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## Insight into the validity of *Leptobrachium guangxiense* (Anura: Megophryidae): evidence from mitochondrial DNA sequences and morphological characters

WEICAI CHEN<sup>\*, 1</sup>, WEI ZHANG<sup>\*, 1</sup>, SHICHU ZHOU<sup>1</sup>, NING LI<sup>1</sup>, YONG HUANG<sup>2</sup> & YUNMING MO<sup>1</sup>

<sup>1</sup>Natural History Museum of Guangxi, Nanning 530012, PR China <sup>2</sup>Guangxi Botanical Garden of Medicinal Plants, Nanning 530023, PR China Corresponding author, Weicai Chen, E-mail: chenweicai2003@126.com Tel.: +86 7712820904, Fax: +86 7712820904 Corresponding address: Natural History Museum of Guangxi, Nanning 530012, PR China. <sup>\*</sup>These two authors contributed equally to this work. E-mail address: WEICAI CHEN, chenweicai2003@126.com WEI ZHANG, zhangwei@nhmg.org SHICHU ZHOU, zhoushichu@nhmg.org NING LI, lining@nhmg.org YONG HUANG, huangykiz@163.com YUNMING MO, moyunming@163.com Abbreviated form of the title: Phylogeny of Leptobrachium guangxiense

## Abstract

*Leptobrachium guangxiense* Fei, Mo, Ye and Jiang, 2009 (Anura: Megophryidae), is presently thought to be endemic to Shangsi, Guangxi Province, China. A molecular phylogenetic analysis and morphological data were performed to gain insight into the phylogenetic position of this species. Maximum parsimony, maximum likelihood, and Bayesian inference methods were employed to reconstruct phylogenetic relationship, using 1914 bp of sequences from mtDNA genes of 12S rRNA, tRNA<sup>Val</sup> and 16S rRNA. Topologies revealed that *L. guangxiense* and Tam Dao (Vietnam) *L. chapaense* lineage (3A) formed a monophyletic group with well-supported values. The uncorrected *p*-distance of ~1.4k bp 16S rRNA data-sets between Tam Dao *L. chapaense* lineage (3A) and *L. guangxiense* is only 0.1%. Morphologically, *L. guangxiense* and Tam Dao *L. chapaense* lineage (3A) shared the same characters, and are distinguishable from "true" *L. chapaense* from the type locality in Sa Pa, Vietnam. Based on morphological characters and mitochondrial DNA, we suggested that the Tam Dao lineages of *L. chapaense* are conspecific with *L. guangxiense*. This represents a range extension for *L. guangxiense*, and a new country record for Vietnam.

Key words: Leptobrachium guangxiense; phylogeny; 16S rRNA; morphological character; genetic distance

## Introduction

*Leptobrachium guangxiense*, a new member of *Leptobrachium*, was discovered and described in 2009 (Fei *et al.* 2009). It is presently known only from Shangsi Country, Guangxi Province, China. Morphologically, the frog is similar to *L. hainanense*, but can be distinguished based on several morphological characteristics, such as rather long limbs, outer palmar tubercle much smaller than inner one, and entire ventrum a purple-brown coloration (Fei *et al.* 2009).

The genus *Leptobrachium* contains 33 species and it is distributed from southern China and India to islands of the Sunda Shelf and the Philippines (Frost 2011; Sondhi & Ohler 2011; Hamidy *et al.* 2012; Stuart *et al.* 2011, 2012; Wogan 2012) (Figure 1). Only 9 species of *Leptobrachium* are known from China: *L. ailaonicum*, *L. boringii*, *L. chapaense*, *L. guangxiense*, *L. hainanense*, *L. huashen*, *L. leishanense*, *L. liui*, and *L. promustache* (Fei *et al.* 2009; Frost 2011). Apart from *L. guangxiense*, the phylogenetic relationships of all the Chinese taxa were recently examined in Rao & Wilkinson (2008), Zheng *et al.* (2008), and Matsui *et al.* (2010). Among them, the evolutionary history of *L. chapaense* was still controversial (Rao & Wilkinson 2008; Zheng *et al.* 2008; Matsui *et al.*