REPTILES & AMPHIBIANS

Oral Structure and Diet of Larval Berdmore's Narrow-mouthed Frogs, *Microhyla berdmorei* (Blyth 1856), in Mizoram, India

H.T. Lalremsanga and Lal Muansanga

Developmental Biology and Herpetology Laboratory, Mizoram University, Aizawl 796004, Mizoram, India (muanapunte16@gmail.com)

The most conspicuous characters that differentiate larval frogs in the family Microhylidae from those in other families are the absence of keratinized jaw sheaths and labial teeth and a single, midventral spiracle (Orton 1953, 1957). Understanding morphology, especially that of the oral disc, is crucial to comprehending the feeding ecology of tadpoles and

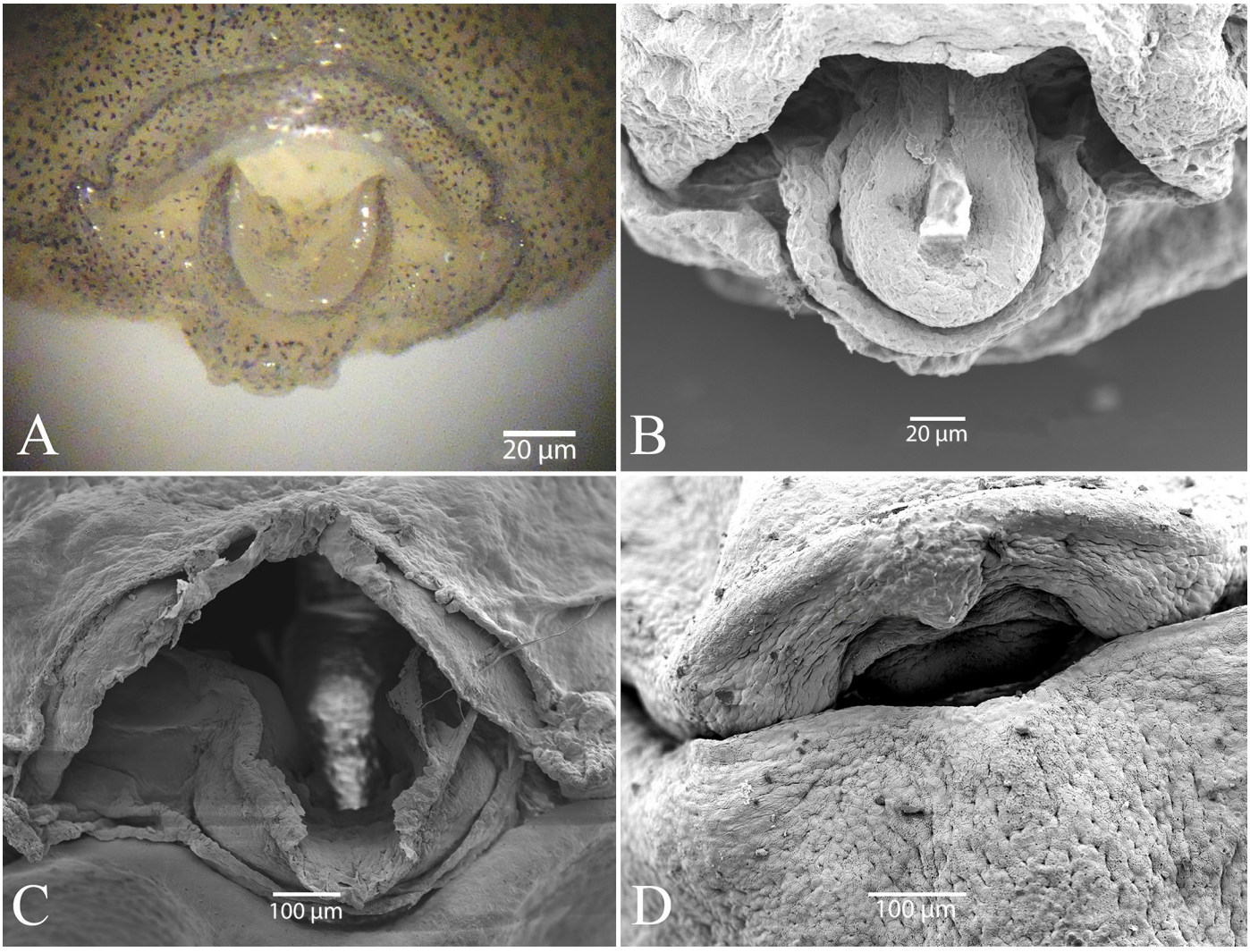

Fig. 1. Oral apparatus of the Berdmore's Narrow-mouthed Frog (*Microhyla berdmorei*) tadpole: Gosner Stage 25 under a light microscope (A); Scanning Electron Microscope images of Gosner Stage 25 (B), Gosner Stage 42 (C), and Gosner Stage 43 (D).

Reptiles & Amphibians ISSN 2332-4961
https://doi.org/10.17161/randa.v29i1.15962

therefore likely is the foremost factor in interpreting most, perhaps all, aspects of their biology (Altig and McDiarmid 1999). External morphological characters and especially internal oral features of tadpoles are very important in the systematics of anuran amphibians (e.g., Wassersug 1980; Chou and Lin 1997; Li et al. 2011). Nevertheless, only scant information is available on the oral structure and even the morphology of tadpoles.

Berdmore's Narrow-mouthed Frog (*Microhyla berdmorei*) occurs in northeastern India, Bangladesh, China, Myanmar, Thailand, Malaysia, Laos, Vietnam, Cambodia, and Indonesia (Frost 2021). Kundu et al. (2021) described genetic diversity within the species. Inthara et al. (2005) provided a brief description of larval morphology and oral features, and Lalremsanga and Hooroo (2012) addressed the remodeling of intestines during development. Herein we present detailed information on oral morphology with respect to diet during larval development in *M. berdmorei*.

We collected tadpoles of *Microhyla berdmorei* in February–May 2019 from a natural pond inside the estate of the Zoram Poultry Development Society Hatchery Farm at Tuvamit, Aizawl, Mizoram (23°74.30'11.10"N, 92°68.00'12.37"E; elev. 964 m asl) and fixed larvae of each stage (Gosner 1960) in a solution comprising 4% buffered formalin and 70% ethanol in equal volume. We examined mouthparts with a light microscope (CETI Star 24) and a scanning electron microscope (JSM-35 CF). For the latter, preserved samples were washed with double distilled water, fixed in 2.5% glutaraldehyde solution (prepared in 0.1M Na-cacodylate buffer) for 1 h and post-fixed in 1% buffered osmium tetroxide (0.1M Na-cacodylate buffer) for 1 h at 4 °C while maintaining the pH of the fixative and buffer at 7.4. Samples were then dehydrated through ascending acetone grades and dried in tetramethylsilane as in Dey et al. (1989) before applying a thin conductive coating of gold using a JFC 1100 (Jeol) ion sputter. We then examined the coated samples at an accelerating voltage of 5 kV and a working distance of 15 mm at tilting angles necessary for viewing the frontal portion of the oral structures. We evaluated the larval diet by examining intestinal contents.

Larval *Microhyla berdmorei* lack keratinized jaws and labial teeth and have a terminally-oriented (as opposed to antero-ventrally oriented; i.e., umbelliform) mouth known as a semicircular labial flap. A U-shaped medial notch protrudes from the mouth between the two semicircular labial flaps (Figs. 1A & B). Infralabial papillae are reduced and lingual papilla are absent. As tadpoles from stage 42 onward ceased to feed, mouthparts gradually changed into the adult configuration (Figs. 1C & D).

Gut contents at stage 25 consist of phytoplankton (*Diatoma*, *Fragilaria*, *Navicula*, *Stauroneis*, *Synedra*, and *Tabellaria* [Bacillariophyceae]; *Cladophora*, *Closterium*, *Cosmarium*, *Spirogyra*, *Staurastrum*, and *Ulothrix* [Chlorophyceae]; *Oscillatoria* [Cyanophyceae]; *Arcella* and *Cryptomonas* [Cryptophyceae]) and a zooplankton (*Lecane*). During hindlimb bud development (stages 26–30), gut contents included phytoplankton (*Cymbella*, *Melosira*, and *Pinnularia* [Bacillariophyceae]; *Mougeotia*, *Oedogonium*, and *Sirogonium* [Chlorophyceae]; *Anabaena* and *Nostoc* [Cyanophyceae]; and *Euglena* [Euglenophyceae]) and a zooplankton (*Euglypha*). Guts of larvae at stages 31–41 contained only *Phacus* (Phacaceae). After the emergence of forelimbs at stages 42–45 and coinciding with a cessation of feeding, the larval oral apparatus began to degenerate. From stage 46 onward, froglets fed on various small invertebrates.

Tadpoles at stages 30–40 have relatively stable external morphological characters (Gosner 1960). The absence of jaw sheaths and keratodonts in larval *Microhyla berdmorei* agrees with observations of Rao (1933), who reported the total absence of teeth in the tadpoles of *Kaloula* and *Microhyla*; Das and Coe (1994), who noted the absence of denticles in *Microhyla rubra*, *M. ornata*, and *Uperodon systoma*; and Inthara et al. (2005), who examined *Kaloula pulchra*, *Microhyla ornata*, *M. butleri*, *M. pulchra*, *Micryletta inornata*, and *Glyphoglossus molossus*. The umbelliform disc of the *Microhyla berdmorei* tadpole has a large, infolded semicircular structure at each corner of the mouth that appears to be derived from the lower labium, as in larval *Microhyla heymonsi* (Altig and McDiarmid 1999) and *M. ornata* (Khan and Mufti 1994). Altig and McDiarmid (1999) reported that an umbelliform or upturned oral disc appears as a convergent trait in tadpoles of some arthroleptids, dendrobatids, hylids, mantelline rhacophorids, megophryids, and microhyline microhylids. Most umbelliform tadpoles occur in backwaters of lotic systems. Tooth rows and jaw sheaths are reduced to absent in these forms, and large ridge-like papillae that project radially from the mouth are common. The absence of keratodonts excludes surface-rasping in some species and, conversely, the presence of robust rostrodonts and multiple tooth rows signals food removal by surface rasping (Candioti et al. 2004).

Larval gut contents consisted mainly of Bacillariophyceae, followed by Chlorophyceae, Cryptophyceae, zooplankton, Euglenophyceae, detritus, and Cyanophyceae. Among food items, Chlorophyceae, *Cladophora*, and *Mougeotia* predominated. Khan and Mufti (1994) noted that sympatric species of tadpoles differ not only in oral disc morphology but also in exploiting different components of the common food base available in the pond ecosystem. For example, in contrast to the sympatric Indo-Burmese Stream Frog (*Sylvirana lacrima*) tadpoles that mainly consume Chlorophyceae, larval *Microhyla berdmorei* fed more frequently on Bacillariophyceae (Lalremsanga 2011). Also, as suggested by Lalremsanga et al. (2017), the reduction in the size of infralabial papillae and the absence of lingual papillae might imply that these structures

are not important in retaining some larger particles in the buccal cavity. This has been observed in *Microhyla butleri*, *M. heymonsi*, *M. ornata*, and *M. steijngeri* (Chou and Lin 1997); *Dermatonatus muelleri* and *Elachistocleis bicolor* (Echeverria and Lavilla 2000); and *Kaloula rugifera* and *K. borealis* (Zhou et al. 2011).

Acknowledgements

We thank Mr. Liandawla (Principal Chief Conservator of Forests), Government of Mizoram, for issuing a permit (No.A.33011/2/99-CWLW/225) to collect herpetofauna in Mizoram, and the Defence Research Development Organisation (DRDO), Ministry of Defence (No. DFTM/07/3606/NESTC/ABR/M/P-01), and the Department of Biotechnology (DBT), Ministry of Science and Technology (No. DBT-NER/AAB/64/2017), Government of India, New Delhi, for financial support.

Literature Cited

Altig, R. and R.W. McDiarmid. 1999. Body plan: Development and morphology, pp. 24–51. In: R.W. McDiarmid and R. Altig (eds.), *Tadpoles, The Biology of Anuran Larvae*. University of Chicago Press, Chicago, Illinois, USA.

Candioti, M.F.V., E.O. Lavilla, and D.D. Echeverria. 2004. Feeding mechanisms in two treefrogs, *Hyla nana* and *Scinax nasicus* (Anura: Hylidae). *Journal of Morphology* 261: 206–224. https://doi.org/10.1002/jmor.10239.

Chou W.H. and J.Y. Lin. 1997. Tadpoles of Taiwan. *National Museum of Nature and Science* 7: 1–98.

Das, L. and M. Coe. 1994. The dental morphology and diet in anuran amphibians from south India. *Journal of Zoology* 233: 417–427. https://doi.org/10.1111/j.1469-7998.1994.tb05274.x.

Dey, S., T.S. Basu Baul, B. Roy, and D. Dey. 1989. A new rapid method of air drying for scanning electron microscopy using tetra-methlysilane. *Journal of Microscopy* 156: 259–261. https://doi.org/10.1111/j.1365-2818.1989.tb02925.x.

Echeverria, D.D. and E.O. Lavilla. 2000. Internal oral morphology of tadpoles of *Dermatonotus muelleri* and *Elachistocleis bicolor*. *Journal of Herpetology* 34: 517–523. https://doi.org/10.2307/1565265.

Frost, D.R. 2021. *Amphibian Species of the World: An Online Reference*. Version 6.1. American Museum of Natural History, New York, New York, USA. <https://amphibiansoftheworld.amnh.org/index.php>. https://doi.org/10.5531/db.vz.0001.

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

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

Khan, M.S. and S.A. Mufti. 1994. Oral disc morphology of amphibian tadpole and its functional correlates. *Pakistan Journal of Zoology* 2641: 25–30.

Kundu, S., H.T. Lalremsanga, L. Biakzuala, H. Decemson, L. Muansanga, K. Tyagi, K. Chandra, and V. Kumar. 2021. Genetic diversity of the Pegu Rice Frog, *Microhyla berdmorei* (Anura: Microhylidae) based on mitochondrial DNA. *Mitochondrial DNA Part B* 6: 1586–1591. http://dx.doi.org/10.1080/23802359.2021.1920504.

Lalremsanga, H.T. 2011. Studies on the ecology, breeding behavior and development of ranid and microhylid anurans prevalent in Mizoram, Northeast India. Unpublished Ph.D. Thesis, North Eastern Hill University, Shillong, Meghalaya, India.

Lalremsanga, H.T. and R.K. Hooroo. 2012. Remodeling of the intestine during metamorphosis of *Microhyla berdmorei* (Anura: Microhylidae). *International Multidisciplinary Research Journal* 2(2): 35–40.

Lalremsanga, H.T., S. Sailo, and R.N.K. Hooroo. 2017. External morphology, oral structure and feeding behaviour of *Kaloula pulchra* tadpoles Gray, 1831 (Amphibia: Anura: Microhylidae). *Science and Technology Journal* 5(2): 97–103.

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. https://doi.org/10.1093/CZOOLO/57.1.93.

Orton, G.L. 1953. The bearing of larval evolution on some problems of frog classification. *Systematic Zoology* 6: 79–86. https://doi.org/10.2307/2411805.

Orton, G.L. 1957. The bearing of larval evolution on some problems in frog classification. *Systematic Zoology*, 6: 79–86. https://doi.org/10.2307/sysbio/6.2.79.

Rao, A.N. 1933. Engystomatid tadpoles. *Current Science* 1: 275–276.

Wassersug, R.J. 1980. The internal oral features of larvae from eight anuran families: Functional, systematic, evolutionary and ecological considerations. *University of Kansas Museum of Natural History Miscellaneous Publication* 68: 1–146. https://doi.org/10.5962/bhl.title.16230.

Zhou, B., P. Li, Y. Lu, J. Lian, and Z. Zhou. 2011. Morphology of *Kaloula rugifera* and *K. borealis* tadpoles (Anura: Microhylidae: *Kaloula verrucosa* group) in China. *Asian Herpetological Research* 2: 169–175. http://dx.doi.org/10.3724/SP.J.1245.2011.00169.