



## Color Polymorphism in Jerdon's Treefrog, Nasutixalus jerdonii (Günther 1876), from Northeast India with Notes on Diet and Distribution in Mizoram, India

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olymorphism is defined as the simultaneous occurrence of two or more discrete, genetically-based phenotypes in a population, in which the frequency of the rarest type is higher than can be maintained by recurrent mutation (McKinnon and Pierotti 2010). Species that exhibit visible polymorphisms are ideal for examining the microevolutionary forces that maintain genetic variation in nature, because phenotypes are easily scored and because color or pattern polymorphisms often exhibit simple Mendelian inheritance (Hoffman and Blouin 2000). In anurans, polymorphism for red, green, or brown/gray dorsal color is a common motif, with some species exhibiting a combination of the above; for example, variation in the Pacific Treefrog (Hyla regilla) includes the presence/absence of dorsal stripes as well as an array of ground colors (Resnick and Jameson 1963). The anuran family Rhacophoridae comprises 440 currently recognized species and the genus Nasutixalus contains three species distributed in India (West Bengal and Arunachal Pradesh) and China (southeastern Xizang) (Frost 2021).

Jerdon's Treefrog (Nasutixalus jerdonii) was first described by Günther (1876) from Darjeeling, West Bengal, and is known to occur in India (West Bengal, Nagaland, Manipur, and Meghalaya), Bhutan, and Myanmar (Putao District, Kachin State) (Frost 2021) (Fig. 1). Sankar et al. (1992) and Sankar and Ray (2006) provided brief accounts for West Bengal and Arunachal Pradesh. Biju et al. (2016) rediscovered the species and studied its biology from Mawphlang Secret Grove in Meghalaya and gave a detailed distribution of the species from India (Nagaland, Manipur, and Meghalaya).

At 2116 h on 10 May 2021, employing the Acoustic Encounter Survey (AES) system, we collected a single male Jerdon's Treefrog (MZMU 2400) during a herpetofaunal survey at Hmuifang Community Reserve Forest, and another male (MZMU 2533) at 2010 h on 12 September 2021 from the same locality (23°27'17.14"N, 92°45'10.76"E; elev. 1,471 m asl). Both were calling from a tree-hole 7 m above the ground in secondary forest in a park constructed by the Department of Local Administration. The tree-hole was 47 mm in diameter, 110 mm in depth, and one-third filled with water. On 13 May 2021, a forest patrol encountered another frog in Murlen National Park, Champhai District, Mizoram (23°39'43.06"N, 93°17'25.47"E; elev. 1,521 m asl). All were preserved and deposited in the Departmental Museum of Zoology, Mizoram University (MZMU). Photographic vouchers were submitted to the Natural History Museum of Mizoram, Mizoram University, Aizawl, Mizoram, India (NHMM/G/9-4). We amplified the partial 16s rRNA gene using primers L02510 (Palumbi 1996) and H03063 (Rassmann 1997) and compared it to 21 congeneric sequences retrieved from the NCBI database. We aligned the

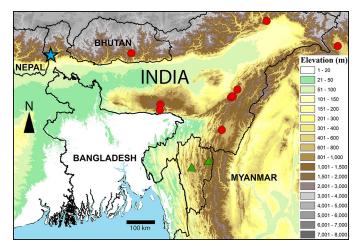


Fig. 1. Map showing the distribution of Jerdon's Treefrog (Nasutixalus jerdonii). The blue star marks the type locality, red dots indicate previously published localities, and green triangles designate records in Mizoram, India.



Fig. 2. Jerdon's Treefrogs (*Nasutixalus jerdonii*) collected in Hmuifang Community Reserve Forest, Mizoram, India (A–C), from Mawphlang, Meghalaya, India (D), and from Myanmar (E). Photographs by Lal Muansanga.

sequences using the MUSCLE algorithm in MEGA 7 (Kumar et al. 2016) and constructed a phylogenetic tree by MrBayes (v. 2.7) using the GTR+G model (Huelsenbeck and Ronquist 2001) using the Mindanao Fanged Frog (*Limnonectes magnus*) (MN759249) as the out-group.

Jerdon's Treefrog has a grayish to black or reddish-olive dorsum with indistinct regular markings, a white venter, throat spotted with brown, and limbs indistinctly barred with black (Günther 1876; Boulenger 1882; Chanda 2002). However, frogs collected in Northeast India (Mizoram and Meghalaya) and Myanmar show variation in color and pattern. MZMU 2400 (Fig. 2C) collected from Hmuifang Community Reserve Forest in Mizoram matches the general descriptions cited above, whereas MZMU 2533 (Fig. 2A), collected on a moss-covered rock beside a stream at the same locality, has light cream to off-white sides, indistinct dark greenish-brown markings on the dorsum, and a stripe below the eyes. A frog from Myanmar (YPX44218; Rahman et al. 2020) (Fig. 2E) has an olive coloration with irregular greenish spots on the back and sides and no stripes on the femur; and a frog from Meghalaya (Fig. 2D) (Biju et al. 2016) is similar to the specimen from Myanmar but has a more tannish-brown

Table 1. The uncorrected K2p distances of Jerdon's Treefrog (*Nasutixalus jerdoni*) and related species based on partial 16S rRNA gene sequences.

Nasutixalus jerdonii MZMU2533																						
Nasutixalus jerdonij MZ413639	0.000																					
Nasutixalus jerdonij K170000	0.000	0.000																				
Nasutixalus jerdonij KU170002	0.000	0.000	0.000																			
Nasutixalus ierdonii KU170003	0.000	0.000	0.000	0.000																		
Nasutixalus ierdonii KU169996	0.003	0.003	0.003	0.003	0.003																	
Nasutixalus ierdonii KU169998	0.010	0.010	0.010	0.010	0.010	0.013																
Nasutixalus jerdonii KU169997	0.010	0.010	0.010	0.010	0.010	0.013	0.000															
Nasutixalus jerdonij MN511838	0.025	0.025	0.025	0.025	0.025	0.023	0.020	0.020														
Nasutixalus vingijangensis MG603590	0.070	0.070	0.070		0.070	0.073	0.070		0.083													
Nasutixalus vingijangensis MG603589	0.070	0.070	0.070		0.070	0.073	0.070	0.070	0.083	0.000												
Nasutixalus medogensis KU170004	0.093	0.093	0.093	0.010	0.093	0.095	0.093	0.093	0.098	0.098	0 098											
Kurixalus berylliniris DQ468669	0.135	0.000	0.000				0.000					0 140										
Kurixalus idiootocus GQ204686	0.140				0.100		0.145	0.145			0.100	0.140	0.065									
Kurixalus wangi DQ468671	0.140	0.110	0.1.10	0.1.10	0.110	0.110	0.143	0.1.10	0.155	0.110	0.140	0.133		0.065								
											0.120	0.1.10			0 000							
Kurixalus eiffingeri AY880492	0.145	0.140	0.140	0.140	0.140	0.148	0.145	0.145	0.158	0.100	0.133	0.148	0.033	0.060	0.038	0.070						
Kurixalus lenguanensis KY768940	0.145	0.145		0	0.145	0	000	000	000	0.153	01100	0.140	0.000	0.038		0.070						
Kurixalus verrucosus KX554485	0.150	0.100	0.150		0.150	0.153	0.155	0.155			0.145	0.143	0.105	0.090	0.098	0.095						
Kurixalus bisacculus KX554535	0.160		0.160		0.160	0.163	0.160	0.160	0.110	0.110	0.143	0.148	0.128	0.115	0.115	0.113	0.118	0.073				
Kurixalus verrucosus MT808303	0.168	0.168	0.168	0.168	0.168	0.170	0.168	0.168	0.183	0.148	0.148	0.153	0.118	0.093	0.100	0.103	0.100	0.048	0.063			
Kurixalus baliogaster KX554537	0.168	0.168	0.168	0.168	0.168	0.170	0.168	0.168	0.178	0.150	0.150	0.153	0.128	0.113	0.113	0.115	0.115	0.073	0.028	0.070		
Kurixalus verrucosus KU589225	0.190	0.190	0.190	0.190	0.190	0.193	0.190	0.190	0.206	0.178	0.178	0.180	0.140	0.115	0.123	0.125	0.123	0.075	0.095	0.033	0.098	
Limnonectes magnus MN759249	0.208	0.208	0.208	0.208	0.208	0.206	0.211	0.211	0.213	0.198	0.198	0.206	0.213	0.231	0.221	0.218	0.233	0.201	0.213	0.201	0.213	0.223

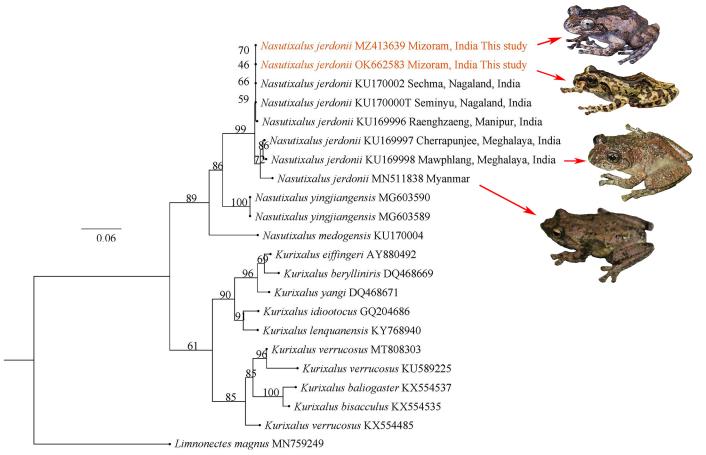


Fig. 3. Bayesian inference (BI) tree derived from the mitochondrial 16S rRNA gene. Numbers above branches are Bayesian posterior probabilities and numbers below branches indicate bootstrap support values for maximum likelihood analyses. GenBank accession numbers and distribution are provided for Jerdon's Treefrogs (*Nasutixalus jerdonii*).

dorsum and faint femoral bands. Moreover, MZMU 2533 (Fig. 2B) has a distinctly colored venter with indistinct dark greenish patches posteriorly, whereas all other individuals have a white venter with spots limited to the throat.

We compared 16S rRNA gene fragments of Nasutixalus jerdonii collected in Mizoram (MZMU 2400 and MZMU 2533; GenBank Accession nos. MZ413639 and OK662583, respectively) with congeners obtained from the NCBI database (Table 1). Interspecific genetic distances within the genus Nasutixalus ranged from 0.00% (Nasutixalus jerdonii) to 9.3% (Nasutixalus medogensis). The intraspecific genetic distance within Nasutixalus jerdonii ranged from 0.00 to 2.5%. The phylogenetic analysis revealed that the genus Nasutixalus formed a distinct monophyletic clade with well supported bootstrap values (Fig. 3). Nasutixalus jerdonii (OK662583) showed no genetic variation from samples from Nagaland, India (KU170000, KU170002, and KU170003) and only a 0.3% difference from N. jerdonii at the type locality in Manipur (KU169996). Therefore, based on the 16S rRNA gene sequence, the presence of N. jerdonii in Mizoram is confirmed despite considerable polymorphism in color and pattern.

Analysis of the gut contents from the frogs from Mizoram revealed that the diet comprises mainly Coleoptera (Fig. 4 A–D) and Hemiptera (Fig. 4 E–F).

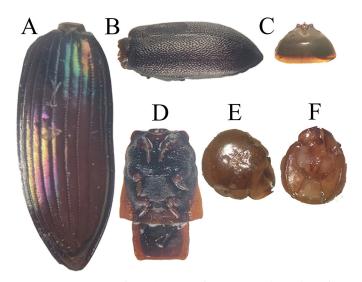


Fig. 4. Gut contents of a Jerdon's Treefrog (*Nasutixalus jerdonii*) from Hmuifang Community Reserve Forest, Mizoram, India (MZMU 2400), comprised coleopterans (A–D) and hemipterans (E–F). Photographs by Lal Muansanga.

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