Herpetological Conservation and Biology 11(2):280–285.
Submitted: 22 December 2015; Accepted: 20 June 2016; Published: 31 August 2016.

A DESCRIPTION OF THE TADPOLE OF *LEPTOBRACHIUM SMITHI* (ANURA: MEGOPHRYIDAE) FROM SOUTH ASSAM, INDIA

PAMMI SINGH[1], MITHRA DEY[2, 4] AND SUNKAM NARAYANA RAMANUJAM[3]

[1,2]*Department of Ecology and Environmental Science, Assam University Silchar-788011, Assam, India*
[3]*Department of Zoology, North Eastern Hill University, Shillong-793022, Meghalaya, India*
[4]*Corresponding author, e-mail: mithradey@gmail.com*

***Abstract.*—Data on the morphology and microhabitat selection are important for understanding the ecological requirements and natural history of tadpoles. We describe the external morphology and the oral apparatus of the tadpoles of *Leptobrachium smithi* (Matsui et al. 1999) that were collected from lotic water at the Rosekandy Tea Estate, Barak Valley, Assam, India. We studied the morphology of tadpoles in stages 25 to 41, and examined the oral apparatus of tadpoles at stage 34 with scanning electron microscopy. The curved and pointed labial teeth that lack cusps are arranged in one row per ridge. The labial tooth row formula (LTRF) was 7(2–7)/6(1–5) at stage 34, and the marginal papillae had a dorsal medial gap.**

Key Words.—amphibian; Smiths Litter Frog; morphology; mouthparts

INTRODUCTION

The megophryid *Leptobrachium smithi* Matsui et al. (1999) was described from peninsular Thailand based on populations that were formerly referred to as *L. hasseltii* Tschudi 1838 (Frost 1985). Variations in the size and shape of the oral apparatus, the papillae at the margins of the oral disc, the shapes of the jaw sheaths, and the number of tooth rows and gaps in rows of teeth are all important features in identifying tadpoles (Duellman and Trueb 1986). Numerous morphological variations of tadpoles reflect adaptations to diverse habitats and their phylogeny (Duellman and Trueb1986; McDiarmid and Altig 1999). The functions of the mouthparts, the morphology of the labial teeth, and the functional significance of structural differences among species are still poorly known (Wassersug and Yamashita 2001).

Variation of labial teeth among species reflects their microhabitat and the feeding habits (Altig and Johnston 1989). Although descriptions of tadpole characteristics are useful in taxonomy, detail descriptions are lacking for a number of species. Tadpole morphology and oral disc structure varies considerably both inter- and intraspecifically. It is hypothesized that as an individual grows larger, the morphological changes in its feeding apparatus, including the number of teeth and gap size allow a wider selection of prey items (Toft 1980; Christian 1982). Data on the morphology and microhabitat selection of tadpoles are relevant to the understanding of the ecological requirements and natural history of frog species (Thomas et al. 2005). Our paper describes the external morphology of *Leptobrachium smithi* tadpoles and gives a description of the oral apparatus examined using a scanning electron microscope.

MATERIALS AND METHODS

We collected tadpoles with a hand net from lotic water at the Rosekandy Tea Estate, Barak Valley, Assam, India. We preserved tadpoles in 10% formalin and deposited them in the Museum of Herpetofauna at the Department of Ecology and Environmental Science, Assam University, Silchar, India. We reared some tadpoles through metamorphosis in laboratory condition for verification of their identification (Fig. 1). We

FIGURE 1. Dorsal (upper), lateral (middle), and ventral (lower) views of a tadpole of *Leptobrachium smithi* at stage 34. (Photographed by Pammi Singh).

TABLE 1. Morphometric measurements (mm) of tadpoles of *Leptobrachium smithi* in different developmental stages, (n = 8; mean ± SD; range in parenthesis). Abbreviations are Char. = Character, TL = Total Length, BL = Body Length, BW = Body Width, IO = Inter-Orbital Distance, IN = Inter-Narial Distance, EN= Eye-Naris Distance, SO = Snout-Orbit Distance, SN = Snout-Naris Distance, SS = Snout-Spiracle Distance, TAL = Tail Length, TH = Tail Height, TMH = Tail Musculature Height, TMW = Tail Musculature Width, and UF = Maximum Height Of Upper Tail Fin.

Char.	Stage 25	Stage 26	Stage 29	Stage 30	Stage 31	Stage 34	Stage 35	Stage 37	Stage 41
TL	37.08 ± 7.18 (22.3–49.1)	43.62 ± 5.37 (36.4–51.1)	53.2 ± 2.86 (50.2–56.8)	47.4 ± 6.2 (39–55.2)	55.8 ± 6.01 (50.3–66.5)	62 ± 4.47 (55.8–67.2)	56.2 ± 7.76 (52–70.1)	55.2 ± 7.29 (50.7–68)	69 ± 2.35 (67–72.3)
BL	16 ± 3.59 (8.1–21.2)	18.5 ± 2.27 (15.2–21)	23.2 ± 1.64 (21.3–25.1)	22.2 ± 2.68 (21.4–26.3)	23.5 ± 2.17 (21.5–26.2)	25.4 ± 4.45 (20.7–30.3)	22.2 ± 3.49 (20.1–28.2)	22.2 ± 3.49 (23.2–28.3)	24.6 ± 1.95 (25.1–29.5)
BW	8.83 ± 3.59 (5.2–16)	12 ± 3.21 (8.3–17.1)	16 ± 3.8 (12–21.4)	14.6 ± 5.94 (8.6–22)	16 ± 3.74 (11.2–22.1)	14.8 ± 4.82 (10.1–20.6)	13 ± 3.32 (10.3–18.7)	12.8 ± 2.39 (11.3–17.4)	17 ± 1.41 (16.1–19.3)
I-O	5.05 ± 0.6 (4.1–6)	5.37 ± 0.74 (4.1–6.6)	6.8 ± 0.45 (6.3–7.1)	6.6 ± 1.52 (5.1–8)	7.5 ± 0.55 (7.2–8)	8.6 ± 1.34 (7.3–10)	8 ± 1.22 (7–10.3)	7.8 ± 0.45 (7–8.1)	10.02 ± 0.11 (9.9–10.2)
I-N	2.58 ± 0.9 (2–4.1)	3.12 ± 0.83 (2–4)	3.8 ± 0.44 (3.1–4)	3.2 ± 0.45 (3–4.2)	3.67 ± 0.52 (3.1–4)	3.8 ± 0.45 (3–4)	3.4 ± 0.89 (3–5.1)	4 ± 0.71 (3.3–5)	4.8 ± 0.45 (4.1–5.2)
E-N	2.08 ± 0.9 (1–3.2)	2.12 ± 0.83 (1–3)	1.94 ± 0.08 (1.8–2)	1.6 ± 0.55 (1.2–2.2)	1.33 ± 0.52 (1.1–2.2)	1.2 ± 0.45 (1–2.4)	2.2 ± 1.3 (1–4)	2.2 ± 0.45 (2.4–3.5)	2.2 ± 0.44 (2.6–3.8)
S-O	3.75 ± 1.14 (2.2–5)	4.75 ± 0.46 (4.1–5.3)	4.2 ± 0.45 (4–5.2)	4.6 ± 0.55 (4.2–5.3)	4.5 ± 0.55 (4–5.3)	4.98 ± 0.34 (4.4–5.5)	5 ± 0.71 (4.2–6.1)	5.2 ± 0.84 (4.5–6.7)	5.4 ± 0.55 (5–6.2)
S-N	1.66 ± 0.49 (1–2)	2.62 ± 0.52 (2.2–3)	2.2 ± 0.44 (2–3.5)	3 ± 0.71 (2.1–4.2)	3.16 ± 0.75 (2.2–4.2)	3.8 ± 0.45 (3–4.3)	2 ± 1 (2.1–4)	3 ± 0.71 (2.3–4.4)	3.2 ± 0.44 (3.2–4.4)
S-S	8.67 ± 2.15 (6–12.3)	10.62 ± 2.07 (8–13.3)	12.6 ± 0.55 (12.2–13)	11.6 ± 0.61 (11–12.4)	12.5 ± 0.84 (11.2–13)	12 ± 1.22 (10.2–13.1)	10.6 ± 1.95 (9.1–14.2)	10.8 ± 0.84 (10.3–12.)	13.4 ± 0.55 (13.1–14)
TAL	21.08 ± 4.19 (14.1–28.2)	25.12 ± 3.48 (21.1–30)	29.2 ± 3.27 (26.2–34)	26.8 ± 4.49 (19.5–30)	32.3 ± 4.5 (28.4–40)	35.6 ± 4.88 (30.3–41)	34 ± 4.69 (30.2–42)	30.6 ± 7.6 (26.5–44)	42 ± 1.22 (41.6–44)
TH	6.5 ± 1.83 (4–9.1)	8.62 ± 1.06 (7–10.1)	7.6 ± 0.55 (7.2–8.2)	8.8 ± 1.1 (8.1–10.3)	9 ± 2 (7.3–12.2)	12 ± 1.22 (10–13.3)	11 ± 1.73 (10.1–14.1)	11.6 ± 2.07 (10.2–15.4)	13.4 ± 0.55 (13.3–14.6)
TMH	4.33 ± 1.37 (3.5–7)	5.25 ± 0.89 (4–6)	5.6 ± 0.55 (5.3–6.4)	5 ± 1 (4–6)	6.67 ± 1.51 (5.3–8.1)	7.6 ± 0.55 (7.2–8.1)	7.6 ± 0.55 (7.3–8.4)	7 ± 0.71 (6.2–8.6)	8.4 ± 0.55 (8.1–9.3)
TMW	2.41 ± 0.67 (2.3–4)	3.75 ± 0.46 (3.1–4.2)	5.8 ± 1.3 (5.3–8.1)	4.2 ± 0.45 (4.5–5.7)	5.17 ± 0.75 (4.2–6.3)	5.8 ± 0.84 (5.1–7.3)	5.8 ± 0.45 (5.3–6.5)	5.4 ± 0.89 (4.2–6.3)	8.2 ± 0.45 (8.2–9.7)
UF	1.75 ± 0.87 (1.2–2.3)	3.25 ± 0.46 (3.2–4.1)	3.6 ± 0.55 (3–4)	3.4 ± 0.55 (3.3–4.1)	3.5 ± 0.55 (3.2–4.5)	4.2 ± 0.84 (3–5.1)	3.6 ± 0.55 (3.2–4.1)	4.2 ± 0.45 (4.1–5.3)	3.8 ± 0.45 (3.2–4.3)

housed tadpoles in aquaria in pond water and fed them with commercially available fish food. We determined the stages of tadpoles according to Gosner (1960), and we used those identified in nine developmental stages (between 25–41) in a morphometric study.

We measured total length (TL), body length (BL), body width (BW), interorbital distance (IO), internarial distance (IN), eye-naris distance (EN), snout-orbit distance (SO), snout-naris distance (SN), snout-spiracle distance (SS), tail length (TAL), tail height (TH), tail musculature height (TMH), tail musculature width (TMW) and maximum height of upper tail fin (UF) in eight tadpoles from each stage with vernier calipers (mean ± SD) in accordance with McDiarmid and Altig (1999). We studied the oral structure of the tadpole at stage 34 with a light microscopy (Olympus SZ51-Olympus Corporation, Tokyo, Japan) and a scanning electron microscope. For scanning electron microscopy, we washed samples with double distilled water and fixed them in 2.5% glutaraldehyde (prepared in 0.1 M sodium cacodylate buffer) for 4 h at a pH of 7.4. We then dehydrated the samples through an acetone series and dried them in tetra methylsilane (Dey et al. 1989). We applied a thin coating of gold to the samples with a Jeol JFC 1100 ion sputter coater, and examined the samples with a Jeol JSM-6360 scanning electron microscope at an accelerating voltage of 20 KV and a working distance of 10 mm. Our descriptions of the oral apparatus and labial tooth row formula (LTRF) are in accordance with Altig (1970).

RESULTS

The external morphology of the tadpoles was based on Gosner stage 34 (Fig. 1). In dorsal view, the body of the tadpoles was oval and the head was depressed (Fig. 1). The mouth was ventral in position. The mean TL was 62.0 mm, mean BL was 25.4 mm, and mean TAL was 35.6 mm. The nares were oval in shape, slightly rimmed, positioned dorsally, directed laterally and closer to the tip of the snout then to the eyes. The mean IO (8.6 mm) was greater than the mean IN (3.8 mm; Table 1).

Herpetological Conservation and Biology 11(2):280–285.
Submitted: 22 December 2015; Accepted: 20 June 2016; Published: 31 August 2016.

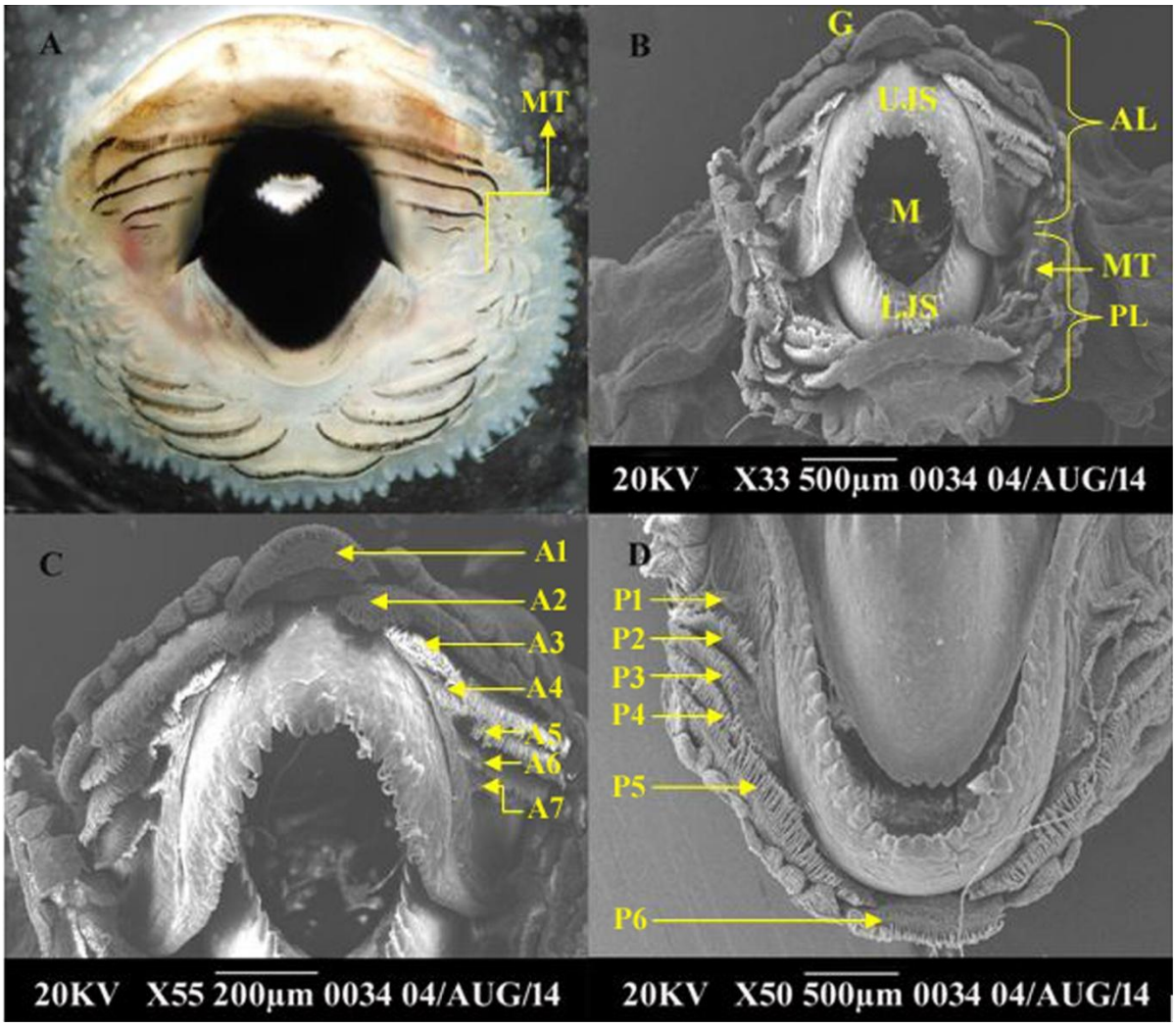

FIGURE 2. Light microscopy and scanning electron micrographs of the oral disc of *Leptobrachium smithi* tadpoles (stage 34). **A:** Light microscopy of oral structure (Photographed by Pammi Singh). **B:** Oral structure showing anterior labium (AL), posterior labium (PL), dorsal gap in marginal papillae (G), upper jaw sheath (UJS), lower jaw sheath (LJS), mouth (M), and marginal teeth (MT). **C:** Oral structure showing anterior tooth rows A1 to A7. **D:** Oral structure showing the posterior tooth rows P1 to P6. (B, C, and D Photographed by Sophisticated Analytical Instrument Facility Unit, North-Eastern Hill University SAIF, NEHU).

The tube of the sinistral spiracle was attached to body wall, but the tip of the spiracle was free, and the spiracular opening was oval and projected posterolaterally (Fig. 1). The dextral vent tube opens at the margin of the ventral fin, and the intestinal coils were clearly visible. The low dorsal fin was taller than the ventral fin, originated near the tail-body junction, and extended parallel to the caudal muscle in its first half (Fig. 1). The tail musculature was robust in its proximal half and tapered gradually in its distal half. The tip of the tail was moderately rounded. The color of living tadpoles was uniformly dark brown, and the tail musculature was light brown. Small and irregular black spots were present on the dorsal and lateral surface of the body and tail (Fig.1).

The upper labium had one continuous tooth row and six rows with medial gaps (Fig. 2C), and the lower labium had five rows with medial gaps and one continuous tooth row (Fig. 2D). All tooth rows were uniserial. The labial teeth were curved, pointed, and devoid of cusps (Fig. 3A). The marginal papillae had a dorsal gap, and the submarginal papillae were absent. The tips of the marginal papillae were rounded. The upper jaw sheath formed a smooth arc, the lower jaw sheath was V-shaped, and both sheaths had pointed serrations (Fig. 3C and 3D). The labial tooth row formula at stage 34 was 7(2–7)/6(1–5).

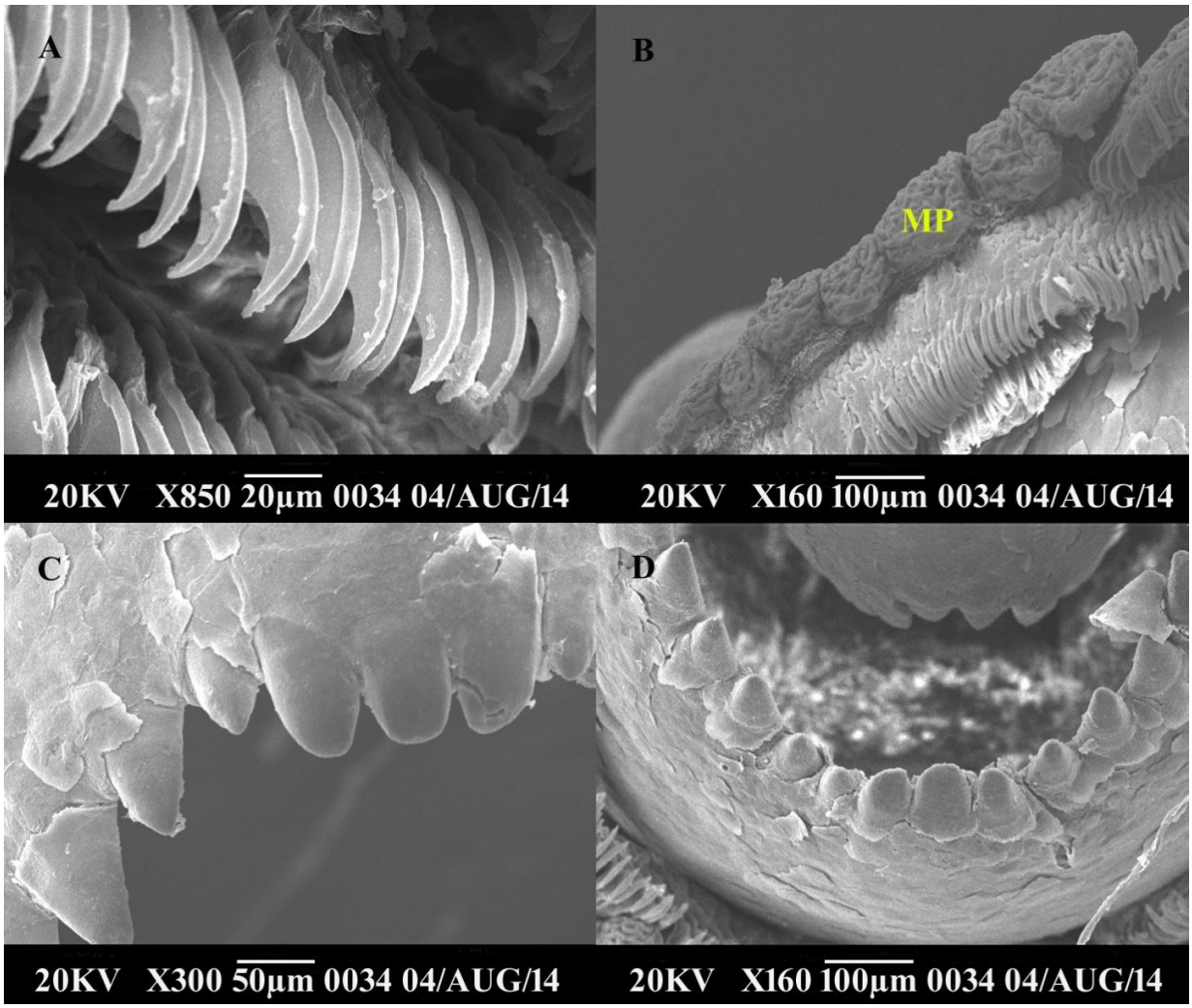

FIGURE 3. Scanning electron micrographs of the oral disc of *Leptobrachium smithi* tadpoles (stage 34). **A:** Labial teeth. **B:** Marginal papillae (MP). **C:** Upper jaw sheath. **D:** Lower jaw sheath. (Photographed by Sophisticated Analytical Instrument Facility Unit, North-Eastern Hill University SAIF, NEHU).

DISCUSSION

To understand the significance of larval characters in taxonomy and systematic, it is necessary to find out the developmental stage at which characters reach their maximum size, form, and color (Grosjean 2005). A number of studies have focused on variations of tadpole characters during development (Dutta and Mohanty-Hejmadi 1984; Tubbs et al. 1993; Hall et al. 1997). Ontogenetic variation has important implications for the use of tadpole characters in taxonomy. The tooth row formula is one of the major characters for identification, although more than one tooth row formula has been reported for some species (Inthara et al. 2005). Matsui et al. (1999) reported the LTRF as 5(2-5)/5(1-5) to7(2-7)/6(1-5) and 7(2-7)/6(1-5) in large larvae of *Leptobrachium smithi* from Thailand. In our study, the LTRF of *Leptobrachium smithi* tadpoles at stage 34 is 7(2–7)/6(1–5), which is similar to the findings of Matsui et al. (1999). Berry (1972), Inger (1983), and Matsui et al. (1999) reported that the body of tadpoles of *Leptobrachium* species is deep and ovoid, eyes are dorsal in position, spiracle is sinistral, vent tube is dextral, mouth is ventral in position, and irregular black spots are present on the body. We found similar arrangements of mouthparts and other morphological characters in the present study. Very little information is available for comparison among the different species of

Leptobrachium; however, Yang et al. (1983) report the labial tooth row formula for *Leptobrachium ailaonicum* of 6(2-6)/5(1-4) with variations for the lower labium including 4+3 or 3+3. However, in this study the labial tooth row formula of *L. smithi* stage 34 tadpoles was 7(2–7)/6(1–5).

Larvae adaped for life in streams are often depressed and elongate, with a robust tail musculature, ventral mouth, and long tooth rows (Orton 1953; Altig and Johnston 1989). The morphological variations in megophryid tadpoles demonstrate a progressive adaptation to changing habitat from fast to slow moving water. In fast moving water, the typical oral apparatus with multiple tooth rows are correlated with lotic-suctorial, benthic feeders that have an anteroventral oral apparatus and large body (Li et al. 2011). Our findings contribute to the knowledge used in making interspecies comparisons among anuran tadpoles, which is useful for phylogenetic and comparative morphological analysis. Our data will also be useful for subsequent studies on selection of food and feeding behavior of tadpoles of different developmental stages.

Acknowledgments.—The authors are grateful to Sophisticated Analytical Instrument Facility Unit (SAIF), North-Eastern Hill University, Shillong, Meghalaya, for the use of the Scanning Electron Microscope. The first and second authors express their gratitude to University Grants Commission, New Delhi, India, for providing financial support. Permission for research has been obtained from the Institutional Ethical Committee (IEC), Assam University Silchar (Approval No. IEC/AUS/B/2013-021 dt-27/11/2013) for which the authors are grateful. The authors are also grateful to the Department of Ecology and Environmental Science, Assam University, Silchar, India, where the work was carried out.

LITERATURE CITED

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

Altig, R. and G.F. Johnston. 1989. Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. Herpetological Monographs 3:81–109.

Berry, P.Y. 1972. Undescribed and little known tadpoles from West Malaysia. Herpetologica 28:338–346.

Christian, K.A. 1982. Changers in the food niche during post metamorphic ontogeny of the frog *Pseudacris triseriata*. Copeia 1982:73–80.

Dey, S., T.S. BasuBaul, B. Roy, and D. Dey. 1989. A new rapid method of air-drying for scanning electron microscopy using tetra-methyl silane. Journal of Microscopy 156:259–261.

Duellman, W.E., and L. Trueb. 1986. Biology of Amphibians. McGraw-Hill Book Company, New York, New York, USA.

Dutta, S.K., and P. Mohanty-Hejmadi. 1984. Ontogeny of teeth row structure in *Rana tigerina* tadpoles. Journal of the Bombay Natural History Society 80:517–528.

Frost, D.R. 1985. Amphibian Species of the World: Taxonomic and Geographical Reference. Allen Press Inc. and the Association of Systematics Collections, Lawrence, Kansas. I-V.

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

Grosjean, S. 2005. The choice of external morphological characters and developmental stages for tadpole-based anuran taxonomy: a case study in *Rana* (*Sylvirana*) *nigrovittata* (Blyth, 1855) (Amphibia, Anura, Ranidae). Contributions to Zoology 74:61–76.

Hall, J.A., J.H. Larsen, Jr., R.E. Fitzner. 1997. Post embryonic ontogeny of the spade foot toad, *Scaphiopus intermontanus* (Anura: Pelobatidae): external morphology. Herpetological Monographs 11:124–178.

Inger, R.F. 1983. Larvae of Southeast Asian species of *Leptobrachium* and *Leptobrachella* (Anura: Pelobatidae). Pp. 13–32 *In* Advances in Herpetology and Evolutionary Biology. Rhodin, A.G.J., and K. Miyata (Eds.). Museum of Comparative Zoology, Cambridge, Massachusetts, USA.

Inthara, C., V. Lauhachinda, J. Nabhitabhata, Y. Chuaynkern, and P. Kumtong. 2005. Mouth part structures and distribution of some tadpoles from Thailand. Journal of Thailand Natural History Museum 1:55–78.

Li,C., X.-G. Guo, and Y.-Z. Wang. 2011. Tadpole types of Chinese megophryid frogs (Anura: Megophryidae) and implications for larval evolution. Current Zoology 57:93−100.

Matsui, M., J. Nabhitabhata, and S. Panha. 1999. On *Leptobrachium* from Thailand with a description of a new species (Anura: Pelobatidae). Japanese Journal of Herpetology 18:19–29.

McDiarmid, R.W., and R. Altig. 1999. Research: material and techniques. Pp. 7–23 *In* Tadpoles. The Biology of Anuran Larvae. McDiarmid, R.W., and R. Altig (Eds.). University of Chicago Press, Chicago, Illinois, USA.

Orton, G.L. 1953. The systematics of vertebrate larvae. Systematic Zoology 2:63–75.

Thomas, M., L. Raharivololoniaina, F. Glaw, M. Vences and D.R. Vieites. 2005. Montane tadpoles in Madagascar: molecular identification and description of the larval stages of *Mantidactylus elegans*, *Mantidactylus madecassus*, and *Boophis laurenti* from the Andringitra Massif. Copeia 2005:174–183.

Toft, C.A. 1980. Seasonal patterns in populations of Panamanian litter frogs and their prey: a comparison of wetter and drier sites. Oecologia 47:34–38.

Tubbs, L.O.E., R. Stevens, M. Wells, and R. Altig. 1993. Ontogeny of the oral apparatus of the tadpole of *Bufo americanus.* Amphibia-Reptilia 14:333–340.

Wassersug, R. J., and M. Yamashita. 2001. Plasticity and constraints on feeding kinematics in anuran larvae. Comparative Biochemistry and Physiology 131:183–195.

Yang, D.-T., D.-S. Ma, H.-J.Chen, and F.-L. Li. 1983. Descriptions of two new pelobatid toad from Yunnan. Acta Zoo Taxonomica Sinica 8:323–327

PAMMI SINGH is a Research Scholar and Project Fellow (University Grants Commission) in the Department of Ecology and Environmental Science, Assam (Central) University, Silchar, Assam, India. She received M.Sc. degree in Ecology and Environmental Science from Assam University, Silchar, in 2010. Her work focuses on researching habitat ecology and ecotoxicological studies on tadpoles. (Photographed by Dulumoni Tamuly).

MITHRA DEY, Ph.D., is Professor in the Department of Ecology and Environmental Science, Assam (Central) University, Silchar, Assam, India. She is actively involved both in teaching and research. Her field of interest is freshwater ecology and ecotoxicology with special interest in Herpetofauna. Her research work includes studies on diversity and ecology of anurans, impact of pesticides and heavy metals on anurans and fish and solid waste management. (Photographed by Pammi Singh).

SUNKAM NARAYANA RAMANUJAM completed Ph.D. in 1982 and is working in the Department of Zoology, North Eastern Hill University, Shillong, Meghalaya, India, for past 12 y. Currently he is the Professor and Head of the Department of Zoology. His main interest of research includes eco-physiology, fish biology and environmental toxicity. He has published 40 papers in national and international journals and presented many papers in national and international conferences. He has supervised eight Ph.D. students. (Photographed by Rajiv Paul).