



A New Locality Record of the Northeast Indian Paddy Frog, *Micryletta aishani* (Anura: Microhylidae), from Mizoram, India

Mara Duasa Hlychho¹, Malsawmdawngliana¹, Ht. Decemson², Vanlalhrauaia², Khawlhing Lalrinzuali³, Mathipi Vabeiryureilai¹, and Hmar Tlawmte Lalremsanga²

¹Cytogenetics Laboratory, Department of Zoology, Mizoram University, Mizoram-796004, India (dawngliana3@gmail.com [corresponding author], m.vabeiryureilai@gmail.com)

²Developmental Biology and Herpetology Laboratory, Department of Zoology, Mizoram University, Mizoram-796004, India (htlrsa@yahoo.co.in)

³Molecular Pathology Laboratory, Mizoram State Cancer Institute, Zemabawk-796017, Mizoram, India

Frogs in the genus *Micryletta* Dubois 1987, which currently comprises 14 recognized species (Frost 2026), have been reported from India, China, Taiwan, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Thailand, and Vietnam (e.g., Poyarkov et al. 2018; Garg and Biju 2019; IUCN SSC Amphibian Specialist Group 2022). *Micryletta inornata* (Boulenger 1890) was reported from Manipur (Mathew and Sen 2009, 2010) and Mizoram (Lalremsanga 2017) in Northeast India. However, recent studies on the species indicated that it comprised five distinct lineages, with only the Sumatran population assigned to *M. inornata* (Alhadi et al. 2019), which in turn led to the description of a new species, *M. aishani*, from Assam, India, by Das et al. (2019). This species is listed as Data Deficient on the IUCN Red List of Threatened Species (IUCN SSC Amphibian Specialist Group 2022).

Methods

At 2031 h on 21 March 2025, during a herpetological expedition in the Dampa Tiger Reserve, Mamit District, Mizoram, we collected an adult *Micryletta* sp. from the Teirei Forest Range (23.4122, 92.2728; elev. 259.6 m asl). According to diagnostic features in Das et al. (2019), we identified the specimen as *M. aishani*.

DNA extraction, PCR amplification, and sequencing—We extracted genomic DNA (gDNA) from liver tissue using the QIAamp DNA Mini Kit (Qiagen Cat. No. 51306) according to the manufacturer's instructions. DNA integrity was assessed using a 0.8% (w/v) agarose gel containing Ethidium bromide. A 20-μL PCR reaction was prepared with 1 X amplification buffer, 2.5 mM MgCl₂, 0.25 mM dNTPs, 0.2 pM forward and reverse primers, 1 μL of gDNA, and 1U Taq DNA polymerase. Primers used were L02510-CGC CTG TTT ATC AAA AAC AT (Palumbi 1996) and H03063-CTC CGG TTT GAA CTC AGA TC (Rassmann 1997).

The PCR thermal regime for amplification was 5 min at 95 °C for initial denaturation, followed by 35 cycles of 1 min at 95 °C for denaturation, 30 sec for annealing at 50.3 °C, elongation for 1 min at 72 °C, and a final elongation for 5 min at 72 °C. We then performed gel electrophoresis of PCR products on a 1.5% agarose gel containing ethidium bromide. Samples were sequenced using Sanger's dideoxy technique in both forward and reverse directions. The partial 16S rRNA sequences generated in this study were deposited in GenBank under accession number PV545500.

Phylogenetic analyses—The phylogenetic relationships of *Micryletta aishani* were assessed using the 16S rRNA gene sequence. Fourteen congeneric sequences were obtained from the NCBI database, and *Amolops indoburmanensis* (MT790757) was used as the outgroup. The newly generated sequences (n = 5) and retrieved sequence (n = 11) were aligned by using the MUSCLE algorithm in MEGA 7 (Kumar et al. 2016). Mean uncorrected genetic distances were calculated under the Kimura 2-parameter (K2P) model (Kimura 1980) in MEGA 7 (Kumar et al. 2016) (Table 1). The final aligned dataset contained 475 bp positions of 16S rRNA gene sequences. Model selection was performed in ModelTest-NG (Darriba et al. 2020), and the GTR+G model was chosen as the best-fitting model according to the lowest BIC scores. Phylogenetic trees were inferred using Bayesian Inference (BI) and Maximum Likelihood (ML) approaches. ML analysis was performed in raxmlGUI-2 with 1,000 bootstrap replicates (Silvestro and Michalak 2012). The BI phylogenetic tree was reconstructed in MrBayes 3.2.5 using GTR+G model. The Markov Chain Monte Carlo (MCMC) was run for 1,000,000 generations by sampling every 100 generations and the burn-in set to 25%. The analysis was terminated when the standard deviation of split frequencies was less than 0.001. The percentage of trees in which the associated taxa

Table 1. Uncorrected K2p-distances of 16S rRNA partial gene sequences between species in the genus *Micryletta* (Anura: Microhylidae).

Sl. No.	Taxon (GenBank accession no.)	K2P distance													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	<i>Micryletta aishani</i> (PV545500)														
2	<i>Micryletta aishani</i> (MK889218)	0													
3	<i>Micryletta aishani</i> (MW035599)	0	0												
4	<i>Micryletta aishani</i> (MW035601)	0	0	0											
5	<i>Micryletta inornata</i> (AB598341)	0.04	0.04	0.04	0.04										
6	<i>Micryletta steinegeri</i> (AB634696)	0.04	0.04	0.04	0.04	0.02									
7	<i>Micryletta lineata</i> (MT609036)	0.03	0.03	0.03	0.03	0.04	0.05								
8	<i>Micryletta subaraji</i> (PQ208546)	0.04	0.04	0.04	0.04	0.05	0.05	0.05							
9	<i>Micryletta erythropoda</i> (PQ570560)	0.04	0.04	0.04	0.04	0.05	0.05	0.02	0.06						
10	<i>Micryletta menglienicus</i> (OK335183)	0.04	0.04	0.04	0.04	0	0.02	0.04	0.06	0.05					
11	<i>Micryletta sumatrana</i> (MN727065)	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.08	0.07				
12	<i>Micryletta nigromaculata</i> (MH756148)	0.05	0.05	0.05	0.05	0.06	0.06	0.05	0.06	0.07	0.06	0.05			
13	<i>Micryletta dissimulans</i> (MT573416)	0.05	0.05	0.05	0.05	0.06	0.05	0.06	0.05	0.07	0.06	0.05	0.05		
14	<i>Micryletta bekouensis</i> (MZ536628)	0.04	0.04	0.04	0.04	0.02	0.01	0.04	0.05	0.04	0.02	0.07	0.06	0.05	
15	<i>Microhyla ornata</i> (AB201187)	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.13	0.13	0.12	0.13	0.11	0.12	0.13

clustered together is shown next to the branches (Ronquist and Huelsenbeck 2003). A phylogenetic tree (Fig. 1) was generated using Figtree v1.4.4 software.

Results

Morphologically, our specimen was assigned to *M. aishani* based on diagnostic features provided in the original description (Das et al. 2019). It has a slender body (SVL = 21.6 mm), snout nearly truncated in both dorsal and ventral views, vomerine teeth absent, adpressed tibiotarsal articulation reaching the axilla, dorsal skin granulated with minute spinules, outer metatarsal tubercles absent, digits with prominent subarticular tubercles, and webbing between fingers and toes absent. The dorsum is reddish-brown with a

faint median band extending from the margins of the upper eyelids to the vent, with scattered blackish-brown spots on the posterior dorsum and near the groin; a prominent blackish-brown streak extends from the tip of the snout to the flanks and limb margins; anterior and posterior surfaces of the limbs are brown with ash-gray mottling; the irises are bicolored, upper half light and lower half dark brown; and the venter is light gray with a purplish tinge.

Our phylogenetic assessment clearly indicated that the Mizoram specimen (MZMU 3697) clustered with the holotype and paratypes of *M. aishani* with an uncorrected *p*-distance of 0.00 (Table 1, Fig. 1), affirming its identity as a male *M. aishani* (Fig. 2). The microhabitat where we found the individual was dry leaf litter along a forest track (Fig. 2),

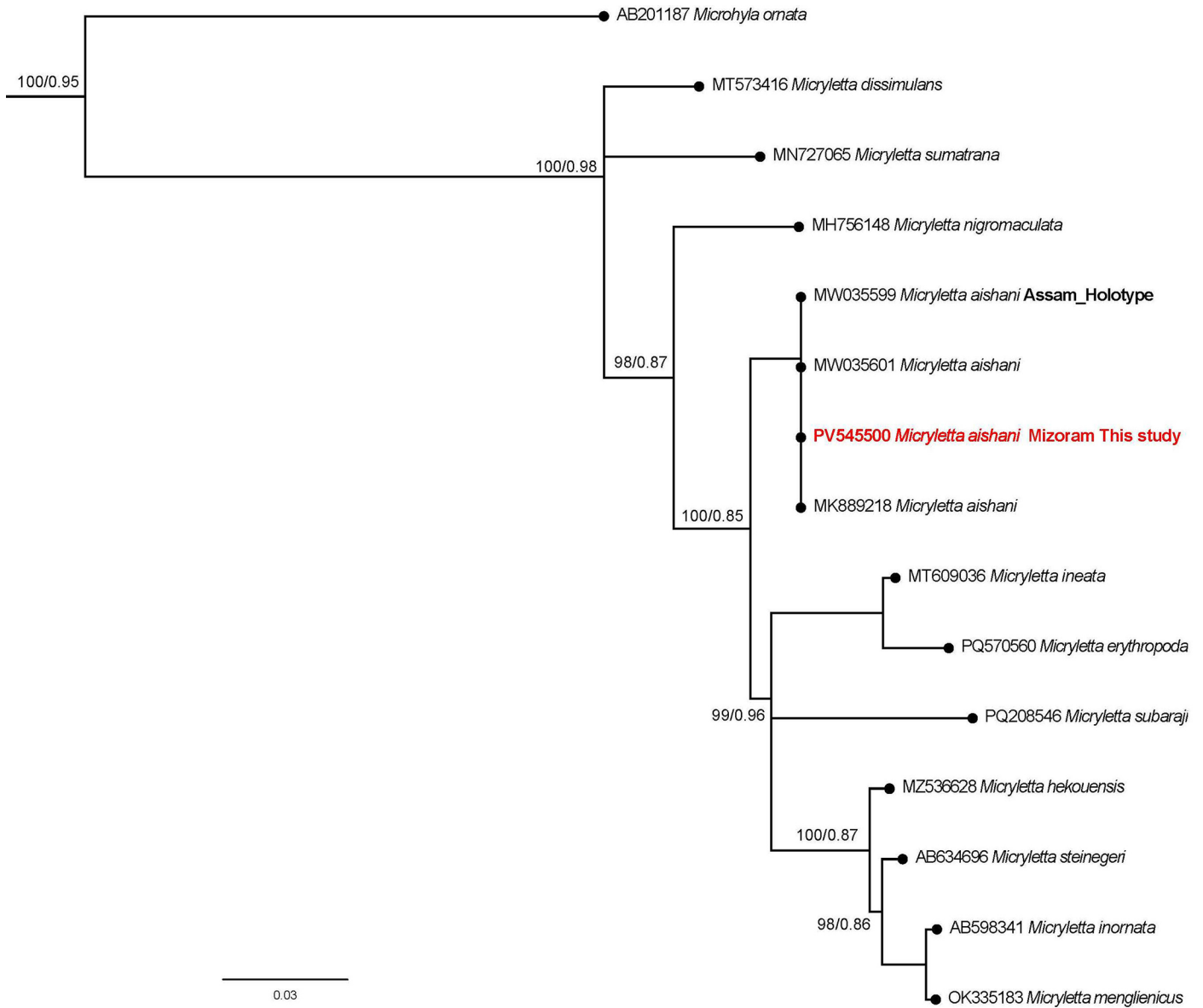


Figure 1. Phylogenetic Bayesian Inference Tree of *Micryletta* spp. based on the 16S rRNA partial gene fragment sequence. Numbers at divergences in tree represent bootstraps/Bayesian posterior probabilities, respectively; values above 90 are considered well-supported and those below 90 considered moderately-supported. GenBank accession numbers are shown at the tips.



Figure 2. An adult male Northeast Indian Paddy Frog (*Micryletta aishani*) (left); microhabitat where the frog was encountered in the Dampa Tiger Reserve in Mamit District, Mizoram, India (center); and gut contents of undigested exoskeletons of ants (right). Photographs by Malsawmdawngliana and Ht. Decemson (left), Kros Muansanga, Wildlife Guard, Teirei Forest Beat (center), and Ht. Decemson, Malsawmdawngliana, and Angshuman Das Tariang (right).

aligning with those reported by Das et al. (2019), Hakim et al. (2020), and Zug (2022). As no information addressed the diet of the species, we examined gut contents, recovering undigested exoskeletons of small hymenopterans (ants) (Fig. 2), which we preserved in 70% ethanol.

Discussion

Our new locality record, 147 km south of the type locality at Subhong, Cachar District, Assam, and 23 km southwest of the previously recorded site at Sihhmui, Aizawl District, Mizoram, is the southernmost point of the species' distribution (Fig. 3). The microhabitat reported here also aligned with those

reported by Das et al. (2019), Hakim et al. (2020) and Zug (2022). This work presents the first documentation on the diet of *M. aishani* as well as molecular confirmation on its occurrence in the state of Mizoram. Further surveys in adjoining Indo-Burma biodiversity hotspot regions, especially the southern parts of Bangladesh and Myanmar, will be necessary to understand the distribution limits of this species.

Acknowledgements

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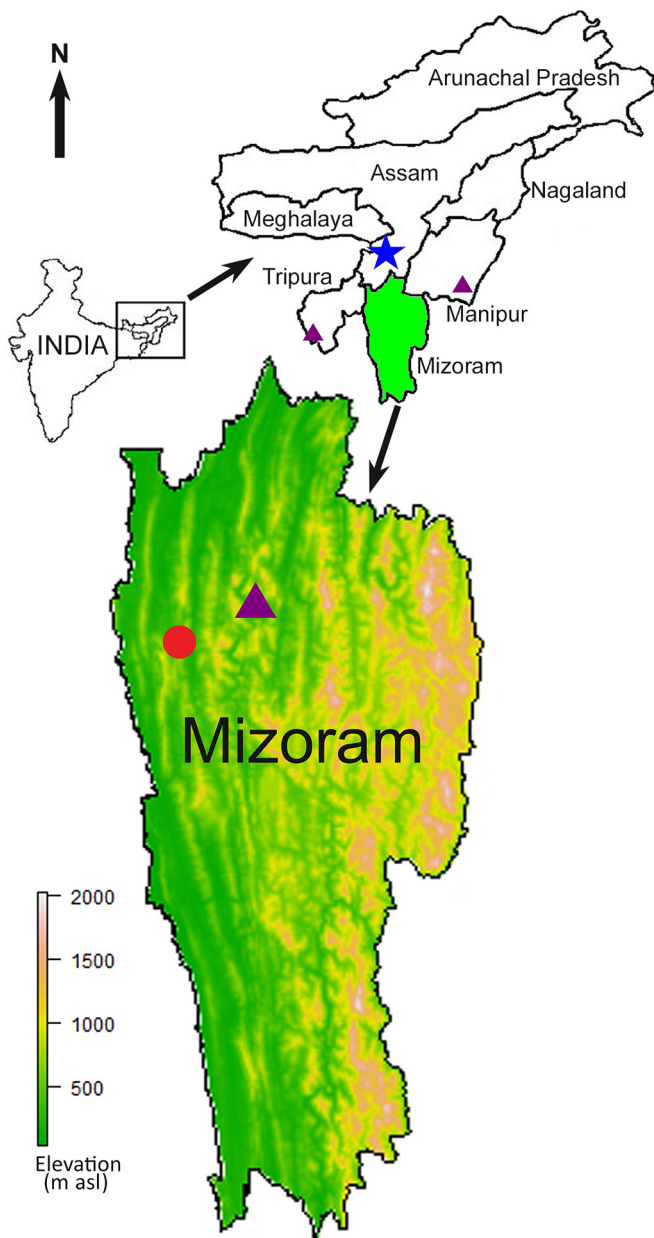


Figure 3. Map showing the known locality records of the Northeast Indian Paddy Frog (*Micryletta aishani*) in Northeast India. The holotype is marked with the blue star, previous records with purple triangles, and the new record from the Dampa Tiger Reserve in Mamit District by the red dot.

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