

On the taxonomic status of *Minervarya granosa* (Kuramoto, Joshy, Kurabayashi & Sumida, 2008) and the distribution of *M. agricola* (Jerdon, 1853) Amphibia: Anura: Dicroglossidae

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ABSTRACT

Analysis of mitochondrial 16s rDNA data from a nearly topotypical specimen of the common Indian cricket frog (*Minervarya agricola*) sequenced during this study revealed a potential conspecificity among samples of *Minervarya* originating from different parts of the Indian subcontinent including Nepal, Bangladesh, Sri Lanka and the Western Ghats. As this includes the type specimens of the supposedly Western Ghats-endemic *Minervarya granosa*, we synonymize *M. granosa* under *M. agricola* and expand the geographic distribution of *M. agricola* throughout most parts of the Indian subcontinent.

Key words: *Minervarya*, *agricola*, *granosa*, synonymy, Western Ghats, distribution, 16s rRNA

The Dicroglossid frog genus *Fjervarya* (s. lat.) has been the subject of extensive research in the recent past (e.g. Howlader *et al.*, 2016; Suwannapoom *et al.*, 2016; Suwannapoom *et al.*, 2017; Garg & Bui, 2017; Sanchez *et al.*, 2018) and has now been split into two genera: the predominantly south Asian *Minervarya* and the predominantly south-east Asian *Fejervarya* (after Sanchez *et al.* 2018). The genus *Minervarya* presently comprises 39 species occurring from India in the west till Thailand in the east (Frost, 2019). Among them, *Minervarya agricola* (Jerdon, 1853) has been a species that had suffered a lot of taxonomic and nomenclatural inconsistencies which were clarified recently by virtue of a neotype designation (Ganesh *et al.*, 2017). We, for the first time, sequenced a nearly topotypical sample of *M. agricola*. Analysis of its molecular data has revealed further insights into systematic status of members of this species group. In this paper, we clarify the systematic status of *Minervarya* populations across the Indian subcontinent by analyzing their genetic data.

One specimen of *Minervarya agricola* (PU EES 001) was collected from Kalapet, Puducherry (12.0194° N, 79.8556° E, 20 m asl.), India. Total genomic DNA was extracted using Himedia® MB506 (India) mammalian DNA extraction and purification kit from liver tissues following the manufacturer's protocol. Quality of the extracted DNA was checked spectrophotometrically using Nanodrop. From the extracted genomic DNA, 16s rRNA gene was amplified using the forward and reverse primers 16sAR-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16sBR-H (5'-CCGGTCTGAACTCAGATCACGT 3') respectively (Kocher *et al.*, 1989). Amplifications were

performed in a 50 µl reaction with 25 µl of P4600 Sigma-Aldrich® master mix, 2 µl each of forward and reverse primers, 3 µl of DNA template and 18 µl of nuclease free water with the following procedure: initial denaturation of DNA at 95° C for 5 min, 35 cycles of: denaturation at 95° C for 1 m, annealing at 55° C for 1 min, extension at 72° C for 1 m and at last, final extension at 72° C for 10 min. The amplicon was checked by running it through an agarose gel electrophoresis for a clear band of the desired region in the amplified PCR product. The amplified PCR product was purified with Himedia® PCR product purification kit MB512 and sent out for sequencing commercially (Eurofins genomics, Bengaluru, India).

The sequence thus obtained was subjected to BLAST search on NCBI, to identify the most similar sequences on the database. Based on the BLAST search, a set of 23 sequences of 16s rRNA of 7 of species of *Minervarya*, four sequences from the genus *Fejervarya* were selected and aligned along with *Limnonectes kuhlii* as the outgroup. The sequences were manually edited by deleting gaps if any and aligned with Muscle using MEGA 6. An alignment consisting of the above sequences was developed, exported in FASTA and MEGA formats. The MEGA alignment was then used to determine uncorrected pairwise genetic distances between the samples with MEGA 6. The FASTA alignment was converted to PHYLIP format in the Alignment Transformation Environment (ALTER) website (<http://sing.ei.uvigo.es/ALTER/>) and was subjected to a maximum likelihood (ML) analysis in RAxML GUI v. 1.3 using the general time reversible model, GTRGAMMA with 500 bootstrapped replicates. The tree

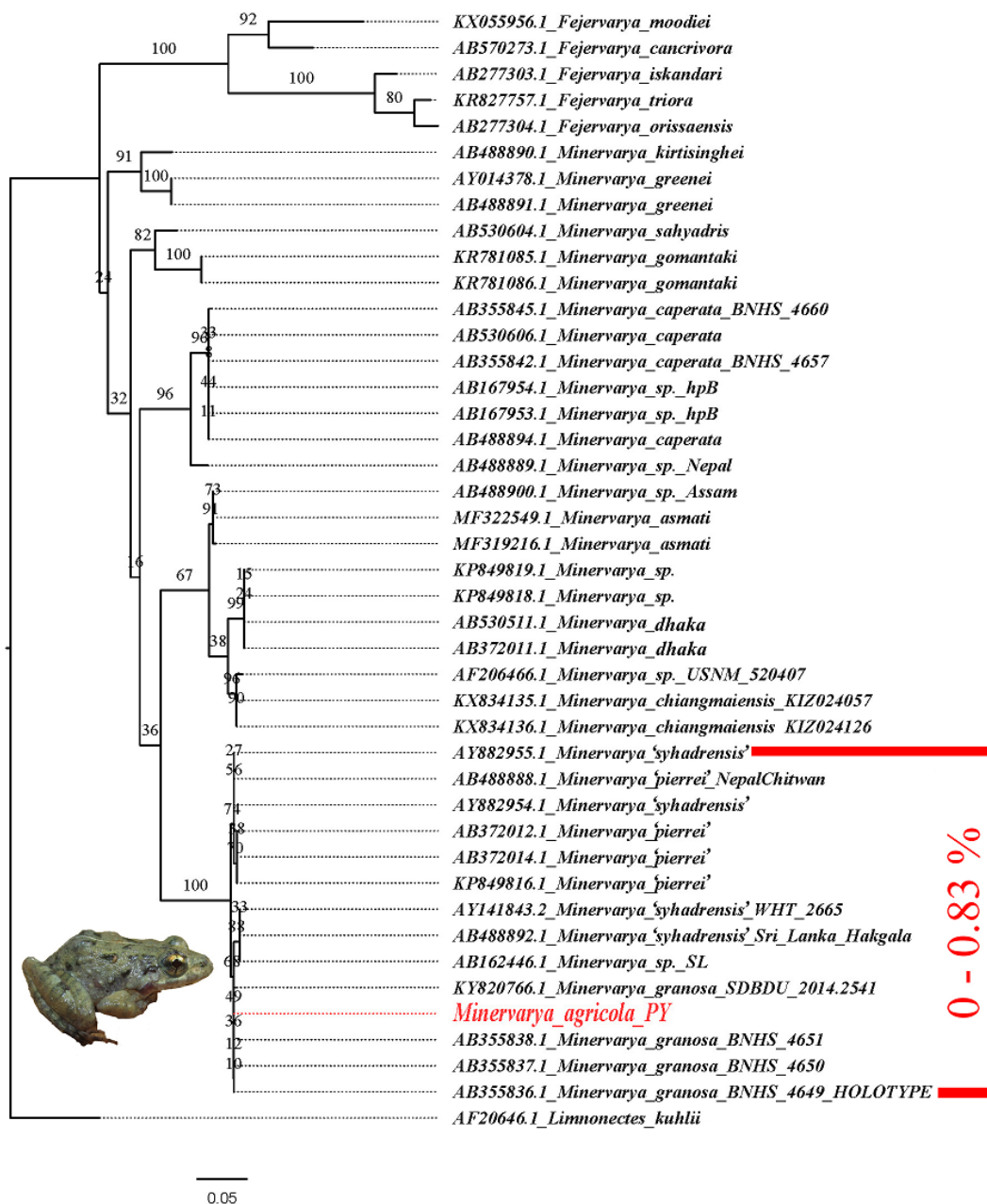


Figure 1. Maximum likelihood phylogenetic tree of *Fejervarya* and *Minervarya* species showing the position of *Minervarya agricola* and *M. granosa*

file generated (Figure 1) was then visualized using Fig Tree v. 1.4.0.

The new sequence of *Minervarya agricola* (MK628927) generated during this study from the Coromandel coastal plains, which is the only available sample from this eco-region, was found to form a well-supported (BS: 100) cluster together with 13 other samples of *Minervarya*, some of which were identified as *M. 'syhadrensis'* and *M. 'pierrei'*, along with three unidentified samples of *Minervarya* and the type specimens of the recently described, *Minervarya granosa*. Intra-specific genetic divergence between the samples in this cluster ranged from 0 – 0.83 % on 16s rRNA gene. The samples comprising this clade were found to originate from a vast geographic region across the Indian subcontinent, spanning from Sri Lanka (Hakgala) in the southern

extremity through the Western Ghats (Talapu, Madikeri: the type locality of *M. granosa*) and the Coromandel Coast (near the type locality of *M. agricola*) extending eastwards till Bangladesh and northwards upto Chitwan in Nepal. Based on genetic homogeneity of these samples, we hereby synonymize the supposedly Western Ghats endemic *Minervarya granosa* with *M. agricola* and expand the distribution of the latter throughout the Indian subcontinent from the coastal plains, all the way upto the higher hills of the Western Ghats and Sri Lanka till the Gangetic flood-plains in Nepal.

***Minervarya agricola* (Jerdon, 1853)**

Minervarya granosa (Kuramoto, Joshy, Kurabayashi & Sumida, 2008) **syn. nov.**

This reveals the fact that *M. agricola* is a very

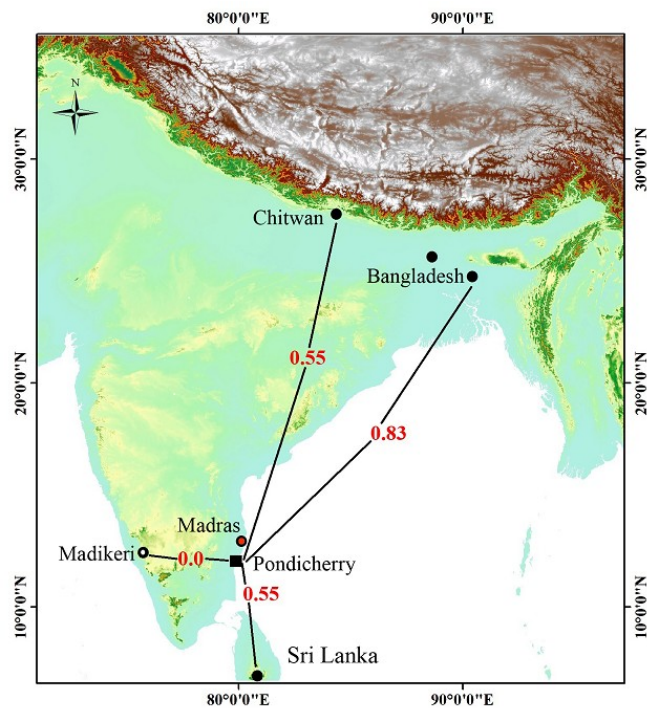


Figure 2. Geographic distribution and the respective genetic divergence (in %) between the samples of *Minervarya agricola* used in the analysis, based on the data from Genbank.

widespread species across the Indian subcontinent and occurs from Sri Lanka in the south all the way till the Gangetic plains in Nepal, and occupies a wide altitudinal gradient of nearly sea level (Pondicherry) till about 1560 m asl. in Hakgala, Sri Lanka and 1130 m in Madikeri, in the Western Ghats and occupies a wide range of habitats from flooded fields and human habitation in the plains all the way till wet forests on the hills (Figure 2). Although a few other members on the clade recovered during this study have been identified and labeled as '*Minervarya syhadrensis*' and '*M. pierrei*', we refrain from commenting on the taxonomic status of these nomina as we have not examined their type specimens or the voucher specimens from which these sequences have been generated. However, in the case of *M. granosa*, the holotype, which forms the very basis of the name, has turned out to be conspecific with *M. agricola* of the eastern coastal plains. Ganesh *et al.* (2017) pointed out the fact that *F. agricola* was most closer morphologically to *F. granosa* but for the degree of toe webbing, which, considering its very wide distribution, could understandably be plastic to some extent. The species name *agricola* was applied to the eastern coastal plains population under the genus *Fejervarya* by Ganesh *et al.* (2017) that was prior to the reclassification of fejervaryan frogs by Sanchez *et al.* (2018). However, even after Sanchez *et al.*'s (2018) recognition of the genus *Minervarya*, a few new species of frogs are being described from peninsular India under the obsolete concept of *Fejervarya* (e.g. Phuge *et al.*, 2019 and references therein). Given that molecular data are available for all such new species, Frost (2019) rightly re-allocated them to *Minervarya*, so as to reflect its monophyly, which we too concur with. DNA barcoding based studies in the recent past have

largely been instrumental in recognizing cryptic diversity and splitting of taxa (e.g., Garg & Biju, 2017). But, the present case involving samples from well-established centers of endemism such as the Himalayan forest slopes, the Western Ghats and Sri Lanka have turned out to be conspecific with each other, highlighting the adaptive abilities of the species to thrive in various ecosystems. This reminds us of a similar scenario in the case of another amphibian *Ichthyophis bombayensis* (see Gower *et al.*, 2007) and underscores the unseen pitfalls in generalization of systems.

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