



Detection of cryptic taxa in *Leptobrachium nigrops* (Amphibia, Anura, Megophryidae), with description of two new species

AMIR HAMIDY^{1,2}, MASAFUMI MATSUI^{1,3}, KANTO NISHIKAWA¹ & DAICUS M. BELABUT^{4,5}

¹Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan

²Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Gd. Widyasatwaloka, Jl. Raya Jakarta Bogor Km 46, Cibinong West Java, Indonesia

⁴Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

⁵Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

³Corresponding author: E-mail: fumi@zoo.zool.kyoto-u.ac.jp

Abstract

We evaluated taxonomic relationships among allopatric populations of *Leptobrachium nigrops* Berry & Hendrickson from Malay Peninsula, Singapore, Indonesia (Belitung), and Borneo (Sarawak). Phylogenetic relationships estimated from the sequence data of mitochondrial 12S rRNA, tRNA^{val}, and 16S rRNA genes, and nuclear NCX1 and SLC8A genes revealed presence of three distinct clades within *L. nigrops*: (1) true *L. nigrops* clade from Singapore and Malay Peninsula, (2) clade from Belitung, Indonesia and coastal area of Sarawak, Borneo, and (3) clade from Kanowit, Sarawak, an inland area of Borneo. Each of these three genetic clades is considered to represent distinct species because they are genetically highly divergent and morphologically distinguishable. We therefore describe the populations from Belitung and coastal area of Sarawak as *L. ingeri* **sp. nov.** and the population from the inland area of Sarawak as *L. kanowitense* **sp. nov.** Ancestral *L. kanowitense* seems to have invaded Borneo Island much earlier than ancestral *L. ingeri*, whose dispersion occurred during the Pleistocene glacial periods.

Key words: *Leptobrachium nigrops*, new species, phylogenetic relationships, Malay Peninsula, Belitung, Borneo

Introduction

The megophryid genus *Leptobrachium* Tschudi consists of 30 species, occurring from Southern China and India to the islands of the Sunda Shelf and the Philippines (Frost 2011; Sondhi & Ohler 2011; Stuart *et al.* 2011, 2012). However, many more cryptic species await description even from Borneo alone (Hamidy *et al.* 2011). Among the named species, eight occur in Sundaland including *L. nigrops* Berry & Hendrickson, which is the smallest species of the genus, with body size of 35–37 mm for males and 37–47 mm for females (Inger 1966). This species is widely distributed on the Malay Peninsula, Singapore, Sumatra, and Borneo (Iskandar & Colijn 2000), and on some islands in the Karimata Strait such as Belitung (Matsui *et al.* 2010b) and Natuna Islands (Brown *et al.* 2009). Like other congeneric species from Southeast Asia, *L. nigrops* was once treated as *L. hasseltii* Tschudi (Taylor 1962), although Taylor (1962: 314) doubted species identification of a specimen from Singapore by adding “?” ahead of species name, *L. hasseltii*. It was Berry & Hendrickson (1963) who described the populations of *L. hasseltii* from Singapore and Peninsular Malaysia as a distinct species, *L. nigrops*, with Singapore as the type locality. They distinguished *L. nigrops* from *L. hasseltii* (type locality: Java), mainly based on the smaller adult body size and different shape of larval oral disk. Later, Inger (1966) noted difference in the shape of finger tips between populations of *L. nigrops* from Borneo and Malay Peninsula, but he retained the two populations as conspecific.

Recent molecular studies by Brown *et al.* (2009) and Matsui *et al.* (2010b) uncovered high levels of genetic divergences among allopatric populations of *L. nigrops*. Matsui *et al.* (2010b) recognized three clades (populations from Malay Peninsula, Belitung, and inland area of Borneo), but, like Brown *et al.* (2009), could not study the population from the type locality, Singapore. This strongly hindered the evaluation of the taxonomic status of those