Description of the larvae of Günther’s toad
*Duttaphrynus hololius* (Günther, 1876) (Anura: Bufonidae) with notes on development and oral ultra-structure

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Morphology of tadpoles of the little known, Peninsular Indian endemic bufonid *Duttaphrynus hololius* (Günther, 1876) is described across different stages in detail, with observations on natural history. The oral apparatus of these benthic feeding tadpoles was examined using scanning electron microscopy and the ultra-structure is illustrated and described in detail. These larvae have a labial tooth row formula of 2(2)/2(2), with about 99 and 97 denticles on the anterior and posterior labia respectively. Novel information on the time taken for its ontogenetic development in the advanced stages is presented.

**INTRODUCTION**

Unlike any other terrestrial vertebrate groups, amphibians have complex life history traits and most of them spend the early stages of their developmental cycle in the aquatic phase, as free-swimming larvae (Duellmann & Trueb, 1994). Though there are exceptions in the form of direct developing taxa that hatch out from the eggs as imagos of the adults (e.g. Rhacophoridae: *Raorchestes*), most species undergo this exhaustive transformation in body form before attaining adulthood. This crucial phase of their life history remains poorly documented for most of the amphibian species in India (Das & Dutta, 2007). Among them, genus *Duttaphrynus* is one of the poorly known genera for which data on larval characteristics exist for a very few species (Das & Dutta, 2007). These include the species *Duttaphrynus melanostictus* (Schneider, 1799), *D. microtymanum* (Boulenger, 1882), *D. scaber* (Schneider, 1799), *D. stomaticus* (Lütken, 1864) and *D. brevirostris* (Rao, 1937) (see Das & Dutta, 2007).

*Duttaphrynus hololius*, one of the poorly known, endemic members of this genus, was reported from Hosur very recently, with additional information on basic morphology (Chandramouli et al., 2011) and subsequently recorded from a few other localities in the Eastern Ghats (Adimalliah et al., 2012; Kalaimani et al., 2012; Srinivasulu et al., 2013). Preliminary data on the larvae and the habitat of this species were briefly presented in Ganesh et al. (2013). Ganesh et al. (2013) provided notes on larval habitat of *D. hololius* and a brief description of the overall morphology of tadpoles across Gosner stages 20-47. However, precise information on morphological characteristics of the larvae belonging to each stage could not be elaborated since their study was entirely based on live, uncollected samples.

The information provided by Ganesh et al. (2013) is supplemented here, based on the collection of new samples and description of their morphology at different phases of metamorphosis. Also, the oral apparatus and details of its ultra-structure are presented herein for the very first time.
MATERIALS AND METHODS

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While on a biodiversity inventory in the Eastern Ghats landscape near the inter state boundary of Karnataka and Tamil Nadu, Southern India (12.38°N, 78.62°E, 677 m asl), bufonid tadpoles were observed in small puddles amidst dry rocky hills. Site characteristics such as the habitat type, forest cover and the number and size of puddles and their attributes such as size and depth were recorded. Four tadpole samples were collected and reared in captive conditions. The tadpoles were reared in captivity and were allowed to feed on the same detritus material collected from the native pool. They were retained for the following eight days before being fixed for preservation. In captivity they were allowed to feed on the organic debris, which was collected from the native pool. In regular intervals, tadpoles were euthanized and preserved in 10% formalin solution across different developmental stages.

The samples were fixed at different stages; i.e., 25 and 42, wherein, they were observed to show considerable variation in the development of the oral apparatus. A piece of the tail muscle tissue was extracted and stored in absolute ethanol from one tadpole prior to fixation. After fixation, the tadpoles were examined under a Leica™ MZ75 microscope for observing oral morphological characteristics. Measurements were done using the microscope. The collected larvae were unambiguously allocated to the species *Duttaphrynus hololis* based on the morphological characters of the metamorphosed toadlets seen along with the tadpoles in the field, namely, rudimentary toe webbing, smooth skin texture, indistinct parotoid glands and the absence of cephalic ridges. An adult female (identified based on the absence of nuptial pads and vocal sac) of this species (identified based on rounded parotoid glands, absence of cephalic ridges and rudimentary toe webbing) was examined, measured in situ and released.

The above diagnostic morphological characters of this species were discussed in detail by Chandramouli et al. (2011). Also, no other anuran species were encountered in the vicinity of the collection site. The specimens are deposited in the collection of the Centre for Ecological Sciences, Bangalore under the voucher number CESF2354. The tadpoles were staged following the key provided by Gosner (1960) and were measured with a

![Figure 1. A. Tadpoles of *Duttaphrynus hololis* seen in a rocky puddle; B. freshly metamorphosed imagos of *Duttaphrynus hololis*; C. adult female.](image-url)
stereo microscope to the nearest 0.01 mm. Following measurements were recorded from the tadpoles – head-body length (HBL), head-body width (HBW), head-body depth (HBD), total length (totL), eye diameter (ED), distance between anterior eye margin and nostril (EN), anterior eye margin to snout tip (ES), tail length (TaL), inter orbital distance (IO), inter narial distance (IN), caudal fin height (CFH), caudal muscle height (CMH), width of the posterior tooth row, width of the anterior tooth row, inter-tooth row distance, oral disc width, upper arm length (UAL), lower arm length (LAL), palm length (PAL), femur length (FEL), tibia length (TBL), tarsus length (TAR) and foot length (FOL). Eco-morphological classification of the larvae based on their microhabitat associations and feeding habits follows Altig & Johnston (1989). Terminologies used in the description of oral apparatus follow McDiarmid & Altig (1999). The adult toad and freshly metamorphosed toadlets encountered in the field were measured in situ with a vernier caliper to the nearest 0.1 mm, followed by release in the place of capture.

Scanning electron microscopy

The oral disc of a stage 25 larva was dissected and kept in absolute alcohol overnight for dehydration. It was then dried completely and mounted on a metallic stub with a thin layer of adhesive. The mounted sample was then desiccated completely and coated with a thin film of gold. The gold coated sample was then subjected to exposure at different levels and under different degrees of magnification in the SEM equipment to study the detailed ultrastructure of the oral apparatus.

RESULTS

Species identification

The tadpoles were allocated to the species *Duttaphrynus hololius* based on the developmental characters of the larvae and morphology of the toadlets observed in the field along with the tadpoles. An intensive search in the immediate surroundings of the pools with tadpoles was successfully able to uncover six freshly metamorphosed imagos (SVL 10.3, 9.6, 11.3, 11.3, 10.3 and 11 mm; mean 10.63 mm) and an adult female of this toad from rock crevices (fig. 1). These toadlets as well as the adult female showed the following diagnostic morphological traits: absence of cephalic ridges, rudimentary toe webbing, rounded parotoid glands and skin without warts. Hence, they were referred to this species.

Ecological notes

The tadpoles of *Duttaphrynus hololius* were observed on 1 June 2013, following a bout of sporadic rains (in the pre-monsoon season), in ephemeral puddles formed by stagnant rain water on rocky hill tops (fig. 1.A). These larvae were found to be benthic feeders and were observed to feed actively on the algal growth at the bottom surface of the pools. Many tadpoles (about 40 per pool) were observed in two adjacent stagnant water pools on an open, rocky hilltop. The puddles were oval in shape, about 1-1.5 m in diameter (at the widest point) with a depth of not more than 10-12 cm.

The immediate vicinity of the collection site was surrounded by rocky outcrops, with sparse vegetation, predominantly with grass clumps and patches of moist soil. No other anuran species were observed in close vicinity of these pools. The tadpoles in these pools were observed to be of different developmental stages, suggesting that the observed individuals could probably belong to more than a single clutch that could have been laid asynchronously with a short lag in between. An adult toad (SVL 27.1 mm) was also sighted in the immediate vicinity. Four larvae were collected and reared in captivity. A brief note on their partial (advanced) ontogenetic development is mentioned below.

Notes on metamorphosis and advanced ontogenetic development

Of the four samples collected on 1 June 2013, three were of stage 24 and one sample was in an advanced stage 27. Upon collection, the tadpoles were uniform dark purplish brown above and pale white below with a translucent skin. After a week of captive husbandry, two larvae were still found to be in stage 25 on 6 June 2013. At this stage 25, they did not show any traces of limb buds while the other, older sample of a slightly advanced stage, reached stage 31 with well developed hind limbs, which, by then, had emerged out of the body capsule. The larvae of stage 25 were of the same overall dark purplish colouration but a feeble, inverted ‘V’ shaped marking on the back had just started developing. There were no traces of forelimb buds at this stage. Upon further captive
maintenance with the same feed, for two more days (i.e., 8 June 2013), the older larva of stage 31 reached stage 43, with the emergence of forelimbs from the body wall, and a slight reduction in the length of the caudal fin. As it was raised further, the caudal fin started decreasing in both height and length, due to the absorption of nutrition for

**Figure 2.** Developmental stages of *Duttaphrynus hololius* (from top to bottom): larvae of stage 25, stage 31, stage 43 and a completely metamorphosed toadlet (all images of living specimens).

**Figure 3.** Structure of the oral apparatus in a *Duttaphrynus hololius* larva of stage 25 CESF2354 a.

**Figure 4.** SEM image of the oral disc of a stage 25 larva CESF2354 b of *Duttaphrynus hololius*, showing the characteristic features.
metamorphosis. At this stage, the larvae had developed intensive pigmentation on the dorsal skin with the pale, inverted ‘V’ shaped marking on the dorsum becoming more evident, and numerous orange colored spots on the dorsum. The venter was more opaque than it was in the earlier stage. Freshly metamorphosed toadlet observed in the field, with its tail completely absorbed, resembled the adult in colouration but with a greater density of orange spots on the body. An evident, light colored inter-orbital band was observed in addition to the inverted ‘V’ marking on the back in the developed toadlets (fig. 2). The larvae of stages 25 and 42 are illustrated and described below in detail.

### Description of larvae of *Duttaphrynus hololius* (Günther, 1875): (tab. 1)

#### Stage 25: CESF2354 a & b

Head-body ovoid, dorso-ventrally depressed; about twice as long as broad (HBW:HBL 0.47), slightly longer than half the length of the tail (TaL:HBL 1.89). Snout blunt and rounded in dorsal view. Tail long and tapering at the end, caudal fins better developed on the ventral than the dorsal part; caudal musculature intensive. Overall colouration: dark purplish on the dorsum without any pattern; venter translucent and pale white. Gut highly coiled and visible through the translucent skin in the posterior ventral part of the head-body.

Spiracle sinistral, vent-tube dextral; oriented posteriorly towards the right side in the ventral region right at the junction of the tail beginning and the end of the posterior end of the head-body. Eyes small (0.4 mm diameter); positioned dorsally; inter orbital space more than twice the eye diameter (IO:ED 2.5); nostrils slightly closer to each other than the eyes (IO:IN 1.17); nostrils positioned dorsally, situated midway between the snout tip and the eyes (EN:ES 0.49); snout rounded in dorsal and ventral views. Dorsal skin densely pigmented; overall colouration dark purplish brown above without any distinct pattern and pale white ventrally. Oral sucker circular

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<th></th>
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and not emarginate; positioned antero-ventrally. Keratodont well developed; labial tooth row formula: 2(2)/2(2). The upper (anterior) labium consists of a total of 99 denticles on the outer row, with 56 on the right half and 43 on the left half, demarcated by a short, interrupted, fleshy diastema at the centre of the left and right halves. The interior row of the upper jaw has 97 denticles arranged in a single, continuous series. Interior tooth row of the lower (posterior) labium separated into two halves, with 34 denticles on the right half and 38 on the left (fig. 3). The exterior tooth row of the lower labium composed of a single, discontinuous row of 73 denticles. The upper and lower jaw sheaths are indicated by a well developed, keratinized beak. The upper jaw sheaths indicated by a
continuous, undivided, serrated ridge.

Lower jaw sheath with an acute bend at the center. Lateral process absent on the upper jaw. Marginal papillae located at the junction of the upper and lower labia arranged in two rows; beak on the upper labium formed by a hard, keratinized ridge. Keratodont constituted by two concentric series, which consist of inwardly curved tooth; those of the outer and inner rows of the upper and lower labia of the same size, made of elongate structures with 6-7 pointed terminal ends (figs. 4-8).

Figure 7. Scanning Electron Microscopic images of: A. the upper jaw sheath; B. keratodont structures in the upper jaw; C. lower labia in a stage 25 larva of Duttaphrynus hololius.
Stage 43: CESF2354 c

Body elongate (SVL 8.11 mm) and moderately slender (BW 3.21 mm); habitus depressed; tail a little shorter (TaL 8.36 mm) after a bit of absorption; fore and hind-limbs well developed with evident differentiation of the digits. Oral apparatus transformed to mouth with the loss of keratodont and beak. Head differentiable from the body and slightly broader (HW 3.88 mm) than the latter (BW 3.21 mm). Position of the eyes shifted from dorsal to lateral; eye larger (ED 1.19 mm) than its distance to the nostril (EN 0.70 mm) and to the snout tip (ES 1.04 mm). Eyes much separated from each other (IO 2.37 mm) than the nares (IN 0.83 mm).

Upper arms (UAL 1.49 mm), about as long as the lower arm (LAL 1.53), palm slightly longer (PAL 1.87 mm) than upper and lower arms. Thigh longer than the shank (FEL:TBL 1.22), tarsus shorter than the foot (TAR:FOL 0.73). Toes basally webbed (fig. 9).

DISCUSSION

Of the nine species of *Duttaphrynus* known from peninsular India (Frost, 2013), larval traits have been documented only in a very few species (Das & Dutta, 2007) among which none existed for the poorly known *D. hololius* till very recently (Ganesh et al., 2013). However, even in the paper by Ganesh et al. (2013), only a general description of larvae of stages 20-47 was presented briefly. Additionally, a stage-wise description of the key morphological characters of the tadpoles could not be elaborated as their study was based only on observations made on live individuals in the field. Hence, our novel data presented here fills in the gap in the existing knowledge on the larval characters of this species by providing a detailed morphological description of the larvae at two major transitional stages, based on vouchered material. Thus, this paper supplements the brief notes on the larvae of this species provided by Ganesh et al. (2013).

Additionally, the data on the time taken for their advanced ontogenetic development is presented. The data on breeding period and habitat associations of the larvae are consistent with those reported by Ganesh et al. (2013).

Morphologically, the tadpoles of *D. hololius* can be differentiated from the congeneric species described as follows. In *D. hololius*, the tadpoles are dark purplish brown without any pattern in the early stages, and with an inverted pale ‘V’ mark in the later stages (vs. black dorsal colour with shiny silvery spots on the body in *D. stomaticus*; uniform black in colour in *D. melanostictus*; brownish tadpoles that are smaller than those of *D. melanostictus* with relatively large nostrils in *D. scaber*) (Daniel, 1963).

It is interesting to note that no other anuran species were recorded to breed syntopically with *D. hololius*, which has a peculiar preference to rocky pools for breeding and oviposition. This observation, again, corroborates
that of Ganesh et al. (2013). Despite being a species found in the open rocky hills, a non mesic habitat, this species has remained poorly known in terms of its biology for a very long time. Additional data on its distribution and natural history being generated, would aid in assessing the conservation status of this taxon, which is currently considered to be data deficient (Biju et al., 2004).

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