

Rediscovery of Doria's Foam-nesting Treefrog, Chirixalus doriae Boulenger 1893 (Anura: Rhacophoridae), from India

Lal Muansanga, Lal Duhzuali, Lal Biakzuala, Vabeiryureilai Mathipi, Saisangpuia Sailo, and H.T. Lalremsanga

Developmental Biology and Herpetology Laboratory, Department of Zoology, Mizoram University, Aizawl, 796004, Mizoram India (muanapunte16@gmail.com)

The 14 currently recognized species in the genus *Chirixalus*, which are distinguished from other Asian rhacophorids by the presence of opposable fingers (Wilkinson et al. 2003), range collectively from southeastern Asia through Malaysia to Borneo (Frost 2020). Doria's Foam-nesting Treefrog (*Chirixalus doriae*), first described by Boulenger (1893) from Karin Bia-po, Myanmar, has a known range that includes northeastern India (Arunachal Pradesh), Bangladesh, Myanmar, Thailand, Laos, Cambodia, and Vietnam (Frost 2020).

Herein, we report the first record of Doria's Foamnesting Treefrog from the Pualreng Wildlife Sanctuary, Mizoram, India (24.2183°N, 92.8025°E; WSG 84; elev. 662 m asl; Fig. 1), which is ca. 104 km east of the nearest locality in Lawachara National Park in Bangladesh (24.3438°N, 91.8291°E; WSG 84) (Hasan et al. 2010; Hakim et al. 2020), and ca. 483 km aerial distance south of the Tenga Valley, Arunachal Pradesh, the only previous record in India

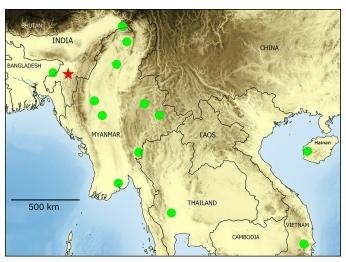


Fig. 1. Map showing documented records of Doria's Foam-nesting Treefrog (*Chirixalus doriae*) in southern and southeastern Asia. Published records are indicated by green dots and the new record in Mizoram, India, is marked by the red star.



Fig. 2. A gravid Doria's Foam-nesting Treefrog (*Chirixalus doriae*) from the Pualreng Wildlife Sanctuary, Mizoram, India. Photograph by H.T. Lalremsanga.

(Annandale 1912). At 2050 h on 20 June 2020, during a nocturnal survey in the buffer area of the Pualreng Wildlife Sanctuary, we collected a gravid female *Chirixalus doriae* (Fig. 2) from a twig ca. 1.5 m above the ground along the edge of a fish pond surrounded by Flowering Banana Trees (*Musa ornata*). We preserved the specimen in 70% ethanol, extracted liver tissue for genetic analysis, and deposited it in the Departmental Museum of Zoology, Mizoram University (MZMU 1781).

The snout-vent length measured 37.71 mm with head longer than broad; snout pointed; canthus rostralis obtuse, loreal region slightly concave; eye large, protruding, pupil horizontal; internarial distance equal to interorbital width; tympanum distinct and round, slightly depressed, half the diameter of the eyes; dorsum smooth and brown with ven-

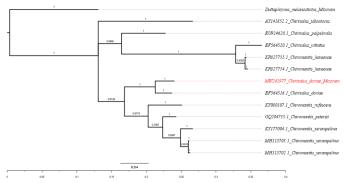


Fig. 3. Bayesian phylogenetic tree showing the relationships of Doria's Foam-nesting Treefrog (*Chirixalus doriae*) with sister species using the 16srRNA gene.

tral surface glandular and slightly granulated; supratympanic fold present; limbs dark brown with indistinct crossbars, fingers 1 and 2 oppose fingers 3 and 4; tips of fingers and toes dilated into prominent discs; fingers free, toes two-thirds webbed, relative finger and toe lengths are III>IV>II>I and IV>V>III>II, respectively; inner and outer metatarsal tubercles present; vomerine teeth absent; heels overlapping when hindlimbs folded at right angles to body; tibiotarsal articulation reaches the middle of eye. Chanda (2002) reported the tibiotarsal articulation reaching the tip of the snout but that of this specimen reached only the middle of the eye.

We amplified the partial 16S rRNA marker gene using the primers L02510 (Palumbi 1996) and H03063 (Rassmann 1997) and compared it to congeneric sequences obtained from the NCBI Database (National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, Maryland, USA). We aligned sequences using the Muscle algorithm in MEGA 7 (Kumar et al. 2016) and generated a phylogenetic tree using MRBAYES (Huelsenbeck and Ronquist 2001) (Fig. 3). From the estimated K2P genetic distances (Table 1), we diagnosed a 5.7% genetic distance between this *C. doriae* (MW242977) and a conspecific sequence (EF564516) from Simao, Yunnan, China. Considering the intraspecies genetic divergence, we presume that the specimen from northeastern India likely represents a distinct population of the taxon. However, we suggest that further sampling for more specimens and genetic data are necessary to illuminate the population status of the species in the region.

Very little is known about the natural history and the distribution of Doria's Foam-nesting Treefrog. The species apparently is rare in India and no specimens have been collected since Annandale's 1912 report (Chanda 2002). Sankar and Ray (2006) also reported this species based on the collection by Annandale in the Fauna of Arunachal Pradesh. This record represents the first specimen found in India after 108 years.

Acknowledgements

We thank the Principal Chief Conservator of Forests (PCCF), Aizawl, Government of Mizoram, for the permit (No. A.33011/2/99-CWLW/225) for herpetofaunal collection in Mizoram; the Defence Research Development Organisation (DRDO), Ministry of Defence, Government of India (No. DFTM/07/3606/NESTC/ABR/M/P-01), and the Department of Biotechnology, New Delhi (No. DBT-NER/AAB/64/2017), for financial support; and Madinga and Dawnga for assistance in the field.

Table 1. Kimura-2 parameter genetic distances of 16S rRNA gene among Chirixalus sp.

Species

EF564516.1 <i>C. doriae</i>													
MW242977 C. doriae, Mizoram	0.057												
AY141852.2 C. idiootocus	0.170	0.155											
EF564520.1 C. vittatus	0.218	0.203	0.224	0.239									
EU924620.1 C. palpebralis	0.176	0.161	0.167	0.173	0.191								
GQ204733.1 C. petersii	0.128	0.116	0.185	0.188	0.200	0.146							
KR827722.1 C. nongkhorensis	0.072	0.054	0.146	0.158	0.200	0.164	0.113						
KR827734.1 C. hansenae	0.212	0.197	0.212	0.227	0.048	0.179	0.185	0.185					
KR827735.1 C. hansenae	0.209	0.194	0.209	0.224	0.045	0.176	0.182	0.182	0.003				
KY080106.1 C. rufescens	0.122	0.131	0.194	0.197	0.215	0.173	0.084	0.134	0.209	0.206			
KY080107.1 C. rufescens	0.122	0.131	0.194	0.197	0.215	0.173	0.084	0.134	0.209	0.206	0.000		
KY177004.1 C. xerampelina	0.140	0.140	0.200	0.206	0.212	0.146	0.057	0.146	0.215	0.212	0.101	0.101	
MH115701.1 C. xerampelina	0.119	0.125	0.188	0.191	0.215	0.134	0.063	0.131	0.206	0.203	0.107	0.107	0.036

Literature Cited

- Annandale, N. 1912. Notes on fauna of Pareshnath Hill, West Bengal. (Reptiles and Batrachia). Records of the Indian Museum 7: 45–48.
- Boulenger, G.A. 1893. Concluding report on the reptiles and batrachians obtained in Burma by Signor L. Fea dealing with the collection made in Pegu and the Karin Hills in 1887–88. *Annali del Museo Civico di Storia Naturale di Genova* 13: 304–347.
- Chanda, S.K. 2002. *Hand Book. Indian Amphibians*. Zoological Survey of India, Kolkata, India.
- Frost, D.R. 2020. Amphibian Species of the World: An Online Reference. Version 6.1 https://amphibiansoftheworld.amnh.org.
- Hakim, J., S.J. Trageser, A. Ghose, S.M.A. Rashid, and S.C. Rahman. 2020. Amphibians and reptiles from Lawachara National Park in Bangladesh. *Check List* 16: 1239–1268. https://doi.org/10.15560/16.5.1239.
- Hasan, M.K., S. Mahony, and M.M. Kabir. 2010. Geographic distribution. *Chiromantis doriae* (Doria's Bush Frog). *Herpetological Review* 41: 103.
- Huelsenbeck, J.P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phy-

- logenetic trees. *Bioinformatics* 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754.
- Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549. https://doi.org/10.1093/molbev/msy096.
- Palumbi, S.R. 1996. Nucleic acids II: the polymerase chain reaction, pp. 205–247.
 In: D.M. Hillis, C. Moritz, and B.K. Mable (eds.), *Molecular Systematics*.
 Second edition. Sinauer Associates Inc., Sunderland, Massachusetts, USA.
- Rassmann, K. 1997. Evolutionary age of the Galápagos iguanas predates the age of the present Galapagos Islands. *Molecular Phylogenetics and Evolution* 7: 158–172. https://doi.org/10.1006/mpev.1996.0386.
- Sarkar, A.K. and S. Ray. 2006. Amphibia, pp. 285–316. In: J.R.B. Alfred (ed.), Fauna of Arunachal Pradesh. Part 1. State Fauna Series, Zoological Survey of India, Kolkata, India.
- Wilkinson, J.A., H. Win, T. Thin, K.S. Lwin, A.K. Shein, and H. Tun. 2003. A new species of *Chirixalus* (Anura: Rhacophoridae) from western Myanmar (Burma). *Proceedings of the California Academy of Sciences* 54: 17–26.