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Description of two new species of *Microhyla* (Anura: Microhylidae) from Bangladesh

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Abstract

Two new frog species belonging to genus *Microhyla* from the southeast, central and northeast regions of Bangladesh are described. Based on a molecular phylogeny derived from mitochondrial DNA sequences, one of the new species forms a clade with *M. fissipes,* while the second new species is sister to this clade. The DNA sequences of the mitochondrial cytochrome *b* gene from these new species are substantially diverged from *M. fissipes* (8.9 and 10.2% [3.6 and 4.2% for 16S ribosomal RNA gene] uncorrected pairwise divergence, respectively), and the estimated phylogenetic splits from their closest relative is in the Pliocene (3.4 Mya) and middle Miocene (10.5 Mya). The first new species (*Microhyla mukhlesuri* sp. nov.) can be diagnosed from its nearest congener (*M. fissipes*) by the following characteristics: SVL: 16.5–21.0 mm, finger length 1 < 4 < 2 < 3, tips of finger and toes not swollen, subarticular tubercles distinct, an inverse U-shaped mark on the anus, and a distinct X-shaped marking on the dorsum. Although the second new species (*M. mymensinghensis* sp. nov.) shares some morphological characteristics with the first new species, it can be readily diagnosed from its close congeners by its longer hindlimbs (HLL/SVL), tibia (TIL/SVL) and forearm width (FAW/SVL), in addition to a combination of the following characteristics: SVL: 14.2–21.3 mm, snout truncate, a crescent-shaped marking on the anus, and an X-shaped marking on the dorsum. The tibio-tarsal articulation extends to the eye in *M. fissipes* but ranges from the eye to the tip of the snout in the two new species.

Key words: *Microhyla mukhlesuri* sp. nov., *Microhyla mymensinghensis* sp. nov., Microhylidae, Mitochondrial DNA, Divergence time, Morphology, Bangladesh

Introduction

Microhylidae is a large anuran family comprising 8% of all frogs (519 species), with *Microhyla* being its type genus. This genus is characterized by various morphological characteristics (e.g., smooth or warty skin, absence of vomerine teeth, a narrow and elliptical tongue, hidden tympanum, free fingers, free or webbed toes, united outer metatarsals, dilated finger and toe tips, absent omosternum, and T-shaped terminal phalanges (e.g., Chanda 2002). Members of this genus show a wide distribution across Asia from the Ryukyu Archipelago in Japan and China to the north, through India to Sri Lanka to the southwest, and through Southeast Asia to Sumatra, Borneo, Java, and Bali to the southeast (Frost 2013). Despite such a large distribution, only 31 *Microhyla* species have been documented (Frost 2013). Especially in Bangladesh, only three nominal *Microhyla* species (*Microhyla ornata*, *M. berdmorei* and *M. rubra*) are known (Kabir *et al*. 2009). Recently, we found three haplotype groups (referred to as the Chittagong, Mymensingh-Sylhet, and Dinajpur haplogroups of *M.* cf. *ornata* by Hasan *et al*. 2012) from Bangladesh genetically distinct from these three species based on mitochondrial 16S ribosomal RNA gene (*16S-rrn*) data. The *16S-rrn* divergence was >5.0% between the Chittagong and Mymensingh-Sylhet haplogroups, and ~14% between the Dinajpur and former groups. When compared with the *Microhyla* taxa from neighboring countries, it became clear that the Dinajpur haplogroup belongs to the Indian *Microhyla* group (including *M. ornata* and *M. rubra*) with the Chittagong and Mymensingh-Sylhet groups included in the Southeast Asian group (e.g., *M. fissipes, M. heymonsi*, and *M. okinavensis*). The Chittagong haplogroup therefore becomes a sister taxon to *M.*

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open fields with some vegetation and slightly wet and loose soil. After dissection, we found many small insect parts, as well as some sand in the gut. Their breeding season is likely June-July, as we caught several females in June from Bangladesh Agricultural University Campus (BAUC) just after light rains, which contained about 40–50 mature ova in the ovaries. Each ovum is very small, approximately 650 µm in diameter.

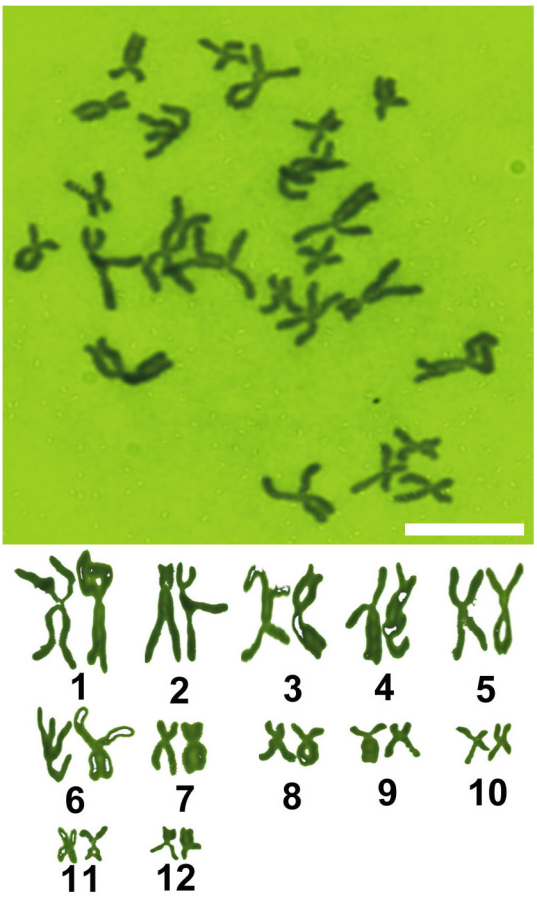

FIGURE 7. Metaphase spread and karyotype from bone marrow cells of *M. mymensinghensis*. Scale bar = 10 µm.

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Appendix 1. Examined specimens list

***Microhyla mukhlesuri*:** Institute for Amphibian Biology, Hiroshima University: IABHU 3956–3960, 3978, 3879–3882.
Collection localities: Raozan, Chittagong, Bangladesh.
***Microhyla mymensinghensis*:** Institute for Amphibian Biology, Hiroshima University: IABHU 4004–4006, IABHU 4117–4120, IABHU 4129–4134, F5012 and IABHU 3898–3899, IABHU 3944–3955.
Collection localities: Bangladesh Agricultural University Campus (BAUC), Mymensingh and Golapganj, Sylhet, Bangladesh.
***Microhyla fissipes*:** Osaka Museum of Natural History: OMNH Am 20028–20042.
Collection localities: Manchou of Pingtung district, Taiwan.