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Identity of larval *Meristogenys* from a single stream in Sabah, Malaysia (Amphibia: Ranidae)

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A morphological and molecular survey was conducted to examine the association between larvae and adults of Bornean torrent frog *Meristogenys* from a stream in Mahua, Crocker Range, Sabah, Malaysia. We identified five discrete morphotypes of larvae, which also showed considerable genetic differentiation. Each larval morphotype was associated through DNA sequence comparisons with adult specimens that had been identified morphologically. One morphotype, identified as *M. orphnocnemis*, was less similar to the larva of this species than to an unidentified larva, both reported previously. Adults of two other larvae were identified as *M. amoropalamus* and *M. kinabaluensis*, but the larval morphotypes differed from previous descriptions of these larvae. Adults associated with another morphotype resembled *M. whiteheadi*, but had longer tibia. This larval morphotype was dissimilar to previous descriptions of the *M. whiteheadi* larva, but was similar to another unidentified larva previously reported. No adult specimens were associated with the fifth larval morphotype, which matched the larva reported as *M. amoropalamus*. From these results, we suspect that either some previous studies include misidentifications or several undescribed, cryptic taxa morphologically resemble known adult species, but differ as larvae. *Zoological Journal of the Linnean Society*, 2007, **151**, 173–189.

ADDITIONAL KEYWORDS: Borneo – cryptic taxa – DNA sequence – larval and adult association – larval morphotype.

INTRODUCTION

The ranid genus *Meristogenys* comprises small to medium-sized, moderately slender, long-limbed frogs. These frogs inhabit primary or secondary forests in hilly or mountainous regions with clear, rocky streams that provide breeding sites. The tadpoles of this and two allied genera [*Amolops* (*s.s.*) and *Huia*] are specialized for life in strong currents, having heavy bodies that are broadly rounded at the snout and flat below. A sizeable oral disc beneath the snout is followed by a large sucker that covers a sizeable portion of the abdomen ('gastromyzophorous' larvae: Inger, 1966). These unique larval organs, first reported by Mocquard (1890) for *Ixalus nubilus* from Borneo, clearly separate the three genera from *Rana*, but the adults are not conspicuously different from some *Rana* species and have been treated variously in terms of genus. Until Yang's (1991) taxonomic revision, *Meristogenys* species had been placed in *Rana* and *Amolops* (*s.l.*).

Inger (1966) separated *Meristogenys* members from *Rana* by grouping *R. jerboa* and *R. cavitympanum* in the genus *Amolops* (*s.l.*), synonymizing *R. whiteheadi* with *A. jerboa*, and describing *A. kinabaluensis*. At the same time, he described the *A. cavitympanum* larva, as well as five gastromyzophorous larval forms (larvae A–E) that he assigned to *Amolops*, but could not identify as to species.

Later, Inger & Gritis (1983) described *A. phaeomerus* and *A. poecilus*, assigned larvae A and B to these respective species, revived *A. whiteheadi* as a species distinct from *A. jerboa*, and added three new gastromyzophorous larvae (F, G and H), whilst discarding larva C. Inger (1985) assigned larva D to *A. kinabaluensis*, based on the lack of outer metatarsal tubercles in advanced stages. Matsui (1986) described *A. amoropalamus*, *A. macrophthalmus* and *A. orph-*

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nocnemis and reported another larval form from one of the paratypic localities of *M. orphnocnemis*.

Yang (1991) split the composite genus *Amolops* (*s.l.*) into three distinct genera, *Amolops* (*s.s.*), *Huia* and *Meristogenys*, mainly on the basis of larval differences. Except for *A. cavitympanum*, which was moved to *Huia*, all *Amolops* (*s.l.*) species from Borneo were placed in *Meristogenys*. *Amolops* (*s.s.*) is confined to species from China and Indochina west to Nepal. Yang (1991) also described the larval forms of six *Meristogenys* species: *M. amoropalamus*, *M. kinabaluensis*, *M. orphnocnemis*, *M. phaeomerus*, *M. poecilus* and *M. whiteheadi*. Although Yang (1991) did not clearly identify his sources, his descriptions of larval *M. phaeomerus* and *M. poecilus* seem to be derived from Inger & Gritis (1983) and Inger (1985), *M. whiteheadi* from Boulenger (1893) and Inger (1966), and *M. kinabaluensis* from Inger (1985). Yang (1991) also newly reported larval *M. amoropalamus* and *M. orphnocnemis*. The gastromyzophorous larva recorded from Borneo for the first time by Mocquard (1890) cannot be assigned to any of these larval forms because of the scarcity of comparable characters described, and we omit this form from further discussion.

In summary, among 11 larval forms reported for *Meristogenys*, six have been assigned to known species, whilst the remaining five [larvae E–H of Inger (1985) and a larval form reported by Matsui (1986)] have not been identified. Previous associations of adults and larvae have been based on advanced-stage larval characters, such as thigh colour patterns, degree of toe web development, and the presence or absence of outer metatarsal tubercles. However, the stability of these characters has never been assessed. To re-examine the previous associations of larval and adult *Meristogenys*, we collected larvae and adults from a stream in Sabah, Malaysia, classified the larvae morphologically, and associated the resultant larval groups with adult specimens through DNA sequence similarities. We then compared our results with those of previous studies.

MATERIAL AND METHODS

Research site

The fieldwork was conducted in August and December 2003, March and August 2005, and March 2006. The research site was located at Mahua on the eastern slope of the Crocker Range National Park, Tambunan District, Sabah State, Malaysia. Our survey was conducted mainly within 5 km north-west of the Mahua campsite along the Mahua River, which varied in width from 5 to 10 m and included three large waterfalls. This river joins with the Pegalan River a few kilometres south-east of our study site, and flows finally into the South China Sea on the west coast of Sabah. We collected adults on the forest floor or perching on rocks and low vegetation near streams at night by hand or net, and gathered tadpoles adhering to rocks in swift currents by net during both daytime and night-time.

Adult specimens

We anaesthetized all frogs collected with acetone–chloroform and preserved small pieces of tissue in 99% ethanol for later DNA analysis. The frogs were then fixed in 10% formalin and stored in 70% ethanol. Except for the adult males with nuptial pads and vocal sacs, all frogs were dissected to determine gender and maturity. Frogs with mature ovaries or convoluted oviducts were treated as female adults, whereas those with immature gonads were omitted from the study. In all, 91 adult specimens from this area were examined. We measured snout–vent length (SVL), head width (HW) and tibia length (TL) and recorded patterns on the rear of the thigh and ventral surface of the tibia, and the degree of development of the toe web. For descriptions of thigh patterns, we followed Matsui (1986): blotched or pied, mottled, and dusted or dotted. The tibia pattern and the toe web development were recorded in four (A–D) and five (A–E) states, respectively, following Inger & Gritis (1983).

Larval specimens

In total, 192 larval specimens from this area belonging to Gosner (1960) stages (st.) 26–41 were examined. We selected this range of developmental stages because tadpoles at st. 25 vary widely in body size and those at st. 42 or older tend to lose some diagnostic characters. All tadpoles were fixed and preserved in 5% formalin. For several specimens that showed unique morphology by gross field inspection, we preserved a small piece of tissue in 99% ethanol before formalin fixation. We measured the following nine characters (Fig. 1): (1) total length (TTL: from tip of the snout to tip of the tail); (2) head–body length (HBL: from tip of the snout to posterior end of the body, not to the junction of the posterior body wall with the axis of the tail myotomes); (3) head–body width (HBW: the maximum width, excluding the spiracle); (4) head–body height (HBH: the maximum height); (5) sucker width (SUW: the maximum width); (6) oral disc width (ODW: the maximum width); (7) snout width (SNW: measured at the level of the angle of the jaws); (8) eyeball diameter (ED: measured longitudinally); and (9) tail height (TLH: the maximum height). The tail length (TLL) was calculated by subtracting the HBL from the TTL.

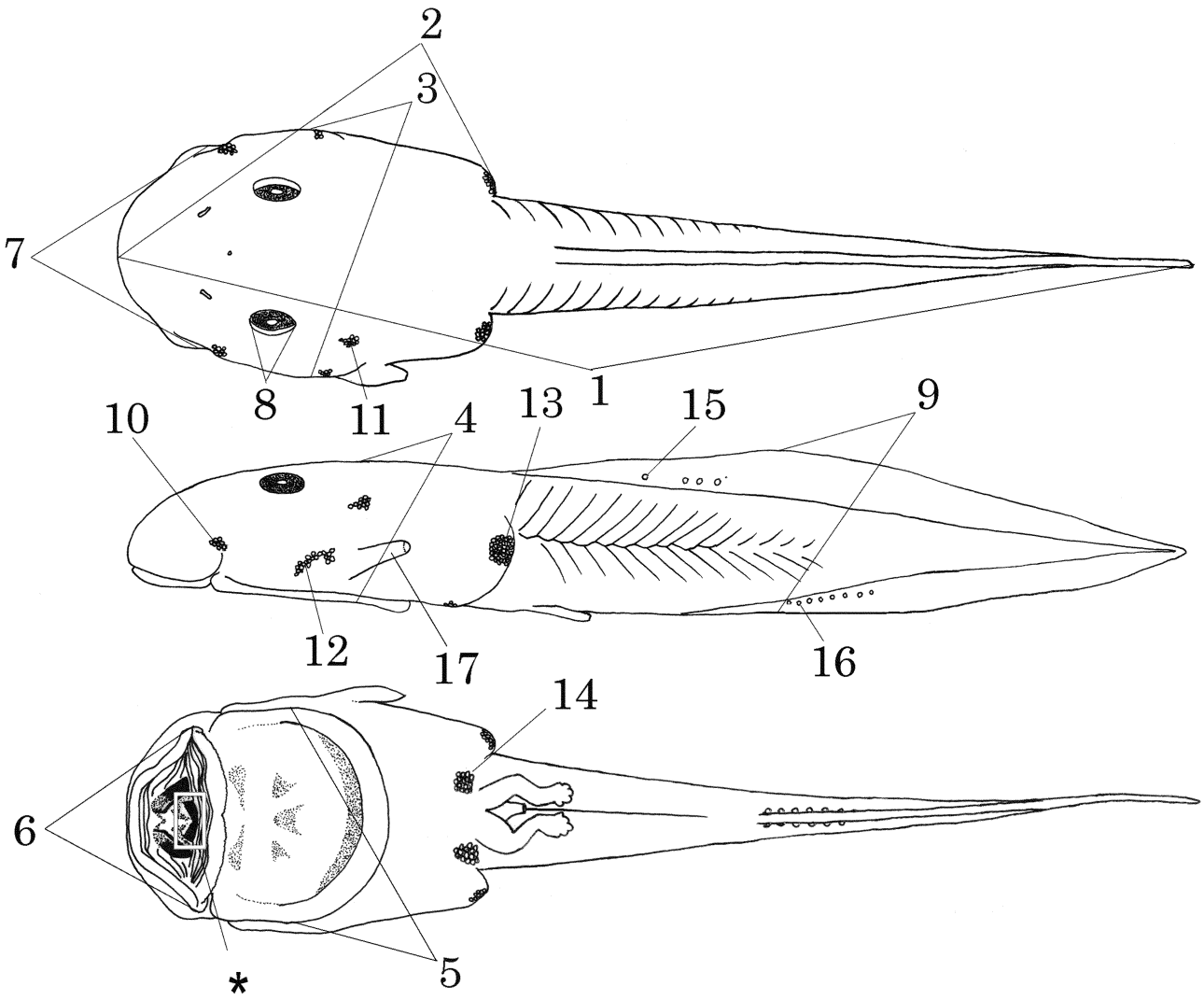

Figure 1. Character dimensions, glands and a spiracle of larval *Meristogenys* (morphotype 4). 1, total length (TTL); 2, head–body length (HBL); 3, head–body width (HBW); 4, head–body height (HBH); 5, sucker width (SUW); 6, oral disc width (ODW); 7, snout width (SNW); 8, eyeball diameter (ED); 9, tail height (TLH); 10, infraorbital glands; 11, postorbital glands; 12, prespiracular glands; 13, posterior midlateral glands; 14, ventral glands; 15, dorsal fin glands; 16, ventral fin glands; 17, spiracle; *the white square indicates the area shown in Figure 3.

Tadpoles of some species of *Amolops* (*s.l.*) have small serous glands on the infraorbital, postorbital, prespiracular, posterior midlateral and ventral areas of the head–body and the dorsal and ventral tail fins (Yang, 1991; Fig. 1). We counted the number of these glands on the left side. Larval *Meristogenys* also have keratinized spinules (surface projections: Fig. 2) on the dorsum that may abate turbulence as water flows over the body (McDiarmid & Altig, 1999). Patterns of these projections were recorded as present on the head and body, present only on the head, or absent.

In addition to describe conventional labial tooth row formula (LTRF: Altig, 1970; McDiarmid & Altig, 1999), we classified labial teeth rows into the following four groups (from anterior to posterior) to facilitate comparisons with previous studies (e.g. Inger & Gritis, 1983): upper (= anterior labium) undivided, upper divided, lower (= posterior labium) divided, and lower undivided rows. In some specimens, the most posterior row had a slight gap, forming a pair. We considered this pair as an immature undivided, rather than a normal divided, row, as the pair seemed to approach each other as the larva grew, and finally connect. In order to avoid confusion, we treated the pair as an undivided row. Lower beak shapes were recorded as right and left beaks divided, undivided, or connected with a thin, grey corneous film (Fig. 3). We counted the number of serrations on the left lower beak of divided

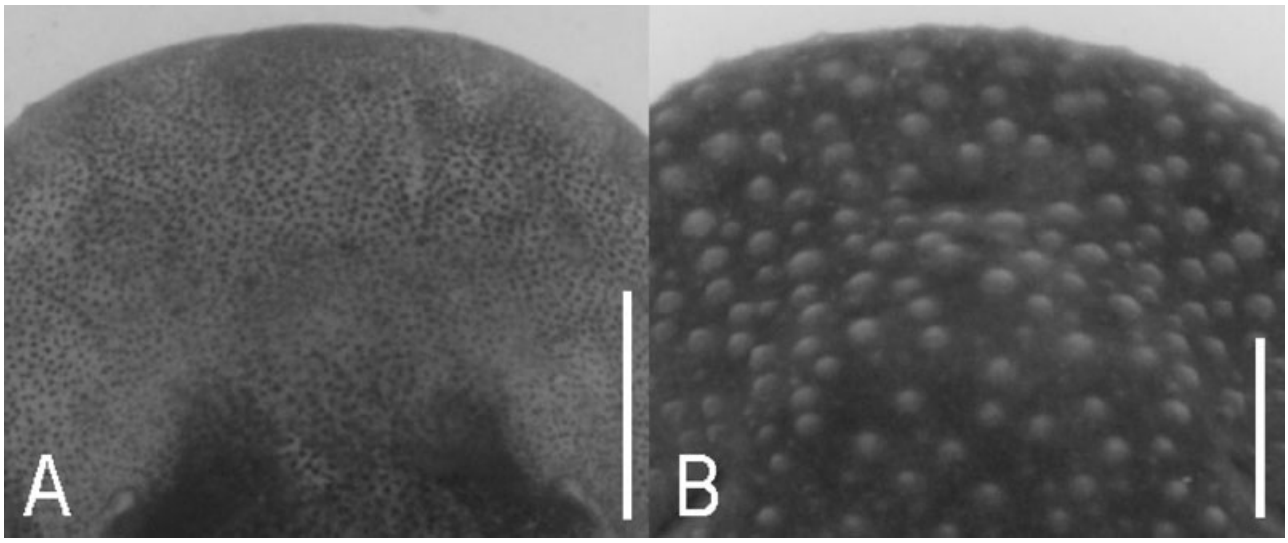

Figure 2. Dorsal views of the snout surface: A, without projections (morphotype 3-a); B, with projections (morphotype 3-b). Scale bar = 1 mm.

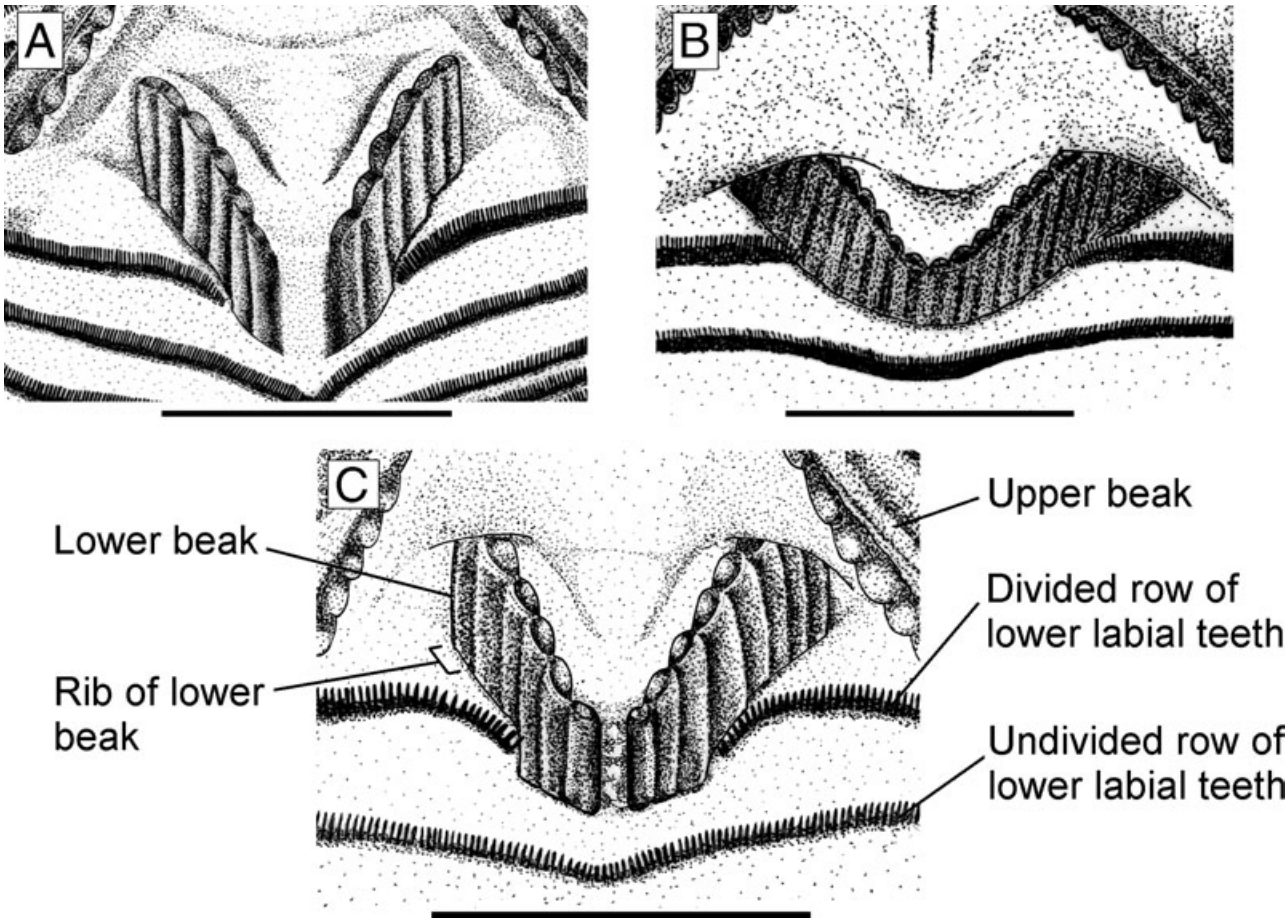

Figure 3. Three types of the lower beaks of larval *Meristogenys*. A, right and left beaks divided (morphotype 3-a); B, undivided (morphotype 4); C, connected with a thin, grey corneous film (morphotype 1). Scale bar = 1 mm.

beaks or on both the left and the right beaks of undivided beaks.

DNA ANALYSIS

We used part of a mitochondrial ribosomal gene (12S rRNA gene) for DNA analysis for two reasons: first, compared with the nuclear genome, the mitochondrial genome houses certain phylogenetically favourable properties, such as the absence of intermolecular genetic recombination (Avise, 2000) and heterozygosity; and second, nuclear genes evolve at a much lower rate; for example, *M.* cf. *orphnocnemis* and *M. kinabaluensis* differ in only 8 bp in 532 bp of partial tyrosinase sequences, but in 40 bp in 553 bp of 16S rRNA gene sequences (Roelants, Jiang & Bossuyt, 2003).

DNA was extracted, using standard phenol–chloroform extraction procedures, from small amounts of tissue preserved in ethanol. We used the primers 12SA-L (Palumbi *et al*., 1991) and H1548 (Matsui *et al*., 2005) to amplify a *c*. 440-bp section of the 12S rRNA gene. The polymerase chain reaction (PCR) cycling, precipitation and sequencing procedures were identical to those of Matsui *et al*. (2006). Newly obtained sequences were deposited in GenBank (AB262538–AB262550).

In estimating the phylogenetic relationships, we used homologous sequences of the following ranine species for hierarchical outgroup rooting: the type species of *Amolops* (*A. marmoratus*: AB211463) and *Huia* (*H. cavitympanum*: AB211466), both of which were once grouped in *Amolops* (*s.l.*), together with *Meristogenys* (see above). We also used a sequence from *Fejervarya limnocharis* (AY158705), which belongs to the Ranidae subfamily Dicroglossinae. We subjected the data to three different methods of phylogenetic reconstruction: maximum parsimony (MP) analysis, with transitions and transversions given equal weight; maximum likelihood (ML) analysis, based on the substitution model and phylogenetic parameters derived from a hierarchical likelihood ratio test (hLRT) in Modeltest 3.06 (Posada & Crandall, 1998); and Bayesian analysis, with the model derived from an hLRT in MrModeltest (Nylander, 2002), with the run using 1000 000 generations, sampling a tree every 100 generations, and discarding the initial 1000 trees for burn-in. We followed Matsui *et al*. (2006) for the MP and ML heuristic methods.

Except for the Bayesian approach, which used MrBayes (Huelsenbeck & Ronquist, 2001), all analyses were conducted using PAUP4.0b (Swofford, 2002). Pair-wise comparisons of corrected sequence divergences [Kimura's two-parameter (K2p) distances (Kimura, 1980)] were also calculated using PAUP. The confidential values of MP and ML trees were tested using bootstrap analyses (Felsenstein, 1985), with 2000 replicates for MP and 500 for ML (Hedges, 1992). Following Matsui *et al*. (2006), we considered bootstrap values of more than 70% and posterior probabilities of more than 95% to be significant.

RESULTS

LARVAL MORPHOLOGY

As a result of morphological observations, the 191 larval specimens were divided into five discrete morphotypes (Table 1), which we named 1, 2, 3-a, 3-b and 4. Each is described below.

Morphotype 1

Four tadpoles in st. 29–31 had the LTRF of 7(4–7)/7(1) or 7(4–7)/8(1) [i.e. four divided upper rows and six or seven undivided lower rows], glands on the dorsal and ventral fins, and lacked surface projections (Fig. 2A). We called this series morphotype 1 (Fig. 4). Although their lower beak appeared at first glance to be divided, the beak parts were connected by a thin sheet of grey corneous film (Fig. 3C). No other tadpoles in our collection showed this incomplete division of the lower beak (Fig. 3A, B). Morphotype 4 tadpoles resembled morphotype 1 in the presence of dorsal fin glands, but differed in having ventral glands and in LTRF [6(4–6)/6(1), i.e. three divided rows of the upper labial teeth].

Morphotype 2

Three tadpoles in stages 26–27 had the LTRF of 7(4–7)/6(1) [i.e. four upper divided and five undivided lower rows], glands on the ventral fin, surface projections and a single lower beak. We called this series morphotype 2 (Fig. 4). The surface projections were far smaller than the size of the narial opening. Morphotype 1 also had four divided rows of the upper labial teeth, but differed in other characters, as described above. Morphotype 4 tadpoles also had undivided lower beaks, but differed in having ventral glands and three divided rows of the upper labial teeth and lacking surface projections.

Morphotype 3

More than two-thirds (133/191) of the tadpoles examined had three rows of divided upper labial teeth [upper LTRF = 6(4–6)/] and two-part lower beaks (Fig. 3A). We classified these as morphotype 3. However, they were highly variable in the number of glands on the ventral fin, the presence of brown surface projections, and the lower LTRF (number of undivided rows of lower labial teeth; see below). Closer

Table 1. Summary of characters of larval *Meristogenys* examined for this study and reported in previous studies

					Rows of denticles						
					Upper		Lower		State of lower beak(s)	Serrae of beaks	
	Reference	*N*	stage	Surface projections*	undiv.	div.	div.	undiv.†		upper	lower‡
morphotype 1	present study	4	29–31	a	3	4	1	6–7	connected with thin film	8–11	7–8
morphotype 2	present study	3	26–27	p	3	4	1	5	undivided	6–7	6
morphotype 3-a	present study	67	26–41	p or a§	3	3	1	5–7	divided	3–11	4–7
morphotype 3-b	present study	66	27–41	p or a¶	3	3	1	5–6	divided	3–8	3–6
morphotype 4	present study	51	26–40	a	3	3	1	5	undivided	6–20	6–18
larva A	Inger (1966)	43	25–42	a	2–3	3–4	1	3–5	divided	6–10	?
'*A. phaeomerus*'	Inger & Gritis (1983)	56	25–42	a	?	3	?	5	divided	5–8	7–8
'*A. phaeomerus*'	Inger (1985)	59	26–40	a	3	3	1	5	divided	5–8	5–8
'*M. phaeomerus*'	Yang (1991)	?	?	?	3	3	1	5	divided	6–8	6–8
larva B	Inger (1966)	24	26–42	p or a	2–3	3–4	1	4–6	divided	5–9	?
'*A. poecilus*'	Inger & Gritis (1983)	17	25–42	p	?	3	?	4–5	divided	6–8	7–8
'*A. poecilus*'	Inger (1985)	21	26–42	p or a	3	3	1	4–5	divided	5–8	4–7
'*M. poecilus*'	Yang (1991)	?	38	?	3	3	1	5	divided	7–8	6
'*R. whiteheadi*'	Boulenger (1893)	?	?	?	2	3	1	4	divided	(4–5)**	(4–5)**
larva C	Inger (1966)	7	29–42	p or a	1–2	3	1	4	divided	?	?
'*M. whiteheadi*'	Yang (1991)	?	?	?	2	3	1	4	divided	?	?
'*M. whiteheadi*'	Malkmus *et al.* (2002)	?	?	?	2	3	1	4	divided	?	?
larva D	Inger (1966)	6	27–41	p	3	3–4	1	5–6	undivided	8–18	?
larva D	Inger & Gritis (1983)	?	(36–41)††	p	?	4	?	6	undivided	16–18	9.5–12
'*A. kinabaluensis*'	Inger (1985)	11	27–41	p or a	3	3–4	1	5–6	undivided	11–19	6–13
'*M. kinabaluensis*'	Yang (1991)	?	?	?	3	4	1	6	undivided	17–20	12
'*M. kinabaluensis*'	Malkmus *et al.* (2002)	?	?	?	3	3–4	1	5–6	undivided	?	?
larva E	Inger (1966)	17	25–40	p or a	3	4	1	5	undivided	4–10	?
larva E	Inger & Gritis (1983)	?	(36–41)††	p	?	4	?	5	undivided	7–9	5.5–8
larva E	Inger (1985)	?	27–41	p	3	4	1	5	undivided	5–9	5.5–8
larva F	Inger & Gritis (1983)	?	(36–41)††	p	?	3	?	5–6	divided	6–9	5–8
larva F	Inger (1985)	?	26–41	p(m)	3	3	1	5–6	divided	4–9	4–8
larva G	Inger & Gritis (1983)	?	(36–41)††	p(m)	?	3	?	5	divided	7–9	6–7
larva G	Inger (1985)	?	29–39	p^4 or a	3	3	1	4–5	divided	5–8	5–7
larva H	Inger & Gritis (1983)	?	(36–41)††	p(m)	?	3	?	5	divided	5–8	6–7
larva H	Inger (1985)	?	26–39	p(m) or a	3	3	1	4–5	divided	5–8	4–7
'*M. amoropalamus*'	Yang (1991)	18	25–41	?	3–4	4	1	5–9	div. or undiv.	6–17	5–13
'*M. amoropalamus*'	Malkmus *et al.* (2002)	?	?	?	3–4	4	1	5–9	?	?	?
'*M. orphnocnemis*'	Yang (1991)	4	38–41	?	3	2	1	5–8	divided	8–10	6–8
'*M. orphnocnemis*'	Malkmus *et al.* (2002)	?	?	?	3	2	1	5–8	divided	?	?
'*A.* sp.'	Matsui (1986)	5	28	p	?	3	?	5	divided	4–5	5
'*Ixalus nubilus*'	Mocquard (1890)	4	(40–42)‡‡	?	?	3?	1	4?	divided	?	?

		Glands						
	Reference	Infraorbital	Postorbital	Prespiracular	Midlateral	Ventral	Dorsal fin	Ventral fin
morphotype 1	present study	4–7	4–7	0–2	2–7	absent	14–18	15–18
morphotype 2	present study	3–4	3–6	3–7	2–5	absent	absent	1–14
morphotype 3-a	present study	1–3	0–3	0–4	0–8	absent	absent	0–16
morphotype 3-b	present study	1–3	0–3	0–4	0–8	absent	absent	0–11
morphotype 4	present study	2–18	3–10	3–24	9–44	1–30	0–9	0–16
larva A	Inger (1966)	absent	present	present	present	absent	absent	absent
'*A. phaeomerus*'	Inger & Gritis (1983)	?	?	?	10–20	?	absent	absent
'*A. phaeomerus*'	Inger (1985)	4–7	2–4	7–10	21–27	absent	absent	absent
'*M. phaeomerus*'	Yang (1991)	present	present	present	present	absent	?	?
larva B	Inger (1966)	present	present	present	present	absent	absent	2–5
'*A. poecilus*'	Inger & Gritis (1983)	?	?	?	2–6	?	absent	2–5
'*A. poecilus*'	Inger (1985)	1–3	2	1–2	2–4	absent	absent	2–6
'*M. poecilus*'	Yang (1991)	present	present	present	present	absent	present (?)	present (?)
'*R. whiteheadi*'	Boulenger (1893)	?	?	?	?	?	?	?
larva C	Inger (1966)	present	present	present	present	absent	?	?
'*M. whiteheadi*'	Yang (1991)	?	?	?	?	?	?	?

Table 1. *Continued*

	Reference	Glands						
		Infraorbital	Postorbital	Prespiracular	Midlateral	Ventral	Dorsal fin	Ventral fin
'*M. whiteheadi*'	Malkmus *et al*. (2002)	?	?	?	?	?	?	?
larva D	Inger (1966)	present	present	present	present	absent	absent	absent
larva D	Inger & Gritis (1983)	?	?	?	?	?	absent	5–8
'*A. kinabaluensis*'	Inger (1985)	3–5	2–6	1–5	0–5	absent	absent	0–7
'*M. kinabaluensis*'	Yang (1991)	present	present	present	present	absent	absent	absent
'*M. kinabaluensis*'	Malkmus *et al*. (2002)	?	?	?	?	?	absent	absent
larva E	Inger (1966)	present	present	present	present	absent	absent	3–5
larva E	Inger & Gritis (1983)	?	?	?	?	?	absent	4–8
larva E	Inger (1985)	3–6	3–4	3–6	5–8	absent	absent	3–7
larva F	Inger & Gritis (1983)	?	?	?	?	?	absent	4–8
larva F	Inger (1985)	2–4	1–2	1–3	5–11	absent	absent	4–8
larva G	Inger & Gritis (1983)	?	?	?	?	?	absent	1–5
larva G	Inger (1985)	2–4	2	1–4	3–6	absent	absent	0–5
larva H	Inger & Gritis (1983)	?	?	?	?	?	absent	3–5
larva H	Inger (1985)	1–3	2	1–2	2–6	absent	absent	3–7
'*M. amoropalamus*'	Yang (1991)	present	present	present	present	absent	present	present
'*M. amoropalamus*'	Malkmus *et al*. (2002)	?	?	?	?	?	present	present
'*M. orphnocnemis*'	Yang (1991)	present	present	present	present	absent	absent	a few
'*M. orphnocnemis*'	Malkmus *et al*. (2002)	?	?	?	?	?	?	?
'*A.* sp.'	Matsui (1986)	?	?	?	?	?	absent	absent
'*Ixalus nubilus*'	Mocquard (1890)	?	?	?	?	?	?	?

*States of surface projections: p, present; p(m), tipped with melanin; a, absent.
†The number of lower undivided rows includes an extra pair of divided rows posterior to them.
‡For tadpoles with a single undivided lower beak, the serrations of the whole beak were counted and divided by two, whereas only the left beak was counted for tadpoles with divided beaks.
§Projections were limited to the head.
¶Projections were observed on the head and body of some individuals (see Fig. 3).
**Determined from pictures on a plate (pl. 11, 4a and 4b).
††The specimens used in this study were at st. 36–41.
‡‡As judged from pictures, at least st. 40 and 42 tadpoles are included.

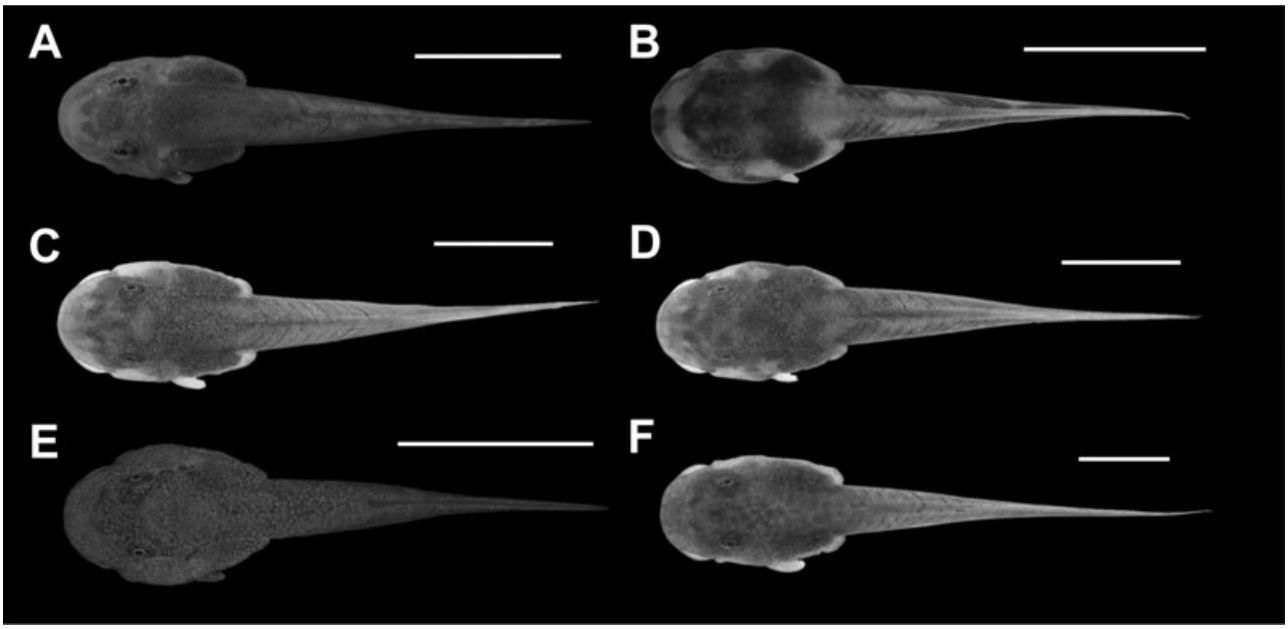

Figure 4. Dorsal views of larval *Meristogenys*: A, morphotype 1 (lineage 1); B, morphotype 2 (lineage 2); C, morphotype 3-a (lineage 3); D, morphotype 3-a (lineage 4); E, morphotype 3-b (lineage 5); F, morphotype 4 (lineage 6). Scale bar = 10 mm.

Table 2. Growth in head–body length (HBL: means ± 2 SE, followed by ranges and number of specimens in parentheses) of five *Meristogenys* morphotypes examined in this study. The grouping of stages (st.) follows Inger (1985)

	st. 26–29	st. 30–33	st. 34–37	st. 38–41
Morphotype 1	11.9 11.5–12.3 (2)	12.6 12.4–12.7 (2)	–	–
Morphotype 2	10.4 9.5–11.5 (3)	–	–	–
Morphotype 3-a	11.2 ± 0.4 10.0–13.0 (16)	12.6 ± 0.3 11.5–13.9 (17)	15.1 ± 0.4 14.0–16.8 (20)	15.9 ± 0.4 14.7–17.1 (12)
Morphotype 3-b	8.7 ± 0.4 7.2–10.2 (13)	11.1 ± 0.4 9.2–13.0 (23)	13.5 ± 0.5 12.6–14.6 (9)	15.5 ± 0.3 14.0–16.5 (21)
Morphotype 4	13.5 ± 0.7 11.2–15.6 (14)	17.2 ± 0.4 15.9–18.7 (13)	19.5 ± 0.3 18.5–20.5 (15)	20.4 ± 0.4 19.4–21.5 (9)

examination of this morphotype led us to split it into two discrete forms. The dorsal head–body of one form (morphotype 3-a) was somewhat rectangular, whereas that of the other (morphotype 3-b) was ovoid (Fig. 4). Of the 133 specimens examined, 67 were classified as morphotype 3-a, and 66 as morphotype 3-b. Each specimen series covered almost the entire range of developmental stages (Table 2).

These two forms also differed in other characters: SNW/SUW was significantly larger in morphotype 3-a than in morphotype 3-b (Mann–Whitney *U*-test, $P < 0.05$; Fig. 5); morphotype 3-a had 5–7 undivided rows of lower labial teeth [lower LTRF = /6(1),/7(1), and /8(1)] and morphotype 3-b had 5–6 rows [lower LTRF = /6(1) and /7(1)], but most of morphotype 3-a had six or seven rows, whereas the majority of morphotype 3-b had five (Fig. 6); morphotype 3-a mostly lacked surface projections or possessed them only on the head, whereas morphotype 3-b tended to have them on the whole body surface after st. 39 (Fig. 7); morphotype 3-a was significantly larger than morphotype 3-b in HBL at all stages, except st. 40–41 (*t*-test, $P < 0.05$; Table 2). The range in the number of ventral fin glands was 0–16 on morphotype 3-a and 0–11 on 3-b.

Morphotype 4

Fifty-one specimens, not assigned to the above four morphotypes, all had a pair of ventral glands that have not been reported in previous studies (Fig. 1; but see the figure from Inger & Tan, 1996). We called this series morphotype 4 (Fig. 4). It comprised st. 26–40 and had the LTRF of 6(4–6)/6(1) (i.e. three divided upper and five undivided lower rows of labial teeth); glands, at least in the ventral fin; a single lower beak (Fig. 3b); and a lack of surface projections. Some individuals of this morphotype had glands in the dorsal fin, like morphotype 1. The number of midlateral glands in this morphotype (9–44) exceeded that in any other morphotype (2–7, 2–5, 0–8 and 0–8 in morphotypes 1, 2, 3-a, and 3-b, respectively).

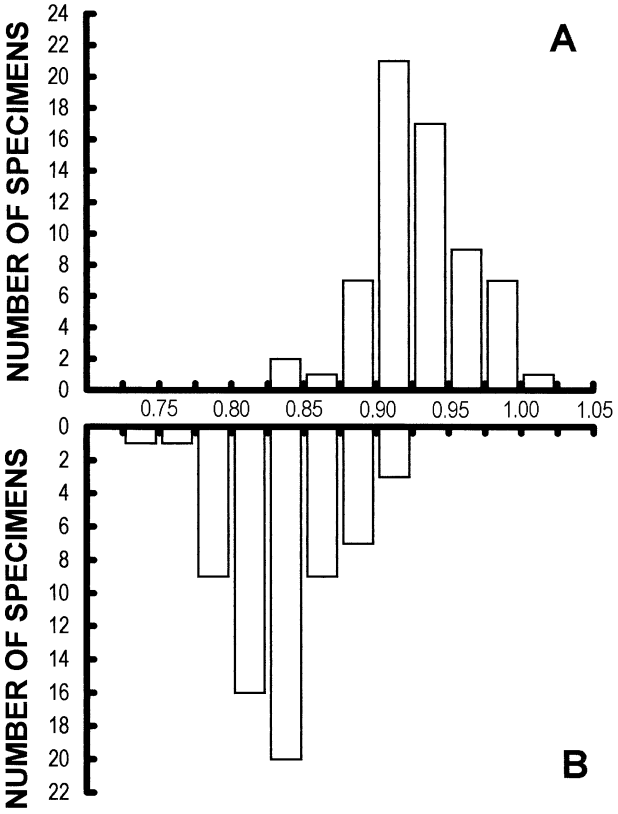

Figure 5. Frequency of snout width/sucker width (SNW/SUW) ratios in morphotypes 3-a (A) and 3-b (B).

Association of adults and larvae

We obtained 449 bp of 12S rRNA gene sequences for 91 adult and 21 larval specimens. The 21 larval DNA

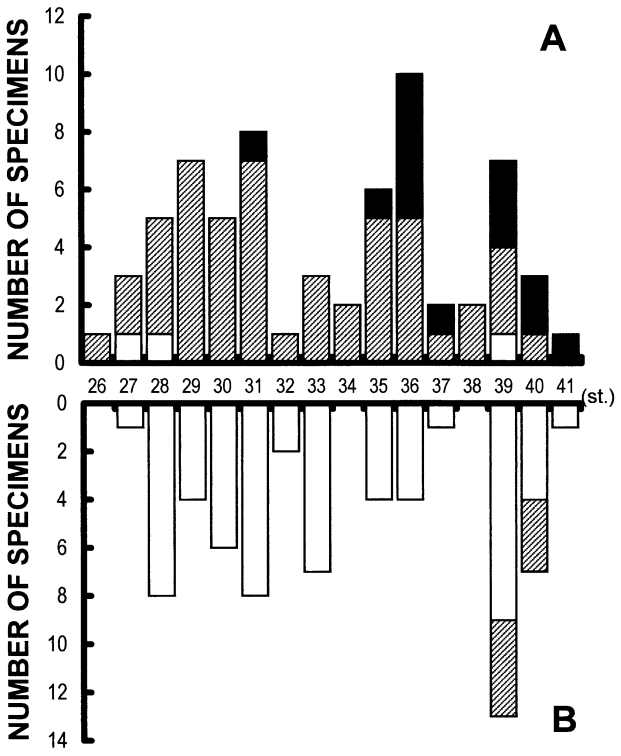

Figure 6. Frequency of the number of undivided rows of lower labial teeth to show ontogenetic changes in morphotypes 3-a (A) and 3-b (B). Open box = 4, hatched box = 5, closed box = 6.

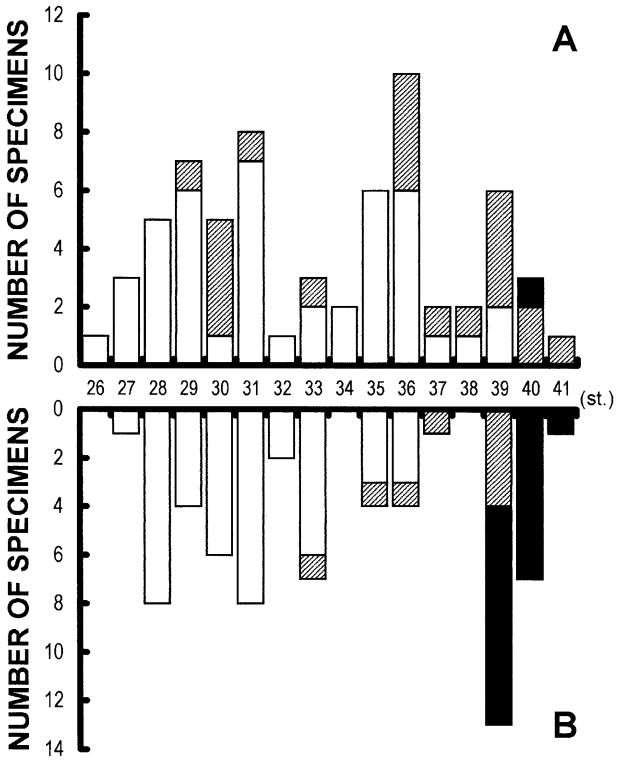

Figure 7. Frequency of the distribution of surface projections to show ontogenetic changes in morphotypes 3-a (A) and 3-b (B). Open box = absent, hatched box = confined to the head, closed box = present on the whole body.

sequences included all five larval morphotypes: three specimens from morphotype 1, one from morphotype 2, five from morphotype 3-a, ten from morphotype 3-b and two from morphotype 4. We found 13 haplotypes amongst the 112 total sequences, which diverged in sequence from 0.002 to 0.139 K2p. We estimated the phylogenetic relationships amongst these haplotypes and three hierarchical outgroups. Of the 449 characters, 188 were variable, and 95 were parsimony-informative. MP searches recovered the five most parsimonious trees of 298 steps (CI = 0.809, RI = 0.737). We used the TrN + $G_{0.386}$ (Tamura & Nei, 1993) and GTR + $G_{0.440}$ (Rodriguez *et al*., 1990) evolutionary models for ML and Bayesian inferences, respectively. The likelihood values of the ML and Bayesian trees were –lnL = 1900.07 and 1900.25, respectively. The results from three phylogenetic inferences were slightly different, but the nodes that were significantly supported were completely shared (Fig. 8). *Huia cavitympanum* and all 13 haplotypes of *Meristogenys* made a monophyletic group against *F. limnocharis* and *A. marmoratus* (with bootstrap values of 100 and 99% in MP and ML, respectively, and a posterior probability of 100%). The monophyly of all 13 haplotypes against *H. cavitympanum* was significantly supported (98, 100 and 100%).

Amongst these 13 haplotypes, haplotype 13 was the first split from all other haplotypes with sufficient support values (74, 99 and 99%). The monophyly of haplotypes 2 and 3 and 6–12 had high support values (91, 100 and 100% for haplotypes 2 and 3, and 100, 100 and 100% for haplotypes 6–12). The genetic distance between haplotypes 2 and 3 (0.002) and amongst haplotypes 6–12 (0.002–0.011) was far less than the distances amongst the other haplotypes (0.025–0.139). Therefore, we treated each of these two groups as a discrete genetic lineage. Thus, haplotypes 1–13 could be grouped into six haplotype lineages (1, 2–3, 4, 5, 6–12 and 13), with unresolved relationships, except between lineage 6 (haplotype 13) and the others. The characteristics of adults and larvae included in each lineage are shown below.

(i) Lineage 1

This lineage included only three tadpoles of morphotype 1, and no adult specimens.

(ii) Lineage 2

This lineage included haplotypes 2 and 3, which one tadpole of morphotype 2 and 11 adult specimens pos-

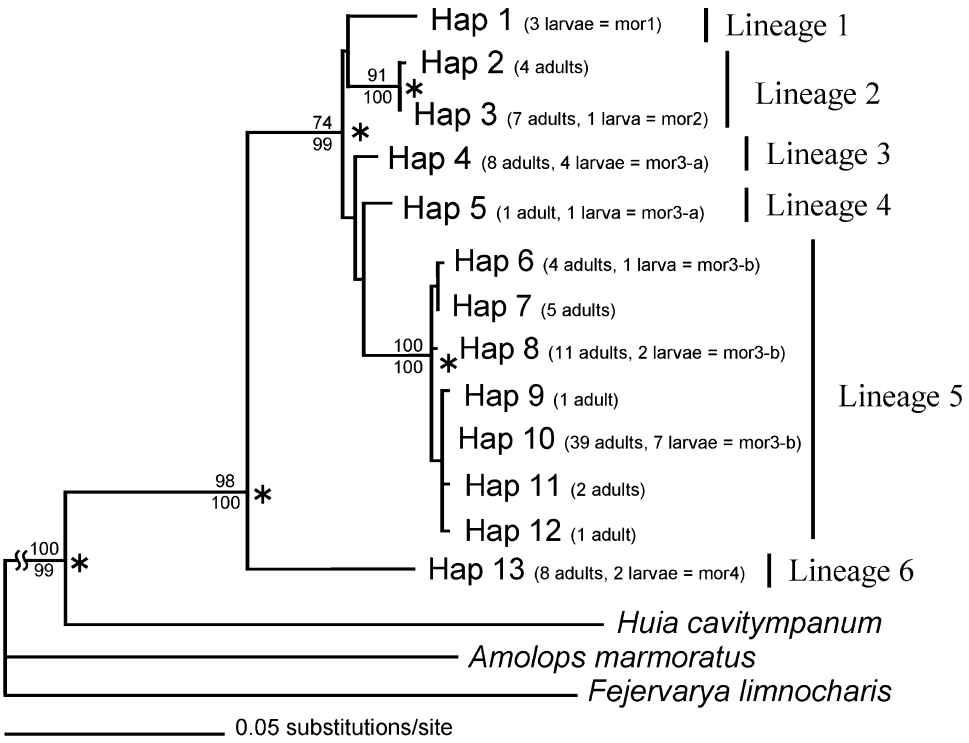

Figure 8. Maximum likelihood tree of a 449-bp sequence of the 12S rRNA gene for haplotypes of *Meristogenys* and related species. Numbers above or below branches represent bootstrap support for the MP (2000 replicates)/ML (500) inference for the respective clade. Nodes with asterisks indicate significant support (≥ 95%) by Bayesian inference. The number of adult and larval specimens of each haplotype is shown in parentheses. Mor = larval morphotype.

sessed. The 11 adults were relatively large for this genus (male SVL ≥ 44.6 mm, female SVL ≥ 74.0 mm) and had mottled or dusted patterns on the rear of the thigh. These characteristics correspond to *M. whiteheadi* (Matsui, 1986), but the relative tibia length of our specimens was much larger than that of *M. whiteheadi* and approached that of *M. poecilus* (Inger & Gritis, 1983; Table 3).

(iii) Lineage 3

This lineage included four tadpoles of morphotype 3-a and eight adults of haplotype 4. The adults, which had broad webs that did not extend beyond the outermost tubercles of the fourth toe, were identified as *M. amoropalamus* (Table 3). Other characteristics that matched that species included: a relatively small body size (male SVL ≤ 34.3 mm, female SVL ≤ 66.9 mm); light brown colour on the rear of the thigh, with a few small, irregular light spots (dusted); and a whitish ventral leg surface, with heavy melanophore pigmentations.

(iv) Lineage 4

One tadpole of morphotype 3-a and one adult of haplotype 5 represented this lineage. The adult specimen, like the eight adults of lineage 3, had characteristics attributed to *M. amoropalamus* (Table 3). However, the two lineages were relatively remote genetically (0.025 in K2p distance), notwithstanding the nearly identical adult and larval morphology.

(v) Lineage 5

Ten tadpoles of morphotype 3-b and 63 adults with haplotypes 6–12 constituted this lineage. The adults were relatively small (male SVL ≤ 39.8 mm, female SVL ≤ 65.0 mm) and had dusted to mottled patterns on the rear of the thigh (Table 3). A broad web extended beyond the outermost tubercle of the fourth toe and to the disc in many specimens. The abdomen and the ventral surfaces of the legs were whitish, with some dot-like melanophores. These characteristics accord with those of *M. orphnocnemis* and *M. phaeomerus*, but the tibial colour pattern and extent of toe webbing in our specimens were closer to *M. orphnocnemis* than to *M. phaeomerus* (Inger & Gritis, 1983). The DNA sequence of haplotype 10 was identical to that of a specimen from Bundu Tuhan (AB211471), the type locality of *M. orphnocnemis* (Matsui, 1986; Matsui *et al.*, 2006).

Table 3. Comparisons of snout–vent length (SVL: means ± 2 SE, followed by ranges in parentheses, in mm), percentage ratios of each of the other character dimensions to SVL (medians, followed by ranges in parentheses), colour patterns, and extent of web in five *Meristogenys* lineages. Notations for tibia pattern and extent of web follow Inger & Gritis (1983)

	Lineage 2		Lineage 3		Lineage 4	Lineage 5		Lineage 6	
	M	F	M	F	F	M	F	M	F
N	6	5	5	3	1	57	6	6	2
SVL	47.5 ± 2.3 (44.6–51.4)	79.2 ± 2.6 (74.0–80.6)	34.0 ± 0.3 (33.5–34.3)	63.3 (61.0–66.9)	62.0	34.0 ± 0.4 (31.2–39.8)	61.8 ± 2.1 (58.7–65.0)	66.9 ± 1.1 (65.2–68.6)	83.4 (81.3–85.5)
HW/SVL	35.8 (33.7–38.7)	36.2 (35.0–37.7)	35.0 (32.7–36.2)	34.9 (34.5–36.2)	39.0	33.5 (31.3–36.5)	34.1 (32.0–35.5)	35.1 (34.3–35.7)	36.7 (35.7–37.6)
TL/SVL	72.4 (71.0–76.2)	72.9 (70.9–77.8)	73.1 (71.4–73.8)	70.1 (69.9–73.1)	75.3	69.0 (65.4–73.7)	69.5 (68.5–71.9)	68.0 (66.0–69.4)	68.9 (67.3–70.5)
Tibia pattern									
A–B	2	3	5	3	1	47	3	6	2
B/C	3	0	0	0	0	4	2	0	0
C–D	1	2	0	0	0	6	1	0	0
Thigh pattern	mottle-dusted		dusted		dusted	mottled-dusted		dusted	
Extent of web									
A–B	3	4	0	0	0	2	1	0	1
C	2	1	1	1	0	11	2	5	1
D–E	1	0	4	2	1	44	3	1	0
Larval morphotype	morphotype 2		morphotype3-a		morphotype3-a	morphotype 3-b		morphotype 4	

(vi) Lineage 6

This lineage included two tadpoles of morphotype 4 and eight adults of haplotype 13. The adults were relatively large (male SVL ≥ 65.2 mm, female SVL ≥ 81.3 mm). The back and top of the head was reddish brown mixed with olive green, and the sides of the head and body were bright olive green, becoming yellow towards the belly. The thighs were covered with small, pale grey dots, and the broad web reached to the toe disc (Table 3). These characteristics identified the frogs as *M. kinabaluensis*, but some of our specimens had outer metatarsal tubercles that are reported to be absent in that species (Inger, 1966).

DISCUSSION

Taxonomic relationships of known larval forms

Some morphological variations observed among each of our larval morphotypes may be ascribed to the intraspecific phenotypic plasticity (Relyea & Auld, 2005). However, because each genetic lineage contained only one morphotype, which in turn was assigned to a discrete adult form, we regard each larval morphotype as a distinct taxon.

Whereas only eight species of *Meristogenys* have been described from adults (Frost, 2004), no less than 11 larval forms have been reported for this genus [*M.* '*amoropalamus*', *M.* '*kinabaluensis*', *M.* '*orphnocnemis*', *M.* '*phaeomerus*', *M.* '*poecilus*', *M.* '*whiteheadi*', larvae E–H, plus a larval form described by Matsui (1986)], indicating several undescribed taxa or excessive splitting of larval forms. We discuss the taxonomic status of previously reported larval forms, as well as problems with some adult forms elucidated from the results of our study on larval and adult *Meristogenys* from a stream in Mahua, Sabah.

(i) Larva A (Inger, 1966), '*Amolops phaeomerus*' (Inger & Gritis, 1983; Inger, 1985) and *Meristogenys* '*phaeomerus*' (Yang, 1991)

These forms were reported to have three divided rows of upper labial teeth and divided lower beaks and to lack surface projections and tail glands. These characteristics coincide with our young morphotype 3-a and 3-b specimens (lineages 3–5), but none of our specimens showed the unique body and tail colour pattern reported for larva A (greenish-yellow with large black spots: Fig. 4; Inger, 1966). Furthermore, larva A was reported to lack surface projections and tail glands throughout its larval development. In contrast, our morphotypes 3-a and 3-b developed projections and glands in the later stages, and after st. 40 all specimens possessed them (Fig. 7). If these differences fall outside the range of geographical variation, larva A (*M.* '*phaeomerus*') is probably absent from our study site in Mahua, Sabah, as we have never collected adults identified exclusively as *M. phaeomerus*.

(ii) Larva B (Inger, 1966), *A.* '*poecilus*' and larva F–H (Inger & Gritis, 1983; Inger, 1985), and *M.* '*poecilus*' (Yang, 1991)

Inger (1966) first recognized larva B, based on specimens from three localities in Sabah, and one in Sarawak. However, Inger & Gritis (1983) associated the Sarawak population with *A. poecilus* and removed the Sabah specimens from the larva B attribution. Although the classification scheme was not clearly stated by the authors, the Sabah specimens seemed to be separated into three groups by localities, with each group raised to a new larval form (larvae F–H). Inger (1985) later noted that he had split larva B (Inger, 1966) and assigned the sample from Liwagu River, Kinabalu (FMNH = Field Museum of Natural History 131243) to larva F and the sample from Kaingeran River (FMNH 109493) to larva G. The sample from Tuaran District (FMNH 140283) originally included in larva B was omitted in the later studies, but another Tuaran sample (FMNH 140213) was assigned to larva H. Because the two localities are not very far apart (about 3 km, judging from the descriptions), and the characteristics of larva H do not contradict those of larva B, the FMNH 140283 and FMNH 140213 specimens may represent an identical taxon. Yang (1991) accepted the association of Inger & Gritis (1983).

According to Inger (1985), larvae F–H and *A.* '*poecilus*' can be differentiated from each other by the combination of body proportions, the number of midlateral glands and undivided rows of lower labial teeth, and the condition of surface projections (Table 4). Some larvae F, H and *A.* '*poecilus*' have surface projections on the head and body, whilst projections on larva G are limited to the head. Furthermore, although Inger & Gritis (1983) noted that projections on some larvae G and H are tipped with melanin, unlike larvae F and *A.* '*poecilus*', Inger (1985) stated that larvae F and H possess tipped projections. All four of these forms lack surface projections in the early stages (Inger, 1985).

Our morphotypes 3-a and 3-b (lineages 3–5) were generally similar to these four larval forms in labial teeth, lower beak and tail glands. However, the HBW/HBL ratios of our samples did not overlap the range of *A.* '*poecilus*.' Similarly, larva H usually has melanin-tipped surface projections, whereas morphotypes 3-a and 3-b generally had protuberances with the same brown colour as the background body surface in advanced stages. Thus, *A.* '*poecilus*' and larva H seemed to be absent from our study site.

Unlike larva H, only a few specimens of larvae F and G have coloured surface projections. From the distribution of projections, our morphotype 3-a seemed

Table 4. Ranges of variations in some characters that may be useful to separate '*A. poecilus*', larvae F, G, H (Inger, 1985), and morphotypes 3-a and 3-b in the present study. Variation ranges of morphotypes 3-a and 3-b are followed by Inger's (1985) larval forms, with overlapping ranges

	A. poecilus	larva F	larva G	larva H	morph. 3-a	morph. 3-b
HBW/HBL (%)	81–89	63–78	75–87	65–68	61–71 F, H	67–69 F, H
HBD/HBW (%)	56–62	48–59	48–60	44–51	49–64 *A. poecilus*, F, G, H	59–62 *A. poecilus*, F, G
EL/HBL (%)	13–15	12–13	11–15	11–12	13–15 *A. poecilus*, F, G	15–16 *A. poecilus*, G
ODW/HBW (%)	52–62	57–65	42–49	60–65	57–73 *A. poecilus*, F, H	55–76 *A. poecilus*, F, H
TLL/HBL (%)	169–190	170–188	134–153	140–183	143–196 *A. poecilus*, F, G, H	174–191 *A. poecilus*, F, H
TLD/TLL (%)	29–35	21–24	34–41	23–29	23–33 *A. poecilus*, F, H	26–29 *A. poecilus*, H
Midlateral glands	2–4	5–11	3–6	2–6	1–7 *A. poecilus*, F, G, H	0–8 *A. poecilus*, F, G, H
Undivided rows of lower denticles	4–5	5–6	4–5	4–5	5–7 *A. poecilus*, F, G, H	5–6 *A. poecilus*, F, G, H
Distribution of projections	head and body	head and body	head	head and body	head and body *A. poecilus*, F, H	head G
Colour of projections	white	tipped with melanin	brown	tipped with melanin	white, brown *A. poecilus*, G	white, brown *A. poecilus*, G

identical to larva G. Morphotype 3-a belonged to lineages 4 and 5, both of which included adults that were identified as *M. amoropalamus*. Therefore, we considered the larva G of Inger & Gritis (1983) and Inger (1985), which was equivalent to part of the original larva B of Inger (1966; see above), to be *M. amoropalamus* or a morphologically very similar cryptic form.

Similarly, we judged larva F to be identical to morphotype 3-b, which was in lineage 5 and included adults identified as *M. orphnocnemis*. Larva F was collected from several streams where *M. orphnocnemis* has been recorded (Kepungit, Mamut, Matukungan and Liwagu rivers; 'Mantukungan' and 'Liwago' in Malkmus *et al*., 2002), which supports the assignment of larva F to *M. orphnocnemis*.

(iii) Larva C (Inger, 1966), '*Rana whiteheadi*' (Boulenger, 1893), *M.* '*whiteheadi*' (Yang, 1991; Malkmus *et al*., 2002).

Boulenger (1893) reported larval '*Rana whiteheadi*' from Bongon, an upper stream of the Baram River, Sarawak, without reliable evidence. He had two types of gastromyzophorous tadpoles from that location, one of which he identified correctly as larval *Huia cavitympanum*. He assigned *R.* '*whiteheadi*' to another form because he assumed that this species must have gastromyzophorous tadpoles like its ally *R. jerboa*, which had been associated with another gastromyzophorous tadpole from Java (probably *H. masonii*).

Inger (1966), whilst synonymizing *A. whiteheadi* with *A. jerboa*, cited Boulenger's (1893) larval form as larva C, because it could be assigned to *A. jerboa* (later shown to be a composite of distinct species) like other larval forms he examined (see above) with equal justification. However, Yang (1991) followed Boulenger (1893) and assigned larva C of Inger (1966) to larval *M. whiteheadi*. Malkmus *et al*. (2002) acceded to this association.

Larva C clearly differs from other known forms, as well as our specimens, in having only one or two undivided rows of upper labial teeth, despite the inclusion of individuals in a wide range of developmental stages [Inger's st. IV–XX (1966), which correspond to Gosner's st. 29–42 (1960)]. In our results, lineage 2 included larval morphotype 2 and adults closely resembling *M. whiteheadi* but with relatively long tibia. Morphotype 2 larvae differed from larva C, not only in having three divided rows of upper labial teeth, but also in the state of the lower beak. If the association of larva C (*sensu* Inger, 1966) to *A. whiteheadi* (Boulenger, 1893; Yang, 1991; Malkmus *et al*., 2002) is correct, our lineage 2 may represent an undescribed cryptic form (see larva E discussion below).

(iv) Larva D (Inger, 1966; Inger & Gritis, 1983), *A.* '*kinabaluensis*' (Inger, 1985) and *M.* '*kinabaluensis*' (Yang, 1991; Malkmus *et al*., 2002)

Inger (1985) associated his larva D (Inger, 1966; quoted by Inger & Gritis, 1983) with *M. kinabaluensis*, based on the absence of an outer metatarsal tubercle. Inger (1966) first described this form as lacking tail glands, but Inger & Gritis (1983) recognized 5–8 glands in st. 36–41 individuals. To explain this reversal, Inger (1985) noted that specimens from some localities had tail glands, whereas others lacked them, and that surface projections and midlateral glands varied geographically (Inger, 1985). Yang (1991) and Malkmus *et al*. (2002) accepted part of Inger's (1985) descriptions.

Thus, we cannot easily compare larva D to our samples. However, amongst our samples, morphotype 4 was most similar to larva D in its possession of an undivided lower beak and a large number of serrations on the beaks. However, morphotype 4 differed decidedly from larva D in its possession of ventral glands. Morphotype 4 formed lineage 6 with adults identified as *M. kinabaluensis*. Because the adult morphology of *M. kinabaluensis* differs very clearly from other congeneric species, morphotype 4 cannot be assigned to other known species. Larva D is highly variable, and Inger (1985) noted that it might contain more than one taxon. Thus, whether our morphotype 4 represents a geographical variation of *M. kinabaluensis* or an undescribed cryptic taxon must be answered in a future study.

(v) Larva E (Inger, 1966, 1985; Inger & Gritis, 1983)

Larva E, as described by Inger (1966), had four divided rows of upper labial teeth, tail glands, an undivided lower beak and surface projections (at least in older stages), all of which we observed in morphotype 2. Although the size of the projections is slightly different, this can be ascribed to developmental stage differences. We therefore considered larva E (Inger, 1966) and our morphotype 2 to represent an identical taxon. Because morphotype 2 formed lineage 2 with adults identified as *M. whiteheadi*, but had much longer tibia relative to SVL than that species, larva E may be a cryptic form currently confused with *M. whiteheadi* (see larva C discussion above).

(vi) Tadpoles from East Kalimantan in Matsui (1986)

Matsui (1986) reported a series of five young tadpoles (st. 28) from Pa Nado in East Kalimantan, where paratypes of *M. orphnocnemis* were obtained. Although the form had a character set not previously reported, Matsui (1986) noted that some characters were a function of early development and did not assign it to any known larval form. These tadpoles are similar to some of our morphotype 3-b, in having three divided rows of upper labial teeth, divided lower beaks, surface projections on the head and body, and a lack of tail glands. As morphotype 3-b is identified as *M. orphnocnemis* (see above), the form reported by Matsui (1986) must also be assigned to this species.

(vii) *M.* '*amoropalamus*' (Yang, 1991; Malkmus *et al*., 2002)

Yang (1991) reported on tadpoles of six *Meristogenys* species and described larval *M.* '*amoropalamus*' and *M.* '*orphnocnemis*' for the first time. These associations were accepted by Malkmus *et al*. (2002), although they omitted some characters. Yang's (1991) *M.* '*amoropalamus*' is similar to our larval morphotype 1, except for the lower beak, which he described as usually undivided, but narrowly divided in some specimens (Fig. 3C). However, in morphotype 1, the divided lower beaks were connected by a thin film, which could be interpreted as an intermediate condition between divided and undivided. In that case, Yang's (1991) larval *M.* '*amoropalamus*' and our morphotype 1 do not differ in this character. Furthermore, some of Yang's (1991) specimens (FMNH 228007) were collected from the Pegalan River (J. Ladonski, pers. comm.), which confluences with the Mahua River and is located about 10 km from our research site. Unfortunately, we had no adult specimens corresponding to larval morphotype 1 (lineage 1) to compare with adult *M. amoropalamus.* As described above, we also consider our morphotype 3-a (lineages 3 and 4) to be a candidate for *M. amoropalamus*. Therefore, if the association by Yang (1991) were correct, three sympatric lineages in Mahua could be identified as *M. amoropalamus*, but would actually include cryptic species.

As noted in the Results section, our lineages 3 and 4 were relatively remote genetically, but were similar in adult and larval morphologies. Three interpretations of this phenomenon should be considered. First, two reproductively isolated, but morphologically similar, taxa may be involved (Malkmus, 1996). Second, these two lineages may have been geographically isolated in the past, but can now reproduce with each other (Goodman *et al*., 2001), and only a trace of the past isolation is retained in the mitochondrial genome. Third, introgression caused by hybridization in the past may have been retained in this population (Vörös *et al*., 2006). To evaluate the validity of these hypotheses, nuclear genome analyses would be necessary.

(viii) *M.* '*orphnocnemis*' (Yang, 1991; Malkmus *et al*., 2002)

Yang (1991) described *M.* '*orphnocnemis*' tadpoles as having two divided rows of upper labial teeth, a condition never reported elsewhere, and Malkmus *et al*. (2002) adopted his description. Except for this character, the tadpoles are similar to our morphotype 3, including morphotype 3-b identified as *M. orphnocnemis*. Because Yang's (1991) specimens (st. 39 and 40) were approaching metamorphosis, their upper divided

rows may already have fallen off. Alternatively, if the unique trait of upper labial teeth is valid, Yang's (1991) specimens may represent a different species than ours.

VENTRAL GLAND EVOLUTION IN *MERISTOGENYS*

As noted above, we found a pair of ventral gland groups just anterior to the base of the tail in morphotype 4 (Fig. 1). These glands differ from those on the head–body or the tail fins in the absence of visible openings, as reported for *Amolops* (*s.s.*) species and *Huia masonii* by Yang (1991). Inger & Tan (1996: fig. 17) included a figure of the glands on the same part of the tail of *M.* '*orphnocnemis*', without any comment. Except for this figure, such ventral glands have been reported only in certain *Amolops* (*s.s.*) and *Huia* species (Yang, 1991). Indeed, Yang (1991) regarded the absence of these glands as one of the characteristics that defined *Meristogenys*. After estimating the phylogenetic relationships of *Amolops* (*s.l.*), Yang (1991) obtained a cladogram, in which ventral glands appeared at the base of *Amolops* (*s.l.*) but were lost in a common ancestor of *Meristogenys* species. Our finding of ventral glands in morphotype 4, which formed a lineage tentatively identified as *M. kinabaluensis* and which was basal to all other species examined [the *M. jerboa* species group of Matsui (1986)], indicates that a common ancestor of *Meristogenys* had the ventral gland, but the ancestor of the *M. jerboa* species group lost it. Although this reduces the synapomorphic characters of the genus *Meristogenys*, the genus still has some unique characters, such as divided upper beaks, with ribs on their outer surfaces (Fig. 3).

CONCLUSIONS

We ascertained as many as six *Meristogenys* lineages at our small site in Mahua: lineage 1, including morphotype 1 larva; lineage 2, including morphotype 2 larva and corresponding to a form similar to *M. whiteheadi*; lineage 3, including morphotype 3-a and identified as *M. amoropalamus*; lineage 4, also including morphotype 3-a and identified as *M. amoropalamus*; lineage 5, including morphotype 3-b and identified as *M. orphnocnemis*; and lineage 6, including morphotype 4 larva and possibly a cryptic taxon related to *M. kinabaluensis*.

Of the larval forms previously reported, larva A (Inger, 1966), '*Amolops phaeomerus*' (Inger & Gritis, 1983; Inger, 1985), *M.* '*phaeomerus*' (Yang, 1991), *A.* '*poecilus*' and larva H (Inger & Gritis, 1983; Inger, 1985), *M.* '*poecilus*' (Yang, 1991), larva C (Inger, 1966), '*Rana whiteheadi*' (Boulenger, 1893), *M.* '*whiteheadi*' (Yang, 1991; Malkmus *et al*., 2002), larva D (Inger, 1966; Inger & Gritis, 1983), *A.* '*kinabaluensis*' (Inger, 1985) and *M.* '*kinabaluensis*' (Yang, 1991; Malkmus *et al*., 2002) were not found in our samples from Mahua.

The remaining forms are associated with our lineages. We considered larva G (Inger & Gritis, 1983; Inger, 1985; = larva B in Inger, 1966) to be *M. amoropalamus* or a morphologically similar cryptic form. We judged larva F (Inger & Gritis, 1983; Inger, 1985; = larva B in Inger, 1966) and tadpoles from East Kalimantan in Matsui (1986) to be *M. orphnocnemis*. Larva E (Inger, 1966, 1985; Inger & Gritis, 1983) is thought to be a cryptic form confused with *M. whiteheadi*, but with relatively long tibia. *Meristogenys* '*amoropalamus*' (Yang, 1991; Malkmus *et al*., 2002) may be *M. amoropalamus* or a cryptic form, and *M.* '*orphnocnemis*' (Yang, 1991; Malkmus *et al*., 2002) may be *M. orphnocnemis* or a cryptic form.

The use of molecular information overcame some difficulties in associating the larvae and adults of previous studies, i.e. comparisons of older larvae with adults. However, the ambiguity of some of our results shows that our procedure still met with many of the obstacles found in the previous studies. Any new methodology must have a good series of larval specimens in different developmental stages and from various localities to diagnose a species throughout its development and clarify its range of geographical variation. Particularly in this genus, the correct identification of adult specimens is problematic. In addition to biochemical determinations of distinct lineages, more

KEY TO *MERISTOGENYS* LARVAE FROM MAHUA, CROCKER RANGE, SABAH

1. Four divided rows of upper labial teeth 2
   Three divided rows of upper labial teeth 3
2. Tail glands on both fins, head and body without surface projections ..... Morphotype 1 (*M.* sp.)
   Tail glands limited to ventral fin, head and body with surface projections ..... Morphotype 2 (cf. *M. whiteheadi*)
3. Lower beak undivided, ventral glands present ..... Morphotype 4 (cf. *M. kinabaluensis*)
   Lower beaks divided, ventral glands absent 4
4. Head and body rectangular, undivided rows of lower labial teeth usually six or seven ..... Morphotype 3-a (*M. amoropalamus*)
   Head and body oval, undivided rows of lower labial teeth usually five ..... Morphotype 3-b (*M. orphnocnemis*)

detailed morphological studies should be conducted to determine diagnostic characters for valid species identifications. Collections should include topotypic specimens, and the taxa thus delimited should be compared with type specimens of described taxa. This is particularly important in *Meristogenys* because as indicated by this study, many taxa in this genus remain undescribed. Further studies are necessary to determine the most efficient gene regions for elucidating substantive taxonomic differences.

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REFERENCES

Altig R. 1970. A key to the tadpoles of the continental United States and Canada. *Herpetologica* **26:** 180–207.

Avise JC. 2000. *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.

Boulenger GA. 1893. Descriptions of new reptiles and batrachians collected by Mr. C. Hose on Mt. Dulit, Borneo. *Proceedings of the Zoological Society of London* **1893:** 522–528.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783–791.

Frost DR. 2004. *Amphibian species of the world.* http://research.amnh.org/herpetology/amphibia/index.php.

Goodman SJ, Tamate HB, Wilson R, Nagata J, Tatsuzawa S, Swanson GM, Pemberton JM, McCullough DR. 2001. Bottlenecks, drift and differentiation: the population structure and demographic history of sika deer (*Cervus nippon*) in the Japanese archipelago. *Molecular Ecology* **10:** 1357–1370.

Gosner K. 1960. A simplified table for staging anuran embryos and larvae, with notes on identification. *Herpetologica* **16:** 183–190.

Hedges SB. 1992. The number of replications needed for accurate estimation of the bootstrap P value in phylogenetic studies. *Molecular Biology and Evolution* **9:** 366–369.

Huelsenbeck JP, Ronquist FR. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

Inger RF. 1966. The systematics and zoogeography of the Amphibia of Borneo. *Fieldiana: Zoology* **52:** 1–402.

Inger RF. 1985. Tadpoles of the forested regions of Borneo. *Fieldiana: Zoology New Series* **26:** 1–89.

Inger RF, Gritis PA. 1983. Variation in Bornean frogs of the *Amolops jerboa* species group, with descriptions of two species. *Fieldiana: Zoology New Series* **19:** 1–13.

Inger RF, Tan FL. 1996. *The natural history of amphibians and reptiles in Sabah*. Kota Kinabalu: Natural History Publications (Borneo) Sdn. Bdh.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16:** 111–120.

Malkmus R. 1996. *Leptobrachium gunungense* sp. n. (Anura: Pelobatidae) from Mount Kinabalu, North Borneo. *Mitteilungen aus dem Zoologischen Museum in Berlin* **72:** 297–301.

Malkmus R, Manthey U, Vogel G, Hoffmann P, Kosuch J. 2002. *Amphibians and reptiles of Mount Kinabalu (North Borneo)*. Koenigstein: A. R. G. Gantner Verlag.

Matsui M. 1986. Three new species of *Amolops* from Borneo (Amphibia, Anura, Ranidae). *Copeia* **1986:** 623–630.

Matsui M, Ito H, Shimada T, Ota H, Saidapur SK, Khonsue W, Tanaka-Ueno T, Wu GF. 2005. Taxonomic relationships within the pan-oriental narrow-mouth toad *Microhyla ornata* as revealed by mtDNA analysis (Amphibia, Anura, Microhylidae). *Zoological Science* **22:** 489–495.

Matsui M, Shimada T, Liu WZ, Maryati M, Khonsue W, Orlov N. 2006. Phylogenetic relationships of the oriental torrent frogs of the genus *Amolops* and its allies (Amphibia, Anura, Ranidae). *Molecular Phylogenetics and Evolution* **38:** 659–666.

McDiarmid RM, Altig R. 1999. *Tadpoles: the biology of Anuran larvae*. Chicago: The University of Chicago Press.

Mocquard F. 1890. Recherches sur la faune herpetologique des Iles de Bornéo et de Palawan. *Nouvelles Archives du Muséum d'Histoire Naturelle, Série 3* **2:** 115–168, pls 7–11.

Nylander JAA. 2002. *MrModeltest V1.0b*. Program distributed by the author. Available from: http://www.ebc.uu.se/systzoo/staff/nylander.html.

Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991. *The simple fool's guide to PCR*, Version 2.0. Privately published, compiled by Palumbi, S., Department of Zoology, University of Hawaii, Honolulu.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

Relyea RA, Auld JR. 2005. Predator- and competitor-induced plasticity: how changes in foraging morphology affect phenotypic trade-offs. *Ecology* **86:** 1723–1729.

Rodriguez F, Oliver JF, Marin A, Medina JR. 1990. The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142:** 485–501.

Roelants K, Jiang JP, Bossuyt F. 2003. Endemic ranid (Amphibia: Anura) genera in southern mountain ranges of the Indian subcontinent represent ancient frog lineages: evidence from molecular data. *Molecular Phylogenetics and Evolution* **31:** 730–740.

Swofford D. 2002. *PAUP*: phylogenetic analysis using parsi-*

*mony (*and other methods)*, Version 4. Sunderland, MA: Sinauer Associates.

Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10:** 512–526.

Vörös J, Alcobendas M, Martínez-Solano I, García-París M. 2006. Evolution of *Bombina bombina* and *Bombina variegata* (Anura: Discoglossidae) in the Carpathian Basin: a history of repeated mt-DNA introgression across species. *Molecular Phylogenetics and Evolution* **38:** 705–718.

Yang D. 1991. Phylogenetic systematics of the *Amolops* group of ranid frogs of Southeastern Asia and the Greater Sunda Islands. *Fieldiana: Zoology New Series* **63:** 1–42.

APPENDIX 1

Larval specimens of *Meristogenys* examined morphologically in this study. BOR: BORNEENSIS Collection, University of Malaysia Sabah; SP: Sabah Parks. Uncatalogued BORNEENSIS are shown by temporal numbers.

Morphotype 1: BOR 03B022, 05B245-47; Morphotype 2: BOR 03B001-03; Morphotype 3-a: BOR 03B005, 03B023a (53 specimens), 03B202-03, 03B256, 03B343, 03B344, 03B346-49, 03B350a, 05B046, SP 3810 (two specimens); Morphotype 3-b: BOR 03B006, 03B009, 03B023b (20 specimens), 03B061-64, 03B255, 03B257-59, 03B345, 03B350b, 05B061-62, 05B063 (29 specimens), 05B067, 05B072, 05B076; Morphotype 4: BOR 03B021, 03B059-60, 03B199-201, 03B271-273, 03B351-57, 05B018, 05B020, 05B234, 05B235 (32 specimens).

APPENDIX 2

Specimens of *Meristogenys* whose DNA sequences were examined in this study. See Appendix 1 for acronyms. BORNEENSIS numbers capped with '03B' or '05B' are temporal numbers of uncatalogued larval specimens. BORNEENSIS specimens without these caps are adults. Two SP specimens are larvae.

Haplotype 1: BOR 05B245, 05B246, 05B247; Haplotype 2: BOR 12435, 12561, 12620, 12622; Haplotype 3: BOR 12433, 12434, 12479, 12512, 12515, 12560, 12562, 03B001; Haplotype 4: BOR 8869, 12476, 12480, 12520, 12621, 12626–28, 03B256, 05B046, SP 3810 (two specimens); Haplotype 5: BOR 12623, 03B203; Haplotype 6: BOR 12522, 12565, 12566, 12583, 03B257; Haplotype 7: BOR 8852, 22611, 22615–16, 22618; Haplotype 8: BOR 8827, 8859, 8861, 12437–39, 12442, 12464, 12478, 12519, 12563, 03B259, 05B072; Haplogype 9: BOR 12444; Haplotype 10: BOR 8854, 8858, 8860, 8862, 8863, 8865–66, 12436, 12440–41, 12443, 12447, 12477, 12511, 12513–14, 12518, 12521, 12559, 12564, 12567–70, 12579–82, 12625, 22590, 22599–600, 22602–04, 22609, 22613–14, 22617, 03B006, 03B009, 03B061, 03B255, 03B256, 05B067, 05B076; Haplotype 11: BOR 12448, 12517; Haplotype 12: BOR 8870, Haplotype 13: BOR 12481–484, 12516, 12629–30, 23485, 03B059, 05B273.