H. T. HARVEY & ASSOCIATES



Ecological Consultants

Introduction

Though the majority of the 24 species Polypedates are within narrowly distributed, six (P. braueri, leucomystax, P. maculatus, P. macrotis, P. megacephalus, and P. mutus) are widely distributed and have been with much taxonomic and fraught distributional confusion (i.e., the P. leucomystax cryptic complex of species). In this state of taxonomic uncertainty, attempted We 10 understand the distributional patterns of species of Polypedates within Myanmar, as Myanmar is situated within the potential geographic confluence of these six widespread species.

Approach

Conducted phylogenetic analysis on specimens collected from throughout Myanmar and limited area in southwest China (Figure 1); considered only clades with high nodal support as operational taxonomic units (OTUs; Figure 2).

Determined consistent within OTU genetic distance limits (turned out to be up to 0.012).

Created histograms of frequencies of pairwise genetic distances and graphed pairwise genetic distances against pairwise geographic distances within and between OTUs following an isolation by distance model (Figure 3).

Expanded analyses to include 1,167 sequences deposited in GenBank from 34 studies throughout Asia (Figure 4), resulting in multiple phylogenies of consistently similar topologies (Figure 5). Clades in Figures 5C and 5D similar to Kuraishi et al. 2013. Zool. Scr. 42:54-70, who provided species names as shown in Figure 5C.

Examined 621 specimens deposited in 7 collections from the same studies for consistent morphological and color pattern differences between OTUs (Figures 6 and 7).

Conducted discriminant function analyses (DFA) on 21 morphometric variables for which OTUs between consistent morphological discrete, differences were not apparent (Figure 8).



Figure 1. Distribution of specimens collected for this study.



Figure 2. Phylogeny of 184 specimens from which 4,237 bases sequenced for genes 12S, 16S, cyt-b, and ND1. Colors match those in Figure 1.

Polypedates leucomystax Species Complex: How Many Species Are There?

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Figure 5. Example phylogeny of 354 specimens from which 1,782 bases sequenced for genes cyt-b and ND1 (A and B). Example phylogeny of 400 specimens from which 2,460 bases sequenced for genes 12S and 16S (C and D).



Figure 3. Example histograms of frequencies of pairwise genetic distances for, and correlations of pairwise genetic to geographic distances between, individuals from two OTUs in Clade B. Large gap between peaks in histogram and parallel line to x axis in graph together indicate separate species.

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Figure 4. Distribution of all 33 OTUs of the *P. leucomystax* complex recognized in this study.



Figure 6. Back of thighs of specimens of OTUs 4 and 19, showing much larger light spotting in OTU 19.



Figure 7. Profiles of specimens of OTUs 19 and 21, showing dark brown spot on tympanum in OTU 21.



Figure 8. Example DFA on specimens of four OTUs in Clade B.

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