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Morphology and ecology of tadpoles of *Ramanella obscura* (Anura: Microhylidae)

Gayan Bowatte and Madhava Meegaskumbura*

Department of Zoology, Faculty of Science, University of Peradeniya, Sri Lanka
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ABSTRACT

External, buccal and chondrocranial morphology, bone development and ecology of the tadpoles of *Ramanella obscura*, a narrow-mouthed frog species endemic to Sri Lanka is described. The tadpoles have a dorsoventrally flattend body, keratinous ridge on lower lip, a postnarial ridge that extends anteriorly between narial papillae and an expanded fenestrated sheet of cartilage associated with the larval otic process and processus lateralis posterior. But they do not possess a medial preglottal papilla in ventral buccal floor as in *Microhyla*. At Gosner stage 25, there is no bone development; but by stage 35, exoccipitals, parasphenoid, frontoparietals and prootics are developed. By stage 40, in addition to the bones at stage 35, nasals are formed. The tadpoles are tree-hole or small-ground-pool dwellers that feed on particulate matter and infusoria both in the substratum and the water column, for which they have morphological adaptations. No other anuran tadpoles were observed in syntopy with *R. obscura* tadpoles.

Key words: Chondrocranium, buccal morphology, tadpole ecology, endemic, Sri Lanka

INTRODUCTION

Ramanella is a species depauperate genus of narrow-mouthed frogs (family Microhylidae), with nine species distributed in India and Sri Lanka (Dutta, 1992; Manamendra-Arachchi and Petiyagoda, 2006). Endemism within this genus is high with Sri Lanka having three endemic species and India five. Only *Ramanella variegata* is shared between the two countries.

All three endemic Sri Lankan species are found within the Wet zone of Sri Lanka (Manamendra-Arachchi and Petiyagoda, 2006). Among these, the commonest is *R. obscura* (the Red Ramanella: grey-brown pug snouted frog) and the rarest is *R. palmata* (due to small range and low population densities). *Ramanella obscura* is widely distributed within the wetter parts of Sri Lanka, up to an elevation of about 1220 m asl (Manamendra-Arachchi and Pethiyagoda, 2006). During the rainy season, males are easily observable calling from breeding sites such as small ground puddles and tree-holes, even during daytime. In contrast, females are difficult to find during daytime, but are found at breeding sites, often at night. Both adult males and females are found in leaf litter, crevices, cavities and under tree bark during daytime, in the dry season (Meegaskumbura, 1999; Meegaskumbura, 2001).

The majority of *Ramanella* species are threatened. According to IUCN Red list (2011), *R. palmata* is Endangered, *R. nagaoi* is Vulnerable, *R. obscura* is Near Threatened and *R. variegata* is of Least Concern. Of the Indian species, *R. anamalaiensis*, *R. minor* are Data Deficient, *R. montana* is Near Threatened, *R. triangularis* is Vulnerable and *R. mormorata* is Endangered.

The natural history and reproductive biology of microhylids are poorly known (Wassersug, 1980; Donnelly *et al*., 1990; Lehr *et al*., 2007). Descriptions of characters useful in taxonomy have only been considered for a very few taxa. Microhylid tadpole morphology varies within taxa (Donnelly *et al*., 1990). Hence, it is important to study tadpole morphology in greater detail to make inter-species comparisons that would be useful for phylogenetic and comparative morphological analyses.

Chondrocrania of only a very few microhylid taxa have been described and this is especially true for old world microhylids. Microhylid chondrocrania of only 12 species from 10 genera have been described (McDiarmid and Altig, 2000) and of these, only three are Old World species, namely, *Kaloula pulchra* (Haas, 1996; 2003), *Microhyla ornata* (Ramaswami, 1940; Khan, 2003) and *Uperodon systoma* (Ramaswami, 1940).

Here we describe the external, chondrocranial and buccal morphology of *Ramanella obscura*. We focus on the mouth location, spiracle location, shape of spiracular

*Corresponding author's email: madhava_m@mac.com

opening, tail morphology and mouthparts, as these features have been identified to show considerable variation within microhylid taxa (Donnelly *et al.*, 1990) and hence are of potential importance in systematics. We also compare the morphology of *Ramanella obscura* with *Microhyla rubra*, another microhylid genus also found in Sri Lanka for which there is now adequate information to highlight the variation between the two genera.

MATERIALS AND METHODS

Several early embryonic stages (Gosner stage 24) were collected from a tree hole (70 15' 31.44" N, 80 36'. 00" E) in the University of Peradeniya premises, Sri Lanka. These were raised in the laboratory, and periodically observed until metamorphosis. Metamorphs were raised up to a month and identified using taxonomic keys devised for adult frogs (Manamendra-Arachchi and Pethiyagoda, 2006). Tadpoles were staged following Gosner (1960).

Grillitsch *et al.*, (1993) and McDiarmid and Altig (2000) were followed for external description of tadpoles. For internal oral anatomy, a combination of Khan (2000) and Wassersug (1976a) were followed. Surgical method delineated by Wassersug (1976a) was used. Following measurements were taken (Fig. 1): maximum height of body (*bh*), maximum width of body (*bw*), maximum diameter of eye (*ed*), internarial distance (*nn*), naro-pupular distance (*np*), interpupular distance (*pp*), rostro-narial distance (*rn*), distance from tip of snout to opening of spiracle (*ss*), distance from tip of snout to insertion of upper tail fin (*su*), snout-vent length (*svl*), total length (*tl*), distance from vent to tip of tail (*vt*), tail muscle height (*tmh*) and tail muscle width (*tmw*).

Morphology was observed using a Motic zoom-stereomicroscope (6 - 50 x magnification). Tadpoles were measured using a digital caliper (measured to the nearest 0.01 mm).

Tadpoles were euthanized using Chlorobutanol and fixed in 10% buffered formalin for two days and preserved in a 1:1 mixture of 10% buffered formalin and 70% alcohol. Tadpole stages are deposited in the collection of the Department of Zoology (DZ), University of Peradeniya, Sri Lanka.

The enzyme clearing and staining technique of Wassersug (1976b), later modified by Newman *et al.* (1983), was used to stain the tadpole skeletons. Specimens were processed synchronously, using the same stock solutions under similar laboratory conditions. Osteological terminology follows Duellman and Trueb (1986), chondrocranium terminology follows mostly Haas (1996; 2003).

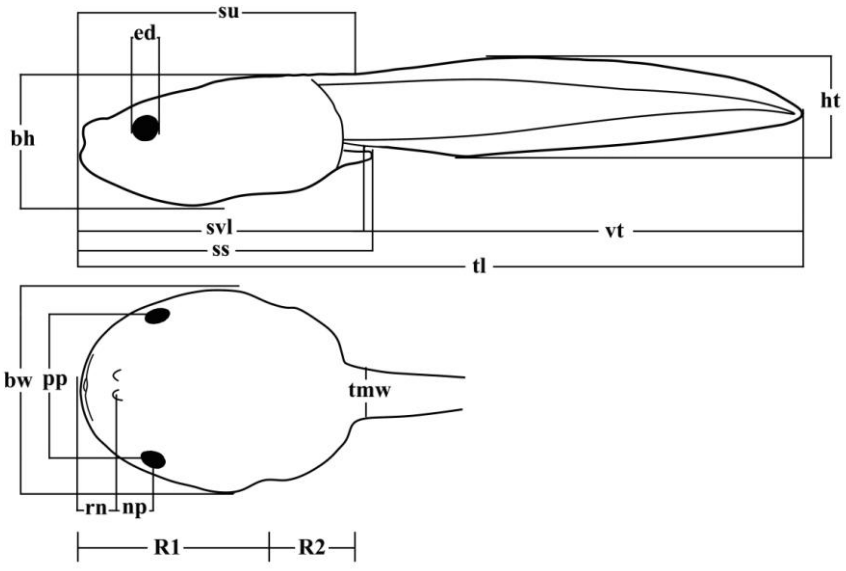

Figure 1. Lateral and dorsal outlines of *Ramanella obscura* showing measurements taken.***bh*** - Maximum height of body, ***bw*** - maximum width of body, ***ed*** - maximum diameter of eye, ***ht*** - maximum height of tail, ***nn*** - internarial distance, ***np*** - naro-pupular distance, ***pp*** - interpupular distance, ***rn*** - rostro-narial distance**,** ***ss*** - distance from tip of snout to opening of spiracle, ***su*** - distance from tip of snout to insertion of upper tail fin, ***svl*** - snout-vent length, ***tl*** - total length, ***vt*** - distance from vent to tip of tail, ***tmh*** - tail muscle height and ***tmw*** - tail muscle width.

RESULTS

Description of tadpole

External morphology: Descriptions based on Gosner stage 35 tadpoles of *Ramanella obscura* (DZ 1038, DZ 1039, DZ 1040), except when explicitly stated. Labial keratinized ridge was present in all individuals examined (Fig. 2).

In dorsal view, body clearly distinguished as two parts, a longer and wider anterior region (R1) and a narrower and shorter posterior region (R2), anterior region almost twice as longer than posterior region (Fig. 1); eyes small (eye diameter/body width = 0.13), snout rounded; slightly concave region immediately posterior to eyes; eyes dorsolateral, but more dorsally directed, not bulbous; nares closed (internarial width/inter-pupullary distance = 0.14); narial depressions visible, oval, dorsally directed, closer to pupils than to snout; nasolacrimal duct apparent; mouth narrow, terminal, lower and upper-lips both visible, lower lip extends beyond upper lip; tail long, tapering. (Figs. 3 and 4).

In profile, R1 elongated, truncated at snout, anterior-dorsal aspect straight, anterior-ventral aspect slightly rounded; R2 ventrally and dorsally slightly convex; gut contained in R2. Spiracle mid-ventral, transparent, cylindrical, ends slightly beyond posterior ventral end of body, dorsally attached to vent tube, ventrally free; vent tube is contained between spiracle and ventral tail fin; ventral tail fin begins at the body, and not contained within the spiracle (Fig. 4A and 4B); tail musculature weak, does not extend to end of tail tip (tail-muscle height/body height = 0.48; tail-muscle width/body width = 0.27); maximum tail height is less than maximum body height; V-shaped myomeres apparent (Fig. 4A and 4B); dorsal tail fin height is equal to ventral tail fin height, dorsal fin originate on body slightly anterior to point of origin of ventral tail fin (Figs. 3, 4A and 4B); tail tip rounded (does not end in a flagellum).

In ventral view eyes and lips not visible; venter slightly lighter in colour compared to dorsum; heart at boundary of R1 and R2; gut is contained with few coils in left side of R2; spiracle is conical shaped.

Oral flaps (labium): upper lip not fleshy (Fig. 2); lower lip has a keratinized ridge (Figs. 2 and 4B).

Colour in life: Body heavily pigmented, appears as dark brown to black in colour. Iridophores absent. In profile, dorsum densely pigmented compared to venter; iris black, markings absent. Tail fins lightly pigmented than the body. Anterior tail musculature more pigmented than posterior. In left profile, dark-brown coiled gut is clearly visible; in right profile, R2 region, pale; in dorsal view, R2 darker than R1. Nasolacrimal grooves are prominent, pigmentation absent or slight (Figs. 4A and 4B).

Colour in preservative: Dorsum dark brown, posterior 1/3 of body is darker than anterior 2/3. Basal area of tail is dark brown, lighter towards tip. Nostrils marked by black or dark-brown pigmentation. In profile body is lighter than dorsal side with lighter pigmentation around eyes. Venter lighter than dorsum, brown colour pigment patches scattered throughout.

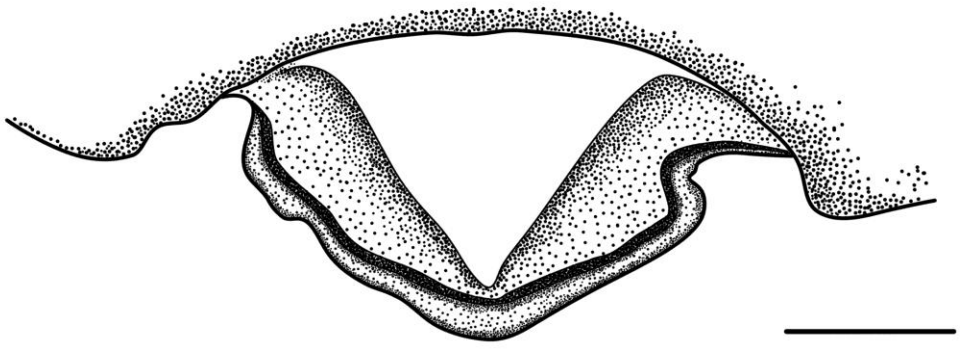

Figure 2. External mouth morphology of *Ramanella obscura* tadpole (Gosner stage 35) showing keratinized ridge at the lower lip. (Scale bar 0.5 mm).

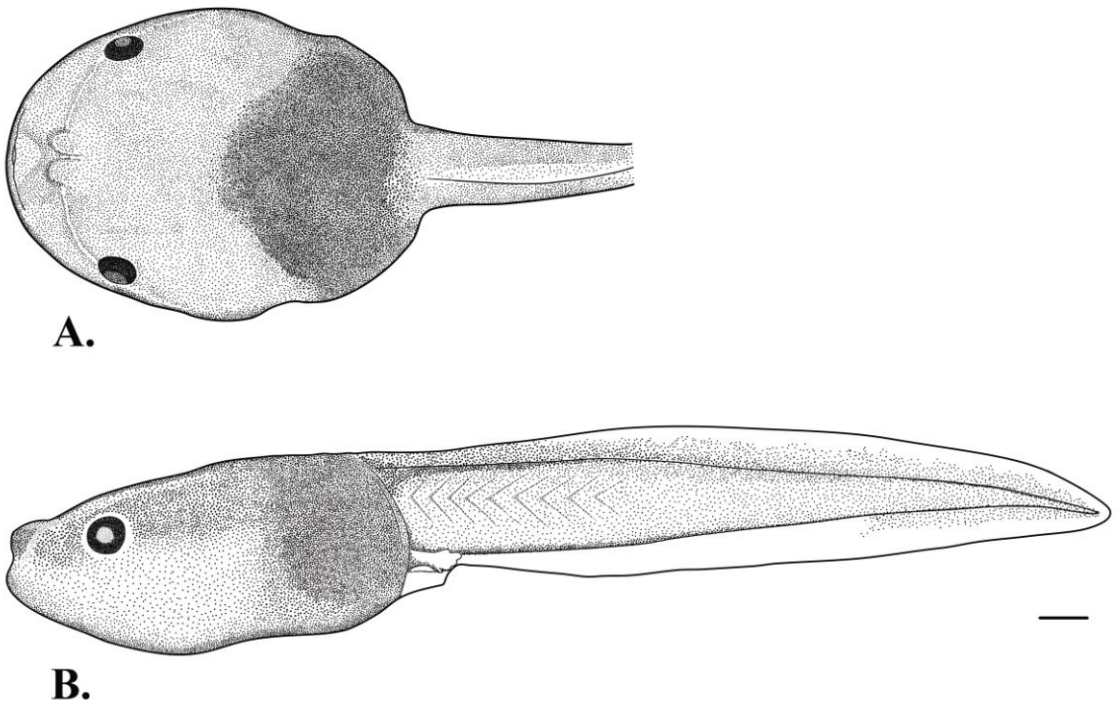

Figure 3. A. Dorsal aspect and **B**. profile of the whole body of *Ramanella obscura* (Scale bar, 1 mm).

Buccal morphology

Ventral buccal region: Prelingual arena V-shaped, length equal to width, pointed portion of V directed anteriorly towards oral aperture; one pair of pre-lingual papillae, flap like, pigmented; continuous medial ridge slightly anterior to pre-lingual papillae; a pair of lateral buccal pockets in anterior region of buccal floor; 4-5 pairs of pocket papillae on anterior wall of pockets; tongue anlage U-shaped, non papillated, pigmented, broader anteriorly, narrower and attached posteriorly, with pigment; buccal floor arena (BFA) triangular, laterally elevated, medially depressed, forming a narrow passage at anterior portion of BFA, posterior end of buccal floor much broader than anterior end; margins of narrow passage and anterior part of BFA pigmented; BFA papillae, nine pairs, closer to glottis are two larger papillae with projections, other papillae with slight projections, all papillae directed anteromedially; medial preglottal papillae absent; buccal pockets long and narrow, blunt and wider at blind end; trachea club-shaped, protruding from base of velum, extending to base of BFA, ending in prominent, pigmented, elevated and pointed lips; broad ventral velum, free margin of velum not smooth, containing several projections above filter plates (Fig. 5A).

Ventral pharynx region: Branchial baskets triangular, 1/3 of filter cavities anterior to velum, all three filter plates distinct; subvelar surface with many secretory ridges (Fig. 5A).

Dorsal buccal region: Choanae open; prenarial arena hourglass shaped; three prenarial papilla, small, blunt, triangular arrangement, posterior one of these placed between narial papilla; narial papillae originate from distal narial wall, twisted, long, flat, robust, edges serrated, anteriorly-directed; postnarial ridge prominent, slightly serrated, medially an extension that ends between narial papillae; 1-3 lateral ridge papillae; buccal roof arena (BRA) triangular, broad anteriorly, two pairs of small and blunt papillae; center region of BRA with many pustulations, unpigmented; (Fig. 5B).

Chondrocranium (at Gosner stage 35):
Chondrocranium is flat and wide; length 0.5 of the total length.

Figure 4. *Ramanella obscura* tadpoles in life. Showing the spiracle, lateral eyes, and long tail fin with a rounded tip. **A**. Gosner stage 30. **B.** Gosner stage 38.

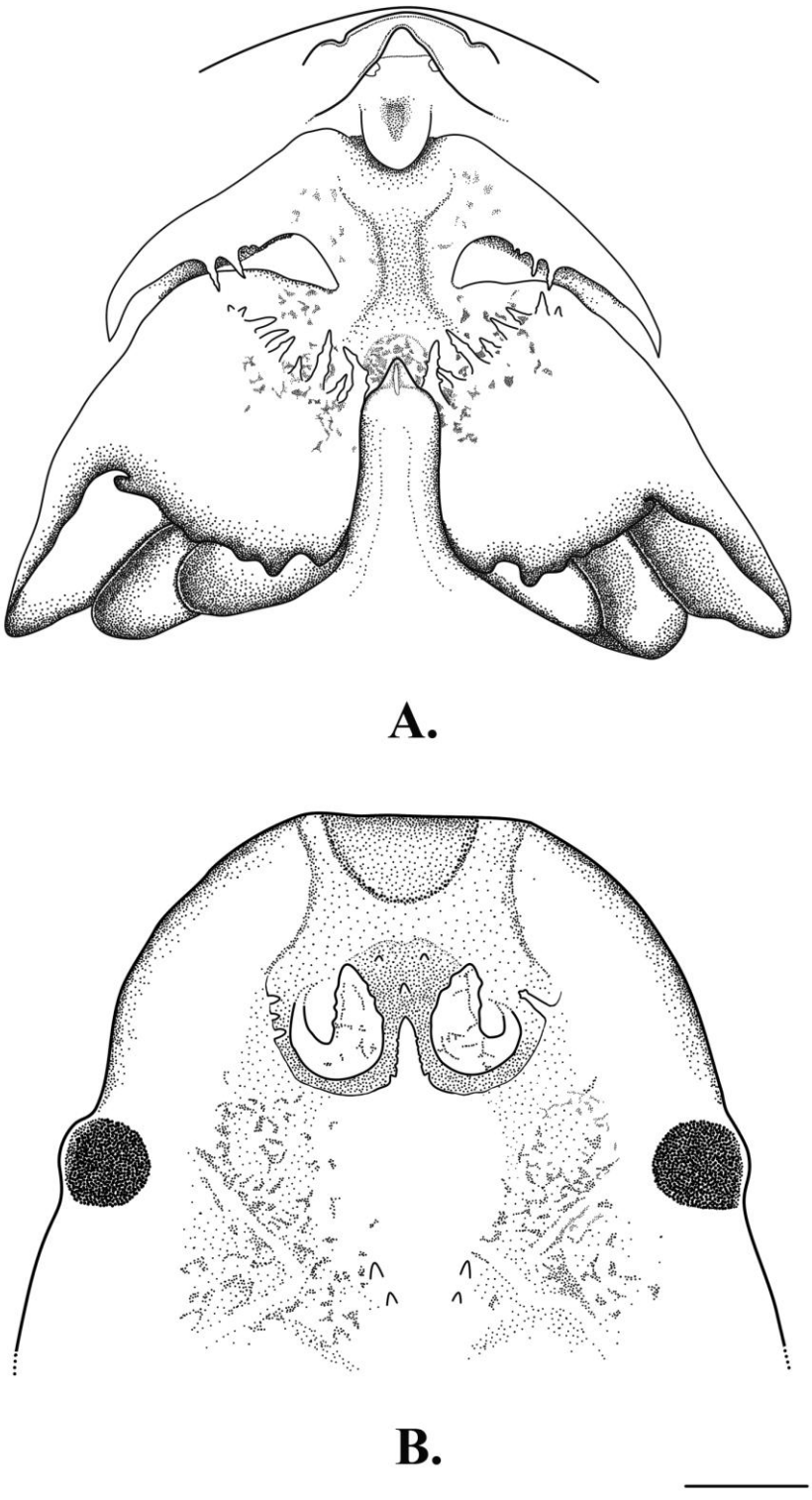

Figure 5. A. Ventral Buccal. **B.** Dorsal Buccal regions of the tadpole of *Ramanella obscura* (Scale bar, 1 mm).

Larvae do not have a keratinized ridge in lower jaw, however, they have cartilago labialis superior and cartilago labialis inferior that support keratinous beaks in other anuran larvae. Cartilago labialis superior, fenestrations are absent; a simple medial notch on ventral surface, and an elaborate W shaped notch on dorsal surface; lateral alae are flat with wide triangular shaped sheets of cartilage. Posses a slight processus dorsalis posterior on posterodorsal margin of each ala. Adrostral cartilage absent (Fig. 6D).

Cornu trabeculae are small ($1/5^{th}$ of total chondrocranial length), curved anterolaterally, forming a V shaped angle of 160^0 in dorsal view. Ends of cornu trabeculae articulate with the lateral alae of the cartilago labialis superior. (Figs. 6A and 6B).

Posterior confluence of cornu trabeculae is continuous with planum trabeculae anticum, which in turn continues with the planum ethmoidale that forms the anterior wall of the braincase (Fig. 6A). Lateral wall of the braincase is formed by the orbital cartilage and bears a large optic foramen (Fig. 6C).

Taenia tecti marginalis is present on dorsal margin of brain case, but taenia tecti transversalis is absent (Fig. 6A).

Posterior margin of frontoparietal fontanelle is demarked by tectum synoticum, a dorsal bridge of cartilage that unites otic capsules. Connection of tectum synoticum to otic capsule is far posterior. Anterior margin of tectum synoticum is wider in middle than at ends, which articulate with otic capsules; medial part of tectum synoticum is flat.

Braincase is closed ventrally, except for two pairs of small foraminae: foramen craniopalatinum and foramen caroticum primarium. Otic capsules are ovoid in shape (Fig. 6B).

Larval otic process extends anteroventrally from the anterolateral wall of the otic capsule. Palatoquadrate unites with brain case posteriorly through processus ascendens. This joins palatoquadrate (arcus subocularis quadrati) anterior to most anterior margin of the otic capsule. At juncture between processus ascendens and palatoquadrate, a cartilaginous process extends laterally and posteriorly. At end of this extension, a sheet of cartilage and the lateral extension of the processus ascendens and palatoquadrate form processus lateralis posterior.

At center of processus lateralis posterior a cartilaginous sheet extends posteriorly and joins with a cartilaginous process that extends laterally from the mid region of the otic capsule. At this juncture two processes are evident: first, a ventrally originating posteriorly directed processus suboticus quadrati, and second, extends posterior laterally and branches out to form fenestrations. A single, thin, rod-like process extends anteriorly and articulates with the processus lateralis posterior, another cartilaginous process extends posterolaterally from the origin of processus suboticus quadrati and articulates with expanded larval crista parotica (Figs. 6A and 6B).

Meckel's cartilages are flat sheets of cartilage, oriented almost perpendicular to main body axis. Lateral edge of Meckel's cartilages is concave while medial part is convex. Medially facing free ends of Meckel's cartilages are pointed (Fig. 6B).

Paired cartilago labialis inferior is an inverted U shaped thin sheet of cartilage which articulates close to anterior end of Meckel's cartilage (Fig. 6B).

The palatoquadrate lies lateral to the braincase. There are three connections to the neurocranium: commissura quadratocranialis anterior, processus ascendens, and processus suboticus quadrati (Figs. 6A and 6B).

Hyobranchial (visceral) skeleton (at Gosner stage 35)**:** Branchial baskets are well developed, deep and wide. Length of hyobranchial skeleton/ length of chondrocranium = 1. Hyobranchial skeleton is wider than length in ventral view. (maximum width > maximum height). One pair of ceratohyals and four pairs of branchial arches form hyobranchial skeleton. Ceratohyals are interconnected by a flexible, comparatively short pars reuniens. Each ceratohyal has four processes: processus anterior and processus articularis on anterior margin, processus lateralis, and processus posterior at posterior margin.

Anterior to pars reuniens lies a small cartilage, the copula. Basibranchials lie posterior to the pars reuniens and are separated from the anterior paired ceratohyla. Processus urobranchialis originates from posterior end of the basibranchial. Plana hypobranchiales articulate with one another along their medial margins. Commissurae terminales connect ceratobranchialia distally (Fig. 6E).

Body Measurements (mm) (Gosner **stage 35):** Maximum height of body (*bh*) = 3.80, maximum width of body (*bw*) = 6.53, maximum diameter of eye (*ed*) = 0.89, maximum height of tail (*ht*) = 3.28, internarial distance (*nn*) = 0.71, naro-pupular distance (*np*) = 1.03, interpupular distance (*pp*) = 4.87, rostro-narial distance (*rn*) = 1.16, distance from tip of snout to opening of

spiracle (*ss*) = 9.08, distance from tip of snout to insertion of upper tail fin (*su*) = 8.11, snout-vent length (*svl*) = 9.058, total length (*tl*) = 23.60, distance from vent to tip of tail (*vt*) = 14.54, tail muscle height (*tmh*) = 1.85 and tail muscle width (*tmw*) = 1.77. Body measurements of tadpoles in Gosner stages 26, 34, 35 and 38 are presented in Table 1.

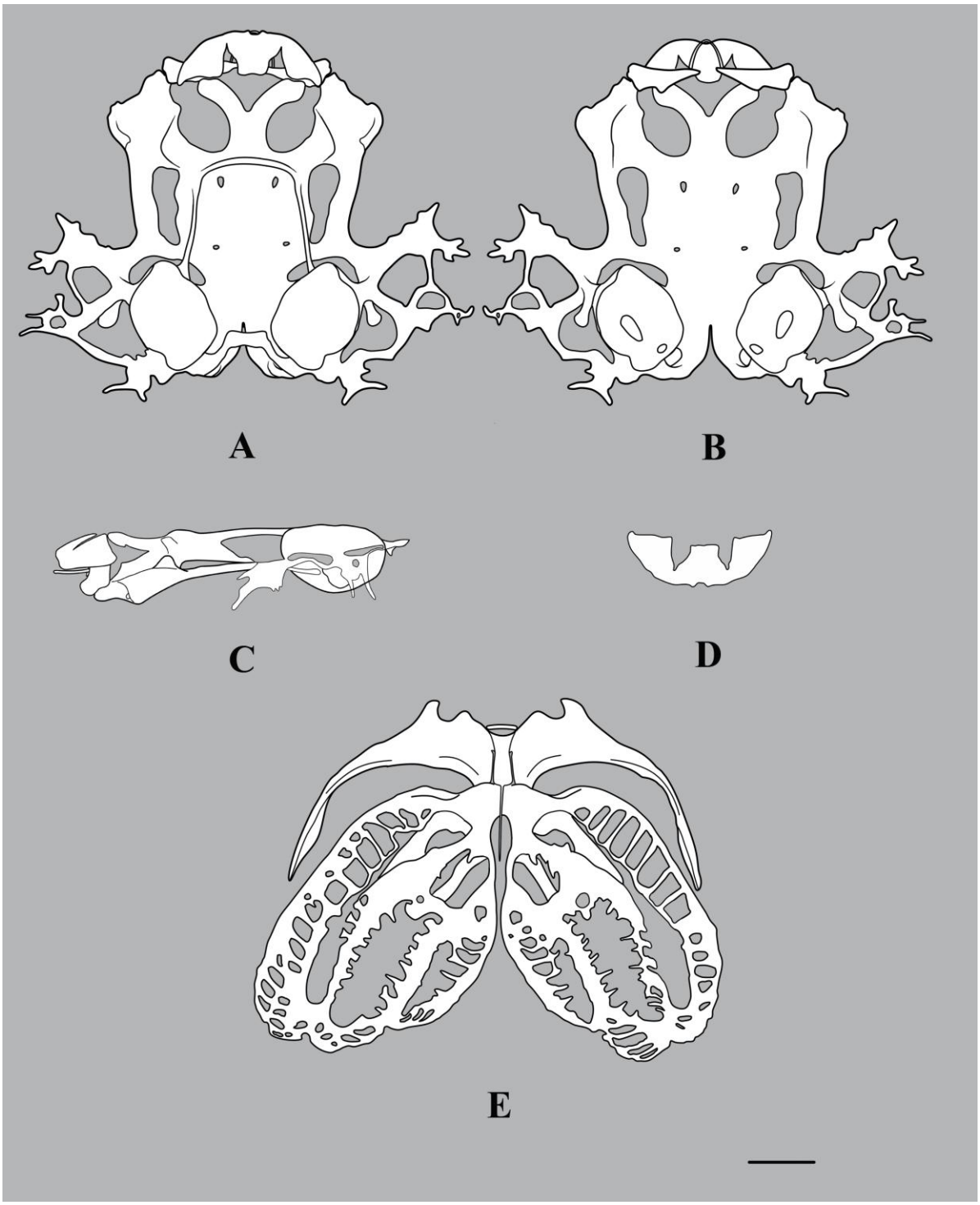

Figure 6. Illustrations of the larval chondrocranium of *Ramanella obscura* (Gosner Stage 35). **A.** Dorsal; **B**. Ventral; **C**. Lateral; **D**. Upper jaw cartilages in frontal view and **E**. Hyobranchial apparatus (Scale bar 1 mm).

Table 1. Tadpole body measurements (mm) of *R. obscura* at various Gosner stages (26, 34, 35 & 38). (n=5).

Measurement	Stage 26	Stage 34	Stage 35	Stage 38
Maximum height of body	3.24	4.26	3.80, 4.33	4.89
Maximum width of body	5.32	6.47	6.53, 7.17	7.69
Maximum height of tail	2.63	3.45	3.28, 3.74	3.91
Interpupular distance	3.54	4.73	4.87, 5.10	5.50
Snout-vent length	7.18	9.37	9.05, 10.17	10.62
Total length	18.25	24.47	23.60, 25.55	27.64
Distance from vent to tip of tail	11.07	15.10	14.54, 15.38	17.02
Tail muscle height	1.39	2.01	1.85, 2.23	2.46
Tail muscle width	1.52	2.30	1.77, 1.99	2.88

Bone Development: At stage 25, there is no bone development. By stage 35, exoccipitals, parasphenoid, frontoparietals and prootics are developed. At stage 40, in addition to the bones at stage 35, nasals are formed.

Ecological notes: We observed egg clutches of *R. obscura* laid on surface of water in tree holes and small ground puddles, mostly in the shade (under canopy cover). Maximum depth of ground pools was about 50 cm, but most tree holes were shallower; surface area ranged from 8-900 cm^2. Water quality of tree hole where the early stage tadpoles were collected, was as follows (at 11.00 am): temperature = 24.7˚ C; dissolved oxygen = 0.87 mg/l; pH = 6.42; conductivity = 121.6 µS; salinity = 0; (NO_3^--N) = 0.113 mg/l; (NH_3-N) = 3.64 mg/l; SO_4^{2-} = 0 mg/l. A total of 100-300 early stage black embryos were observed (two tree holes and one water puddle were considered). No other anuran tadpoles were observed in syntopy with *R. obscura* tadpoles.

DISCUSSION

Ramanella obscura tadpoles have a terminal mouth, an arrangement that is also so far seen in all so far new world microhylids. Considering the species so far studied, 21 of the old-world microhylids have terminal mouths, only 11 species have dorsoterminal mouths (Donnelly *et al.*, 1990). The two other *Ramanella* species endemic to Sri Lanka, *R. palmata* and *R. nagoi* have dorsoterminal mouths (Kirtisinghe, 1958; Manamendra-Arachchi and Pethiyagoda, 2001; Ukuwela *et al*., 2010).

The keratinized ridge on lower jaw in *Ramanella obscura* is also observed in *R. variegata* (Rao, 1918). In contrast, all New World microhylids lack keratinized mouth parts (Donnelly *et al.*, 1990). The other microhylid species that have horny ridges or beaks on lips is *Scaphiophryne gottlebei* (Mercurio & Andreone, 2006).

Among the *Ramanella* species in Sri lanka, there appears to be considerable variation in the flaps on upper and lower lips. Though upper lip flaps are completely absent in *R. obscura* and *R. palmata*, ornate flaps are present in *R. nagaoi* (Manamendra-Arachchi and Pethiyagoda, 2001). The lower lip is U shaped in both *R. obscura* and *R. palmata*, however, there are no infralabial prominences, which are apparent in *R. nagaoi*. In contrast, *Ramanella obscura*, *Microhyla rubra* and *Microhyla ornata* have prominent oral flaps (Rao, 1918; Kirtisinghe 1957; 1958; Parker, 1934; Khan 2000; Bowatte and Meegaskumbura, 2011). *Ramanella obscura* lack papillae on proximal end of lower lip.

However, papillae are present in several other species, such as *R. variegata* and *Uperodon systoma*. In all other Sri Lankan microhylids, this feature remains unassessed.

Vent-tube is placed between spiracle and lower tail fin in *R. obscura*. This arrangement is similar to that of *R. palmata* (Kirtisinghe, 1958). In both these species, vent-tube opens anterior to the spiracle. However, the arrangement in *R. nagoi* is different, in that the vent tube appears to be located in tail, and the spiracle ends anterior to the vent-tube opening. However, location of spiracular opening may not be a useful taxonomic character as it sometimes varies even within populations (see Donnelly *et al.*, 1990).

Though only a few New-World genera have nasolacrimal grooves, these are prominent in *R. obscura* and *R. var*iegata. Their presence is not discussed for *R. palmata* and *R. nagaoi*. *Microhyla rubra* also has nasolacrimal grooves (Bowatte and Meegaskumbura, 2011), suggesting the fact that this feature is present in at least some Old World microhylids.

Though most microhylid tadpoles have pointed tail tips, tadpoles of *Ramanella* have more or less rounded tail tips. *Ramanella palmata* appears to have a well-rounded tail tip, and *R. obscur*a has an elongate tail tip. In all *Ramanella* species, the tail muscles end just before the tail tip.

In many microhylids ventral fins are placed higher than the dorsal fin. But in *Ramanella* tadpoles, except in *R. variegata* (Rao, 1918), both fins are about the same in height. *Microhyla rubra* and *M. ornata* tadpoles have deeper ventral tail fins.

Metamorphosis is longer in *R. obscura* and takes 70 - 100 days (8.83 - 10.07 mm svl, emergent juveniles) (Fig. 7). This period is very short in *R. variegata* tadpoles that live in ephemeral pools. According to Rao (1918) it takes fourteen days. Though *R. obscura* lives in small puddles and tree holes, they start breeding only after three days of heavy rain (Meegaskumbura, 1999), ensuring the commencement of the rainy season. In the Dry zone, where *R. variegata* inhabits, it is evolutionary advantageous to metamorphose as quickly as possible.

Internal oral morphology has been described only for a few species of Old World microhylids, namely: *Microhyla berdmorei*, *M. heymonsi* (Wassersug, 1980), *M. ornata*, (Wassersug, 1980; Khan, 2000), *M. rubra* (Bowatte & Meegaskumbura, 2011), *Chaperina fusca* and *Microhyla borneensis* (Inger, 1985). This is the first description of the internal oral morphology of the genus *Ramanella*. *Ramanella* does not possess a medial preglottal papilla in the ventral buccal floor as in *Microhyla.* The glottis is situated far anterior to the rear margin of the velum, a feature that both *Ramanella* and *Microhyla* share.

At the dorsal buccal, a distinguishing character is observed, the median ridge which extending anteriorly between narial papillae. This feature is absent in all described species of *Microhyla*. This type of medial ridge is found in the New World microhylids, *Dermatonotus muelleri*, *Elachistocleis bicolor* (Echeverría and Lavilla, 2000) and *Hypopachus aguae* (Savage, 1955)

Figure 7. Newly emerged froglet of *Ramanella obscura* (SVL = 9.2 mm).

Chondrocrania of only three Old World microhylid species has been described, namely, *Microhyla ornata*, (Ramaswami, 1940; Khan, 2003), *Kaloula pulchra* (Haas, 2003; 1996) and *Uperodon systoma* (Ramaswami, 1940). The chondrocranium of *R. obscura* is similar to that of *Kaloula pulchra*. However, a significant feature of *R. obscura* is that it possesses an expanded, fenestrate sheet of cartilage associated with the larval otic process and processus lateralis posterior. Both *Uperodon* and *Microhyla* lack such an elaboration of the larval chondrocranium (de Sa and Trueb, 1991). Apart from *Ramanella* such an elaboration is found only in New World Genera *Hamptophryne* and *Otophryne* (de Sa and Trueb, 1991).

Ramanella obscura lays its eggs as a raft on the water surface, often in shade. If disturbed by frogs or by rain, the egg raft sinks to the bottom, but will still develop. However, the breeding pair, if left undisturbed, will not disturb the floating egg mass. The egg membranes are sticky and capture various small invertebrates, this may provide food for the developing tadpoles later (Meegaskumbura, 1999).

Ramanella obscura only lay their eggs in small and shallow ground-pools and tree-holes. This may be due to the high competition for resources in larger pools. In a constricted tree-hole habitat, dissolved oxygen concentration is very low (DO=0.87 mg/l), and salinity reaches zero, however, the ammoniacal nitrogen concentration ((NH_3-N = 3.64 mg/l) is high, and these factors may also prevent other frogs from utilizing this habitat.

We observed about 100-300 eggs in three nests during the rainy season. So far, during a single rainy season, 38-338 eggs have been observed being laid by a single female (Meegaskumbura, 1999), with the egg count diminishing as the season progresses. This may be an adaptation to balance mortality and growth rates.

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