REPTILES & AMPHIBIANS

A New Record of the Agasthyamalai Bushfrog (*Raorchestes agasthyaensis*) from Devikulam, Munnar, Kerala, India

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The Western Ghats of India, recognized as one of the world's eight "hottest hotspots" of biodiversity (Gunawardene et al. 2007), harbor an exceptional level of endemism, especially of amphibians. This is largely attributed to extensive elevational gradients that create distinct climatic zones and specific microhabitat conditions and biogeographical barriers, both of which play vital roles in speciation in the Western Ghats (Vijayakumar et al. 2016).

One such biogeographical barrier, the Shencottah Gap in the southern Western Ghats, is known for its role in the speciation of amphibians and other taxa (Bossuyt et al. 2004; Robin et al. 2015). Notably, this pattern is particularly evident in several genera of endemic frogs (Vijayakumar et al. 2014; Van Bocxlaer et al. 2012). However, exceptions to this biogeographical pattern of speciation have been reported, as in the case of Myristica Swamp Treefrog (*Mercurana myristicapalustris*) (Gopalan et al. 2020).

Bushfrogs in the genus *Raorchestes*, among the most diverse amphibian genera in the Western Ghats, exhibit direct development and a high degree of microhabitat specificity (Garg et al. 2021). The Agasthyamalai Bushfrog (*Raorchestes agasthyaensis*), described from the Agasthyamalai Hills near Bonacaud, Kerala, has been reported from only a few locations south of the Shencottah Gap: Kakachi and Kodayar in Tirunelveli District, Tamil Nadu; Athirimala and Pandipath in Thiruvananthapuram District; and Shendurney in Kollam District, Kerala, with no records beyond the Agasthyamalai landscape (Garg et al. 2021). We herein report a northward range extension of *R. agasthyaensis* to the high-elevation shola forests of Devikulam, Munnar in Idukki District, Kerala.

On 28 July 2019 in a patch of shola forest at Devikulam, Munnar, Kerala, India (1,569 m asl) (Fig. 1), Agasthyamalai Bushfrogs (*Raorchestes agasthyaensis*) were active from about 1830 h. We collected an actively calling male (Fig. 2A–B) perched on shrubs about 45 cm above the ground. A toe-clip was removed and preserved in 100% ethanol and stored at –20 °C. The specimen was vouchered in 70% ethanol and has been deposited at the Natural History Museum,

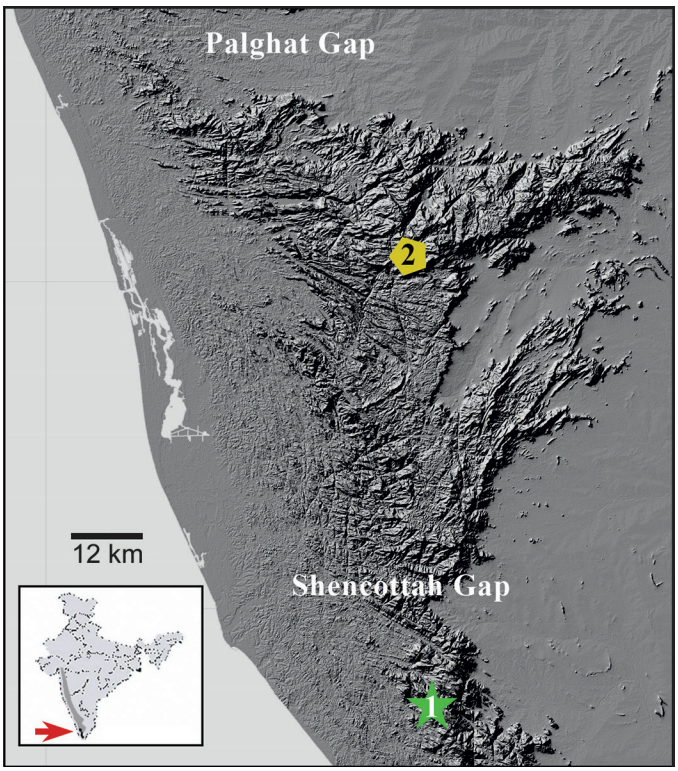

Figure 1. Map showing known localities for the Agasthyamalai Bushfrog (*Raorchestes agasthyaensis*) in the southern Western Ghats, Kerala, India. The type locality (Bonacaud Estate, Thiruvananthapuram) and nearby localities are indicated by the green star and the new locality in Devikulam, Munnar, is marked by the yellow pentagram. The approximate extent of the Western Ghats is shown in the inset map in gray and the smaller black area corresponds to the area shown in the larger map.

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Thiruvananthapuram, Kerala (TNHM.RA249.129). The following year, we observed and photographed additional individuals calling in the same area (Fig. 2C–D).

Molecular phylogenetics

Total genomic DNA was isolated from the toe-clip using DNeasy Blood and Tissue Kit (Qiagen). The amplification and sequencing of the 16S rRNA was performed following Gopalan et al. (2014). The new sequence is deposited in GenBank under accession number (MW817160).

We used 16S rRNA reference sequences of *R. agasthyaensis* (JX092723.1), and the sister species of *R. agasthyaensis* (*R. kadalarensis*, KM596554.1; *R. luteolus*, EU450005.1; *R. chlorosomma*, EU450017.1; and *R. travancoricus*, EU450029.1) available in GenBank along with the sequences generated in the present study for comparison. Additional sequences of *Pseudophilautus amboli* (KU169982.1), *Pseudophilautus kani* (EU449994.1), and *Pseudophilautus wynaadensis* (KU169983.1) from GenBank were used as outgroups. Sequences were aligned using ClustalW, built-in BIOEDIT (Thompson et al. 1994; Hall 1999). The best-fit nucleotide substitution model was selected as GTR + G + I using the model test implemented in MEGA ver. 5 (Tamura et al. 2011); phylogenetic position of the species was determined using the maximum-likelihood method as implemented in MEGA ver. 5 (Tamura et al. 2011), and branch support was evaluated using 1,000 bootstrap replicates (Felsenstein 1985). Pairwise genetic distances between the species were calculated using the Kimura 2-parameter method in MEGA ver. 5 (Tamura et al. 2011).

Specimens collected on 28 July 2019 and in the following year matched the description of *R. agasthyaensis* in Zachariah et al. (2011), with key taxonomic features including: a light grayish-brown dorsum with a dark brown inverted V-shaped mark and scattered dark patches; a dark brown line connecting the eyelids; irregular bands on the forelimbs, fingers, hindlimbs, and toes; a dark brown iris with dense golden speckling, horizontally divided into a lighter upper half, a darker lower half, a dark brown iris periphery, and an ash-gray sclera (Garg et al. 2021) (Fig. 2). The 16S rRNA sequence generated from the sample clustered with the *R. agasthyaensis* with a high bootstrap value (Fig. 3). Since the 16S rRNA ref-

Figure 2. Agasthyamalai Bushfrog (*Raorchestes agasthyaensis*) Dorsal and Lateral view (A &B): Male vocalizing from a branch perch in natural habitat (C &D). Photographs by Sujith V Gopalan (A–B) and Renjith Jacob Mathews (C–D.

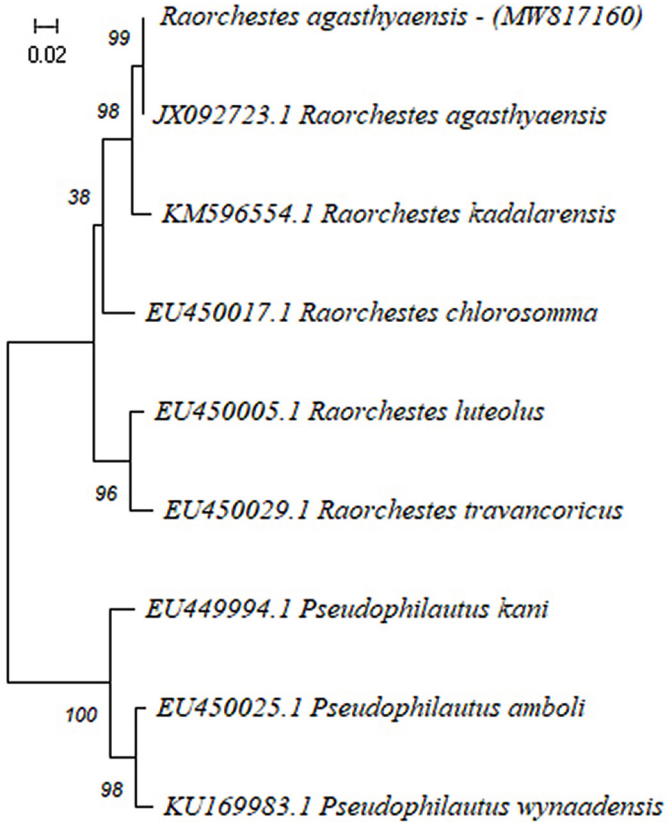

Figure 3. Phylogenetic tree (ML) showing relationships of the Agasthyamalai Bushfrog (*Raorchestes agasthyaensis*) and related species based on 16S rRNA sequence. The phylogenetic position of the species was determined using the maximum-likelihood method as implemented in MEGA ver. 5 (Tamura et al. 2011).

erence sequence of *R. agasthyaensis* showed 100% homology with the generated sequence (Table 1), we confidently identified the specimen as *R. agasthyaensis*.

Raorchestes agasthyaensis was described from the Bonacaud Estate in Thiruvananthapuram District and subsequent studies suggested that the species was restricted to an area of approximately 30 km^2 in and around its type locality (Zachariah et al. 2011; Garg et al. 2021). Many species of Bushfrogs, such as *R. crustai*, which occupies an elevational range of about 600–1,500 m asl, similar to those of *R. agasthyaensis* and *R. bobingeri*, also have restricted distributions south of the Shencottah Gap. For these species, the Shencottah Gap (70–180 m asl) acts as a geographical barrier. However, results from the current study suggest that *R. agasthyaensis*, previously known only from south of the Shencottah Gap, now has a substantially extended range over 120 km northward across the Shencottah Gap.

The 100% genetic homology observed in the 16S rRNA region between the sampled individual from Devikulam and the reference sample from south of the Shencottah Gap suggests a surprising lack of genetic divergence despite the considerable geographic separation. This absence of molecular differentiation could indicate either recent dispersal or historical connectivity between the northern and southern populations that has not yet resulted in lineage divergence. It also indicates that not all species respond uniformly to biogeographical barriers, as has been shown in a number of other species (e.g., Nair et al. 2012; Gopalan et al. 2014; Gopalan et al. 2020)

The study points to the lack of targeted amphibian surveys in underexplored areas of the Western Ghats. The current study also suggests that the species has a limited distribution around the Devikulam region. Surveys conducted by the same team across the Munnar landscape following the discovery failed to yield any additional records. The isolated and patchy nature of high-elevation habitats in the Western Ghats implies that such populations are still susceptible to local extinctions, particularly in the face of anthropogenic pressures such as tourism development, forest fragmentation, and climate change.

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Table 1. Pairwise genetic distances (%) between the Agasthyamalai Bushfrog (*Raorchestes agasthyaensis*) and related species calculated using the Kimura 2-parameter method in MEGA ver. 5 (Tamura et al. 2011).

		1	2	3	4	5	6	7	8
1	MW817160.1 *R. agasthyaensis*								
2	JX092723.1 *R. agasthyaensis*	0.0000							
3	EU450017.1 *R. chlorosomma*	0.0483	0.0483						
4	KM596554.1 *R. kadalarensis*	0.0236	0.0236	0.0593					
5	EU450005.1 *R. luteolus*	0.0691	0.0691	0.0573	0.0716				
6	EU450029.1 *R. travancoricus*	0.0619	0.0619	0.0690	0.0691	0.0319			
7	EU450025.1 *P. amboli*	0.1322	0.1322	0.1277	0.1440	0.1349	0.1425		
8	EU449994.1 *P. kani*	0.1269	0.1269	0.1226	0.1361	0.1294	0.1321	0.0468	
9	KU169983.1 *P. wynaadensis*	0.1323	0.1323	0.1278	0.1389	0.1270	0.1374	0.0251	0.0511

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