

Systematics of the Shrub Frogs of the *Pseudophilautus popularis* Complex

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Alpha taxonomy and species delimitation are cornerstones of biodiversity conservation. However, species delimitation can be convoluted in certain instances, making it relatively difficult to determine the geographic limits and population status of certain species. This situation is exemplified by *Pseudophilautus popularis* and *Pseudophilautus regius*, two morphologically similar endemic rhachophorid shrub frogs of Sri Lanka, which are difficult to distinguish in the field. Hence, the species boundaries of these two species were evaluated using an integrative approach. Specimens, tissue samples and bioacoustic data were collected from four distinct locations covering all bioclimatic zones of Sri Lanka where the two species are known to occur naturally. Species identification was done using their original descriptions. A total of eighteen morphological features and thirty morphometric measurements were taken from the collected specimens and type specimens of the two species. An approximately 540 base pair fragment of mitochondrial 16S rRNA gene was sequenced from the tissue samples and phylogenetic analysis was conducted using both Maximum Likelihood and Bayesian methods. The statistical analysis of morphometric data and bioacoustic properties showed no clear distinction between the two species. Similarly, the phylogenetic analyses showed no monophyletic clades corresponding to the two species and the pairwise genetic distance between the locations, ranged between 0.51-1.59%. Thus, integrative evidence provided here does not support the presence of the two distinct species, *P. popularis* and *P. regius*. The study highlights the significance of using integrative approaches in delimiting species limits.

Keywords: Amphibian, Sri Lanka, species delimitation, DNA barcoding, bioacoustics