



## Lineage delimitation and description of nine new species of bush frogs (Anura: *Raorchestes*, Rhacophoridae) from the Western Ghats Escarpment

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### Abstract

Bush frogs of the genus *Raorchestes* are distributed mainly in the Western Ghats Escarpment of Peninsular India. The inventory of species in this genus is incomplete and there is ambiguity in the systematic status of species recognized by morphological criteria. To address the dual problem of taxon sampling and systematic uncertainty in bush frogs, we used a large-scale spatial sampling design, explicitly incorporating the geographic and ecological heterogeneity of the Western Ghats. We then used a hierarchical multi-criteria approach by combining mitochondrial phylogeny, genetic distance, geographic range, morphology and advertisement call to delimit bush frog lineages. Our analyses revealed the existence of a large number of new lineages with varying levels of genetic divergence. Here, we provide diagnoses and descriptions for nine lineages that exhibit divergence across multiple axes. The discovery of new lineages that exhibit high divergence across wide ranges of elevation and across the major massifs highlights the large gaps in historical sampling. These discoveries underscore the significance of addressing inadequate knowledge of species distribution, namely the “Wallacean shortfall”, in addressing the problem of taxon sampling and unknown diversity in tropical hotspots. A biogeographically informed sampling and analytical approach was critical in detecting and delineating lineages in a consistent manner across the genus. Through increased taxon sampling, we were also able to discern a number of well-supported sub-clades that were either unresolved or absent in earlier phylogenetic reconstructions and identify a number of shallow divergent lineages which require further examination for assessment of their taxonomic status.

**Key words:** Species delimitation, *Raorchestes*, Multi-criteria, Wallacean shortfall, Taxon sampling, Western Ghats

geographical and behavioral. In the case of well-supported clades, the comparison is restricted to the sister lineages, while for lineages with poorly supported close relatives, comparisons are made within a larger pool of related lineages nested within the next well-supported clade to which the target lineage can be assigned.

This approach towards lineage diagnosis deviates from earlier studies and explicitly uses multiple variables and incorporates geography as one of the prominent axes in discerning lineages, especially shallow divergent lineages. Though we recovered a large number of potential lineages through our hierarchical method, we have adopted a conservative approach towards describing species, and have, at this time, only described highly divergent lineages and shallow divergent lineages that exhibited deep divergences in morphological and/or acoustic axes. Since we only used mitochondrial markers in delimiting lineages, one important caveat is the problem of mitochondrial introgression and incomplete lineage sorting that could potentially affect recently divergent complexes (Vences and Wake 2007). However, given our conservative approach to species described, we believe that the mitochondrial introgression and incomplete lineage sorting are less likely to affect the lineages recognized here.

The historical pattern of frog species discovery in the Western Ghats is marked by episodes of sudden discovery with intervals of long stasis (Aravind *et al.*, 2004). The bush frog discovery patterns follow this general trend, with a number of species described during latter part of the 18<sup>th</sup> century and early part of the 19<sup>th</sup> century followed by a long gap of several decades before researchers initiated fresh collections (eg. Kuramoto & Joshi, 2003; Biju & Bossuyt, 2005a; 2006; Gururaja, 2007; Seshadri *et al.*, 2012; Padhye *et al.*, 2013). The use of molecular based phylogenetic tools can be considered as one important reason for the recent burst in species discovery (Biju & Bossuyt, 2009). Though these tools facilitated our work, the incorporation of spatial sampling is equally or more important in increasing taxon sampling. The significance of this factor in the Western Ghats (and other similar regions of the world) cannot be overstated given the high topographic heterogeneity and the underlying geological and climatic history that has created ample opportunities for speciation in amphibians. The results here exemplify the significance of spatial sampling, and geographical range of species, that were used effectively in conjunction with recent molecular based phylogenetic tools and classical morphological variables, in uncovering deeply divergent unknown lineages.

## Acknowledgements

We thank the following agencies—Council for Scientific and Industrial Research (CSIR) grant-in-aid scheme 37(1371)/09/EMR\_II, Critical Ecosystem Partnership Fund (Western Ghats), and Ministry of Environment and Forests (MoEF)—for funding. The state forest departments of Kerala, Karnataka, Tamil Nadu and Maharashtra provided permission for fieldwork. We thank Riya Menezes and Aditi Jayarajan for helping conduct the laboratory analysis. We acknowledge Mayavan, Saunak Pal, Varun R. Torsekar, K.A. Subramanian, M.S. Chaitra, Giby Kuriakose, K.V. Gururaja and members of local communities for providing support during various field expeditions. KPD is thankful to DBT and SERB for fellowship and financial assistance to conduct part of this work. We sincerely thank the anonymous reviewers and Miguel Vences for their critical comments which greatly aided in improving the manuscript.

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