REPTILES & AMPHIBIANS

A New Record of the Bangladeshi Cricket Frog, *Minervarya asmati* (Howlader 2011) from Manipur, India, with Comments on the Occurrence of the Paddy Frog, *Fejervarya multistriata* (Hallowell 1861) (Anura: Dicroglossidae) in Mizoram, India

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The Bangladeshi Cricket Frog (*Minervarya asmati*) was originally described from Hathazari, Chittagong District, Bangladesh, and later recorded from Dhaka District, Bangladesh (Sarker et al. 2012), and Nazipur, Rajshahi Division, Naogoan District, Bangladesh (Ahmad and Alam 2013). In India, the species has been recorded from Aizawl District (Lalronunga et al. 2017) and the Dampa Tiger Reserve, Mamit District (Decemson et al. 2021), in Mizoram. The conservation status of this species has not been assessed for the IUCN Red List of Threatened Species (Dinesh et al. 2020). Some of the diagnostic features of *M. asmati* include a line on both sides of the venter, absence of a rictal gland at the mouth commissure, smooth skin with minute warts or folds, lips more or less barred, fingers free of webbing, toes not fully webbed, male snout-vent length (SVL) 29.1–33.4 mm, and butterfly-shaped vocal marking present in males (Howlader 2011a). Herein, we provide a new record of *M. asmati* from Manipur in northeastern India.

At about 2000 h on 28 January 2021, HD collected a single male *M. asmati* (Fig. 1) from a thigh-deep littoral bank of relatively turbid marshy water along the Chakpi River, Manipur, India (24.192380°N, 93.591316°E; elev. 881 m asl; Fig. 1). The specimen was initially placed in 70% ethanol and liver tissue was extracted and stored in 95% ethanol for DNA isolation. The specimen was preserved in 4% formalin in the Departmental Museum of Zoology, Mizoram University, Aizawl–796004, Mizoram, India (MZMU 2239).

We extracted genomic DNA from the liver tissue sample using QIAamp DNA Mini Kit (Cat. No. ID: 51306) following the manufacturer's protocol, amplified the gene fragments of mitochondrial 16S rRNA using the primers forward (L02510-CGC CTG TTT ATC AAA AAC AT) (Palumbi 1996) and reverse (H03063-CTC CGG TTT GAA CTC AGA TC) (Rassmann 1997), and sequenced the sample using the Sanger dideoxy method, carrying out the reactions in both directions on a sequencer (Barcode Bioscience, Bangalore, India). The generated partial 16S rRNA sequences were deposited in GenBank (GenBank MW687119). We compared our sample to 15 previously published sequences of 16S rRNA (Kuramoto et al. 2007; Kotaki et al. 2008, 2010; Dinesh et al. 2015; Howlader et al. 2016; Suwannapoom et al. 2017) retrieved from the NCBI database, using one

Fig. 1. A male Bangladeshi Cricket Frog (*Minervarya asmati*) (MZMU 2239) collected from along the Chakpi River, Manipur, India. Photographs by Hmar Tlawmte Lalremsanga (frog) and Ht. Decemson (habitat).

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sequence of the Mountain Horned Frog (*Megophrys monticola*) (KY679895) as an out-group. Other sequences used in the analysis included those from specimens sampled from four districts in Mizoram: Ralie, Tokalo Wildlife Sanctuary, Siaha District (MZMU 1645; GenBank # MT790756); Thenzawl, Serchhip District (MZMU 1768; GenBank # MW165452); Pualreng Wildlife Sanctuary, Kolasib District (MZMU 1778; GenBank # MW165468); Palak National Wetland (MZMU 1825; GenBank # MW165473); and the Mizoram University (MZU) campus (MZMU 1711; GenBank # MT790764) from Aizawl District. We also included our generated sequences of the Paddy Frog (*Fejervarya multistriata*) from the Chakpi River, Manipur (MZMU 2234; GenBank # MW687118) and from the MZU campus, Mizoram (MZMU 1360; GenBank # MT799715).

All sequences were aligned using the MUSCLE algorithm in MEGA X (Kumar et al. 2018); and Kimura 2-parameter (K2P) genetic distances (Kimura 1980) were calculated using MEGA X (Kumar et al. 2018). The Bayesian Inference (BI) phylogenetic tree was constructed in MrBayes 3.2.5 using the GTR+I+G model (Huelsenbeck and Ronquist 2001). We ran the MCMC (one cold and three hot chains) for 20,000,000 generations by sampling every 1,000 generations and setting the burn-in to 25%. The analysis was terminated when the standard deviation of split frequencies was less than 0.01. The percentage of trees in which the associated taxa clustered is shown next to the branches (Huelsenbeck and Ronquist 2001). The generated phylogenetic tree was further illustrated using Interactive Tree Of Life (iTOL) version 5 software (Letunic and Bork 2021).

The male *M. asmati* from the Chakpi River (SVL 32.44 mm) matched the original descriptions by Howlader (2011) and the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) showed a genetic sequence similarity. In the aligned dataset of 16S rRNA gene fragments (549 bp), 345 sites were conserved and 120 sites were parsimony-informative. From the estimated K2P genetic distances (Table 1), we determined the overall mean for intraspecies genetic divergences within *M. asmati* and *F. multistriata* to be 0.2% and 9.6%, respectively. The male *M. asmati* from the Chakpi River (MZMU 2239) (GenBank # MW687119) showed 0.0% genetic divergence from specimens from Assam, India (GenBank # AB488900); Mizoram, India (Murlen NP, GenBank # MT632250; MZU campus, GenBank # MT790764); and Dhaka (Bangladesh) (GenBank # KP849815). Genetic divergence was 0.2% from the other Mizoram specimens sampled from Tokalo WLS (GenBank # MT790756), Thenzawl (GenBank # MW165452), and Palak NWL (GenBank #: MW165473); and 0.4% from the specimen from Pualreng WLS (GenBank # MW165468). Our sequence of *F. multistriata* from Manipur, India (GenBank # MW687118) diverged 0.0% from the sequences from Husa, China (GenBank # AB488884) and Mizoram, India (GenBank # MT102377), and 0.4% with our generated sequence of the frog from the MZU campus, Mizoram, India (GenBank # MT799715). Interspecific genetic distances of 13.6–14.2% existed between *F. multistriata* sampled from Manipur (India) and the sequences of *M. asmati*. In our 16S rRNA marker-gene-based Bayesian inference (BA) phylogeny, both *M. asmati* and *F. multistriata* formed well-supported distinct clades. Strongly supported in the BA topology, our generated sequence of *M. asmati* from Manipur (India) is clearly nested among conspecific sequences from Mizoram (India) and Dhaka (Bangladesh) (Fig. 2). Likewise, our samples of *F. multistriata* from Manipur and Mizoram (India) also can be grouped with specimens sampled from Mizoram (India) and Husa (China) by significant Bayesian posterior probabilities.

Our 16S rRNA-based phylogenetic reconstruction and the estimated genetic divergences of *M. asmati* from related species reveal its occurrence in Manipur, India, and extend the distribution by ca. 145 km aerial distance northeastward from the closest recorded location in Aizawl, Mizoram (Fig. 3). In addition, we noted that *M. asmati* is widely distributed in sympatry with *F. multistriata* in Mizoram, and both of the species were encountered throughout the year. Furthermore, the much needed verification of the earlier record of *F. multistriata* (MZMU 1046; SVL 48.10 mm; GenBank # MT102377) from Mizoram, India, by Lalbiakzuala and Lalremsanga (2019) is confirmed (See Frost 2021). However, the sampling site at Chakpi River is surrounded by intact habitat suitable for anurans, and encountering other amphibians is virtually guaranteed. Extensive explorations in the region are recommended to better understand the herpetofaunal diversity of Manipur, northeast India.

Table 1. Estimated evolutionary divergence based on the uncorrected K2P distance using 16S rRNA partial genes of *Minervarya asmati* and *Fejervarya multistriata*. Sequences generated in this study are indicated with an asterisk.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	*M. asmati* MW687119 Chandel, Manipur, India*																						
2	*M. asmati* AB488900 Assam, India	0.000																					
3	*M. asmati* MT632250 Murlen NP, Mizoram, India	0.000	0.000																				
4	*M. asmati* KP849815 Dhaka, Bangladesh	0.000	0.000	0.000																			
5	*M. asmati* MT790764 MZU Campus, Mizoram, India*	0.000	0.000	0.000	0.000																		
6	*M. asmati* MT790756 Tokalo WLS, Mizoram, India*	0.002	0.002	0.002	0.002	0.002																	
7	*M. asmati* MW165452 Thenzawl. Mizoram, India*	0.002	0.002	0.002	0.002	0.002	0.004																
8	*M. asmati* MW165473 Palak NWL, Mizoram, India*	0.002	0.002	0.002	0.002	0.002	0.004	0.004															
9	*M. asmati* MW165468 Pualreng WLS, Mizoram, India*	0.004	0.004	0.004	0.004	0.004	0.006	0.006	0.006														
10	*M. kudremukhensis* AB355841	0.073	0.073	0.073	0.073	0.073	0.076	0.075	0.075	0.078													
11	*M. gomantaki* KR781087	0.075	0.075	0.075	0.075	0.075	0.075	0.077	0.077	0.079	0.099												
12	*M. syhadrensis* AB488893	0.078	0.078	0.078	0.078	0.079	0.078	0.080	0.080	0.083	0.086	0.040											
13	*M. muangkanensis* MF166918	0.098	0.098	0.098	0.098	0.098	0.102	0.100	0.100	0.102	0.104	0.119	0.109										
14	*M. mudduraja* AB488896	0.108	0.108	0.108	0.108	0.109	0.108	0.110	0.110	0.113	0.122	0.128	0.119	0.133									
15	*M. rufescens* MF176666	0.106	0.106	0.106	0.106	0.106	0.110	0.108	0.108	0.110	0.113	0.122	0.108	0.101	0.129								
16	*M. keralensis* GQ478322	0.119	0.119	0.119	0.119	0.119	0.116	0.121	0.121	0.123	0.128	0.133	0.128	0.136	0.063	0.134							
17	*F. multistriata* MW687118 Chandel, Manipur, India*	0.138	0.138	0.138	0.138	0.136	0.142	0.140	0.140	0.142	0.148	0.154	0.133	0.143	0.154	0.150	0.147						
18	*F. multistriata* MT799715 MZU Campus, Mizoram*	0.138	0.138	0.138	0.138	0.136	0.142	0.140	0.140	0.142	0.153	0.159	0.138	0.148	0.159	0.150	0.152	0.004					
19	*F. multistriata* AB488884 Husa, China	0.138	0.138	0.138	0.138	0.136	0.142	0.140	0.140	0.142	0.148	0.154	0.133	0.143	0.154	0.150	0.147	0.000	0.004				
20	*F. multistriata* MT102377 Tamdil NWL, Mizoram, India	0.162	0.162	0.162	0.162	0.162	0.168	0.166	0.166	0.169	0.174	0.192	0.163	0.170	0.178	0.175	0.172	0.000	0.000	0.000			
21	*F. iskandari* AB277303	0.159	0.159	0.159	0.159	0.157	0.159	0.157	0.162	0.164	0.173	0.178	0.169	0.152	0.144	0.168	0.142	0.115	0.119	0.115	0.147		
22	*F. orissaensis* AY882958	0.168	0.168	0.168	0.168	0.166	0.166	0.166	0.171	0.173	0.187	0.180	0.168	0.176	0.166	0.183	0.172	0.130	0.135	0.130	0.159	0.062	
23	*Megophrys monticola* KY679895	0.258	0.258	0.258	0.258	0.256	0.264	0.261	0.255	0.264	0.260	0.285	0.278	0.282	0.287	0.291	0.288	0.267	0.267	0.267	0.280	0.302	0.315

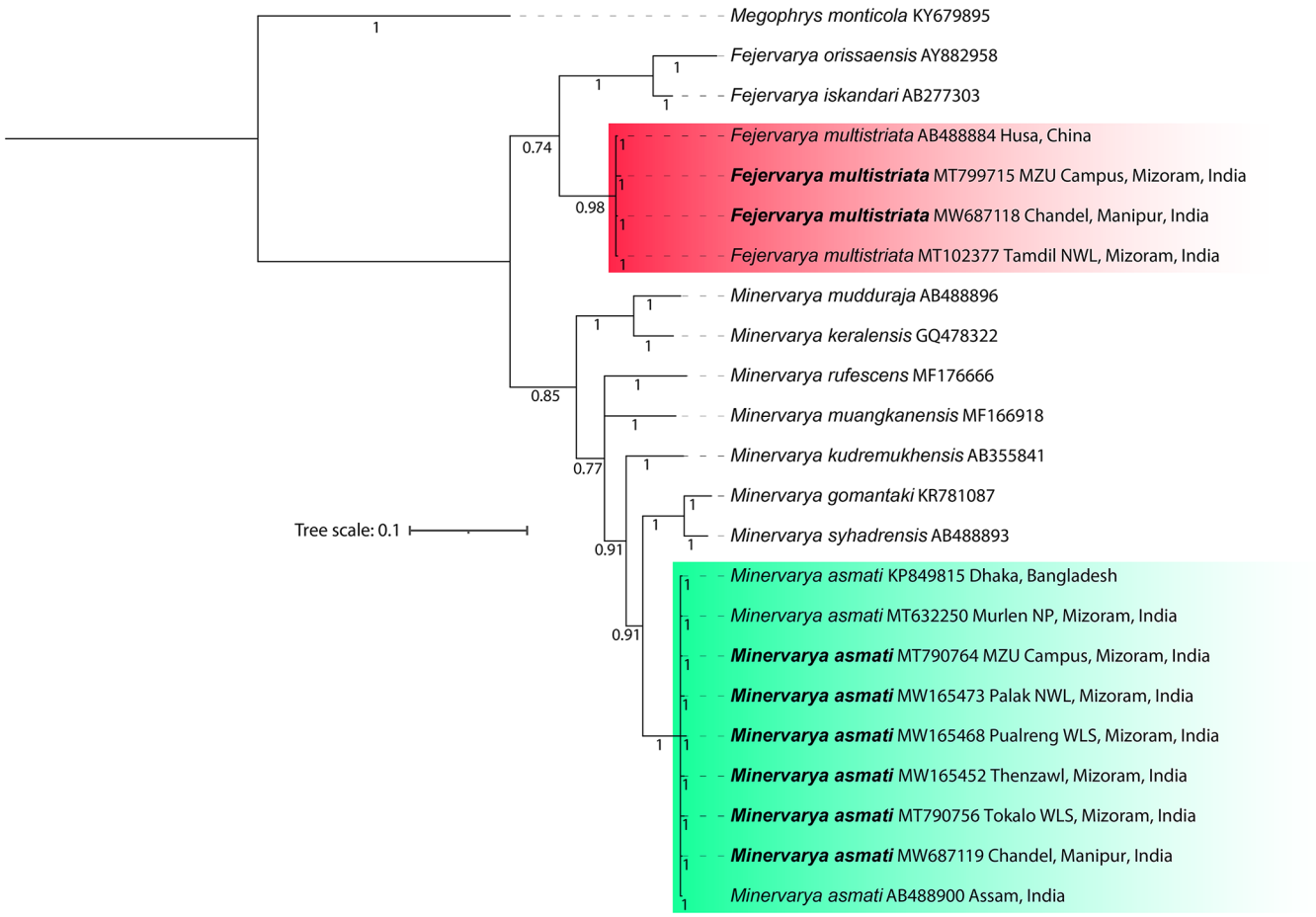

Fig. 2. Bayesian inference phylogenetic tree derived from the partial DNA sequence of 16S rRNA partial genes. Numbers at the nodes represent Bayesian posterior probabilities. Sequences generated in this study are shown in bold.

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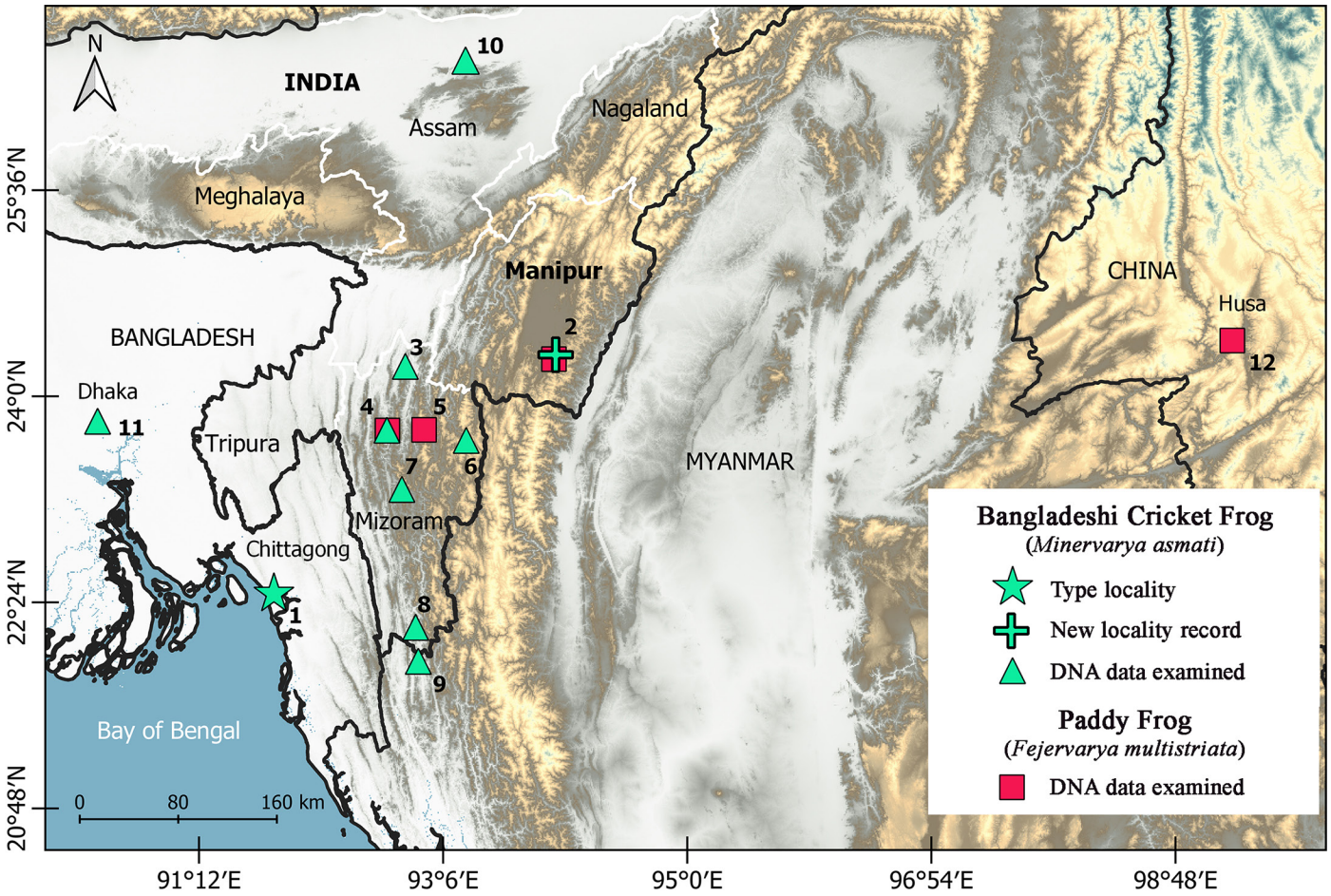

Fig. 3. Map showing collection sites of Bangladeshi Cricket Frogs (*Minervarya asmati*): (1) Type locality at Chittagong University Campus, Chittagong (Bangladesh) (green star); (2) a new record from Chandel, Manipur (India) (green cross); and additional sites from which DNA data were examined (green triangles): (3) Pualreng WLS, Mizoram; (4) MZU Campus, Mizoram;(6) Murlen NP, Mizoram; (7) Thenzawl, Mizoram; (8) Palak NWL, Mizoram; (9) Tokalo WLS, Mizoram; (10) Assam (India); (11) Dhaka (Bangladesh). Locations for Paddy Frogs (*Fejervarya multistriata*) from which DNA data were examined (red squares): (2) Chandel, Manipur (India); (4) MZU Campus, Mizoram (India); (5) Tamdil NWL, Mizoram (India); (12) Husa (China).

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