



Two new additions to the amphibian fauna of Manipur, India

Manipur has one of the least well-known amphibian faunas in the northeastern states of India. Sen (2004) recorded 17 species, Sarkar *et al.* (2005) recorded 14, and Frost (2024) listed 42 species of amphibians. Ningombam & Bordoloi (2007) recorded 25 species of amphibians from in and around Loktak Lake, Manipur. Manipur has all three orders of Amphibians, Urodela (salamanders: 1 species), Anura (frogs and toads: 39 species), and Apoda (caecilians: 2 species) (Frost 2024). A recent description of a salamander *Tylototriton zaimeng* (Decemson *et al.* 2023) illustrates the opportunities there are for the discovery of new herpetofaunal species in Manipur.

Through our preliminary survey of the Senapati District, based on morphology and genetic data, we report here two new amphibian records for Manipur State. The study was conducted in Tungjoy (25.475058°N, 94.252953°E; alt. 1,600 m a.s.l.) Senapati District of Manipur, India. It was conducted using a randomized survey (Lambert 1984) and a visual encounter survey (Heyer *et al.* 2014). Two individual frogs were collected for this study. After collection, the samples (ADBUG5 and ADBUR4) were fixed in 10% formaldehyde solution and were deposited in the Lab of the Department of Zoology, Assam Don Bosco University, Sonapur, Assam, India. All measurements were recorded in mm which were taken with the help of Mitutoyo digital calipers to the nearest 0.1 mm.

DNA isolation was done using the Phenol: Chloroform: Isoamyl alcohol method followed by 0.8% agarose gel electrophoresis and visualized under UV light using Biorad Gel

Imager Gel Documentation Unit. The 16S rRNA gene was amplified using gene-primers pair AH-16S_S 5'-CGC CTG TTT ACC AAA AAC ATC GCC T-3' and AH-16S_R 5'-TGC GCT GTT ATC CCY RGG GTA ACT-3' following Caranza & Arnold (2006). Sequence alignment was done using MUSCLE (Edgar 2004) in MEGA7 (Tamura & Nei 1993, Kumar *et al.* 2016) with default parameter settings.

A Maximum Likelihood (ML) phylogenetic tree was reconstructed using an unpartitioned dataset in IQ-TREE (Nguyen *et al.* 2015) with the substitution model TIM2+F+I+G4 selected based on the BIC scores by Model Finder (Kalyanamoorthy *et al.* 2017). The ML analysis was run with an ultrafast bootstrap option (Minh *et al.* 2013) for 1000 iterations to assess clade support. The Uncorrected pairwise p-distance was calculated in MEGA7 (Kumar *et al.* 2016) with pairwise deletions of missing data and gaps.

The Maximum Likelihood (ML) tree (Fig. 1) constructed using 16S rRNA sequences, indicated that one specimen belonged to *Polypedates braueri* and the other to *Duttaphrynus stuarti*. The *Polypedates braueri* specimen clustered closely with other samples of the same species from Mizoram (India), Myanmar, and China. The Manipur specimen formed a sister clade with *Polypedates braueri* from Mizoram (see Lalronunga *et al.* 2020), with an uncorrected p-distance of 0.013 (Sup. Table 1). Similarly, *Duttaphrynus stuarti* was found to group with other samples of *Duttaphrynus stuarti* from Myanmar and China, showing uncorrected p-distances ranging from 0.017–0.019 (Sup. Table 1).

Polypedates braueri (Fig. 2) is a tree frog found in Taiwan, China, Myanmar, and Mizoram. Although previously reported in various regions of India, its presence in the Senapati District of Manipur was not

documented until now. The frog was found near running streams in the forests of Tungjoy Village on 30 May 2022 around 13:00 hr when the temperature was 22°C with 75% humidity. The collected sample is characterized by an SVL of 58.54 mm. Manipur is the second state in India to record *Polypedates braueri* after Mizoram (Sailam Village, Aizawl District (23.3497°N, 92.7981°E, alt. 1,370 m) and Reiek Community Reserved Forests of Mamit District (23.6772°N, 92.6030°E, alt. 1,229 m, see Siammawii *et al.* 2024). *Duttaphrynus stuarti* (Fig. 3) is a species in the Bufonidae family. The frog was found on

the dry mud of a fish farm near a human settlement in June 2022 at around 15:00 hr when the temperature was 22°C with 70% humidity. The collected sample had an SVL of 54.06 mm. The species was known to occur in the states of Arunachal Pradesh, Sikkim, and Meghalaya (Frost 2024), but this is the first record for Manipur. This study expands the known amphibian diversity of Manipur with the first records of *Polypedates braueri* and *Duttaphrynus stuarti*, highlighting the unexplored potential for higher biodiversity in northeastern India.

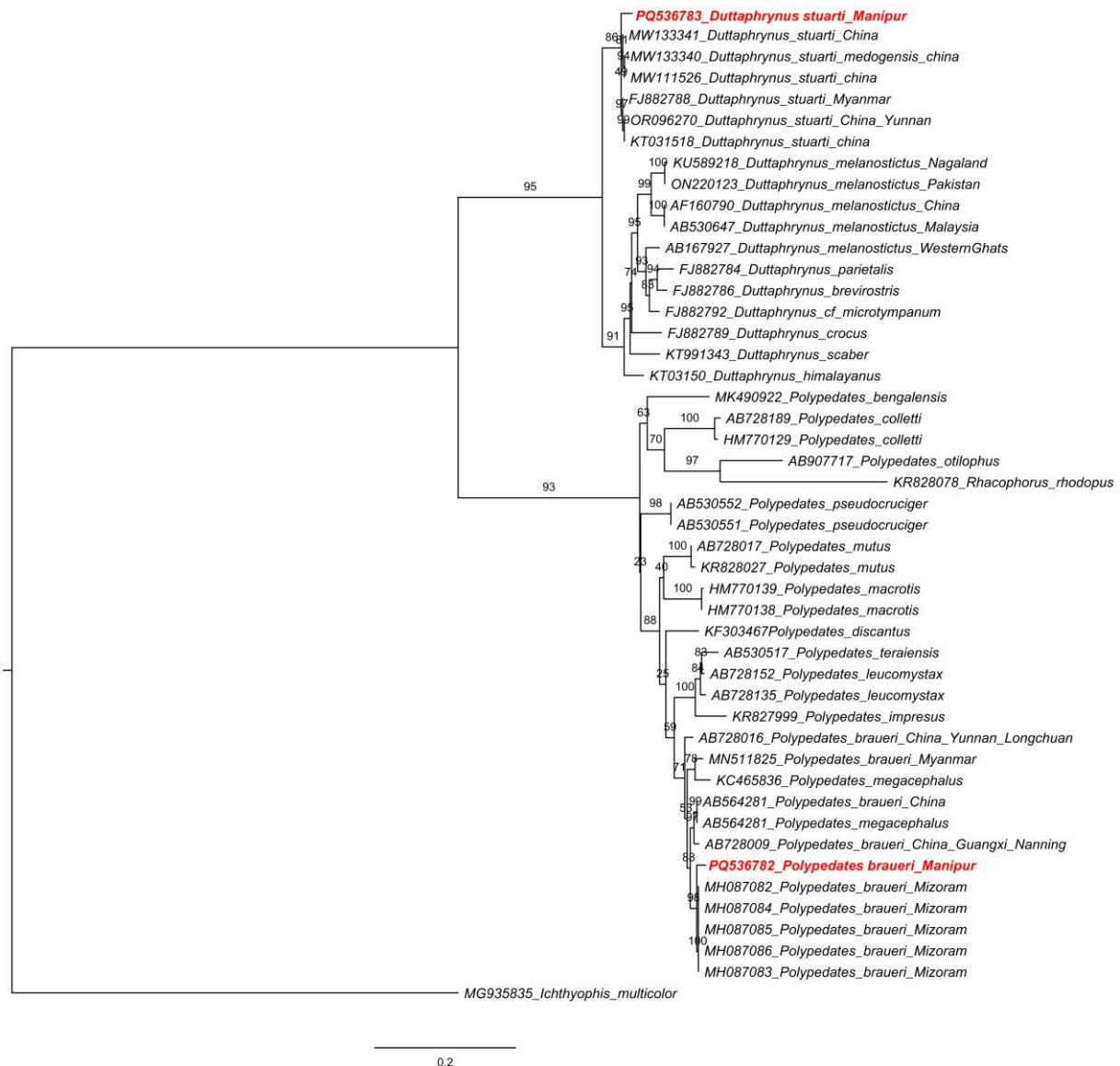


Figure 1. A Maximum Likelihood (ML) tree showing the relation of collected specimens of *Polypedates braueri* and *Duttaphrynus stuarti* from Tungjoy in Senapati District of Manipur (in red)

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Figure 2. *Polypedates braueri* in life from Tungjoy in Senapati District of Manipur, India



Figure 3. *Duttaphrynus stuarti* in life from Tungjoy in Senapati District of Manipur, India

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