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LARVAL DESCRIPTIONS OF SOME POORLY KNOWN TADPOLES FROM PENINSULAR MALAYSIA (AMPHIBIA: ANURA)

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***ABSTRACT.* –** The diagnostic larvae of six anuran species (two microhylids, one ranid and three rhacophorids) occurring in Peninsular Malaysia are described. The tadpole of *Microhyla annectens* Boulenger, a montane species, is described for the first time. The tadpole of *Microhyla berdmorei* (Blyth) is redescribed. A unique, endotrophic (non-feeding) larva is positively assigned to *Limnonectes laticeps* (Boulenger). Confirmations of the tadpoles of *Rhacophorus appendiculatus* (Günther) and *R. cyanopunctatus* Manthey & Steiof are provided. The tadpole of *Rhacophorus tunkui* Kiew is described for the first time and comparisons made with its congener, *R. prominanus* Smith.

***KEY WORDS.* –** Peninsular Malaysia, anuran larva, tadpole, *Microhyla annectens*, *Microhyla berdmorei*, *Limnonectes laticeps*, endotrophy, *Rhacophorus appendiculatus*, *Rhacophorus cyanopunctatus*, *Rhacophorus bimaculatus*, *Rhacophorus tunkui*, *Rhacophorus prominanus*.

INTRODUCTION

In Peninsular Malaysia, at least 88 species (in five families) of anurans have been recorded. However, the status of their larval identities was only reviewed recently, in which a scoring system was devised in order to gauge how thoroughly or feebly known their tadpoles are (Leong, 2002). The scores for each frog or toad species may range between 0 to 2.0; whereby 0 – completely unknown, 0.5 – doubtfully known (with degree of uncertainty), 1.0 – basic, minimal description and/or illustration, 1.5 – significantly more detailed descriptions/illustrations; 2.0 – detailed descriptions/ illustrations plus insights into its internal oral morphology (buccopharyngeal anatomy); 0* – descriptions of the diagnostic larvae in the midst of being prepared. This would help focus research on the species with scores of 0 and 0.5 especially, in order to determine their true identities. However, species with scores of 1.0 are still deserving of redescriptions nevertheless, so as to obtain a clearer picture of their larval morphology (and elevate the score up to 1.5 at least).

Not long after the publication of this tadpole identity checklist, the diagnostic larvae of various species in the peninsula have gradually been brought to light. These include the ranids *Limnonectes nitidus* (Smedley), *L. tweediei* (Smith), *Rana miopus* Boulenger and a rhacophorid *Theloderma asperum* (Boulenger), whose tadpoles were previously unknown otherwise (scores of 0) (Leong & Yaakob, 2002; Leong & C. F. Lim, 2003; Leong & K. K. P. Lim, 2003). There has also been a reassignment of larval identity, whereby the tadpole previously thought to belong to *Rana glandulosa* Boulenger (Berry, 1972) was actually that of the newly described *Rana banjarana* Leong & B. L. Lim, 2003. The present paper hopes to further contribute to the better understanding of Peninsular Malaysian anuran larvae with descriptions of six tadpole species, of which three are being revealed for the first time with certainty (*Microhyla annectens*, *Limnonectes laticeps* and *Rhacophorus tunkui*). The tadpole of *Microhyla berdmorei* (earlier basic description/simplistic illustration by Smith, 1924) is described and figured for greater precision. The larval identity of *Rhacophorus appendiculatus* (assigned, with uncertainty, by Inger, 1966, 1985) is confirmed and a Malayan developmental series is matched with Inger's descriptions.

For *Rhacophorus cyanopunctatus* [new name given for Sundaic forms previously referred to as *R. bimaculatus*, now regarded as a Philippine endemic (see Manthey & Steiof, 1998)], the first delegation of a provisional larval assignment was by Inger (1985). This larval form was previously assigned to the Bornean ranid *Staurois latopalmatus* (cf. Inger, 1966). The name *R. bimaculatus* continued to be used in reference to larval *Rhacophorus* occupying the microhabitats of rainforest riffles in Borneo (Inger, 1986). Later on, this larval form of *R.* '*bimaculatus*' was formally verified to be that of *R. gauni* (Bornean endemic) instead,

rendering the true tadpole type of *R. bimaculatus* (now *R. cyanopunctatus*) unaccounted for (Inger & Tan, 1990). Moreover, it was not indicated as to which species of *Rhacophorus* the former *R.* '*gauni*' tadpole (cf. Inger, 1985: 82-84) should belong. Thereafter, the larvae of *R. gauni* were correctly referred to, while that of *R. cyanopunctatus* remained unknown (Inger, 1992; Inger & Stuebing, 1997; Leong & Tan, 2002). Subsequently, a small collection of larvae was obtained from two forest streams in Johor (southernmost state of Peninsular Malaysia) and found to fit precisely the descriptions of larval *R.* '*gauni*' (cf. Inger, 1985) in terms of morphology and microhabitat. Along these streams, adult vouchers of *R. cyanopunctatus* were also collected. In Peninsular Malaysia, *R. cyanopunctatus* is the only species of *Rhacophorus* consistently encountered along the banks of small forest streams. The other Malayan species would normally be found to aggregate/breed around stagnant pools/wallows, with their larvae developing in such microhabitats. Thus, the tadpole previously described as belonging to *R.* '*gauni*' (cf. Inger, 1985) is now reassigned to *R. cyanopunctatus*, with descriptions of Malayan larvae provided here.

The rhacophorid, *Rhacophorus tunkui* Kiew, 1987 was first described from the lowland forests of Peninsular Malaysia, with the holotype collected from Sungai Jasin, Ulu Endau, Johor and paratypes from Kuala Tahan, Taman Negara, Pahang. It belongs to the group of Jade Treefrogs, whose members display a translucent jade to turquoise green colour in life. Other members include *R. prominanus* (Malay Peninsula, Sumatra) and *R. dulitensis* (Sumatra, Borneo). While the larvae of both species have previously been described by Berry (1972) and Inger (1985), respectively, that of *R. tunkui* had remained unknown till now. However, the nomenclatural status of *R. tunkui* has recently changed and considered to be a junior synonym of *R. prominanus* (Harvey et al., 2002). Unfortunately, the justifications provided by Harvey et al. (2002) were insufficient and unconvincing, especially when type material of both *R. prominanus* and *R. tunkui* were not even examined. Upon examination and comparisons of specimens (adults and larvae) identified as belonging to these two species, the author is convinced that *R. tunkui* is distinct from *R. prominanus* and should be duly removed from synonymy.

MATERIALS AND METHODS

The larvae were obtained from various localities throughout Peninsular Malaysia and the detailed locality/microhabitat data are provided for each species respectively. Where possible, vouchers of adults were collected from the vicinities of the microhabitats where their larvae were found. Photographs of the live tadpoles were taken for most of the species. Larvae were preserved in 10% formaldehyde. Staging is in accordance with Gosner (1960). Morphometric measurements were taken using slide verniers (to 0.1 mm). These include BL (Body Length: measured from snout tip to body-tail junction), TAL (Tail Length: from body-tail junction to tail tip), TL (Total Length: from snout tip to tail tip), MTH (Maximum Tail Height: greatest distance between dorsal and ventral fin margins), IOD (Inter-Orbital Distance: between centres of the pupils), IND (Inter-Narial Distance: between centers of narial apertures); abbreviations and definitions follow Altig & McDiarmid, 1999. In addition, BW (Body Width: widest part of body) and BH (Body Height: measured at mid-body) were also taken. Description of oral apparatus also follows Altig & McDiarmid (1999), with standard denotations for specific labial tooth rows (eg. A-1 and P-1 refers to the first labial tooth row of the anterior and posterior labia respectively). Where necessary, SVL (Snout Vent Length) of metamorphs and adults were measured. Labial tooth row formula (LTRF) is in accordance with Altig, 1970. Specimens of adults and larvae are currently deposited at the Raffles Museum of Biodiversity Research of the National University of Singapore (ZRC). Representative samples will subsequently be deposited at the herpetology collections of the Department of Wildlife and National Parks, Peninsular Malaysia (DWNP) and the Field Museum of Natural History, Chicago (FMNH).

LARVAL DESCRIPTIONS

Microhyla annectens Boulenger, 1900

(Fig. 1, Table 1)

Larval microhabitat. **–** Presumably, this montane species would breed in temporary to semi-permanent forest pools or puddles, although the larval series was found in a square concrete drainage corner (ca. 80 x 80cm). The water was ca. 30cm deep, with abundant submerged leaf litter, which gave the water a clear brown tinge. There was an abundance of dipteran larvae throughout the water column, and the entire substratum was teeming with tubificid worms. This artificial breeding habitat was located at the summit of Gunong Batu Brinchang (4°31'N, 101°23'E; 2,032m asl.), Cameron Highlands, just beside the telecommunications tower. At night, the calls of adults were heard from the undergrowth

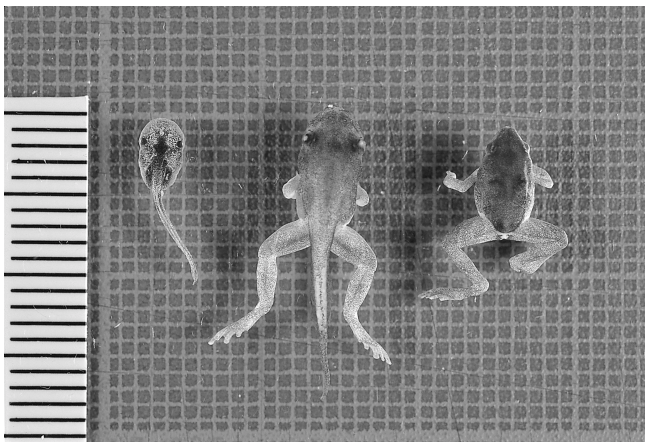

Fig. 1. Dorsal aspect of larval *Microhyla annectens*; depicting (from left to right) early (Stage 31), advanced (Stage 42) and emergent (Stage 46). Note anteriorly directed mouth (lower labium not expanded) and lack of thread-like terminal tail filament.

Table 1. Developmental changes in BL (body length) and TL (total length) of larval *Microhyla annectens* (ZRC.1.5194-5198; n = 5, Stages 31-46).

Gosner Stage	No.	BL (mm)	TL (mm)
31	1	4.1	10.7
42	2	6.9-8.0	12.5-17.3
43	1	7.0	11.6
46	1	SVL = 6.7mm	

surrounding this pool, but could not be detected. Adults of this species have been sighted previously at this vicinity (Jeet Sukumaran pers. comm.). At the time of discovery, the larvae were found in low numbers and only five specimens were obtained (ZRC.1.5194-5198; coll. T. M. Leong, 25 Jun.2000). No other anurans were observed in the immediate vicinity, although the resonant and distinctive "poop" calls of *Metaphrynella pollicaris* (another montane microhylid) were audible in surrounding montane forest.

***Larval diagnosis.* –** A small tadpole (TL not more than 18mm); colour in life reddish brown; mouth anteriorly directed, lower labium not expanded; spiracular flap with crenulate margin; tail without terminal filament. As with all larvae in the genus *Microhyla*, labial teeth, mandibles are absent from the oral disc.

***Larval morphology.* –** (Fig. 1) Body elliptical, BL 1.44-1.47 of BW, dorsum rather flat, venter rounded, BH 0.73-0.76 of BW; snout rounded, nostril equidistant between eye and snout tip; eyes laterally directed, IOD 4.37-4.42 of IND; spiracle median, spiracular margin crenulated, snout-spiracle 0.71-0.75 of BL; vent median, continuous with ventral fin, opening directed ventrally. Tail gradually tapering towards a narrowly pointed tip, without terminal filament, dorsal fin as deep as ventral fin, TAL 1.58-1.62 of BL, MTH 0.23-0.26 of TAL.

***Colour/Markings.* –** In life, dorsum and flanks reddish brown; venters largely free of pigment; tail muscle and dorsal fin uniform dark gray, ventral fin largely unpigmented, except for posterior end.

***Oral Disc.* –** Mouth directed towards anterior, lower labium not expanded; papillae, labial teeth or jaw sheaths absent.

***Developmental changes.* –** In the metamorph, the characteristic reddish brown colour and dark scapular markings are prominent, but fade upon preservation. The size changes of the small developmental series are presented in Table 1.

Microhyla berdmorei (Blyth, 1856)

(Fig. 2, Table 2)

***Larval microhabitat.* –** As with all larvae of *Microhyla*, this species inhabits temporary, stagnant pools of water. The developmental series was collected from a choked drain (ca. 200cm long by 30cm wide by 15cm deep) within the regenerated forest of the Forest Research Institute of Malaysia (FRIM; 3°14'N, 101°38'E), Kepong, Selangor, in the vicinity of Sungai Kroh (ZRC.1.7192-7432, coll. T. M. Leong & Norsham Yaakob, 18 Feb.2001). Larvae were mostly suspended at midwater level. They were also found together with the larvae of *Rana chalconota* (ZRC.1.6916-6930) and *Bufo parvus* (ZRC.1.6931-7191). In yet another locality within FRIM, a large number of larvae (ZRC.1.11161, coll. T. M. Leong, 21 Nov.2003) were collected from a rain-flooded pool (ca. eight metres diameter, 80cm deep) along the Engkabang Trail. Adult vouchers of this species (ZRC.1.11170-11174, coll. T. M. Leong et al., 22 Nov.2003) were also collected on the ground, among leaf litter, from around this temporary pool.

***Larval diagnosis.* –** A small tadpole (TL not exceeding 23mm); colour in life yellowish, fins largely clear; mouth facing anterior, lower labium not expanded, with distinct median 'U'-shaped arch; spiracular flap with smooth, curved margin, not crenulated; tail without terminal filament.

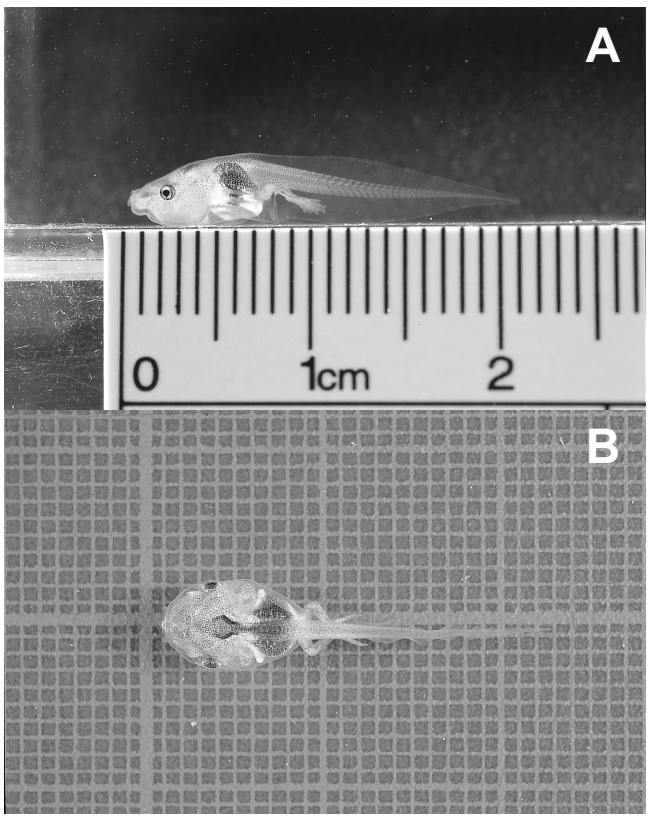

Fig. 2. Lateral (A) and dorsal (B) aspects of *Microhyla berdmorei* larva (Stage 39). Note anteriorly directed mouth (lower labium not expanded) and absence of terminal tail filament.

Table 2. Developmental changes in BL (body length) and TL (total length) of larval *Microhyla berdmorei* (ZRC.1.7192-7432; n = 241, Stages 25-46).

Gosner Stage	No.	BL (mm)	TL (mm)
25	5	3.5-3.8	9.4-10.1
26	13	4.8-5.2	10.8-11.8
28	14	5.1-5.4	13.2-13.9
29	7	5.7-6.2	12.9-13.7
30	5	5.5-6.3	13.8-14.6
31	7	5.9-6.2	14.5-15.5
32	8	6.3-6.7	14.9-15.6
33	20	6.2-6.6	15.0-15.9
34	98	6.3-7.4	16.3-18.7
35	19	6.5-7.1	16.7-17.9
36	2	7.0-7.9	16.8-18.7
37	1	6.8	17.3
38	3	7.1-7.5	17.4-18.8
39	5	7.3-7.8	17.7-19.1
40	16	7.7-8.1	17.9-21.8
42	5	6.9-7.6	16.7-18.2
43	10	6.1-6.6	8.4-12.3
44	2	6.2-6.4	6.4-6.6
46	1	SVL = 6.7mm	

***Larval morphology.* –** (Fig. 2) Body elliptical, BL 1.62-1.68 of BW, dorsum rather flat, venter rounded, BH 1.02-1.09 of BW; snout rounded, nostril nearer snout tip than eye; eyes laterally directed, IOD 4.97-5.21 of IND; spiracle median, spiracular margin a smooth convex, snout-spiracle 0.83-0.88 of BL; vent median, continuous with ventral fin, opening directed ventrally. Tail gradually tapering towards a narrowly pointed tip, without terminal filament, ventral fin deeper than dorsal fin for proximal half, TAL 1.29-1.34 of BL, MTH 0.28-0.32 of TAL.

***Colour/Markings.* –** In life, dorsum and flanks yellowish, body wall translucent, tail muscle whitish, tail fins clear or very lightly pigmented.

***Oral Disc.* –** Mouth anteriorly directed, lower labium not expanded, centre portion arched in a 'U'-shape; papillae, labial teeth or jaw sheaths absent.

***Developmental changes.* –** By stage 43, the symmetrical patterns of the dorsum and dark band on flanks are clearly distinct. The almost complete webbing in the hindfeet (characteristic of this species) is also visible at this stage onwards. The changes throughout development are reflected in Table 2.

Limnonectes laticeps (Boulenger, 1882)

(Figs. 3, 4, Table 3)

***Larval microhabitat.* –** A total of five larvae were encountered in a small, shallow creek (ca. 20cm wide, 2cm deep, sandy substrate) flowing slowly down hill slopes of the Canopy Trail within FRIM (3°14'N, 101°38'E) (ZRC.1.10808-10812; coll. T. M. Leong & Gary H. S. Lim, 29 May.2003). Adult, sub-adult and recent emergent vouchers found within this exact microhabitat were collected in the same night (ZRC.1.10804 – adult male, SVL 44.3mm; ZRC.1.10805 – adult female, SVL 42.1mm; ZRC.1.10806 – subadult, SVL 16.0mm; ZRC.1.10807 – emergent, SVL 7.4mm). As no other anuran species were found in this immediate vicinity, it was deduced that the larvae belonged to this parental species. This was subsequently confirmed with the rearing of a larva to an advanced stage.

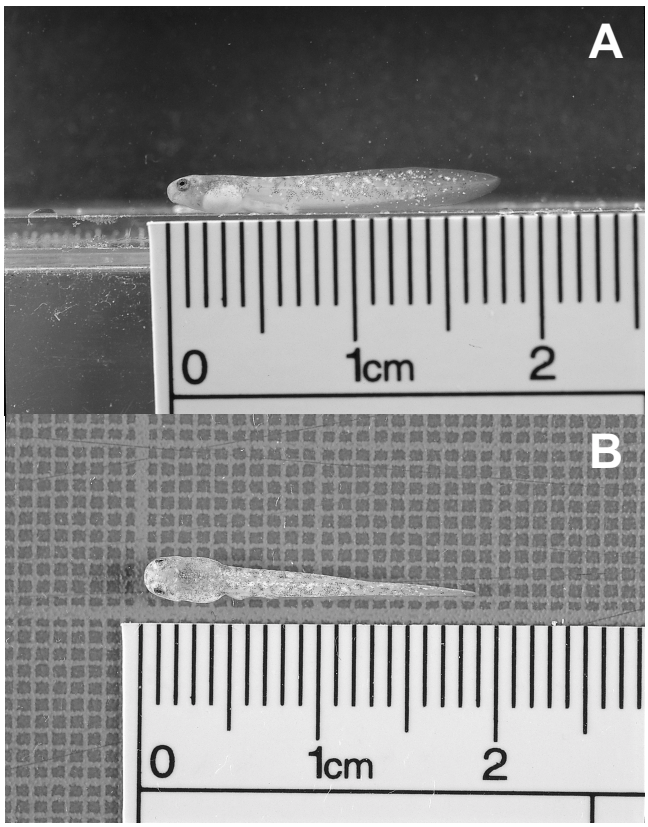

Fig. 3. Lateral (A) and dorsal (B) aspects of *Limnonectes laticeps* larva (Stage 37). Note persistence of pronounced yolk sac, despite well developed hind limb buds.

Table 3. Developmental changes in BL (body length) and TL (total length) of larval *Limnonectes laticeps* (ZRC.1.10807-10812; n = 6, Stages 36-46).

Gosner Stage	No.	BL (mm)	TL (mm)
36	2	4.0	15.7-16.5
37	2	3.9-4.1	16.8-17.5
41	1	4.0	16.1
46	1	SVL = 7.4mm	

***Larval diagnosis.* –** A small, sedentary tadpole (maximum TL ca. 18mm); nostrils located at upper edge of snout tip, opening towards anterior; mouthparts consisting only of a pair of papillae on upper rim of oral aperture; prominent yolk sac (visible from dorsal perspective) up to advanced stages; nutritional mode endotrophic (non-feeding); vent median; tail fins low, dorsal fin originating after proximal 1/4 of tail.

***Larval morphology.* –** (Figs. 3, 4) Body elliptical, BL 1.45-1.53 of BW, slightly depressed dorsoventrally, cross-section almost cylindrical, BH 0.85-0.94 of BW; snout rounded from dorsal perspective, truncate from lateral perspective, nostrils located at upper edge of snout tip, directed anteriorly; eyes lateral, IOD 2.48-2.61 of IND; spiracle sinistral, not projecting as a free tube, tapered towards the opening, opening directed backwards, spiracle visible from above and below, snout-spiracle 0.41-0.46 of BL; vent median, tapering towards narrow opening directed posteriorly, exceeds margin of ventral fin. Tail almost straight for entire length, except for abrupt tapering towards a round tip, dorsal fin originating after proximal 1/4 of tail, tail muscle deeper than either fins for proximal 3/4. TAL 3.18-3.27 of BL, MTH 0.18-0.19 of TAL. No observable lateral line pores or glandules.

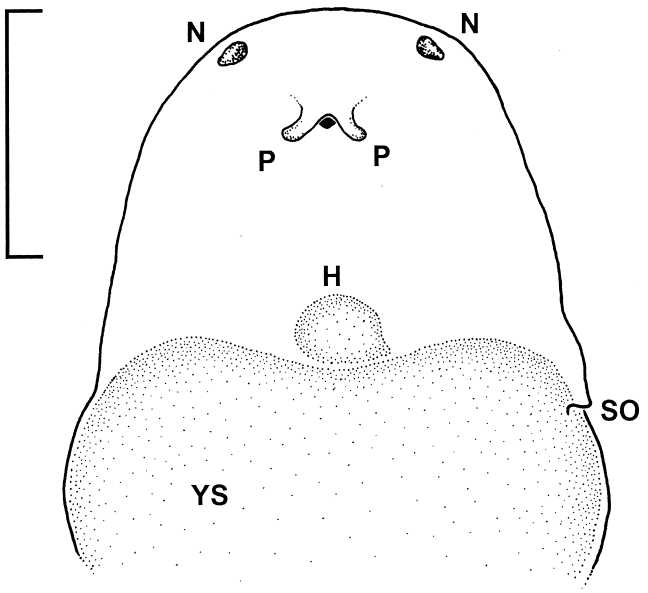

Fig. 4. Ventral aspect of larval *Limnonectes laticeps* (Stage 37). Note single pair of elongate papillae (P) trailing from upper rim of mouth; complete absence of anterior or posterior labia (and associated marginal/infra-marginal papillae), labial tooth rows, or jaw sheaths. Nostrils (N) visible at upper edge of snout; yolk sac (YS) extensive; H = heart; SO = spiracular opening. Scale bar = 1mm.

***Colour/Markings.* –** In life, dorsum and flanks light beige to buff, lightly speckled with orange brown and whitish pigments; yolk sac creamy yellow; tail muscle translucent, tail fins clear, entire tail with scattered pigments.

***Mouth.* –** (Fig. 4) Entirely lacking any fleshy anterior/posterior labia and associated labial teeth/jaw sheaths; a miniscule oral aperture (width ca. 1/10th of BW) flanked only by a pair of elongate papillae originating from upper rim of mouth, possibly serving the function of preventing particulate matter from entering buccal cavity while breathing.

***Developmental changes.* –** Although attempts to rear the larvae to complete metamorphosis were unsuccessful, the single most advanced larva (Stage 41, died 14 days after initial collection) was already beginning to exhibit characteristics of its parental species. These include slightly expanded toe tips, incomplete webbing of hindfeet and a dark, inverted chevron, scapular marking. Despite the advanced stage of this particular larva, the amount of yolk sac remaining still occupied just under half the volume of the entire body cavity. This larva was observed to swim to the water surface for gulps of air with increasing frequency as it matured. However, like the other larvae, it remained motionless at the bottom unless disturbed. The measurements of this small, but crucial, series are given in Table 3.

Rhacophorus appendiculatus (Günther, 1858)

(Figs. 5, 6, Table 4)

***Larval microhabitat.* –** Temporary forest pool (ca. 5 x 2m, 5cm deep; recently rain-filled) in lowland forest at foothills of Gunong Panti, south east Johor (1°51'N, 103°23'E) (ZRC.1.10284-10323; coll. T. M. Leong et al., 14 Dec.2002). Adults of this species were seen and heard at this site, with vouchers collected (ZRC.1.10239-10246). Other rhacophorids encountered here include *Polypedates leucomystax* and *P. macrotis*. However, their larvae were not to be seen in the pool at that time.

***Larval diagnosis.* –** A small, benthic tadpole (maximum TL ca. 27mm, BL ca. 11mm); light patches/spots present along tail muscle; advanced hindlimbs exhibiting pale areas on knee and heel; LTRF 4(2-4)/3(1).

***Larval morphology.* –** (Fig. 5) Body elliptical, BL 1.63-1.67 of BW, slightly depressed dorsoventrally, BH 0.65-0.72 of BW; snout rounded, nostril equidistant between eye and snout tip; eyes dorsolateral, IOD 2.31-2.41 of IND; spiracle sinistral, not projecting as a free tube, oval opening directed upwards

Table 4. Developmental changes in BL (body length) and TL (total length) of larval *Rhacophorus appendiculatus* (ZRC.1.10284-10323; n = 40, Stages 34-46).

Gosner Stage	No.	BL (mm)	TL (mm)
34	2	7.2-7.5	16.4-16.9
35	2	7.8-8.1	18.7-20.7
36	11	8.2-8.7	22.8-24.3
37	2	8.9-9.3	25.7-26.9
39	1	9.4	25.5
40	1	9.5	24.2
41	11	9.1-10.3	23.9-26.5
42	3	8.7-9.2	16.0-19.2
46	6	SVL = 8.2-9.3mm	

and backwards, snout-spiracle 0.54-0.58 of BL; vent dextral, fused with ventral fin, opening directed towards posterior. Tail tapering only at distal 3/4 towards a narrowly rounded tip, fins sub-parallel for anterior half of tail, dorsal fin slightly deeper than ventral fin at midpoint of tail, TAL 1.78-1.92 of BL, MTH 0.23-0.26 of TAL. Naso-lacrymal groove and lateral line pores present.

***Colour/Markings.* –** In life, dorsum, flanks and tail muscle grayish brown; small, pale flecks on body, but larger spots/ patches on tail muscle; tail fins with lightly scattered dark gray pigments; hind limbs in advanced larvae (Stages 39 and beyond) with pale areas on the knee and heel.

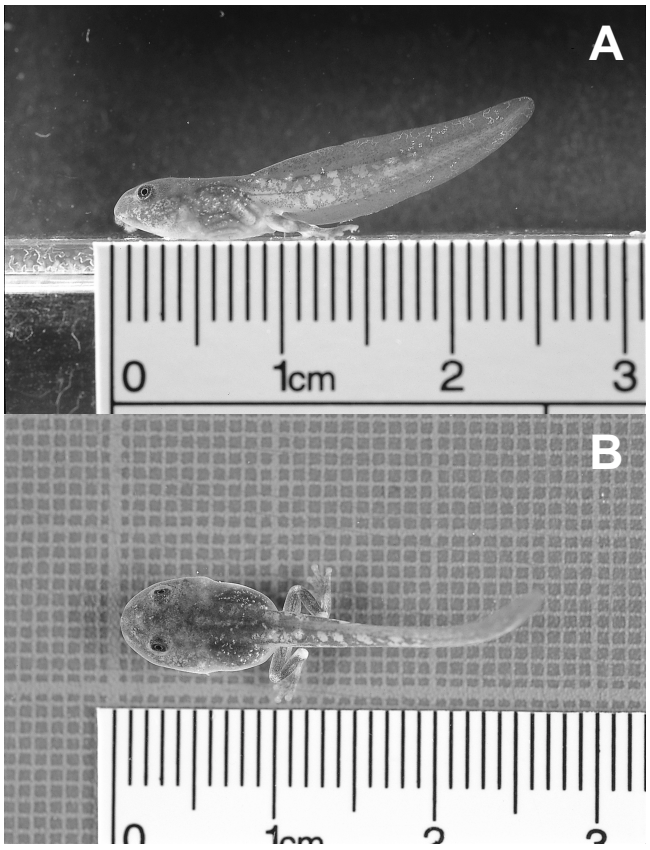

Fig. 5. Lateral (A) and dorsal (B) aspects of *Rhacophorus appendiculatus* larva (Stage 40). Note presence of pale spots/ blotches along tail muscle.

***Oral Disc.* –** Marginal papillae on anterior labium confined to single row at lateral corners, a continuous row of marginal and another row of infra-marginal papillae on posterior labium; margin of upper jaw sheath straight in the centre, without median convexity, lower jaw sheath a typical 'V' arch, both jaw sheaths keratinised and serrated at the margins; ODW 0.48-0.56 of BW.

***LTRF.* –** 4(2-4)/3(1); P-1 separated in centre (discontinuous), although gap not always noticeable; size of labial teeth in P-3 smallest.

***Developmental changes.* –** In the emergents, additional pale areas are evident at the axillary region, upper arm to elbow and at the vent (Fig. 6). No dermal fringes along the outer edges of the limbs are noticeable at this stage. Their SVL does not exceed 10mm (Table 4).

Rhacophorus cyanopunctatus Manthey & Steiof, 1998
(Fig. 7, Table 5)

***Larval microhabitat.* –** A small collection of larvae was obtained from two different streams in south eastern Johor (ZRC.1.8627-8630, Kahang, 2°16'N, 103°36'E, coll. T. M. Leong et al., 24 Sep.2001; ZRC.1.10254-10255, Gunong Panti foothills, 1°51'N, 103°23'E, coll. T. M. Leong et al., 15 Dec.2002). However, the larvae were collected from

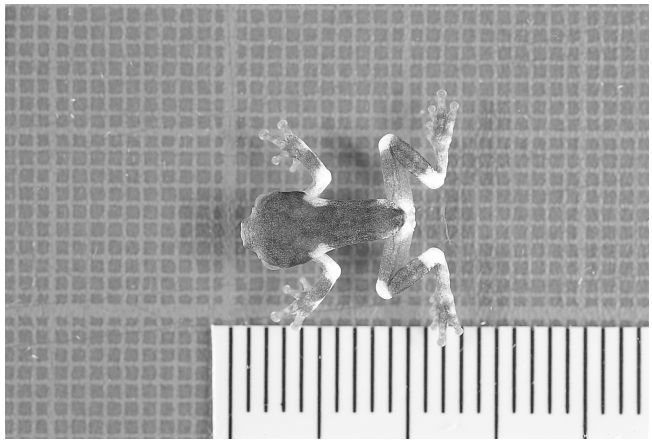

Fig. 6. Dorsal aspect of recent emergent of *Rhacophorus appendiculatus* (Stage 46). Note symmetrical pale areas on axilla, upper arm to elbow, knee, heel and around vent.

Table 5. Developmental changes in BL (body length) and TL (total length) of larval *Rhacophorus cyanopunctatus* (ZRC.1.8627-8630, 10254-10255; n = 6, Stages 25-37).

Gosner Stage	No.	BL (mm)	TL (mm)
25	1	4.6	15.3
28	3	6.3-6.9	18.6-20.2
31	1	6.8	20.9
37	1	9.7	28.2

similar situations of leaf drifts on the sides of the streams where current was minimal and leaf litter had accumulated. The streams were not more than two metres wide and half a metre deep, with sandy substrate. At night, adult vouchers were collected from both localities, perched on vegetation flanking the stream, with adult males calling (ZRC.1.8609-8611, Kahang; ZRC.1.10237-10238, Panti foothills). At the second site, the larvae of *Limnonectes blythii* (ZRC.1.10251), *Rana chalconota* (ZRC.1.10252) and *Rana signata* (ZRC.1.10253) were collected from the same patch of leaf drift as the *R. cyanopunctaus* larvae.

Larval diagnosis. **–** A small, stream-dwelling tadpole (maximum TL ca. 29mm); body elongated; nostril and eye elliptical; spiracle projecting as free tube; body and tail muscle black in life, fins largely clear; oral disc assuming cup-shape; LTRF 9(5-9)/3(1).

Larval morphology. **–** (Fig. 7) Body elliptical, BL 2.16-2.21 of BW, slightly depressed dorsoventrally, BH 0.61-0.69 of BW; snout rounded, nostril equidistant between eye and snout tip, nostril an oval slit, opening directed laterally; eyes dorsolateral, oval shaped; IOD 1.93-2.02 of IND; spiracle sinistral, low on left side, projecting as a short, free tube, tapered towards the end, opening directed backwards, spiracle visible from above and below, snout-spiracle 0.55-0.58 of BL; vent dextral, conical, continuous with ventral fin, ending as a free flap beyond margin of ventral fin. Tail weakly convex at midpoint, dorsal fin slightly deeper than ventral fin, tail tip narrowly rounded, TAL 1.91-2.35 of BL, MTH 0.13-0.16 of TAL. Lateral line pores faintly discernible, naso-lacrymal groove indistinct.

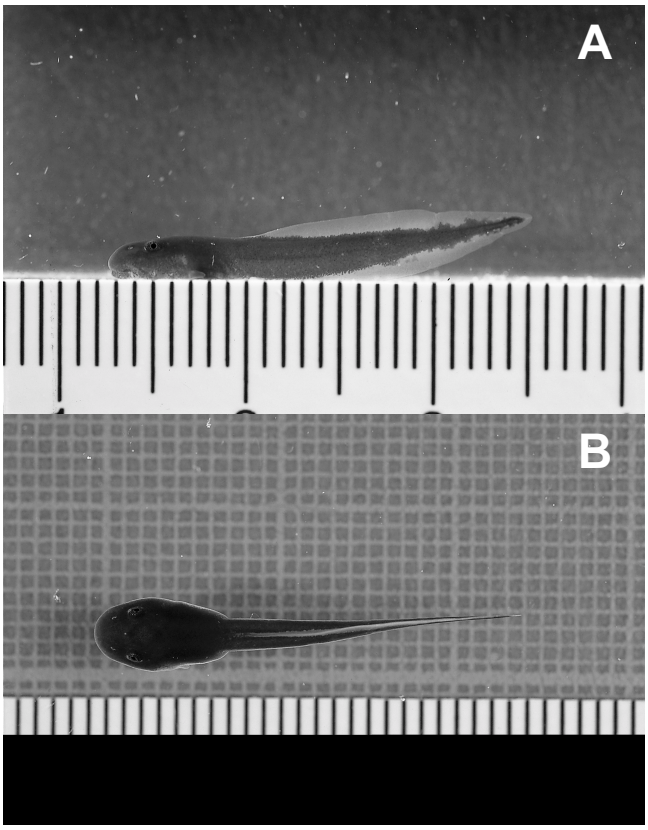

Fig. 7. Lateral (A) and dorsal (B) aspects of *Rhacophorus cyanopunctatus* larva (Stage 31). Note uniformly black head-body and tail muscle, fins largely unpigmented.

Colour/Markings. **–** In life, body and tail muscle jet black, venters light gray; tail fins mostly clear, except for areas immediately adjacent to dorsal and ventral margins of tail musculature.

Oral Disc. **–** Pronounced cup-shape formed by protuberant anterior and posterior labia, marginal papillae of anterior labium occupying lateral thirds on both sides, size (length) of individual papillae increasing from inner towards outer, additional infra-marginal row at lateral quarters of anterior labium; marginal papillae of posterior labium continuous, with 2-3 rows of evenly spaced/sized infra-marginal papillae; margin of upper jaw sheath curved downwards, lower jaw sheath broadly arched, both jaw sheaths keratinised and serrated at their margins, degree of keratinisation on lower jaw sheath more extensive (wider) than that of upper; ODW 0.57-0.62 of BW.

LTRF. **–** 9(5-9)/3(1) in advanced larvae (Stage 37); 8(5-8)/3(1) in earlier stages; size of labial teeth decreasing from P-1 to P-3.

Developmental changes. **–** A stage 25 larva was still with yolk sac, but later stages already exhibit the typical coiled intestines. In the stage 37 larva, expanded toe discs may be seen and finger discs visible through the translucent skin of venter. A summary of the measurements is presented in Table 5.

Remarks. **–** An additional larval specimen from Borneo [ZRC.1.4072, Stage 36, LTRF 9(5-9)/3(1); East Kalimantan, Kayan Basin, Sungai Bako, tributary to Sungai Kayan. Coll. H. H. Tan, 23 Nov.1999] was also examined and found to belong to this species. It was collected in similar microhabitats as described above and found together with larvae of *Rana signata* (ZRC.1.4044-4065) and *Limnonectes* sp. (ZRC.1.4066-4070).

Table 6. Developmental changes in BL (body length) and TL (total length) of larval *Rhacophorus tunkui* (ZRC.1.10607-10722; n = 128, Stages 28-46).

Gosner Stage	No.	BL (mm)	TL (mm)
28	2	5.5-5.7	14.6-14.9
29	5	6.3-6.8	17.8-19.5
30	4	7.1-7.9	19.3-20.1
31	4	7.3-7.8	18.7-20.3
34	4	7.6-8.2	19.0-22.4
35	12	8.8-9.6	23.1-26.1
36	28	9.2-9.9	23.9-25.6
37	37	9.7-10.7	26.7-27.8
38	4	10.5-11.4	27.3-27.9
39	1	10.9	28.4
40	2	10.5-10.7	27.9-29.3
41	5	10.2-10.8	26.1-29.9
42	7	9.9-11.1	18.4-26.9
43	1	10.6	18.5
44	3	9.6-10.5	10.2-12.4
46	9	SVL = 8.6-10.2mm	

***Rhacophorus tunkui* Kiew, 1987**
(Figs. 8-11, Table 6)

***Larval microhabitat.* –** A developmental series was reared from a freshly deposited clutch of ova (unpigmented), encountered on the night of 4 Jan.2003 within lowland forest at the Gunong Panti foothills (1°51'N, 103°23'E), south east Johor (coll. T. M. Leong et al.). The foam nest (ca. 8 x 4cm) was attached to the underside of a leaf of a shrub in the middle of a flooded pool (ca. 5 x 2m, 80cm deep). At this site, adult males were seen and heard calling from vegetation overhanging the pool, of which voucher specimens were obtained (ZRC.1.10363-10374). Other anurans found here were *Polypedates macrotis*, *Rhacophorus appendiculatus*, *Kaloula baleata*, *Microhyla borneensis*, *M. palmipes*. Attention was initially drawn to the foam nest by the presence of a juvenile keelback snake (*Xenochrophis trianguligerus*; ZRC.2.5623, total length 28.2cm), which was observed to be probing for ova and feeding from the foam nest (Fig. 8). The leaf was carefully removed to allow further embryological development ex-situ, with initial signs of hatching after ca. 36 hours. The hatching embryos were allowed to drop into a receptacle of water placed ca. 30cm beneath (Fig. 9).

Fig. 8. In-situ ovophagy by a juvenile *Xenochrophis trianguligerus* (ZRC.2.5623, total length 28.2cm) on the freshly deposited ova (unpigmented) of *Rhacophorus tunkui*. Photographed by Andrew Tay (04 Jan.2003).

***Larval diagnosis.* –** A small tadpole (maximum TL ca. 30mm); body and tail grayish green in life; tail muscle with two to three closely spaced, dark spots (actually ocelli when viewed under microscope) along midline immediately posterior to body-tail junction; LTRF 6(2-6)/3.

Fig. 9. Freshly hatched embryos of *Rhacophorus tunkui* dropping into receptacle of water provided for them below.

***Larval morphology.* –** (Fig. 10) Body elliptical, BL 1.72-1.85 of BW, mildly depressed dorsoventrally, BH 0.75-0.81 of BW; snout rounded, nostril nearer to snout tip than eye; eyes dorsolateral, IOD 2.38-2.42 of IND; spiracle sinistral, continuous with body wall, oval opening directed upwards and backwards, spiracle visible from above and below, snout-spiracle 0.61-0.65 of BL; vent dextral, tubular, fused with ventral fin, opening directed towards posterior. Tail originating from body-tail junction, rising to slight convex at midpoint and gradually descending to rounded tip, dorsal fin slightly deeper than ventral fin, TAL 1.42-1.83 of BL, MTH 0.24-0.27 of TAL. Naso-lacrymal groove and lateral line pores present.

***Colour/Markings.* –** In life, body and tail grayish green; greenish tinge increasingly pronounced in later stages; distinct spots (ocelli) on tail muscle black.

***Oral Disc.* –** Marginal papillae on anterior labium confined to lateral corners, continuous row of marginal and single row of infra-marginal papillae on posterior labium; margin of upper jaw sheath curved, lower jaw sheath with 'V' arch, both jaw sheaths keratinised and serrated at their margins; ODW 0.47-0.52 of BW.

***LTRF*. –** 6(2-6)/3 in advanced larvae, 5(2-5)/3 in earlier stages; size of labial teeth in P-3 smallest.

***Developmental changes.* –** The diagnostic tail spots already show from an early stage but the full set of three only becomes obvious in later stages. In the emergent, the pale jade green colour is reminiscent of the adult colouration, and its flesh is translucent, enabling some of its limb bones to be seen (Fig. 11). The red markings on the webbing of its hindfeet are not developed as yet. The emergents did not exceed 11mm in SVL (Table 6).

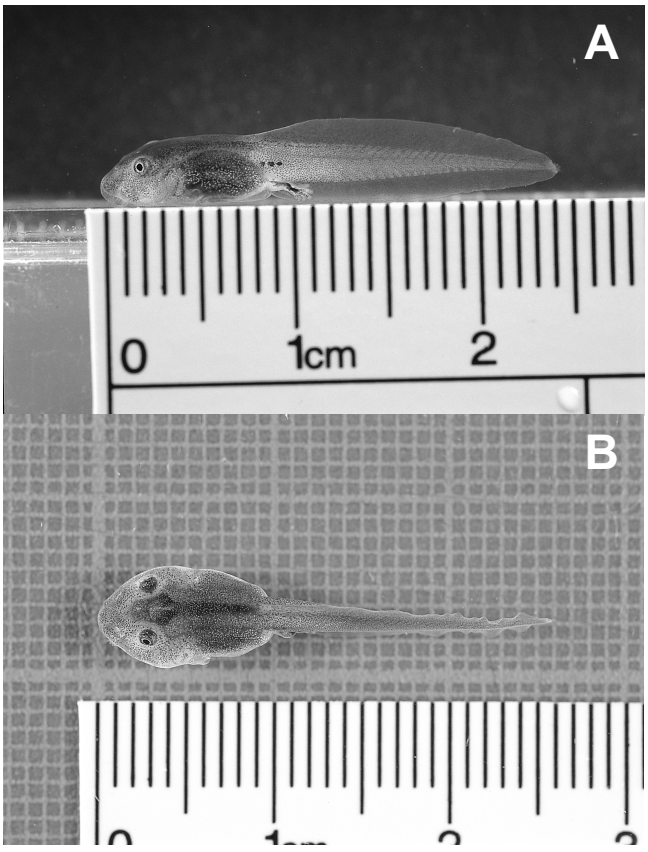

Fig. 10. Lateral (A) and dorsal (B) aspects of *Rhacophorus tunkui* larva (Stage 37). Note presence of three distinct dark spots on midline of tail muscle, immediately posterior to body-tail junction.

DISCUSSION

Among the known tadpoles in the genus *Microhyla* Tschudi within Peninsular Malaysia, two distinct morphotypes are recognised: (a) surface feeder with lower labium expanded, mouth dorsally directed; or (b) midwater feeder with lower labium not modified, mouth anteriorly directed. Both *Microhyla annectens* and *M. berdmorei* larvae fit into the second category and may be distinguished from those of *M. borneensis* and *M. heymonsi*, which belong to the first category (Leong & Chou, 1997, 1999); also from those of *M. butleri* and *M. ornata* by their presence of terminal tail filaments (absent in *M. annectens* and *M. berdmorei*) (Heyer, 1971; Chou & Lin, 1997). The larva of *M. palmipes* lacks such a filament but a more detailed diagnosis/description/illustration will have to be necessary for better comparisons (Iskandar, 1998). The larval identity of *M. superciliaris* remains unknown (Leong, 2002).

From a historical perspective on tadpole illustrations, there has been a significant contrast in terms of proportion and detail. Case in point would be to compare the accurately and exquisitely painted tadpoles of *Microhyla butleri* and *M. ornata* (Flower, 1899, Pl. LX, Figs. 1 & 2) with the simplistic sketch of *M. berdmorei* (Smith, 1924, Pl. VII, Fig. 1). The brief written description accompanying this was subsequently repeated by Parker (1934) and Bourret (1941), without noticeable improvements. Hence, the redescription in this paper.

Within the ranid genus *Limnonectes* Fitzinger in Peninsular Malaysia, all previously known larval forms are by far exotrophic and dependant on external food sources for growth and metamorphosis, with the exception of *L. hascheanus* (Stoliczka), which has been considered to be in

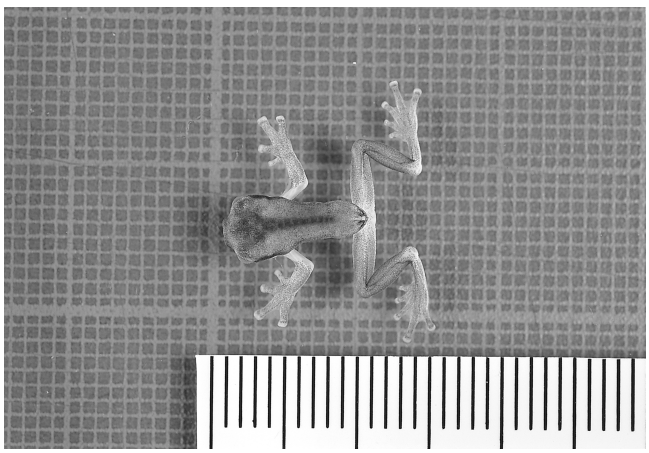

Fig. 11. Dorsal aspect of recent emergent of *Rhacophorus tunkui* (Stage 46).

its own genus *Taylorana* Dubois, 1986. This species is considered unique because of its direct development mode, which was first reported by Taylor (1962). Although the presently known larva of *L. laticeps* is free-swimming, it is clearly an endotroph, with abundant yolk and drastically reduced mouthparts. This mode of endotrophy is also witnessed in at least two other anurans from the region, namely *Pelophryne brevipes* (Bufonidae) and *Kalophrynus pleurostigma* (Microhylidae) (Thibaudeau & Altig, 1999). In Borneo, two feeding modes (endotrophic and exotrophic forms) were reported for *Rhacophorus gauni* (Rhacophoridae) (Inger, 1992). The earlier larval assignment to *L. laticeps* had been uncertain (Inger, 1985), and the description provided actually bore a closer resemblance to the larval types of the *L. blythii*/*macrodon* group instead.

Outside of Southeast Asia, examples of ranids exhibiting endotrophic larvae have been recorded in two Japanese species, namely *Rana tagoi* and *R. sakuraii* (Kusano & Fukuyama, 1989; Maeda & Matsui, 1989). In species that have such larval forms, the females usually employ the reproductive strategy of laying a small number of large eggs, instead of large numbers of very small eggs (Sanderson & Kupferberg, 1999). This trend was supported upon dissection of three adult female specimens of *Limnonectes laticeps* from FRIM, Selangor, Peninsular Malaysia. The specimen ZRC.1.7463 (SVL 44.2mm) was found to have 21 enlarged ova (diameter to 2.7mm), ZRC.1.9872 (SVL 38.0mm) had 12 enlarged ova (diameter to 2.5mm), ZRC.1.10805 (SVL 42.1mm) had 20 enlarged ova (diameter to 2.6mm). All ova were unpigmented.

The 'tentative' larval assignment for *Rhacophorus appendiculatus* (cf. Inger, 1966, 1985) based on Bornean specimens is cleared of its uncertainty and confirmed with comparable specimens from Peninsular Malaysia as described/illustrated in this paper. The larval form previously described and assigned to *Rhacophorus gauni* (cf. Inger, 1985) is now confirmed to be that of *R. cyanopunctatus* instead. As noted by Inger (1985), the microhabitat (streams) and morphology (elongated body, cup-like mouth) of these larvae (*R. bimaculatus* Group) are markedly different from the other *Rhacophorus* larvae, which live in stagnant pools, have more rounded body forms and non-specialised oral discs instead.

In Peninsular Malaysia, the two representative species of jade treefrogs are *Rhacophorus prominanus* and *R. tunkui*, of which the larvae of the former were first described by Berry (1972). Although the live colouration of tadpoles in both species is similar, they are distinguishable nevertheless. Firstly, larval *R. tunkui* is smaller in size (ca. 80% of *R. prominanus*) when comparing maximum attainable body lengths (BL) at Stage 36 for example [9.9mm in *R. tunkui* (Table 6, this paper) vs. 12.4mm in *R. prominanus* (Table 1, Berry, 1972)]. Secondly, *R. tunkui* is easily diagnosed by the presence of three small, dark spots (ocelli) on its proximal tail muscle, which are absent from larvae of *R. prominanus*. There are no differences in their LTRF.

The distinct differences between the larvae of these two species of *Rhacophorus* further undermine the synonymy of *R. tunkui* under *R. prominanus* by Harvey et al. (2002). In their opinion, Harvey et al. (2002) believed that differences between *R. tunkui* and *R. prominanus* were "the result of sexual dimorphism and individual variation within a single species". Although Kiew (1987) did not specify the gender in his type series or mention differences between males and females in his original description, Harvey et al. (2002) eventually assumed that the two adjacent specimens published in Plate 1 of Kiew (1987: 420) belonged to separate sexes of the same species, instead of *R. tunkui* (right) and *R. prominanus* (left) as originally stated. Their assumption was based upon examination of female specimens of *R. prominanus*, "eg. FMNH 189972 with a SVL of 47.61mm".

After examination of *R. prominanus* specimens at the ZRC, it was found that females of this species actually attain a much greater SVL than that indicated by Harvey et al. (2002). An adult female (ZRC.1.11001) had an SVL of 76.0mm. Adult males of *R. prominanus* may reach an SVL of ca. 62.0mm. In contrast, adult males of *R. tunkui* attain an SVL of up to 41.8mm (ca. 67% that of adult male *R. prominanus*). In addition, the degree of red pigmentation in the webbing of the hindfeet is also noticeable between the two species. In *R. prominanus*, the red extends between the third and fifth toes. In *R. tunkui*, the red band is confined to between the fourth and fifth toes. One other significant difference between both species is in their altitudinal distribution. *R. tunkui* is confined to lowland primary forest, whereas *R. prominanus* is found from sub-montane to montane forest (600m asl and above). Thus, *R. tunkui* deserves to be revalidated and removed from synonymy under *R. prominanus*. The comparative material examined is summarised in the appendix.

With the verification and description of these three species of *Rhacophorus* larvae (*R. appendiculatus*, *R. cyanopunctatus* and *R. tunkui*) in this paper, the last remaining Malayan member in this genus whose tadpole remains to be uncovered would be that of *R. robinsoni*, a rarely encountered treefrog. In addition, the tadpole of *R. reinwardtii* would also warrant an improved description, as and when larvae become available.

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APPENDIX

COMPARATIVE MATERIAL EXAMINED

Rhacophorus prominanus

[Adult males]: ZRC.1.899 (Jor, Batang Padang, Perak), ZRC.1.3273 (Fraser's Hill, Pahang), ZRC.1.9044, 11124 (Genting Highlands,

Selangor), ZRC.1.11193 (Gunong Telapak Burok, Negri Sembilan). [Adult females]: ZRC.1.9045 (Genting Highlands, Selangor), ZRC.1.11001 (The Gap, Fraser's Hill, Pahang). [Larvae]: ZRC.1.3456, 3461-3463, 3661-3664 (Fraser's Hill, Pahang), ZRC.1.11191, 11192 (Gunong Telapak Burok, Negri Sembilan).

Rhacophorus tunkui

[Adult males]: ZRC.1.10162 (Taman Negara, Pahang), ZRC.1.10363-10374 (Panti forest, Johor). [Larvae]: ZRC.1.10607-10722 (Panti forest, Johor).