Zootaxa 1843: 24–34 (2008)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Detection of two cryptic taxa in *Meristogenys amoropalamus* (Amphibia, Ranidae) through nuclear and mitochondrial DNA analyses

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Abstract

We identified three distinct sympatric lineages of frogs among specimens previously considered a single species (*Meristogenys amoropalamus* Matsui), based on 909 bp of mitochondrial DNA (12S rRNA and cytochrome b). To seek evidence of reproductive isolation between these lineages, we first analyzed a 249-bp fragment of the nuclear proopiomelanocortin (POMC) gene and found five haplotypes, of which two were limited to lineage 1 and three belonged to lineages 3 and 4. In a subsequent phylogenetic analysis of a 1313-bp fragment of nuclear POMC, Rag-1, and rhodopsin, lineage 1 was again distinct, while lineages 3 and 4 could not be differentiated. The results of the nuclear gene analyses suggest that lineage 1 is strongly isolated reproductively from lineages 3 and 4, which are not isolated from each other. This conclusion conforms to groupings based on larval morphology. These results indicate that frogs morphologically identified as *M. amoropalamus* should be split into two sympatric species, one of which contains two mitochondrial lineages that have presumably been retained via deep coalescence.

Key words: cryptic taxa, intraspecific polymorphism of mitochondria, larval morphology, *Meristogenys amoropalamus*, Borneo

Introduction

The number of recognized amphibian species is increasing at an astonishing rate (Köhler *et al*. 2005). One factor contributing to this proliferation is the improvement of molecular tools that reveal large numbers of morphologically cryptic species within taxa that were previously considered a single species (Köhler *et al*. 2005). However, such molecular studies sometimes face incongruence among hypotheses based on different tools, such as allozymes, mitochondrial (mt) DNA, and nuclear (nu) DNA (Sota and Vogler 2001; Gonçalves *et al*. 2007). This discordance among evolutionary trees may be caused by some genomic events, such as gene duplication, recombination, introgressive hybridization, and incomplete lineage sorting (Maddison 1997). In particular, the mitochondrial and nuclear genomes differ in many ways, including genome size, ploidy, mode of inheritance, degree of recombination, number of introns, effective population size, mutation rate, and repair mechanisms (Scheffler 1999). These differences are important when attempting to make inferences about the biology of the whole organism, and it is risky to infer general patterns from mtDNA alone (Ballard and Whitlock 2004; Fouquet *et al.* 2007).

 Accepted by M. Vences: 26 Jun. 2008; published: 6 Aug. 2008