A little frog leaps a long way: compounded colonizations of the Indian Subcontinent discovered in the tiny Oriental frog genus *Microhyla* (Amphibia: Microhylidae)

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ABSTRACT

Frogs of the genus *Microhyla* include some of the world's smallest amphibians and represent the largest radiation of Asian microhylids, currently encompassing 50 species, distributed across the Oriental biogeographic region. The genus *Microhyla* remains one of the taxonomically most challenging groups of Asian frogs and was found to be paraphyletic with respect to large-sized fossorial *Glyphoglossus*. In this study we present a time-calibrated phylogeny for frogs in the genus *Microhyla*, and discuss taxonomy, historical biogeography, and morphological evolution of these frogs. Our updated phylogeny of the genus with nearly complete taxon sampling includes 48 nominal *Microhyla* species and several undescribed candidate species. Phylogenetic analyses of 3,207 bp of combined mtDNA and nuDNA data recovered three well-supported groups: the *Glyphoglossus* clade, Southeast Asian *Microhyla* II clade (includes *M. annectens* species group), and a diverse *Microhyla* I clade including all other species. Within the largest major clade of *Microhyla* are seven well-supported subclades that we identify as the *M. achatina*, *M. fissipes*, *M. berdmorei*, *M. superciliaris*, *M. ornata*, *M. butleri*, and *M. palmipes* species groups. The phylogenetic position of 12 poorly known *Microhyla* species is clarified for the first time.

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These phylogenetic results, along with molecular clock and ancestral area analyses, show the *Microhyla—Glyphoglossus* assemblage to have originated in Southeast Asia in the middle Eocene just after the first hypothesized land connections between the Indian Plate and the Asian mainland. While *Glyphoglossus* and *Microhyla* II remained within their ancestral ranges, *Microhyla* I expanded its distribution generally east to west, colonizing and diversifying through the Cenozoic. The Indian Subcontinent was colonized by members of five *Microhyla* species groups independently, starting with the end Oligocene—early Miocene that coincides with an onset of seasonally dry climates in South Asia. Body size evolution modeling suggests that four groups of *Microhyla* have independently achieved extreme miniaturization with adult body size below 15 mm. Three of the five smallest *Microhyla* species are obligate phytotelm-breeders and we argue that their peculiar reproductive biology may be a factor involved in miniaturization. Body size increases in *Microhyla—Glyphoglossus* seem to be associated with a burrowing adaptation to seasonally dry habitats. Species delimitation analyses suggest a vast underestimation of species richness and diversity in *Microhyla* and reveal 15–33 undescribed species. We revalidate *M. nepenthicola*, synonymize *M. pulverata* with *M. marmorata*, and provide insights on taxonomic statuses of a number of poorly known species. Further integrative studies, combining evidence from phylogeny, morphology, advertisement calls, and behavior will result in a better systematic understanding of this morphologically cryptic radiation of Asian frogs.

Subjects Biodiversity, Evolutionary Studies, Taxonomy, Zoology
Keywords Molecular phylogeny, Biogeography, Miniaturization, Narrow-mouthed frogs, Southeast Asia, Microhylinae, Species delimitation, Indian collision, Cryptic species, Glyphoglossus

INTRODUCTION

The tropical areas of South and Southeast Asia include biogeographic regions recognized as global centers of biodiversity (*Myers et al., 2000*; *Bain et al., 2008*; *Stuart, 2008*; *De Bruyn et al., 2014*). Understanding processes that sculpted this diversity is hampered by a highly complex geological and climatic history of this region. Combining data on tectonics, paleoclimate, and phylogenetics has proved to be a powerful instrument for examining patterns of diversification within clades and understanding processes involved in the assembly of high biodiversity in regions like South and Southeast Asia (*De Bruyn et al., 2014*).

The tectonic collision between the Indian subcontinent (ISC) and the Eurasian landmass during the Early Cenozoic is widely recognized as a key event that caused significant geologic and climatic changes, such as the rise of the Himalaya, uplift of the Tibetan plateau, and a general drying of Central Asia (*Harrison et al., 1992*; *An et al., 2001*; *Guo et al., 2002*; *Molnar, 2005*; *Solovyeva et al., 2018*). This tectonic event also induced a major biotic interchange between the ISC and Eurasia and is widely regarded as a major driver of biotic diversification (*Wilkinson et al., 2002*; *Roelants, Jiang & Bossuyt, 2004*; *Li et al., 2013*; *Garg & Biju, 2019*). Numerous studies have demonstrated that floral and faunal elements reached and colonized tropical Asia from Gondwanaland via the