunung Murud, Pulong Tau National Park, Sarawak, East Malaysia (Borneo), altitude 2,152 m asl (N 03°54.721', E 115°29.954, GPS WGS 84), coll. Y. M. Pui, A. Jankowski and S. T. Hertwig, 28 February 2012. Adult female.

**Paratype:** NMBE 1066153, field number SH13–291 (Fig. 3C, D), GenBank accession number KJ488547, 25 m away from the locality of holotype at the summit trail, coll. Y. M. Pui, T. Einecke, T. Keilholz and S. T. Hertwig, 10 March 2013. Adult female.

**Diagnosis.** The new species is assigned to the genus *Ansonia* based on its relatively slender body, long legs, indistinct subarticular tubercles, membranous digital webbing, the absence of parotoid glands, tympanum visible (Inger 1960, 1966) and a robustly supported phylogenetic hypothesis (Fig. 1).

*Ansonia vidua* sp. nov. can be differentiated from all other congeners by the following combination of characters: SVL 33.5 and 34.3 mm in the two females known; body not flattened; canthus rostralis slightly swollen; two low longitudinal interorbital ridges present; dorsum of body, flanks and upper surfaces of the limbs covered with numerous small, regular, low, rounded warts, resulting in a velvet-like appearance, only warts on scapular and temporal regions slightly enlarged; warts on dorsum, head and limbs terminating in a single fine, keratinous spine; first finger short, not reaching tip of second finger when adpressed; no sharp tarsal ridge; no skin flaps on posterior thigh near vent; third and fifth toe with 2½ phalanges free of web medially; colouration of the dorsal surface of head and body uniformly black-brown; limbs dark brown with lighter areas on joints of the limbs and phalanges; ventral surface uniformly dark grey to yellowish brown; dorsal and ventral parts of body and limbs completely patternless.

**Description of holotype.** Habitus slender; body as wide as head (Figs. 2–3A, B), body not dorsoventrally flattened; head slightly longer than wide (Table 2); HWAJ/TBL 0.62; rostrum truncate in dorsal view, tip of snout rounded, longer than canthus, oblique in lateral view, projecting beyond tip of mandible, longitudinal, medial groove on the dorsal face of snout, rostral length greater than eye diameter; nostril lateral, closer to snout tip than



**FIGURE 1.** Phylogenetic relationships of *Ansonia vidua* sp. nov. according to a RaxML-based Maximum Likelihood analysis of partial sequences of the mitochondrial 16S rRNA gene. Node labels above branches show the bootstrapping support of ML analysis (only values >50), below branches the posterior probability values of BI (only values >70).

eye (Fig. 4); distinct vertical ridge between tip of snout and centre of maxilla (Figs. 3A, B, 4); canthus swollen with rounded edge, slightly arcuate in lateral view; lores vertical and concave; eye diameter greater than eye-nostril distance; interorbital distance subequal to upper eyelid (Table 2); tympanum distinct, eye diameter 1.9 times greater than tympanic diameter (Fig. 4, Table 2); two low, slightly curvestridges on head forming a parenthetical shape “( )”extends from the anterior aspect of the interorbital region to a point approximately in line with the centre of the tympani (Fig. 3A), one supratympanic ridge in parotoid region (Fig. 2 and 3A).

**TABLE 2.** Measurements of holotype and paratype of *Ansonia vidua* sp. nov., for details and abbreviations see text.

	holotype NMBE 1061645	paratype NMBE 1066153		holotype NMBE 1061645	paratype NMBE 1066153
SVL	33.51	34.28	HTYD	1.99	2.01
BW	12.07	10.93	VTYD	2.13	2.26
AGD	17.7	16.4	TBL	15.96	15.99
HL	11.4	11.36	THL	16.26	15.54
HWAE	4.69	4.9	HND	9.18	9.14
HWAJ	9.88	10.1	FOT	12.88	12.69
HD	4.63	4.46	TI	1.68	1.7
SL	4.16	4.23	TII	2.49	2.58
NS	1.79	1.48	TIII	3.79	4.0
EN	2.44	2.49	TIV	7.3	7.53
IN	2.85	2.51	TV	4.76	4.81
ED	3.73	3.85	FI	2.96	3.08
UE	4.27	4.29	FII	3.63	3.89
IO	4.1	4.2	FIII	6.63	7.25
TED	0.49	0.41	FIV	4.31	4.29

Tips of fingers IV and V weakly expanded, spatulate, without distinct discs; first finger (II) much shorter than second (III), only reaching middle of second phalange of second finger; subarticular tubercles low; palm smooth with large, distinct rounded palmar tubercle. Tips of all toes rounded; fifth toe projecting farther than third; toes not fully webbed, first toe with one phalange free of web, second with 1½ phalanges free of web medially, third and fifth with 2½ phalanges free of web medially, fourth toe with 4 phalanges free of web medially; subarticular tubercles indistinct; two metatarsal tubercles, both oval and slightly raised (Fig. 5); no tarsal fold (Fig. 3). Left foot slightly deformed, toes shortened (Fig. 3A, B).

Skin of sides, back and dorsal surfaces of limbs uniformly covered with small, low, rounded tubercles; shagreened skin has a characteristic velvet-like appearance; tubercles not arranged in distinct rows, tubercles on top of head and eyelids smaller (Figs. 2–4), slightly enlarged tubercles on scapular and temporal region and posterior of tympanum (Figs. 2–4); one row of enlarged, spineless tubercles with darker pigmentation beneath mandibular symphysis (Fig. 3B), followed posteriorly by few smaller, spineless, irregularly arranged tubercles (Fig. 3B); tubercles on dorsal surfaces of head, body, and limbs tipped with one small, regularly shaped dark brown spine, tubercles on sides, venter and throat without spines (Fig. 3A); no skin flaps on posterior thigh near vent (Fig. 3A, B).

Colour in preservative uniformly dark brown above, slightly lighter areas on sides and on joints of limbs and phalanges; venter uniformly brownish grey, throat and chest uniformly yellowish brown (Fig. 3A, B). Colour in life uniformly black-brown above, limbs dark brown with lighter areas on joints of the limbs and phalanges (Fig. 2). Iris black with small irregular intense red spots (Fig. 2).

**Variation.** In the paratype (Table 2, Fig. 3C, D), skin on sides is slightly smoother, abdomen and throat finely granular, tubercles smaller and indistinct, only a single row of enlarged tubercles with darker pigmentation present posterior of the mandibular symphysis. Skin fold on rear thigh near vent is a preservation artifact (Fig. 3C, D). Lighter areas on sides larger in preservative, colour of throat changes from light yellowish grey to yellowish brown anteriorly (Fig. 3C, D).

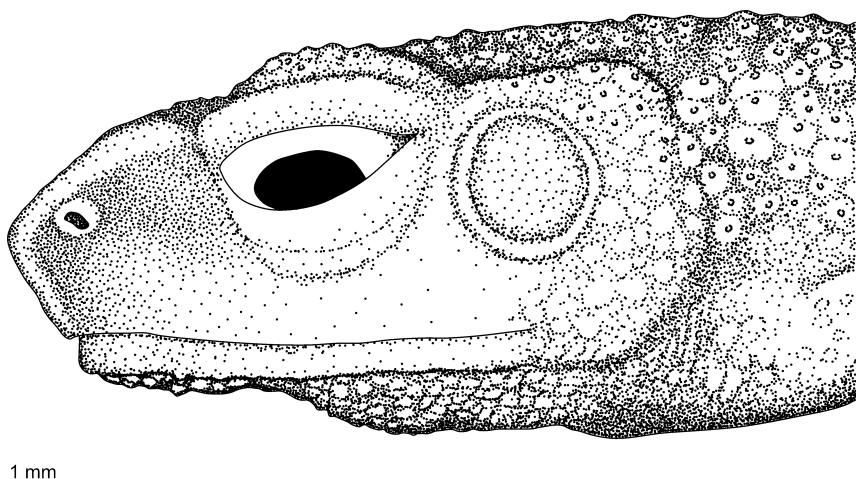


**FIGURE 2.** Holotype of *Ansonia vidua* sp. nov. (NMNH 1061645) in life.

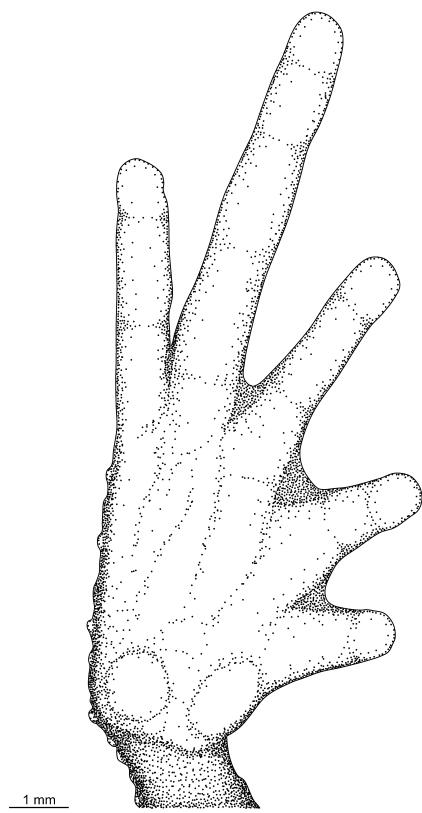
**Comparisons** *Ansonia vidua* sp. nov. can be distinguished morphologically from the 12 described congeneric Bornean species on the basis of an apomorphic combination of diagnostic characters (see ‘Diagnosis’). Bornean congeners differ from *A. vidua* as follows: *A. albomaculata* Inger, 1960: interorbital ridges absent; tympanic annulum partially hidden by thickened skin; sharp tarsal ridge present; toes almost fully webbed; light band running from the posterior corner of the eye to the axilla, often with a subocular light spot (Inger 1966; Inger & Stuebing 2005); *A. echinata*: interorbital ridges absent; dorsum with rounded tubercles both small and large; large ventrolateral tubercles (up to half diameter of tympanum) tipped with small black spines; spinose tubercles on top of snout; third and fifth toe with 1–2 phalanges free of web; limbs with dark crossbars (Inger & Stuebing 2009); *A. fuliginea*: larger body size (females 38–44 mm); interorbital ridges absent; snout vertical in profile and not projecting; tympanum ca. 50% of eye diameter; submandibular spines arranged in a double row (Inger 1960, 1966; Inger & Stuebing 2005); *A. guibei* Inger, 1966: interorbital ridges absent; interorbital distance much greater than width of upper eyelid; tympanum diameter 25–33% of eye diameter; first finger reaches tip of second finger when adpressed; web reaches tips of first, second, third, and fifth toes; dorsal and dorsolateral warts topped with clusters of dark spinules; oblique flap of skin present on each side of vent; upper surfaces dark brown with irregular lighter spots on top of head and back, underside light grey with indistinct dark spots (Inger 1966; Inger & Stuebing 2005); *A. hanitschi*: body flattened; narrower head (HWAJ/TBL 0.50–0.56); interorbital ridges absent; third and fifth toe with 1.5–2 phalanges free of web; skin on back with heterogeneous small rounded warts and a few oval warts, a few warts form short, slender ridges on the back; dark greenish-grey to reddish-brown colouration with dorsal markings, limbs with black crossbars, venter with pattern of dark blotches (Dring 1983; Inger 1966; Inger & Stuebing 2005); *A. latidisca*: larger body size (females 52.1–59.8 mm); canthus rostralis sharp, straight; interorbital ridges absent, but two rows of conical interorbital tubercles; tympanum diameter half of eye; tips of fingers dilated into truncate discs (that of third finger equal in width to tympanum); green colouration above with a distinct pattern of irregular brown spots, abdomen brown with scattered yellowish spots (Inger 1966; Matsui *et al.* 2012); *A. leptopus*: larger body size (females to 60 mm); interorbital ridge absent; tip of first finger reaches swollen tip of second; chest and belly grey with darker mottling (Inger 1966; Inger & Dring 1988; Inger & Stuebing 2005);



**FIGURE 3.** Preserved type material of *Ansonia vidua* sp. nov.: A. dorsal view of holotype (NMNH 1061645); B. ventral view of holotype; C. dorsal view of paratype (NMNH 1066153); D. ventral view of paratype.



**FIGURE 4.** Head of holotype of *Ansonia vidua* sp. nov. in lateral view.



**FIGURE 5.** Foot of holotype of *Ansonia vidua* sp. nov. in ventral view.

*A. longidigita*: much larger size (females to 65 mm); canthus rostralis sharply angular; tip of first finger reaches swollen tip of second; third and fifth toe with one to two phalanges free of web; abdomen with faint dark spots on a pale background or dark with obscure pale spots, limbs with obscure light crossbars (Inger 1966; Inger & Dring 1988; Inger & Stuebing 2005); *A. minuta*: smaller size (females 23–28 mm); interorbital ridges absent; interorbital distance 1.3 width of upper eyelid; distinct tarsal ridge; toes are three-fourth webbed in females; dorsum brown with irregular small orange or yellowish dots, side of head with dark and light bars with a whitish area below eye, abdomen pale with black spots (Inger 1960, 1966; Inger & Stuebing 2005); *A. platysoma*: smaller size (females 20–25 mm); head and body flattened; interorbital ridges absent; skin with heterogeneous warts; 1–1.5 phalanges free of web on third toe; abdomen with light and dark mottling; limbs with light crossbars (Inger 1960, 1966;

Malkmus *et al.* 2002); *A. spinulifer*: larger body size (females to 40–45 mm); interorbital ridges absent; tip of first finger reaches swollen tip of second; dorsal and dorsolateral warts elevated, large and tipped with one to three substantial black spines; top of head and body black with a yellowish or whitish oval patch between shoulders and a light streak along flanks, venter black with small cream-coloured spots or black marbling (Inger & Dring 1988; Inger & Stuebing 2005); *A. torrentis* Dring, 1983: interorbital ridges absent; dorsum covered with numerous heterogeneous rounded tubercles; tips of tubercles without spines, except on temporal and scapular region; blackish in colour with yellowish to pale brown markings on flanks, limbs with light crossbars, abdomen with large grey-brown blotches separated by pale areas (Dring 1983).

*Ansonia vidua* sp. nov. is unambiguously separated from *A. endauensis*, *A. inthanon*, *A. jeetskumarani*, *A. kraensis*, *A. latifrons*, *A. latirostra*, *A. malayana*, *A. mcgregori*, *A. muelleri*, *A. penangensis*, *A. siamensis* and *A. tiomanica* by phylogenetic signal of sequence data (Fig. 1). The recently described species *A. lumut* Chan, Wood, Anuar, Muin, Quah, Sumarli, and Grismer is the sister taxon to a clade consisting of *A. jeetskumarani*, *A. malayana* and *A. penangensis* (Chan *et al.* 2014) and, therefore, also not directly related to *A. vidua* sp. nov..

**Etymology.** The species name *vidua* means 'widow' and refers to the uniform brownish-black colouration in life of this species, which is traditionally the colour of choice by widows. We suggest the English vernacular name 'Murud Black Slender Toad' for the species.

**Ecological notes.** The type series of *Ansonia vidua* sp. nov., consisting of two female individuals, was collected on two expeditions to the Pulong Tau National Park in 2012 and 2013. Males and larval stages of the new species are, as yet, unknown. Both specimens were found at night, resting on the leaves of lower vegetation at the edge of the summit trail to Gunung Murud, ca. two hours walk from Church Camp, a few meters from the edge of a small stream. The type locality is situated between this unnamed small side stream and the Sugei Murud that runs eastward from near the summit of Gunung Murud down to the plateau of the Kelabit Highlands. The type locality is situated just below the summit ridge of the Gunung Murud massif. In this undisturbed montane mossy forest (Beaman 1999; Beaman & Anderson 1997; Mjöberg 1925), the following species of frogs have been recorded in sympatry: *Limnonectes* cf. *kuhlii*, *Philautus mjobergi*, *P. cf. petersi*, *Pelophryne murudensis* and *Leptobrachium montanum*. The amphibian fauna of Gunung Murud has been described by Das (2005, 2008). The discovery of a new and possibly endemic species from the Gunung Murud massif underlines the importance of the Pulong Tau National Park in the protection of the highly diverse montane amphibian fauna of Sarawak and that of Borneo.

## Discussion

The uncorrected genetic distance of 4.3–5% between *Ansonia vidua* sp. nov. and the undescribed taxon *A. sp. 3* from Bario, Kelabit Highlands (Matsui *et al.* 2010) at the 16S rRNA fragment examined in this study to the undescribed taxon *A. sp. 3* from Bario, Kelabit Highlands is well within the range of distances found between other valid Bornean *Ansonia* species (see above). Bario is located close to the type locality of *A. vidua* (ca. 15–20 km linear distance), and the Gunung Murud massif is the summit of the Kelabit Highlands. Unlike other widespread amphibian species on Borneo (Haas & Das 2008; Hertwig *et al.* 2011), the genetic variation observed cannot be explained, therefore, in terms of isolation by distance. Future field studies are clearly needed to investigate the distribution of *A. vidua* and *A. sp. 3* in the Pulong Tau National Park and the adjacent Kelabit Highlands, especially with regard to the presence or absence of a contact zone or distribution gap at intermediate elevations.

As well as being genetically distinct, the new species displays a combination of morphological characters that is unique in *Ansonia*. Beside the uniformly black colouration of the body and the absence of color patterns, the shagreened skin structure distinguishes the new species from congeners on Borneo. In *A. echinata*, however, only males have been described so far and no tissue samples for this species are available. The striking difference between the skin structure of *A. vidua* sp. nov. and *A. echinata* (tubercles both small and large on the dorsum and large spinose tubercles ventrolaterally; Inger & Stuebing 2009) is unlikely to be explained solely by sexual dimorphism, because a comparably pronounced morphological difference between males and females has not been described in this genus so far. Moreover, *A. echinata* is known exclusively from Bukit Kana, Bintulu Division, Sarawak, which is a hill near the coast of Sarawak, and the type material was collected at an elevation of ca. 250 m asl (Inger & Stuebing 2009). In contrast, the two available specimens of *A. vidua* sp. nov. were found at 2,100 m asl, in the montane climate of Gunung Murud.

An enigmatic Bornean species of the genus *Ansonia* is *A. torrentis*, described by Dring (1983) from the Sugei

Tapin in the Gunung Mulu massif. The linear distance between Gunung Mulu and Gunung Murud is ca. 75 km. Dring used the slightly longer legs, slightly smaller size and narrower head to distinguish this species from the syntopic *A. hanitschi*. According to the original description, *A. torrentis* is similar to *A. vidua* sp. nov. in size, webbing and the absence of a sharply angular canthus rostralis. However, the heterogeneous skin structure in *A. torrentis* (tubercles both small and large, dorsal tubercles without spines) and the color patterning on the dorsum and venter distinguish this species from *A. vidua* sp. nov..

Surprisingly, in our phylogenetic analysis the sample identified as *A. torrentis* by Matsui *et al.* (2010, AB435296) clustered with a sample identified by Frost *et al.* (2006) as *A. longidigita* from Sipitang District, Sabah (DQ283341) (uncorrected p-distance 0.3%). Given the possibility that one or both of these samples may have been erroneously determined, the validity of *A. torrentis* needs to be addressed in future studies. Similarly, we found *A. latiffi* (Wood *et al.* 2008), to be both morphologically and genetically similar to *A. leptopus* (uncorrected genetic distance of just 1–2%) and suggest that the validity of these taxa requires additional attention.

## Acknowledgements

The Economic Planning Unit, The Prime Minister's Department, Malaysia, especially Munirah Abd. Manan, helped by issuing permission to conduct research in Malaysia. We thank the Sarawak Forest Department and Sarawak Forestry Corporation, in particular Nur Afiza Binti Umar, Dayang Nuriza Binti Abang Abdillah, Mohamad bin Kohdi, Engkamat Anak Lading Datu Haji Ali Yusop and Mohd. Shabudin Sabki, for providing advice and issuing permits (NCCD.907.4.4 (Jld.VI)-106, NCCD.907.4.4 (Jld.9)-20; Park Permit 56/2011, 038/2012; export permits 09813, 10644). We are grateful to Andre Jankowski, Toralf Keilholz, Tobias Einecke and our local guides, including Gelawat Kadir, Sulaiman Penguran, Singa Kadir, Lasung Nailo, Gukang Labo, Balang Abai, Mutang Bawat, for assistance and companionship in the field. Our expeditions benefited significantly from Ch'ien C. Lee and Agong Patan's knowledge and organization. Beatrice Blöchliger and Manuel Schweizer performed technical tasks with admirable skill. We are grateful to Leder Fuchs, Göppingen, for their contribution to our fieldwork. For permission to examine material under their care we thank Colin McCarthy (BMNH), Alan Resetar and Robert Inger (FMNH), Margarita Naming (SBC), Charles Leh Moi Ung (SM), David Edwards and Helen Pang (UBD) and Peter Kee Lin Ng and Kelvin Kok Peng Lim (ZRC). Lucy Cathrow improved the language of this manuscript. Finally, we wish to express our gratitude to the anonymous reviewers and Jodi Rowley who contributed helpful comments for the improvement of this paper.

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#### **APPENDIX 1.** Comparative material examined.

***Ansonia albomaculata* Inger, 1960.** FMNH 81975 (holotype) and SM (uncatalogued), in Bottle 51 (paratype), from “1,400–2,000 feet above sea level, in the headwaters of the Baleh River, Third Division, Sarawak”; UBD 266, 309, 318, 337, 389, 396, 412, 472, 476, 481, 487, 508, 539, 617, Batu Apoi Forest Reserve, Temburong District, Brunei Darussalam.

***Ansonia guibei* Inger, 1966.** UNIMAS 7746; 8058, 8060. Mesilau, Gunung Kinabalu Park, Sabah, Malaysia.

***Ansonia hanitschi* Inger, 1960.** UNIMAS 8050, Liwagu Trail, Gunung Kinabalu Park, Sabah, Malaysia; UNIMAS 8055, ZRC 1.11911, Mesilau, Gunung Kinabalu Park, Sabah, Malaysia; NMBE 1056271-1056280, Sungai Tapin, Gunung Mulu, Sarawak, Malaysia.

***Ansonia latidisca* Inger, 1966.** UNIMAS-OJJ 009–011, NMBE 1061497, NMBE 1061498, Gunung Penrissen, Sarawak, Malaysia.

***Ansonia leptopus* (Günther, 1872).** UBD 289, 382, 293, 397, 420, 506, 510, 517, 618–19, Batu Apoi Forest Reserve, Temburong District, Brunei Darussalam; NMBE 1056582, 1056591, 1057156, 1057168, 1057169, 1057172, 1059721-1059723, UNIMAS 8393, 8402, Kubah National Park, Sarawak, Malaysia; NMBE 1056281-1056283, Camp 5, Gunung Mulu National Park, Sarawak, Malaysia; UNIMAS 8725, Gunung Santubong, Sarawak, Malaysia.

***Ansonia longidigita* Inger, 1960.** BMNH 99.8.19.12 (holotype), “..4,200 feet on Mount Kina Balu, North Borneo”; UNIMAS 7925–26. Gunung Santubong, Sarawak, Malaysia; UBD 90, 94–98, 135, 160, Batu Apoi Forest Reserve, Temburong District, Brunei Darussalam; NMBE 1056284-1056288, ZMH A09368, ZMH A09371, ZRC 1.12012–13, Camp 2, Gunung Mulu National Park, Sarawak, Malaysia; ZMH A09370 8th Mile, Crocker Range Park, Sabah, Malaysia.

***Ansonia minuta* Inger, 1960.** NMBE 1056601, 1057163, ZMH A10003–06, Sungai Rayu, Kubah National Park, Sarawak, Malaysia; ZRC 1.2215, Summit Trail off Sungai Bawang, Kubah National Park, Sarawak, Malaysia.

***Ansonia platysoma* Inger, 1960.** ZMH A10007–09, ZRC 1.12004, 1.12007, Camp 2, Gunung Mulu National Park, Sarawak, Malaysia.

***Ansonia spinulifer* (Mocquard, 1890).** SBC A.00001, Gunung Meraja, Bau, Sarawak, Malaysia; SBC A.00032, Gunung Pambor, Bau, Sarawak, Malaysia; SBC A.00045, Gunung Ropih, Bau, Sarawak, Malaysia; SBC A.00065–67, Gunung Tai Ton, Bau, Sarawak, Malaysia; SBC A.00093, Gunung Batu Payong, Bau, Sarawak, Malaysia; SBC A.00165–66, Gunung Umbut, Bau, Sarawak, Malaysia; SBC A.00288, Gunung Podam, Bau, Sarawak, Malaysia; UNIMAS 7875, Anna Rais, base of Gunung Penrissen, Sarawak, Malaysia; UNIMAS 7020–22. Ranchan Pool, Serian, Sarawak, Malaysia; UNIMAS 7580. Gunung Gading, Sarawak, Malaysia; NMBE 1057085, 1057086, 1057171, Kubah National Park, Sarawak, Malaysia.

***Ansonia torrentis* Dring, 1983.** NMBE 1056296-1056298, Sungai Tapin, Gunung Mulu, Sarawak, Malaysia.