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First record of the Burmese Whipping Frog, *Polypedates mutus* (Smith, 1940), from Manipur State, India, with the description of its tadpole

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The Burmese Whipping frog (*Polypedates mutus*) was originally described from N'Chang Yang, Kachin State, in northern Myanmar (25.8333°N, 94.7999°E, elevation 789 m) (Smith, 1940). Kuraishi et al. (2012) stated that its range included mainland and peninsular Myanmar (Bago, Kachin, Shan, and Tanitharyi States) into southern China, northern Laos, northern Thailand, and Vietnam. From India, it has so far only been reported from Khawzawl District in Mizoram State (Siammawii et al., 2023).

Manipur State in Northeast India is situated at the convergence of the Himalayan and Indo-Burma hotspots, with the states of Assam to the east, Mizoram to the south, and Nagaland to the north, and with Myanmar to the east and southeast (Ningombam and Bordoloi, 2007). Manipur's amphibian fauna has been the subject of few publications and awaits more thorough explorations. Chanda (2002) documented 14 species of amphibians from the state, and Sarkar et al. (2002) also reported 14 species. Subsequently, Sen (2004) included 17 species, and Shah et al. (2022) listed 27. Most recently, Decemson et al. (2023) described a new species of *Tylototriton* and Devi et al. (2025) added *Fejervarya limnocharis* and *Minervarya sengupti* to the list, for a total of 30 as of this writing.

Tadpoles exhibit many distinct differences from adult frogs (McDiarmid and Altig, 1999), and the identification of tadpoles is often facilitated by examining the external structure and morphology of their mouthparts (Altig and Pace, 1974; Lee, 1976; Inthara et al., 2005). There is considerable variation in the size, shape, and number of keratodont rows in tadpole oral structures. However, information on the mouthparts of many species, particularly those of the Family Rhacophoridae, remains limited (Bowatte and Meegaskumbura, 2011). Here, we described the tadpoles of *P. mutus* and the ultrastructure of their oral apparatus with a discussion of the phylogenetic relationship amongst congeners.

Materials and Methods

Specimen collection. We carried out this study in Kangchup, Manipur State, India (23.8863°N, 93.8064°E) during the months of June and July in 2023 and 2024. Wassersug (2000) suggested that the climax stage of larval ontogeny corresponds to Gosner Stages 26–36, while Grosjean (2005) stated most of the characters relevant for identification and characterization occurred in Stages 32–40. We considered tadpoles of *P. mutus* at Stage 32 best suited for representing taxonomic characters. Tadpoles were collected using a 1 × 1 m dip net and 20 individuals were obtained for our study. Three tadpoles were euthanized and kept in > 94% ethanol at −40°C for molecular study while the remaining tadpoles were preserved in 10% formalin. To assess the morphological differences among the collected specimens, a total of 13 parameters (see Devi et al., 2025) were measured to an accuracy of 0.1 mm using Mitutoyo CD–6"ASX digital callipers. Character abbreviations are provided in Table 1.

DNA extraction, sequencing, and data analysis. DNA was extracted from tadpole tail muscles preserved in alcohol using the phenol-chloroform method following (Marmur, 1961). The 16S rRNA gene was amplified using the forward primer AH-16S_S (5'–GGC CTG TTT ACC AAA AAC ATC GCC T–3') and the reverse primer AH-16S_R (5'–TGC GCT GTT ATC CCY RGG GTA ACT–3') following Grosjean et al. (2015). PCR thermal cycling involved initial denaturation at 95°C for 30 s, annealing at 50°C for 30 s, elongation at 72°C for 45 s, and a final extension at 72°C for 3 min.

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Table 1. Measurements (mean ± standard deviation, in mm) and relative lengths (Rel Length; percentages of total length) of *Polypedates mutus* tadpoles from Manipur, India, at Gosner Stage 32.

Parameters	Measurements	Rel Length
total length (TL)	35.3 ± 5.0	n/a
snout–vent length (SVL)	14.4 ± 2.0	41
tail length (TAIL)	24.8 ± 3.5	70
body width (BW)	7.2 ± 1.2	20
body depth (BD)	6.8 ± 1.4	19
tail depth (TD)	8.0 ± 1.0	22
oral disc length (ODL)	1.2 ± 0.3	3
oral disc width (ODW)	3.4 ± 0.4	9
eye-to-nostril distance (EN)	4.4 ± 0.7	12
nostril-to-snout distance (NS)	2.0 ± 0.3	5
internarial distance (INS)	3.2 ± 0.5	8
interorbital distance (IOS)	7.4 ± 0.9	21
eye diameter (ED)	1.7 ± 0.3	5

The amplified DNA was analysed using electrophoresis on a 0.8% agarose gel and visualized under UV light. Sequencing in both directions was carried out using the same primers as in PCR on an ABI3730xl automated sequencer (Applied Biosystems, Foster City, California, USA), at the Barcode Bioscience facility in Bangalore, India.

Sequence chromatograms were quality-checked, edited, and assembled into contigs using Sequence Scanner v1.0 (Applied Biosystems). Comparative 16S rRNA sequences from *Polypedates* species were retrieved from GenBank. Sequence alignment was conducted using MUSCLE (Edgar, 2004) in MEGA7 (Tamura and Nei, 1993; Kumar et al., 2016) with default settings. PartitionFinder v2 (Lanfear et al., 2017) was used to determine the optimal partitioning scheme for the dataset. A Maximum Likelihood (ML) phylogenetic tree was constructed in IQ-TREE (Nguyen et al., 2015) using an unpartitioned dataset, with the TIM2+F+G4 substitution model selected based on Bayesian Information Criterion scores from Model Finder (Kalyaanamoorthy et al., 2017). ML analysis was performed with 1000 iterations of ultrafast bootstrap resampling to evaluate clade support (Minh et al., 2013). The uncorrected pairwise *p*-distance was calculated in MEGA7, with pairwise deletion of gaps and missing data.

Scanning Electron Microscopy. Tadpole mouthparts were fixed in 3% glutaraldehyde, washed with 0.1-M sodium cacodylate buffer, and dehydrated in increasing acetone grades before drying in tetramethylsilane. Specimens were mounted on brass stubs with adhesive tape and coated with gold using a JFC 1100 ion sputter to enhance conductivity and prevent radiation damage. Observations were conducted at 20 KV using a Jeol JSM 6360 Scanning Electron Microscope in secondary electron emission mode at SAIF, North-Eastern Hill University, Shillong.

Results

Phylogenetic analysis. The tadpoles from Manipur grouped with *P. mutus* from Myanmar (type locality) and Mizoram (Fig. 1). The uncorrected *p*-distance between the specimen from Manipur and the other samples ranged from 0.000–0.003, showing no or very low genetic divergence between these localities (Table 2). This allows us to confirm the presence of *P. mutus* in Manipur for the first time.

External tadpole morphology. The dorsal view of a tadpole (Fig. 2A) shows an elliptically elongated body. The ventral side (Fig. 2B) is translucent and four-and-a-half coils of the intestine are visible. The tadpoles (n = 20) are of moderate size (SVL = 14.4 ± 2.0 mm) and the snout is blunt and flattened in both dorsal and ventral views (BW:BD = 1.07 ± 0.13). The SVL is less than half of total length (SVL:TL = 0.41± 0.01). Body width is moderate (BW:SVL = 0.49 ± 0.02). Large eyes (ED:SVL = 0.11 ± 0.01) are placed laterally (Fig. 2C), and their size is less than one-fourth of the interorbital distance (ED:IOS= 0.22 ± 0.03). The nostrils are closer to the tip of the snout than the eyes (NS:EN = 0.46 ± 0.06).

The nostril opening is oblong, widely spaced (Table 1). Internarial distance is 43% of interorbital distance on average, with a narrow nasal base compared to the space between the eyes (INS:IOS = 0.43 ± 0.05). Sinistral spiracle. The tail (Fig. 2D) is long (TAIL:TL = 0.70 ± 0.02) with well-developed musculature, tail tip slight pointed. The tail fins are slightly transparent and whitish in colour with well-developed musculature, broad at the junction of the body and tail, gradually tapering towards the end. The fins are relatively tall, with the dorsal fin beginning just before the body-tail junction and being nearly the same height as the ventral fin. The vent tube is dextral, slightly tilted to the right axis of ventral fin. Glands on the body surface are absent.

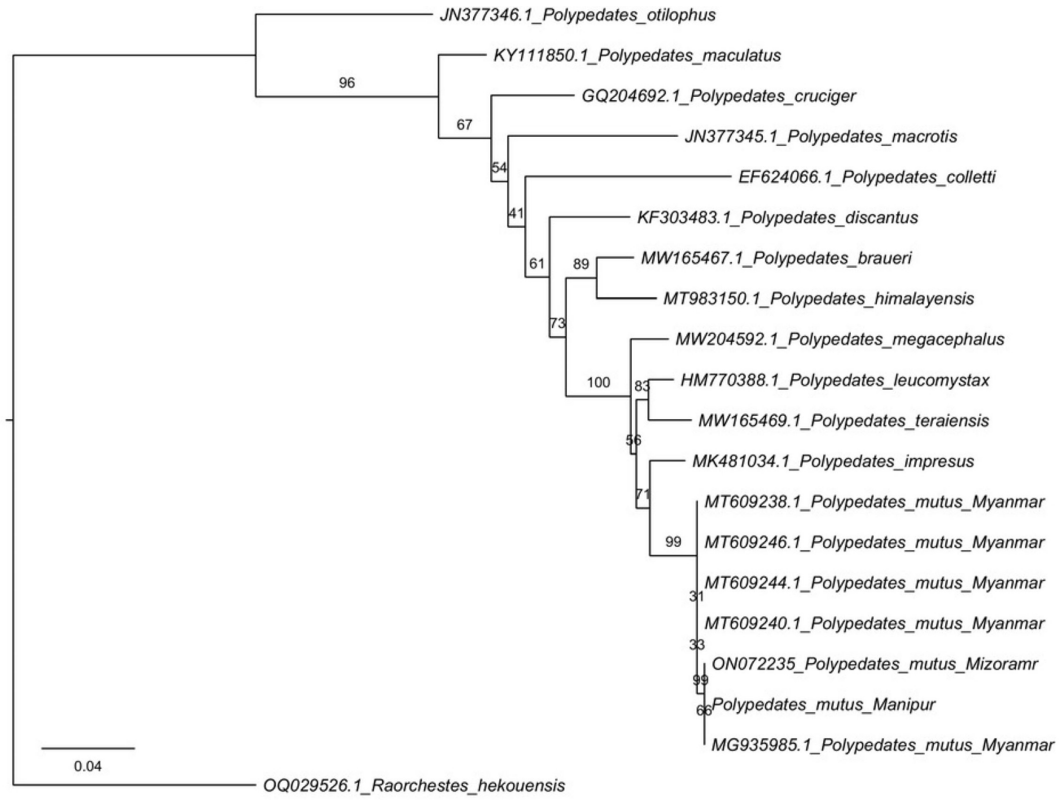

Figure 1. Maximum Likelihood tree (16S rRNA) showing members of *Polypedates* species used in this study. The number preceding the species name is the GenBank accession number. The GenBank accession number of *P. mutus* from Manipur is PV254821. Numbers on the branches represent bootstrap support values (%).

Table 2. Estimates of evolutionary divergence based on pairwise *p*-distances among populations of *Polypedates mutus* based on a phylogenetic analysis using the partial 16S rRNA gene.

No.	Sample	1	2	3	4	5	6
1	Manipur, India						
2	Mandalay, Myanmar (MG935985)	0					
3	Mizoram, India (ON072235)	0	0				
4	Tanintharyii, Myanmar (MT609246)	0.003	0.003	0.003			
5	Tanintharyii, Myanmar (MT609238)	0.003	0.003	0.003	0		
6	Tanintharyii, Myanmar (MT609240)	0.003	0.003	0.003	0	0	
7	Tanintharyii, Myanmar (MT609244)	0.003	0.003	0.003	0	0	0

Oral morphology. The mouth is positioned anteroventrally. The oral apparatus (Fig. 2E) is non-protruding, and the oral disc is emarginate. It is composed of anterior (upper) labium and posterior (lower) labium (Fig. 3). The oral disc is much wider than long (ODL:ODW = 0.36 ± 0.07). The upper jaw sheath forms a large arch shape, and the lower jaw sheath is continuous with the upper jaw sheath and also curved.

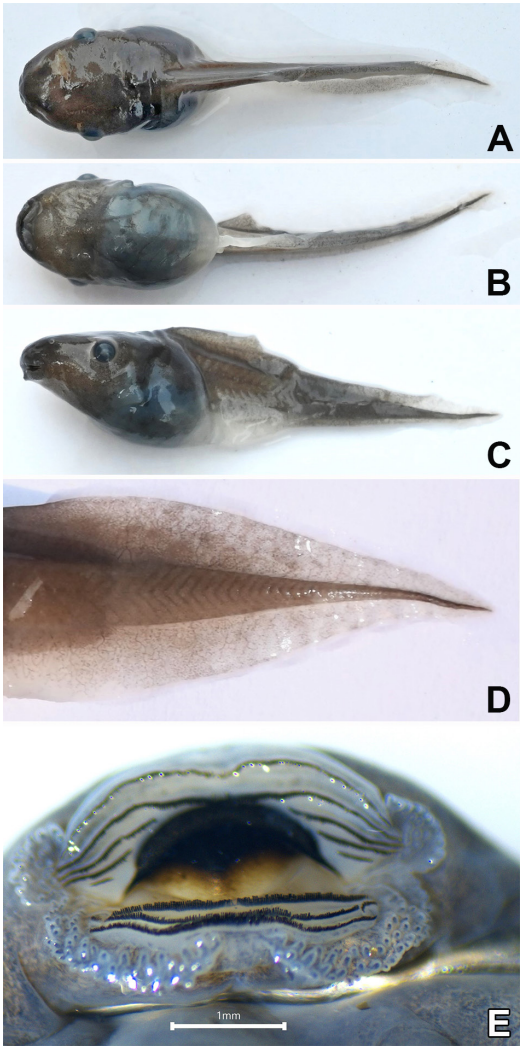

Figure 2. (A) Dorsal, (B) ventral, (C) lateral views of a Gosner stage 32 *Polypedates mutus* tadpole from Manipur, India, taken using a Nikon Z50 mirrorless camera fitted with a Nikkor ZDX 50–250 mm lens. (D) Tail fin, showing light brown and black marble speckling. (E) Oral disc of photographed on a Stereozoom Microscope with Micron Optic Digital camera attachment. Photos by Dhiraj Das.

The upper and lower jaw sheaths are cuspate and pointed, with a thick and broad beak. The jaw sheath exhibits serrations, featuring a single row of thin marginal and sub-marginal papillae posteriorly. The oral disc structure is characterized with two continuous and three discontinuous (three left and three right side) teeth rows in the upper labium. The lower labium has three continuous teeth row. Both the infrarostrodont and suprarostrodont have a pointed tip. The arrangement of keratodont rows is biserial, with a labial toothrow formula (LRTF) of 5(2–5)/3. Order of length of denticle rows is A2 > A1 > P3 > P2 > P1 > A3 > A4 > A5.

Colour in life. Tadpoles are extremely dark brown in colour, sometimes almost black with a light grey ventral abdomen revealing coils of the gut. We could not easily differentiate the nares in dorsal view.

Colour in preservative. During the time in preservative, tadpole colour changed to light brown.

Habitat. *Polypedates mutus* tadpoles were observed among aquatic vegetation in shallow, stagnant water pools near a flowing river. The pools have a muddy bottom with maximum water depth of approximately 67 cm.

Discussion

The phylogenetic analysis showed that the *P. mutus* population in Manipur is deeply nested among *P. mutus* populations from Myanmar and other localities. The tadpoles of all *Polypedates* species have an ovoid body (i.e., BW/BH about 100%), lateral eyes, nares placed nearer to the snout than eyes, and a flagellum at the tip of the tail (Chakravarty et al., 2011). The general morphology of the *P. mutus* tadpole generally matches the tadpole descriptions for *P. leucomystax* (Gravenhorst, 1829), *P. teraiensis* (Dubois, 1987), *P. maculatus* (Gray, 1830), *P. pseudocruciger* Das & Ravichandran, 1998, and *P. braueri* (Vogt, 1911) suggesting close relationships (Alcala, 1962; Chakravarty et al., 2011; Raj, 2023; Raj et al., 2023; Siammawii et al., 2025). It differs from *P. himalayensis* (Annandale, 1912), whose eyes are dorsolateral with nostrils closely placed (Rangad, 2021). According to Raj et al. (2023), tadpoles of *Polypedates* species have a compressed and rounded body, which contrasts with our observation of an ovoid body. Tadpoles of *P. braueri* from Mizoram (Siammawii et al., 2025) are more or less similar except for a minor difference in LTRF, 5(2–5)/3 in *P. mutus* and 4(2–4)/3 in Stage 30 tadpoles of *P. braueri*. Furthermore, the LTRF of *P. mutus* was found to be 5(2–5)/3 in our study, whereas Grosjean (2004) recorded it as 5(2–5)/(1)3, or

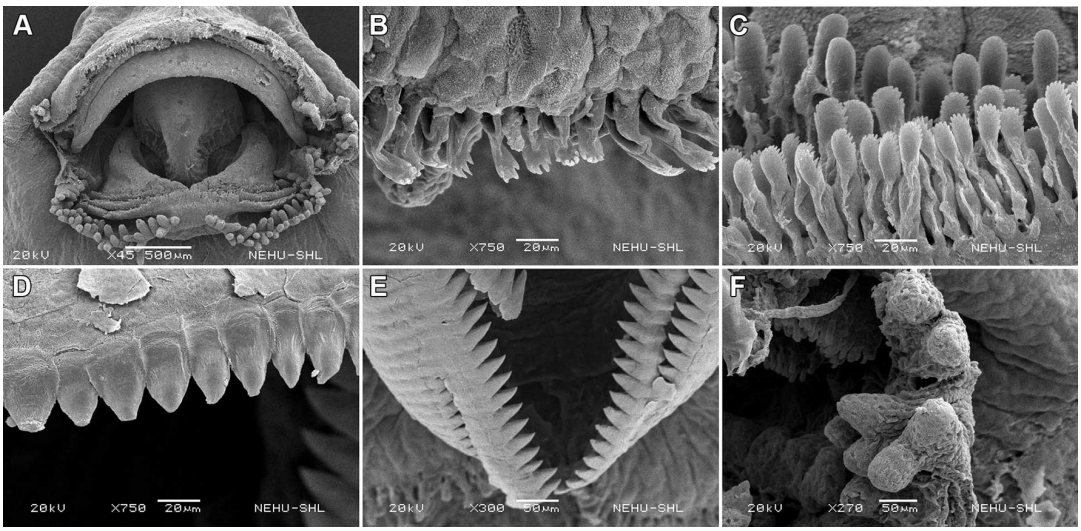

Figure 3. Scanning electron micrographs of the oral apparatus of Gosner stage 32 *Polypedates mutus* tadpoles from Manipur, India. (A) Oral disc showing upper and lower jaw sheaths with lateral emargination. (B) Upper labial teeth. (C) Lower labial teeth. (D) Serration of the infrarostrodont. (E) Serration of the suprarostrodont. (F) Marginal papillae. Photos courtesy of SAIF, NEHU, Shillong.

1:4+4/1+1:2 when expressed in our terms. Grosjean also reported the presence of a clear dot on the snout, which we did not observe.

The most common configuration is an LTRF of 2/3, but these numbers can range from 0/0–17/21, in many different combinations (Altig, 2007). Although most tadpoles have a single tooth row per ridge (uniserial), others have been seen to have bi-, tri-, or even multiserial rows (e.g., dicroglossids; McDiarmid and Altig, 1999). The LTRF of *Polypedates* tadpoles exhibits considerable variation across different geographic and developmental stages. In Stages 26–34, reported LTRFs include 4(2–4)/3 or 4(2–4)/3(1) for *P. braueri* (Siammawii et al., 2025), 5(2–5)/3(1) for *P. pseudocruciger* (Raj, 2023), and 5(2–5)/3(1) for *P. leucomystax* (Inger, 1956). In contrast, at Stage 35–40, the LTRF has been documented as 4(3)/3(1) for *P. maculatus* (Rout et al., 2022), 4(2–4)/3 or 4(2–4)/3(1) for *P. leucomystax* (Alcala and Brown, 1956; Alcala, 1962), and 4(3–3)/3(1) for *P. teraiensis* (Chakravarty et al., 2011). These variations highlight the influence of both ontogeny and regional factors on LTRF patterns within *Polypedates* species.

The anteroventral orientation of the oral disc indicates that these tadpoles are generalized feeders (Khan and Mufti, 1994). The lower labium of *P. mutus* has three rows with a narrow gap between the first and the second row. We observed a unique trait in their oral apparatus where the second and third row of the lower labium almost adhered to each other in the centre. A narrow gap between the first and second row was also observed in *P. pseudocruciger* (Raj, 2023), while the lower labium of *P. maculatus* has a large gap in the first row. Such a gap in the posterior jaw sheath has also been reported (Inger, 1985; Tesia et al., 2017) for *P. macrotis* (Boulenger, 1891), *P. colletti* (Boulenger, 1890), and *P. otilophus* (Boulenger, 1893), as well as *P. megacephalus* Hallowell, 1861, which do not conform to *P. mutus*.

The external morphology of tadpoles is conserved within a group (genus) and by the incorporation of supplementary features, such as the buccopharyngeal area, would aid the enhancement of species identification and addressing issues with anuran ecosystems (Wassersug, 1976; Das and Coe, 1994). Knowing the morphology and ecology of larval forms can help determine the region's anuran conservation priorities, which will contribute to biota conservation.

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