

Larval identities of *Ansonia hanitschi* INGER, 1960 (Amphibia: Bufonidae) and *Polypedates colletti* (BOULENGER, 1890) (Amphibia: Rhacophoridae) from East Malaysia (Borneo)

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Abstract. We describe the tadpoles of *Ansonia hanitschi* INGER, 1960 and *Polypedates colletti* (BOULENGER, 1890) from East Malaysia, Borneo. The morphological description is supplemented by photos of living specimens and SEM images of external and internal features. Identification was based on morphological and genetic characters (16S rRNA). The tadpole of *A. hanitschi* is among the largest tadpoles described for the genus *Ansonia* from Borneo. This suctorial-rheophilous tadpole has been collected from fast-flowing rocky streams, in association with foaming currents. The tadpole of *P. colletti* is redescribed due to uncertainties about its identity and lack of photographic evidence in previous descriptions. It is an inhabitant of stagnant waters, living in and on flooded leaf litter accumulations.

Key words. Bufonidae, Rhacophoridae, *Ansonia hanitschi*, *Polypedates colletti*, tadpole description, larva, larval morphology, oral disk.

Introduction

At present 5,574 anuran species have been described (www.amphibiaweb.org). New species continue to be discovered from even well-explored regions of the earth, but the inventory and description of larval forms of the known species are far from complete. However, recognition of the larval stages and descriptions of tadpoles are essential for many purposes and research questions, such as surveys, habitat inventories, studies on resource use in habitats, interspecific competition studies, and conservation efforts. Tadpoles are so different anatomically and ecologically from the adult stage that they deserve separate treatment (McDIARMID & ALTIG 1999). Interest in tadpoles has increased in the recent past, and various studies have attempted to summarize regional tadpole faunas (for example, CHOU & LIN 1997, LEONG & CHOU 1999, ANSTIS 2002).

For the island of Borneo, and especially the East Malaysian states of Sabah and Sarawak, ROBERT F. INGER laid a solid foundation for tadpole research in a series of papers, espe-

cially INGER (1966, 1983, 1985, 1992) and INGER et al. (2006). In a recent study (DAS & HAAS 2006), we summarized the literature that provides data on larval identities of Bornean amphibians. According to this survey, approximately 55% of the Bornean amphibian fauna have known larvae; since then, one new description has become available (INGER et al. 2006). Even when descriptions are available, these are often superficial or in abbreviated form, sometimes without drawings, often without images (photographs), preventing unequivocal identification of larvae in the field or laboratory and the reproduction of the former. Furthermore, the descriptions surveyed by DAS & HAAS (2006) were frequently without deposited well-documented voucher specimens, making identities of described tadpoles uncertain. This is especially a problem with old species names that are now considered to contain two or more valid species, e.g., *Leptobrachium montanum* FISCHER, 1885 and *Hylarana signata* (GÜNTHER, 1872) (see MALKMUS et al. 2002, BROWN & GUTTMAN 2002).

The present work describes two larval

forms: the tadpoles of *Ansonia hanitschi* INGER, 1960 (Bufonidae) and *Polypedates colletti* (Boulenger, 1890) (Rhacophoridae). *Ansonia hanitschi* is a stream toad endemic to Borneo. Adults have been reported from streams within submontane and montane forests above 950 m above sea level (INGER & STUEBING 2005). The species is known from Gunung Kinabalu and the adjacent Crocker Range (Sabah), Gunung Mulu (northern Sarawak) and from the north-eastern parts of Kalimantan (MALKMUS et al. 2002, INGER & STUEBING 2005). The tadpoles of *A. hanitschi* were mentioned briefly in MALKMUS et al. (2002) and INGER & STUEBING (2005), describing these tadpoles as "... black and teardrop shaped. Maximum length is 20 mm." This description is so general that it could fit several *Ansonia* species and it conflicts with our findings. Hence we doubt that this description is valid for *A. hanitschi* and consider the tadpole of *A. hanitschi* as formally undescribed.

Polypedates colletti (BOULENGER, 1890) is a widespread species in lowland dipterocarp forests, alluvial forests, and peat swamps. It shares its habitat preference with, for example, *Ingerophrynus quadriporcatus* (BOULENGER, 1887), *Hylarana baramica* (BOETTGER, 1900) and *H. glandulosa* (BOULENGER, 1882), and is often found in syntopy with these species. The inaccessibility of the swamps in lowland Borneo and the difficulties in finding tadpoles in the dark brown, leaf litter filled waters may be reasons why the tadpoles of swamp species have rarely been collected and described; only recently were the tadpoles of *I. quadriporcatus* (LEONG & CHOU 1999) and *H. glandulosa* (INGER et al. 2006) described from such habitats. INGER (1966) described the larva of *P. colletti*, however, without providing drawings or photos. Later, INGER (1985: 71) concluded for other samples that some "... factors leave the identification uncertain". Also INGER (1985) described samples from Sabah and Sarawak as differing markedly in tail tip shape and colouration, and deviating

from his earlier account (INGER 1966). This uncertainty and the lack of drawings or photographs in Inger's accounts warrant a re-description of the *P. colletti* tadpole.

Materials and methods

Tadpoles of *A. hanitschi* were collected at Sungei Silau-Silau, Gunung Kinabalu National Park (N 06°00.639', E116°32.418'; 1,450 m asl), Sabah. Calling adults and breeding congregations of *A. hanitschi* were present in large numbers at the collection sites at the time of collecting. No adults of other *Ansonia* species were encountered at Sungei Silau-Silau during the two seasons of collecting. One adult voucher was collected from Mesilau (also within the Gunung Kinabalu massif, ca. 2,000 m asl; voucher ZRC 1.11911; ex ID 8161) and tissue was taken for DNA sequencing.

Tadpoles of *P. colletti* were collected in Sarawak at (1) Sama Jaya Nature Reserve (N 01°31.258', E110°23.248') from a leaf litter filled shallow depression next to the trail (field number: 189), (2) Gunung Santubong (field number: 135), Summit Trail, from leaf litter filled depression next to trail, and (3) Gunung Mulu National Park, Camp 5, Kerangas Forest Trail, approx. 50 m off trail, pool from an uprooted tree in Kerangas forest vegetation (field number: 384). Living tadpoles were photographed and subsequently anaesthetized and preserved in either 4% neutral-buffered formalin or absolute ethanol. Adults from two sites were sampled for liver and muscle tissue (absolute alcohol): one adult from Loagan Bunut National Park (ZRC 1.11912, ex-ID 8094) and one from Mulu National Park, Camp 5, Deer Pond (ZRC 1.11914, ex ID 8571).

Both species in this study were identified from a series of metamorphic and post-metamorphic specimens and DNA sequence matching. Metamorphs of *A. hanitschi* had slightly dilated finger tips. In metamorphs, the tip of the first finger did not reach the

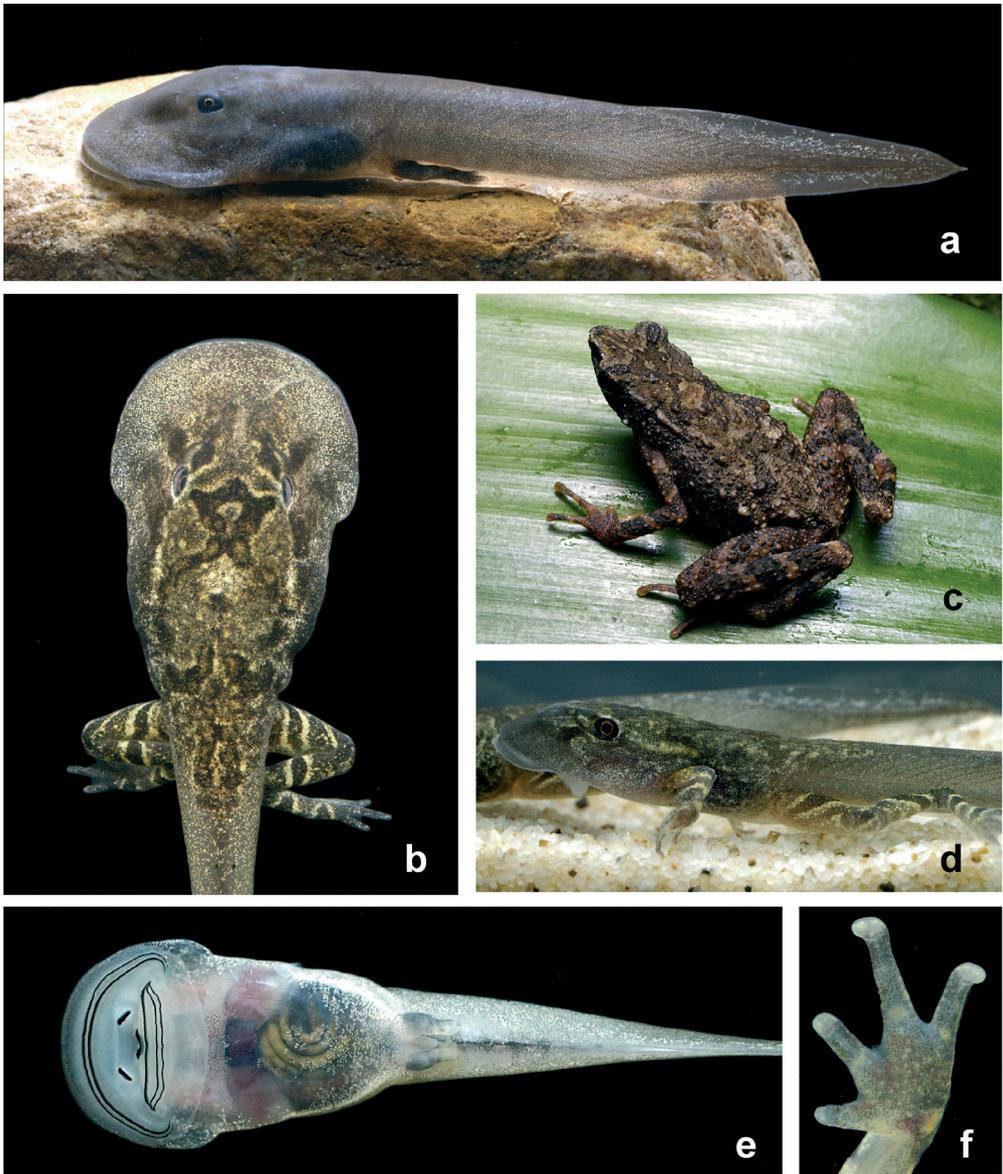


Fig. 1. Colouration of larval *Ansonia hanitschi* in life (various specimens): a) lateral view of fully grown tadpole (stage 38), note the short acuminate tip; b) dorsal view of metamorph (stage 41) with emerging juvenile dorsal pattern on dorsum and legs; c) adult individual from Sg. Silau-Silau; d) metamorph; e) ventral view, note the length of upper lip keratodont rows curving laterally around lower lip keratodont rows, LTRF 2/3, widely separate upper jaw sheaths; f) hand (ventral view) of stage 42 specimen.

disk of the second when adpressed (INGER, 1966) and the typical adult dorsal pattern was present with congruence in even subtle details of the patterns on the head and shoulder parts (Fig.1).

Genetic matching of tadpoles with adults was based on the partial mitochondrial 16S rRNA gene. DNA was extracted from macerated muscle or liver tissue according to standard methods (HILLIS et al. 1996) and was stored at -20°C. DNA amplification of partial 16S rRNA gene sequences was done with peqGOLD PCR-Master-Mix Y (Peqlab) according to the manufacturer's guidelines. The sense primer (16SC) 5'- GTRG-GCCTAAAAGCAGCCAC - 3' and the anti-sense primer (16SD) 5'- CTCCGGTCT-GAACTCAGATCACGTAG - 3' were chosen (RAFE BROWN pers. comm). They amplified a >830 bp long 16S rRNA fragment that overlaps broadly with 16S DNA sequences obtained from other primer pairs (e.g., VENCES et al. 2005). The cycling conditions for the amplification were: denaturation at 94°C for 2 min; 35 cycles at 94°C for 0:30 min, 48.2°C for 0:30 min, and 72°C for 1:00 min; then one extension cycle at 72°C for 5:00 min, stop at 4°C. The PCR products were purified using a Quiagen gel extraction kit. Single strand sequencing was done by a contractor (Agowa Berlin; www.agowa.de) with the 16SC (forward) primer. The DNA sequences obtained were aligned automatically using Clustal X (THOMPSON et al. 1997) and then checked visually.

GenBank accession numbers: *Ansonia hanitschi*: EF433427 (tadpole, voucher ZMH 09248, Kinabalu) and EF433428 (adult, voucher ZRC 1.11911, ex-ID 8094, Kinabalu). *Polypedates colletti*: EF566974 (tadpole, ZMH A09362, Kuching), EF624065 (tadpole, ZMH A09363, Mulu), EF624066 (adult, ZRC 1.11914, Mulu), and EF566973 (adult, ZRC 1.11912, Loagan Bunut).

Adult and larval *A. hanitschi* matched 99.1%. The match between adult and larval *P. colletti* sequences ranged from only 95% (EF566974 vs. EF566973 sequence overlap 816

bp, 41 non-matching sites) to 100% (EF624065 vs. EF624066). A Genbank BLAST search yielded *P. colletti* (AF215354.1, by M. Vences) as best match, differing from EF566974 in 7 base pairs in the overlapping 528 bp region (98.7%). Additional tadpole voucher specimens were deposited at the Zoological Museum, Hamburg (ZMH, see Table 1).

We apply the classification of FROST (2007) based on suggestions of FROST et al. (2006). We used terms for tadpole descriptions from INGER (1985), ALTIG & MCDIARMID (1999), and ANSTIS (2002). Terminology for internal oral features was adopted from WASSER-SUG (1976). Tadpole stages were recorded in using GOSNER's table (1960). Measurements were taken from digital images using ImageJ software (National Institute of Health). For scanning electron microscopy, tadpoles were transferred from 4% formalin preservation (buffered) to an alcohol series of increasing concentration, and finally critical-point dried. No post-fixation was applied. The dried specimens were mounted and sputter-coated (GEA 004S) and were examined and digital images taken with a Leo 1525 scanning electron microscope.

Results

Ansonia hanitschi

Colour in life (Fig. 1, stages 33–40). Viewed in the habitat from a distance, the animals look dark grey dorsally without distinct pattern in premetamorphic stages (Fig. 1a). In close-up the flanks, dorsum, and tail have a fine "salt-and-pepper" pigmentation pattern (iridocytes and melanocytes) (Figs. 1a). The skin on the flanks is dark pigmented and opaque. Dorsal epidermal melanocytes are small and more or less circular in general shape, but fringed with short irregular cytoplasmatic processes. In advanced stages (Fig. 1b-d, Stage 40) the post-metamorphic dorsal pattern emerges: dark markings with light brown borders appear on dorsum and thighs. The venter is mostly unpigmented; gut coils are visible in

Tab. 1. Measurements of *Ansonia hanitschi* and *Polypedates colletti* larvae. BL, body length (head-trunk); BS, body end to spiracle distance; HT, maximum tail height; LF, ventral fin height at HT position; NE, naris to eye distance; NN, internarial distance; No, specimen number; PP, interorbital distance; RN, rostrum-narial distance (lateral projection); SS, snout-spiracle distance; TL, total length; UF, dorsal fin height (at HT position); ZMH, Zoological Museum Hamburg. All measurements in mm.

Species	ZMH voucher lot	Field Indiv. ID	Stage No	TL	BL	SS	BS	RN	NE	HT	UF	LF	NN	PP	
<i>Ansonia hanitschi</i>	ZMH Ao8803	187F	1	33	30.4	10.6	7.56	3.07	2.95	0.75	3.92	0.00	0.30	1.79	2.45
	ZMH Ao8805	187F	2	34	28.4	10.0	7.66	2.38	2.96	0.73	3.82	0.00	0.38	1.65	2.28
	ZMH Ao8801	187F	3	34	30.6	10.5	7.99	2.54	3.20	0.75	3.72	0.30	0.51	1.81	2.63
	ZMH Ao8801	187F	4	31	29.3	10.2	7.68	2.53	3.11	0.75	3.94	0.20	0.49	1.75	2.32
	ZMH Ao8803	187F	5	29	28.4	9.8	7.21	2.62	3.20	0.72	3.92	0.11	0.35	1.70	2.42
	ZMH Ao8802	187P	1	38	32.8	11.7	8.46	3.19	3.41	0.94	4.16	0.78	1.03	1.9	2.81
	ZMH Ao8802	187P	2	41	32.4	11.5	8.67	2.78	2.95	1.05	3.93	0.72	1.3	1.84	3.07
<i>Polypedates colletti</i>	ZMH Ao8807	189P	1	40	31.8	11.0	6.78	4.20	1.26	1.91	5.54	1.27	1.21	1.93	4.36
	ZMH Ao8808	189F	1	40	32.8	10.7	5.73	4.97	0.73	1.29	6.46	2.10	1.99	1.92	4.46
	ZMH Ao8808	189F	2	40	32.7	11.4	6.81	4.58	1.15	1.55	6.28	2.10	1.87	1.80	4.40
	ZMH Ao8807	189F	3	41	31.9	9.8	6.09	3.74	0.50	1.39	5.08	1.50	1.49	1.63	4.62
	ZMH Ao8806	135F	1	32	30.3	11.0	6.91	4.08	1.05	1.78	5.91	1.94	1.45	2.13	3.92
	ZMH Ao8806	135F	2	34	30.9	10.5	6.12	4.35	1.13	1.89	5.31	1.73	1.43	1.97	3.83

ventral view. Some „salt-and-pepper“ pigmentation extends from the flanks to the venter posterolaterally to the oral disk and at the posterior venter (Fig. 1e). The gills and the heart shine in bright red colour through the ventral skin. The center of the gut coil is visible left of the mid-sagittal plane. There is deep (sub-integumental), dark brown pigmentation that lines the braincase, anterior vertebral column, and also pericardium and peritoneum, but only partially obscures the heart, liver and lateral gut coils in lateral view. The iris is black except for a narrow golden ring around the pupil. The skin of the oral sucker is mostly without pigmentation (there is some pigmentation on the backside of lower lip and along base of upper lip). The tail has the same „salt-and-pepper“ pattern of round melanophores with interspersed iridophores. Colouration of the tail is lightest in the middle part of the tail. The tail fins bear melanophores, increasing in density towards the tail tip and along the margin of the fins.

A streak of condensed (sub-integumental) melanophores is formed ventrally in the anterior half of the tail covering the vena caudalis ventralis (Fig. 1e). The ventral skin of the tail itself is devoid of pigmentation between body and beginning of ventral fin. The tail blood vessels are inconspicuous in this species but the vena caudalis dorsalis was visible in early, less dark pigmented specimens. The vein curves ventrally over the tail musculature on the left side of the tail and disappears at the root of the tail.

Colour in preservation (4% formalin) is similar to colouration in life, except that the silvery iridophores have disappeared in preservation: consequently the „salt-and-pepper“ pattern is gone and ventral parts of the animal appear even more translucent. All dense brown melanocyte pigmentation remains. The iris is all black.

External morphological features. Moderately large tadpole, up to 32.8 mm total length (Tab. 1). The body contour in dorsal view is

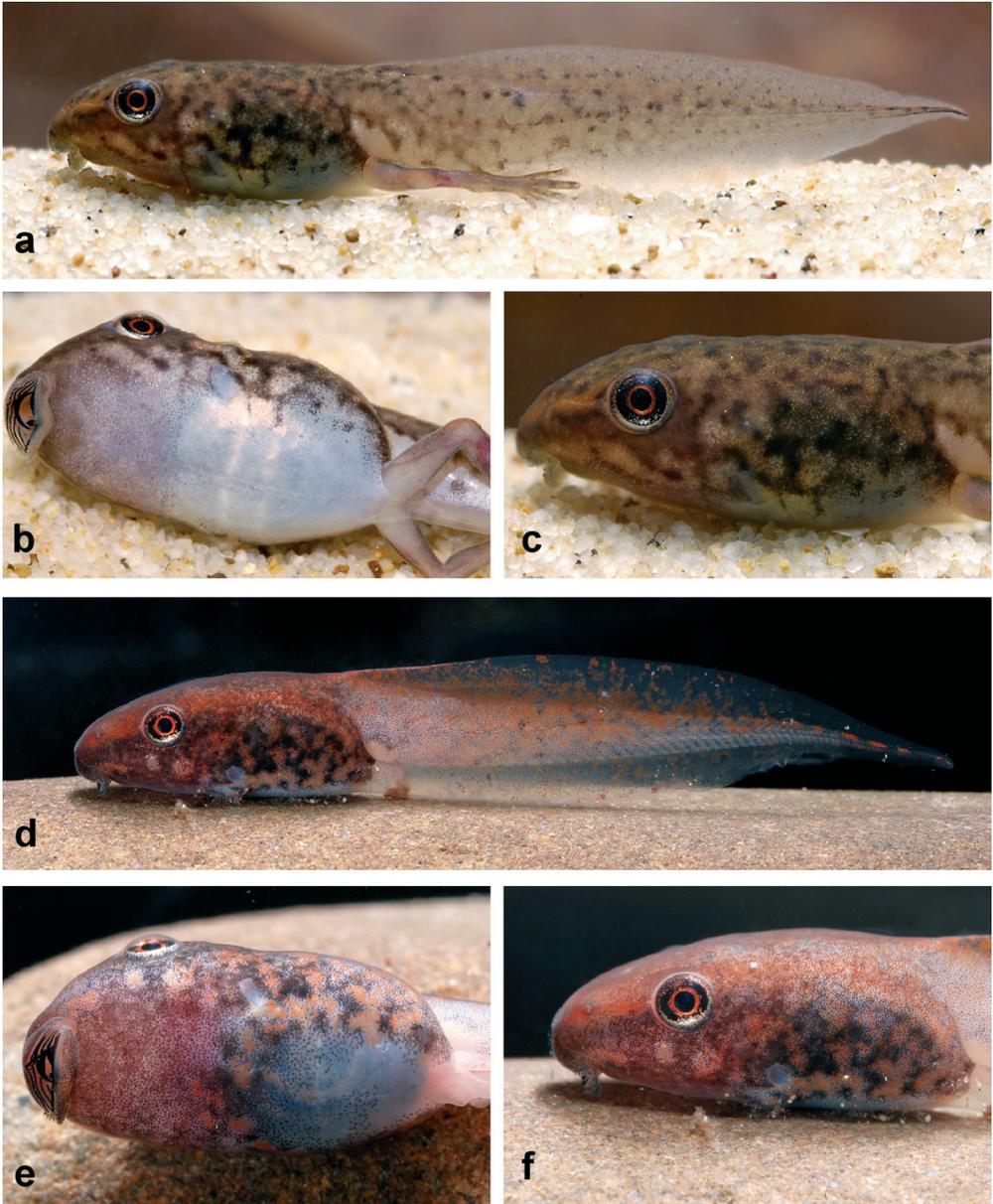


Fig. 2. Colouration of *Polypedates colletti* tadpoles in life: a) lateral view of fully grown tadpole (stage 40; Sama Jaya Nature Reserve, Kuching; 189P#1); b) ventral view close-up, note silvery-white, opaque venter colouration, dusted buccal and gular area, size and orientation of oral disk, and lateral eye position; c) lateral view close-up, note the dorsal dark mottling, replaced by mottling of light spots ventrally. d–f) specimen 384P from Gunung Mulu National Park to show colour variation in comparison to distant Sama Jaya population.

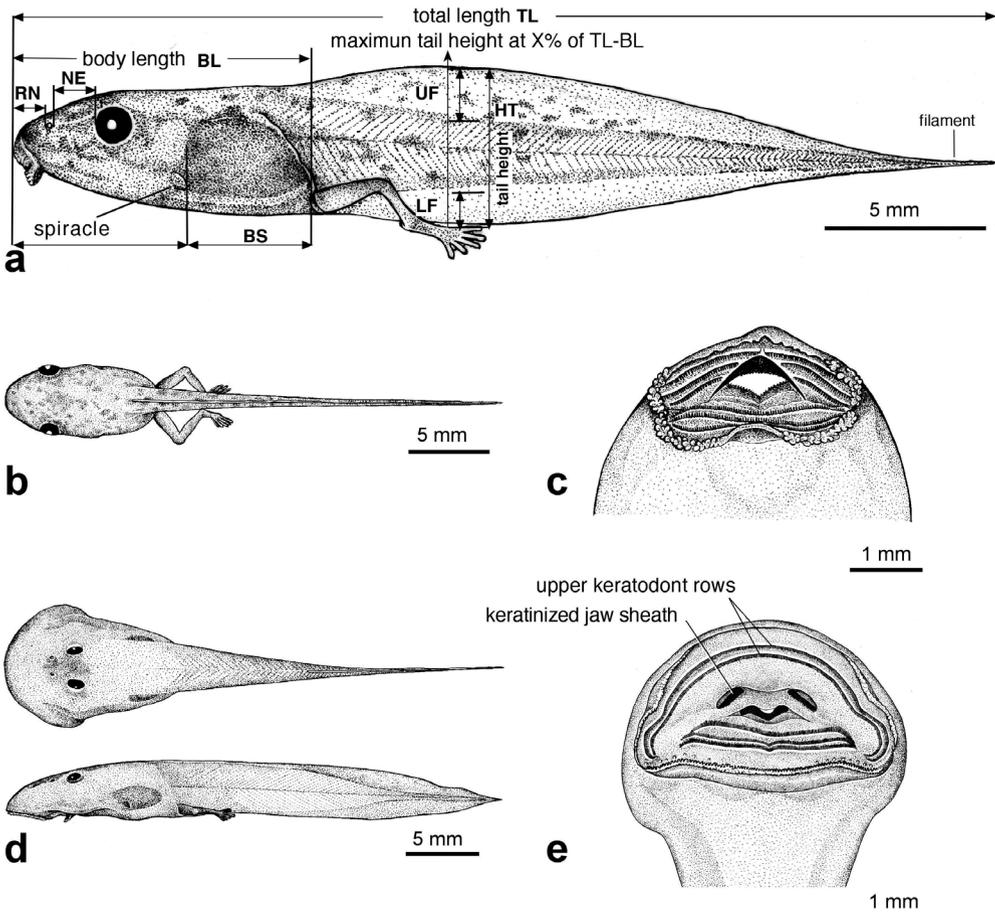


Fig. 3. a) Tadpole of *Polypedates colletti* (stage 40, 189P#1) lateral view with indication of measurements taken in Table 1; b) *P. colletti*, dorsal view, showing approximately parallel sided body contour and lateral eyes; c) *P. colletti* mouthparts showing keratodont rows and marginal papillae; d) *Ansonia hanitschi*, dorsal (above) and ventral (below) views; note the pear-shaped body contour in dorsal view, broadest at eye level; e) *A. hanitschi* ventral view of the head showing oral sucker and oral disk structures.

pear- or tear drop shaped. In dorsal view, the body is broadest approximately at eye level. The body is wider than deep. There is a constriction of the body contour behind the level of the eye, coinciding ventrally with the end of the oral sucker (Figs. 1b,e; 3d–e). The snout is extremely extensive. In life and adhering to the substrate, the anterior snout may be slightly concave in profile. The eyes are dor-

sally far away from the body contour in dorsal view. The narrowly spaced external nares are much closer to the eyes than to the snout (Fig. 1c). The spiracle is sinistral and the spiracular orifice is fused to the body wall, but exhibits an almost complete margin. The spiracle is directed posterodorsally, opening close to the substratum when the tadpole is attached by its sucker.

The oral disk is ventral and very wide, wider than the trunk. The marginal papillation of the oral disk is only present on the lower lip; the margin of the upper lip is fleshy and devoid of papillae (Fig. 4a-b). Marginal papillae are arranged uniserially. Papillae are short, blunt and adjoining (Fig. 4a). Submarginal papillae are present on the lower lip in form of 2–3 irregular rows of broad flat knobs (Fig. 4b). The oral disk margins possess inconspicuous lateral indentations (end of papillae row). The labial ridges bear uniserial keratodont rows. The Labial Tooth Row Formula (LTRF) is 2/3. The upper lip keratodont rows extend caudally beyond lower lip keratodont rows and bend medially at their lateral ends (Figs. 1e, 3e, 4e). The keratodonts are spoon-shaped with >20 incisions along the edge (Fig. 4b). Keratodont shape is different on the different rows (Figs. 4c–d). The upper beak is reduced to two short, widely spaced serrated edges. The lower beak is flat V-shaped and serrated along its edge.

The dorsal tail fin starts only at about 45% of the tail length. The narrow tail fins taper posteriorly with sharp angle and straight edges. The tail ends in a short, acuminate tip (Fig. 1a). Dorsal and ventral fins are approximately of the same height. The maximum height of the tail was in the purely muscular part, i.e., anterior to the tail fin. The anal siphon is located medially, embedded in a flap of skin extending from the posterior end of the body between the limb anlagen. The tail musculature is very strong; the height of the muscular tail at the base of the tail is almost equivalent to body height (Fig. 1a).

Variation. Colouration in stages earlier than shown in Fig. 1, e.g. stage 26 tadpoles, is lighter along the flanks and the muscular part of the tail. These early stages are dark pigmented on the dorsal side of the head, especially between the eyes, with dark marginal areas already present on the tail fin. The terminal acuminate tail tip appears somewhat less distinct in early stages. No variation in keratodont rows was observed.

Internal oral features. The choana (Fig. 4e,

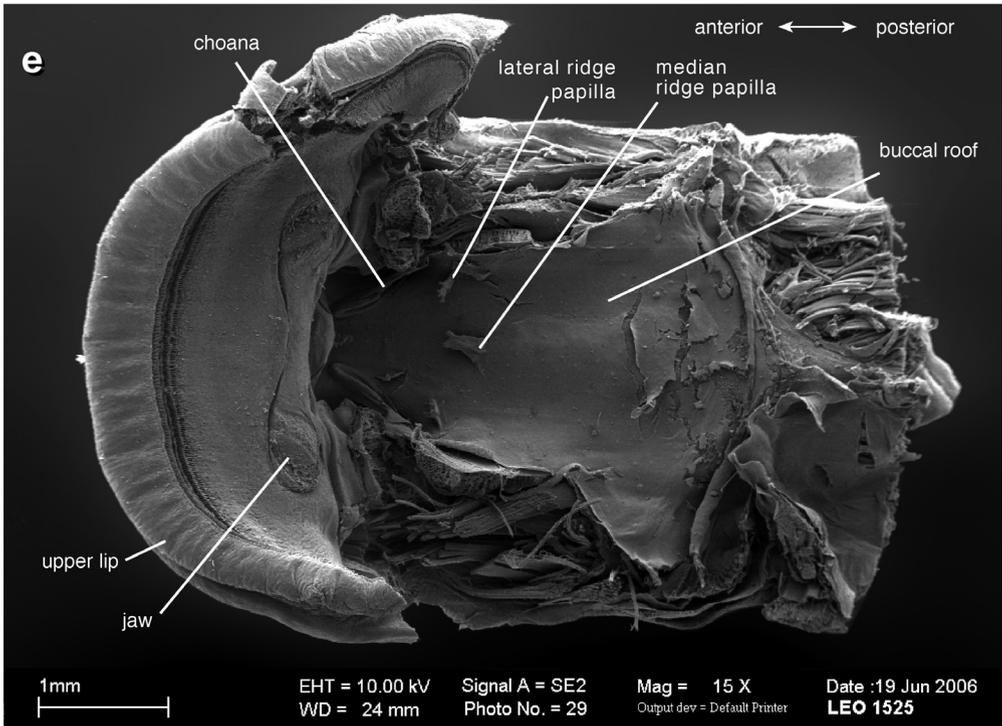
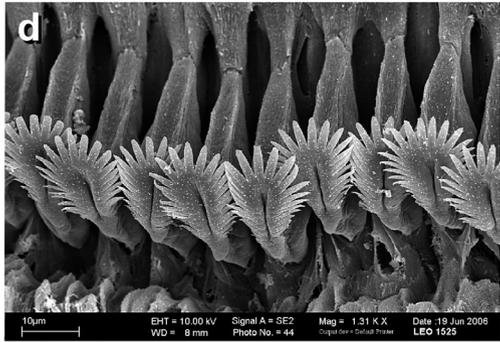
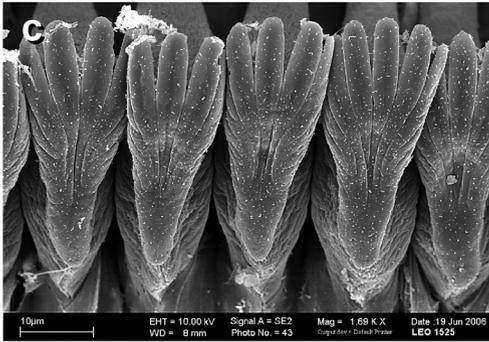
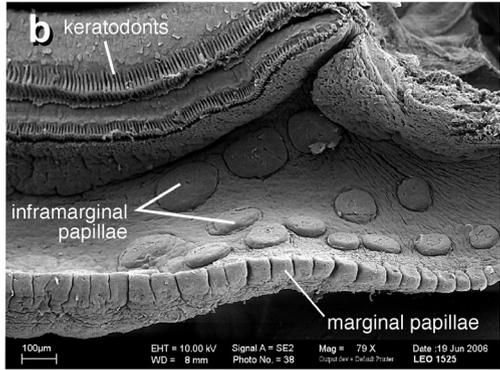
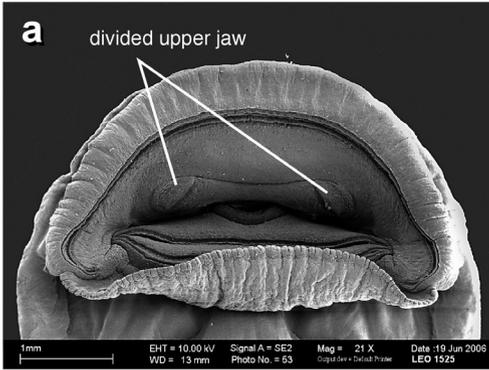
specimen 187#1, stage 33) is long, obliquely oriented and bordered with flaps that lack papillae. The postnarial papilla is absent. One long, slender lateral ridge papilla is present on each side. The median ridge separates the narial arena from the buccal roof arena. The median ridge is present in form of a moderately long, triangular, projecting flap. The buccal roof arena is parallel-sided anteriorly and widens posteriorly. Posterior to the medial ridge, it is free of papillae or large pustules (Fig. 4e).

In the oral floor region (187#4, stage 31), one broad, flat infralabial papilla with 5–6 fingerlike processes is present on each side, each reminiscent of a hand. Two lingual papillae, unbranched and elongate, are present medially on the anterior buccal floor. They are set relatively widely apart. A tongue pad was absent at this stage. Prelingual papillae are absent. The buccal floor arena is bordered laterally by only 5–6 moderately long, simple papillae (and some short pustules). Prepocket papillae are absent. Buccal pocket ridge papillae are absent. The surface of the buccal floor arena and further posteriorly onto the ventral velum is smooth (pustules absent). The margin of the ventral velum lacks projections.

Ecological notes. We collected *Ansonia hanitschi* tadpoles from smooth rock faces in cascades or waterfalls with foaming water during the day and by night. Most individuals were collected from vertical rock faces, but some were seen grazing algal overgrowth from more horizontally inclined rock surfaces. Tadpoles sometime emerge from the water and graze in the spray zone above the water line.

Fig. 4 (right page). SEM photographs of larval *Ansonia hanitschi* (187F#1). a) Overview of the oral sucker, ventral view (specimen 187F-5, stage 29); b) close-up of oral sucker lower lip showing the marginal and submarginal papillae; c) 2nd lower lip keratodont row; d) 3rd lower lip keratodont row, e) buccal roof.

Larval identities of *Ansonia hanitschi* and *Polypedates colletti*



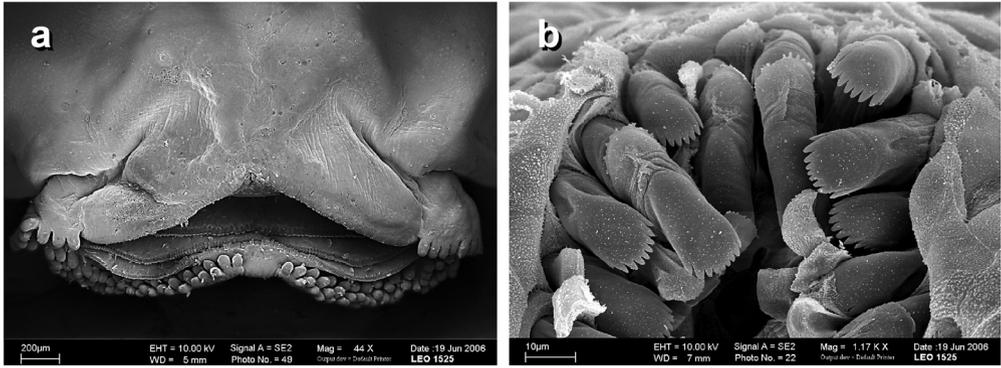


Fig. 5. SEM photographs of larval *Polypedates colletti*. a) Overview of oral disk, anterodorsal view (specimen 189F-1, stage 40); b) close-up of median notch in upper lip with first upper keratodont row.

Polypedates colletti

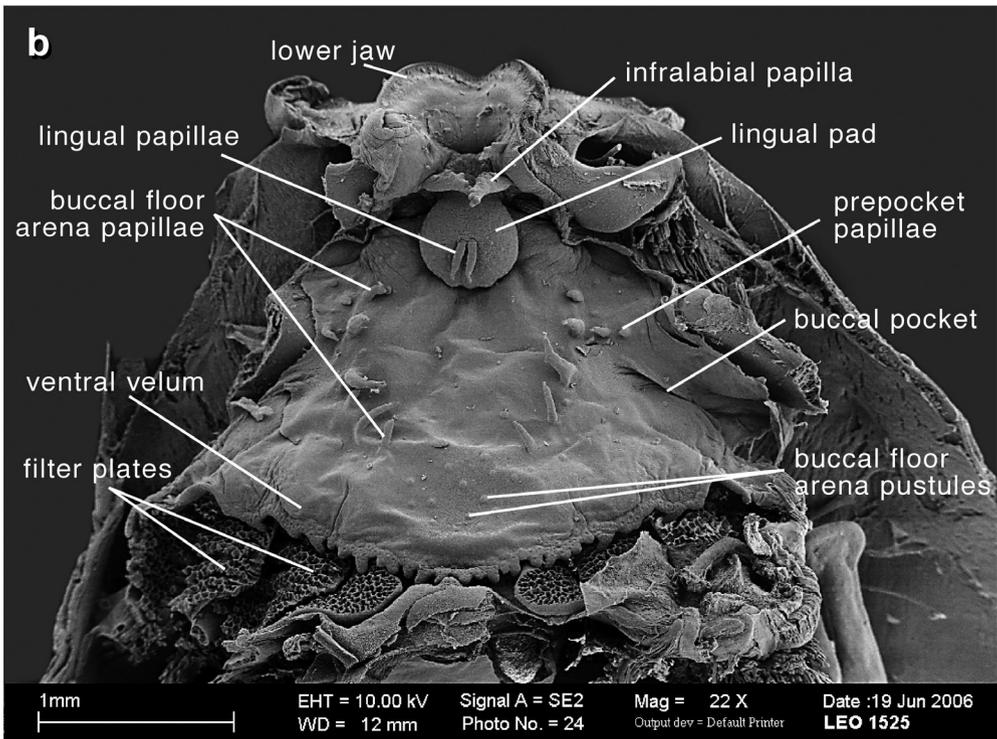
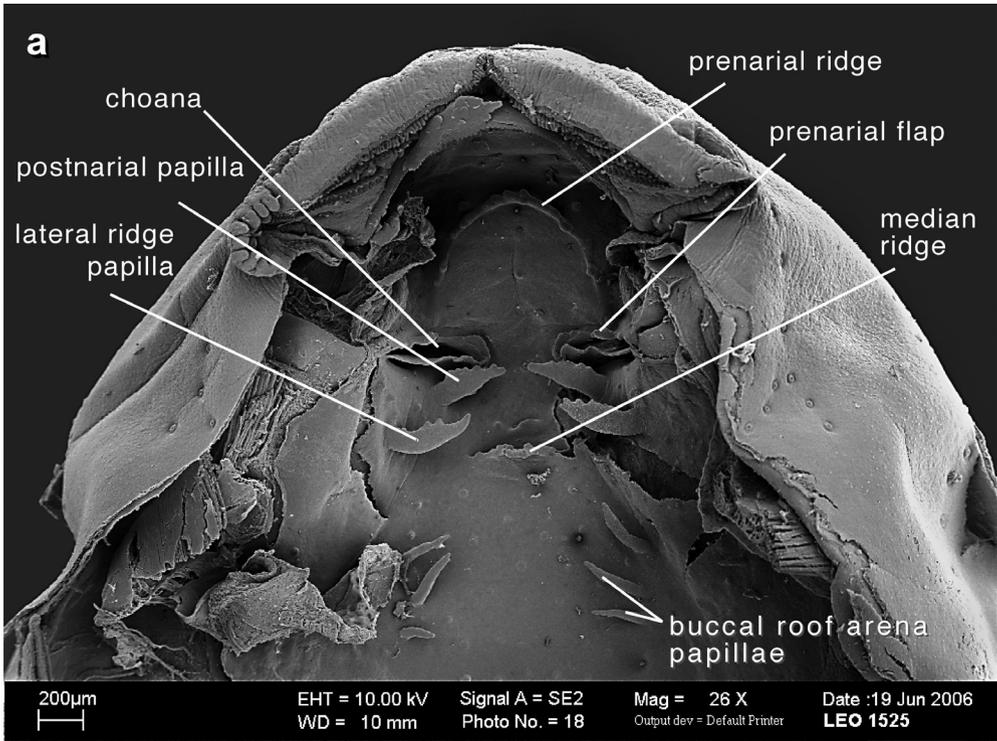
The following description is based on the sample from Sama Jaya Nature Reserve. Colour in life (Fig. 2, 189P#1, stage 40). Viewed from a distance in daylight, the tadpoles appear dark brown dorsally. In closer view (Fig. 2a) the body has a mottling of dark brown, indistinct spots on a lighter brown background. On the flanks the pattern is reversed to light spots on dark background in a distinct high contrast pattern. Ventrally the pattern transforms in a narrow gradient to the white venter colouration. The ventral side is white to silver in the trunk region but finely pigmented in the head region (buccal and gular) gradually becoming darker towards the oral disk (Fig. 2b). The skin of the oral disk is mostly without pigmentation. The viscera are fully covered, neither the gut coils nor the gills are visible through the skin. The marbling of the trunk continues caudally onto the tail, however, with decreasing contrast and intensity. Spots are mostly on the muscular part of the tail but extend onto the fins. Epidermal melanophores are small and round. The elongate flagellum of the tail is marked with a longitudinal stripe. The iris is very distinct: black background with dusted brown pigment in the upper half, silver-white pigment in the lower half, and a narrow cop-

per-red ring around the pupil. Beyond the iris, the pattern continues onto the eye sclera (brown dorsally, silver-white ventrally). The caudal blood vessels are inconspicuous.

Colour in preservation (4% formalin) is similar to colouration in life, but specimens bleached rapidly and the epidermis became brittle in some individuals. Preserved specimens lighten up and can become almost entirely white, the tail fin fully translucent. The venter remains milky and the gut coils and entrails are faintly but not clearly visible. The iris is dark brown, the sclera black.

External morphological features. *Polypedates colletti* has a moderately large tadpole, reaching 32.8 mm in total length (Tab. 1). The body shape in dorsal view is parallel-sided and cylindrical (Fig. 3). Body approximately as wide as deep. The snout is narrow and rounded in dorsal view and moderately convex in lateral view. The eyes are positioned laterally, the cornea projecting beyond the body contour. The widely spaced external nares are closer to the snout than to the eye in lateral view (Fig. 3a). The spiracle is sinistral. The posterior spiracular orifice is fused to the body, with only an anterior crescentic margin at the orifice. The spiracle is directed

Fig. 6 (right page). SEM photographs of larval *Polypedates colletti*. a) buccal roof; b) buccal floor.



posteriorly, opening ventral to the longitudinal body axis in lateral view. The oral disk is anteroventral. The marginal papillation of the oral disk has a broad gap on the upper lip and a narrower gap on the lower lip (Figs. 3c, 5a). Lateral oral disk indentations are present. The upper lip has a notch medially, through which the first keratodont row passes (Figs. 5a–b). The oral disk marginal papillae are arranged in double to triple rows (Fig. 5a). Some submarginal papillae are located in lateral areas of the disk. Marginal papillae are short and terminate in blunt tips. The labial ridges bear uniserial keratodont rows. The Labial Tooth Row Formula (LTRF) is $4(2-4)/3$. Keratodonts are spoon-shaped with 10–12 relatively shallow serrations along the edge (Fig. 5b). Keratodonts of the distal (peripheral) tooth rows on upper and lower lip, respectively, are smaller than those on the inner rows. Upper and lower beaks are undivided, well developed, dark pigmented, and serrated along their edges.

The dorsal tail fin begins at the body-tail junction. The tail fins are moderately arched and taper posteriorly with slightly concave contours into a distinct elongate flagellum. Dorsal and ventral fin are approximately of the same height (Tab. 1). The maximum height of the tail is in the anterior half of the tail length. The anal siphon is located at a dextral position, at the base of the ventral tail fin. The height of the muscular tail at the base of the tail is only slightly less than body height (Fig. 2d).

Variation. A fifth (proximal) very short, divided keratodont row may be present on the upper lip in some specimens. Specimens from the Kerangas site at Gunung Mulu National Park deviated from the above description in: (1) red-brown instead of brown body pigmentation background (Figs. 2d–f), (2) extension of the flank marbling ventrally across the belly behind the gill region (Fig. 2e), and (3) less distinct dark markings on tail.

Internal oral features. The prenarial area of the buccal roof bears an arched ridge. The

choana (Fig. 6a) is transversely oriented. Its margins are bordered with flaps of epithelium but without projections. A prenarial papilla is present (removed by cut in Fig. 6a), similar in shape to the postnarial one. The single postnarial papilla is long and flat. It is followed posteriorly and more ventrally by a large, flat lateral ridge papilla. The median ridge is low. The buccal roof arena is oval; longer than wide. The lateral border of the buccal roof arena is marked by three papillae, whereas the center of the arena is covered with flat pustules.

In the oral floor region (Fig. 6b), one infralabial papilla is present on each side posterior to the lower jaw. The tongue anlage is formed as a round pad in advanced stages. It is medially located on the anterior buccal floor. The pad bears two slender lingual papillae. Anterior to the lingual papillae a pair of prelingual papillae is present (not visible in Fig. 6b). The buccal floor between and posterior to the buccal pockets has a series of 5–8 moderately long buccal floor arena papillae on each side. The most anterior ones are short. Prepocket papillae (2–3) are present laterally to the buccal floor arena papillae. At the posterior end of the buccal floor, epithelial pustules are scattered in the central area. The ventral velum is without median notch and bears a series of blunt short projections along its margin (Fig. 6b).

Ecological notes. *Polypedates colletti* tadpoles were collected from shallow (< 50 cm) leaf filled depressions (uprooted tree in one site) holding peaty brown water. We could not see tadpoles during the day, but at dusk, tadpoles emerged from their place of concealment under leaves. At night, tadpoles rested on the leaves with only occasional slow movements and maneuvering by undulation of their tail filament. They can hover in the water column almost motionless, using the tail filament for turning slowly or holding position in the water column. When disturbed they dashed under leaf debris at the bottom.

Discussion

For any inventories of amphibian fauna, it is essential to be able to identify tadpoles. Identification is often relatively easy at the generic level and reliable determination keys are available (INGER, 1985; for East Malaysian frogs). However, within genera, identification of species can be difficult or ambiguous, especially in regions where new species can be expected to be discovered. If available at all, keys mostly rely on traditional characters from preserved specimens, but often neglect other features that permit quick unequivocal identification in the field, such as colouration and colour patterns. Recently, genetic barcoding, i.e., the identification of specimens by gene sequences, has been adopted as a tool in amphibian taxonomy and is particularly useful in matching tadpoles to adults (e.g., THOMAS et al. 2005, VENCES et al. 2005, HAAS et al. 2006). Genetic matching allows unequivocal identification in the laboratory, but is of little help in the field; there still remains the need to identify tadpoles quickly and accurately in the field, and determination keys for Bornean species await completion.

On the island of Borneo, 12 species of the genus *Ansonia* have been described, and additional species may be recognized in the future. Of these, tadpoles of four species are unknown and four appear in the literature only with abbreviated description (no figures) (DAS & HAAS 2006). Among the latter is the tadpole of *A. hanitschi*. Available accounts in the literature (MALKMUS et al. 2002, INGER & STUEBING 2005) are brief and do not mention features of *A. hanitschi* differentiating it from other *Ansonia* tadpoles. Furthermore, they are not congruent with our data from morphologically and genetically identified *A. hanitschi* specimens. Thus we think that these sources do not describe *A. hanitschi* and consider the tadpole of *A. hanitschi* as formally undescribed.

However, INGER (1985) described large (up to 30.8 mm) tadpoles from the Mt. Kina-

balu as *Ansonia* sp. that fit our description of *A. hanitschi*. He speculated that the tadpole could belong to either *A. hanitschi* or *A. platysoma*. He rejected both options because he suspected that these moderate to small sized toads should not have such large tadpoles. In a subsequent paper, INGER (1992) assigned a sample of large *Ansonia* tadpoles from Sungei Silau-Silau, Gunung Kinabalu, to *A. longidigita* INGER, 1960. The description of these tadpoles is in many details in conflict with his previous description of *A. longidigita* tadpoles (INGER 1985), and also with a third description (INGER & STUEBING 2005). We suspect that the „*A. longidigita*“ tadpoles in INGER (1992), collected at Silau-Silau and Sungei Liwagu, Mt Kinabalu, could be *A. hanitschi* tadpoles as inferred from morphological congruence with our data. In our samples, *A. longidigita* could be ruled out, because (1) in our specimens the first finger was much shorter than the second in metamorphosing specimens (Fig. 1f) (INGER 1966, INGER & STUEBING 2005), and (2) > 99% genetic match of *A. hanitschi* larvae and adults from the same mountain range.

The lack of details or uncertain assignments of larvae to species in the genus *Ansonia* makes it impossible to give all diagnostic differences between all *Ansonia* species for unequivocal field identification at this point. However, the tadpole of *A. hanitschi* stands out as one of the largest *Ansonia* tadpoles so far described (INGER 1985, 1992; INGER & STUEBING 2005) and as one that prefers strong laminar or foaming currents as microhabitat. The current evidence allows preliminary comparisons between the tadpole of *A. hanitschi* and the larvae of other *Ansonia*:

Following the description in INGER (1985: 17; „Assignment [...] is tentative“), *A. albomaculata* INGER, 1960 is (1) much smaller (max. total length 11.8 mm) than *A. hanitschi*, (2) has a distinct colour pattern with well-defined light and dark areas, (3) lacks infralabial papillae, and (4) the tail tip is rounded.

Ansonia anotis INGER, TAN & YAMBUN,

2001 (INGER et al. 2001; tentative species assignment of larva) reaches a similar size (10–13 mm head-body length), but markedly differs from *A. hanitschi* by possession of an abdominal sucker. Recently, it has been suggested to remove the taxon from *Ansonia* and to place it under *Sabaphrynus* (MATSUI et al. 2007).

Ansonia guibei INGER, 1966 (MALKMUS & KOSUCH 2000) differs from *A. hanitschi* by (1) smaller maximum body size; (2) more rhomboidal, angular body contour in dorsal view; (3) more extensive dorsal tail fin reaching body-tail junction; (4) tapering tail that lacks a terminal acuminate tip; (5) preference for slower water currents (pers. obs.); upper keratodont rows not curved around lateral ends of lower rows; (8) lower jaw sheath divided.

The assignment of tadpoles to *A. longidigita* is currently ambiguous (see above).

Ansonia leptopus GÜNTHER, 1872 (INGER 1992, INGER & STUEBING 2005) differs from *A. hanitschi* by (1) smaller maximum body size; (2) conspicuous colouration (characteristic dark markings on light background); (3) more extensive tail fins; (4) lack of terminal acuminate tail tip (pers. obs.); (5) preference for leaf litter (versus rocks in *A. hanitschi*) in sluggish waters (pers. obs.).

According to INGER (1985) *A. minuta* larvae are smaller than *A. hanitschi*. *Ansonia minuta* larvae measured only 6 mm head-body length at stage 27. *A. minuta* is restricted to lowland habitats.

Ansonia spinulifer MOCQUARD, 1890 as described in INGER (1992) can be diagnostically separated from *A. hanitschi* by (1) smaller size; (2) upper lip keratodont rows not curved around lower lip keratodont rows; (3) lack of acuminate tail end; (4) double row of labial papillae (INGER 1992: Fig. 2).

Tadpoles of the other Bornean *Ansonia* species (*A. fuliginea* [MOCQUARD, 1890]; *A. latidisca* INGER, 1966; *A. platysoma* INGER, 1960; and *A. torrentis* DRING, 1984) remain unknown (DAS & HAAS 2006).

The tadpole of *P. colletti* can be diagnosed

by the combination of (1) median notch in upper lip; (2) small median gap in lower lip papillation; (3) elongate tail filament; (4) lateral eyes; (5) lack of conspicuous skin gland fields; (6) LTRF 4(2–4)/3; (7) unique mottled pattern; (8) distinct iris and eyeball colouration in life (bright red pupil ring in combination with scattered red-brown pigment cells in upper and white pigment cells in lower parts of eye).

The combination of these characters allow discrimination against potentially syntopic species, in particular *Hylarana raniceps* and *H. glandulosa* (possess extensive gland fields), *I. quadriporcatus* (lacks tail filament, broad ventral gap in papillation, no red pupil ring, small larvae), or species of *Limnonectes* (dorsolateral eyes, no filament). Lateral eyes, mottled pattern and an elongate tail filament are present in *H. erythraea*, which differs, however, from *P. colletti* in having a relatively higher tail fin, larger size at similar stage, different iris colouration (red-brown band across iris), different LTRF: 1/2(1), and lack of gap in lower lip papillation. Our account of the *P. colletti* tadpole differs most notably from INGER's (1985) account in the presence of a narrow median gap in the lower lip papillation (Fig. 5).

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