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More of the same: a diminutive new species of the *Limnnectes kuhlii* complex from northern Vietnam (Anura: Dicroglossidae)

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Abstract

A new species in the dicroglossid genus *Limnnectes* known only from Ha Giang province, Vietnam is described. Analysis of DNA sequence data from the mitochondrial 12S and 16S gene regions places the species within the *Limnnectes kuhlii* Complex and demonstrates it to be the sister taxon to an Indochinese clade containing *L. isanensis*, *L. jarujini*, *L. megastomias*, and *L. taylori*. The new species occurs in syntopy with *L. bannaensis*. Both molecular and morphological data support the recognition of this lineage as a new species. Notably, the relatively diminutive size of this species distinguishes *Limnnectes nguyenorum* **sp. nov.** from all other members of the *L. kuhlii* Complex.

Key words: dicroglossid, fanged frog, *Limnnectes nguyenorum* **sp. nov.**, mitochondrial DNA, morphology, species complex

Introduction

Limnnectes Fitzinger comprises 63 currently recognized species (Frost 2014). The genus is characterized by the presence of odontoid processes (hence the colloquial name of “fanged frogs”), male-biased size dimorphism, and male parental care (Emerson *et al.* 2000). Frogs of the genus *Limnnectes* are distributed throughout east and Southeast Asia, most are tied to forest stream habitats, and it is not uncommon to observe two or more congeners occurring in syntopy (e.g., McLeod 2008, 2009). The generotype, *Limnnectes kuhlii* Tschudi, historically considered to be a single, broadly distributed species, was the focus of two recent molecular phylogenetic studies (Matsui *et al.* 2010; McLeod 2010) that highlighted a considerable amount of diversity hidden within this species complex.

McLeod (2010) presented a robust molecular phylogeny of the *L. kuhlii* Complex using mtDNA sequences from individuals representing approximately 63 populations across its known distribution. The results corroborated previous phylogenetic treatments of the *kuhlii* Complex (Emerson *et al.* 2000; Evans *et al.* 2003; Matsui *et al.* 2010; Zhang *et al.* 2005). Furthermore, McLeod (2010) demonstrated that *L. kuhlii*, which historically had been recognized as a single species, is a complex of more than 22 well-supported evolutionary lineages (*viz.*, species), many of which remain subsumed under the nominal *L. kuhlii*. Tschudi (1838) designated the island of Java as the type locality for *L. kuhlii*. McLeod (2010) followed this designation and restricted all individuals from Java to retain the name *L. kuhlii*. Additionally, the study also uncovered several cases of sympatric/syntopic lineages, and in no case were co-occurring lineages each other’s closest relatives (McLeod 2010).

In 2000, a series of anuran specimens was collected from Vi Xuyen District, Ha Giang Province, Vietnam by Raoul Bain and Nguyen Quang Truong (Bain & Nguyen 2004). These specimens were identified as *Limnnectes kuhlii* and deposited in the American Museum of Natural History and Institute for Ecology and Biological Resources (Hanoi, Vietnam). At the time of collection, a remarkable difference in the size of two gravid females from the same location was noted in the expedition field notes (R. Bain & T. Nguyen, pers. comm.). In 2008, these

(McLeod 2010), a single sample consistently fell out in the “wrong” place in preliminary trees. When the voucher specimen for this sample (AMNH A-163944) was examined along with a series 16 other specimens of *L. bannaensis* from Vietnam (three from the same specific locality) the morphological differences were obvious. Communication with the collectors (R. Bain and T. Nguyen, pers. comm.) revealed that this small specimen was first considered a juvenile in the field, and that its unique appearance warranted notation in the field records. Upon subsequent investigation it was identified as a gravid female, though notably smaller than other gravid females collected at the same time. The importance of taking both tissue samples and whole voucher specimens during field studies cannot be emphasized enough. In this case, had there been a tissue sample only, it seems likely that the identity of the single AMNH sample would have been impossible to confirm, that the sequence data might have simply been considered to be “poor quality” and discarded, and ultimately that real diversity would have been overlooked.

During the development of this manuscript, two subsequent field trips to Ha Giang province were made to look for additional specimens of this small frog. These follow-up expeditions yielded 18 specimens of *L. bannaensis* but only 3 of *L. nguyenorum*. These data suggest that *L. nguyenorum* is either less abundant, more rarely encountered, or both. From a conservation standpoint both “rare” and “rarely encountered” are significant descriptors. Given the rapid loss of tropical Southeast Asian forests (e.g., Sodhi *et al.* 2009), and the threat that this poses to forest biota, it is critical that we attempt to identify and understand the true biodiversity of this region before it is lost. Recognizing diversity is only the first step towards developing informed conservation and management strategies. In the case of *L. nguyenorum*, we have made that first step herein. Unfortunately we do not yet understand this species: we do not know its real geographic distribution, its true natural history, ecology, or the status of this species in nature. Future field studies will be necessary if we are to determine whether *L. nguyenorum* is an endemic species on the brink of extinction and “in need of conservation” or if it is abundant and thriving elsewhere and that our limited knowledge renders it “data deficient”? Serendipity may have led to the discovery of this frog, but our understanding of this species and its future fate should not be left to chance.

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APPENDIX. Specimens examined (morphology)

Limnnectes nguyenorum: VIETNAM: Ha Giang Province: VNMN A.2015.1, A.2015.3, IEBR A.2015.3, AMNH A163944
Limnnectes bannaensis: VIETNAM: Ha Giang Province:, VNMN A.2015.41–48, IEBR A.2014.32, A.2014.33–40, AMNH A163945, 163953, 163955; Ha Tinh Province: AMNH A 161196, 161204, 161646; Lao Cai province: AMNH A168707–713; Quang Binh Province: AMNH A 161202, 161203; CHINA: Guangxi Province: KU 311786, 311788, 311790–795, LAO PDR: Huaphahn Province: FMNH 255140–149; Phongsaly Province: FMNH 258519, 258520, 258522.
Limnnectes taylori: LAO PDR: Bokeo Province: MNHN 1997.3902, 1997.3904, 1997.3916, 1997.4104; Phongsaly Province: FMNH 258517–18, 258521, 258523–26; MYANMAR: Shan State, CAS 230947–48, 235470; THAILAND: Chiang Mai Province: CUMZ(A) 2003.6–8, 2003.13, 2003.29–30, 2003.32–33.