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Morphological and genetic discordance in two species of Bornean *Leptobrachium* (Amphibia, Anura, Megophryidae)

Amir Hamidy [a,b], Masafumi Matsui [a,*], Tomohiko Shimada [a,c], Kanto Nishikawa [a], Paul Yambun [d], Ahmad Sudin [e], Mirza D. Kusrini [f], Hellen Kurniati [b]

[a] Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan
[b] Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Gd. Widyasatwaloka, Jl. Raya Jakarta Bogor Km 46, Cibinong West Java, Indonesia
[c] Faculty of Education, Aichi University of Education, Kariya, Aichi 448-8542, Japan
[d] Research and Education Division, Sabah Parks, P.O. Box 10626, Kota Kinabalu 88806, Sabah, Malaysia
[e] Institute for Tropical Biology and Conservation, University Malaysia Sabah, Kota Kinabalu 88999, Sabah, Malaysia
[f] Faculty of Forestry, Bogor Agricultural Institute, Bogor, West Java, Indonesia

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ABSTRACT

Recent phylogenetic studies of Southeast Asian megophryid *Leptobrachium*, while clarifying (1) distinct specific status of three Philippine populations and (2) high genetic diversities within Bornean *Leptobrachium montanum*, posed two questions, (1) relationships and divergence histories of two Philippine species and Bornean *Leptobrachium gunungense*, and (2) possible discordance between phylogenetically and morphologically defined lineages. In order to solve these questions, and especially reviewing current taxonomy of Bornean species, we estimated the phylogenetic relationships of endemic Bornean species together with their putative relatives from Philippines and Sumatra, using 2451 bp sequences of the 12S rRNA, $tRNA^{val}$, and 16S rRNA of mitochondrial DNA genes. With respect to *Leptobrachium hasseltii* and *Leptobrachium chapaense*, lineages from Borneo, Philippines, and Sumatra formed a monophyletic group with *Leptobrachium lumadorum* from Mindanao as the basal clade, while two other Philippine species from Palawan and Mindoro formed a clade and nested in Bornean lineages. Sister species relationship of the two Philippine species and *L. gunungense* is not supported, rejecting the hypothesis of Philippine origin of *L. gunungense*. Phylogeny does not conform to morphological variation, and the topotypic *L. montanum* and one lineage of *Leptobrachium abbotti* are genetically very close despite their clear difference in ventral color pattern. Furthermore, each of these species forms a paraphyletic group and contains several lineages, each of which is a candidate of good species. These results clearly indicate that current taxonomy of Bornean species based on several morphological characteristics requires complete revision. Detailed studies on adult and larval morphology, as well as acoustic characteristics, are necessary to evaluate the taxonomic status of all lineages recovered.

1. Introduction

Of the megophryid genus *Leptobrachium* Tschudi, 1838, five species of the nominate subgenus (Matsui et al., 2010b) are known from Borneo Island: *Leptobrachium montanum* Fischer, 1885, *Leptobrachium abbotti* (Cochran, 1926), *Leptobrachium gunungense* Malkmus, 1996, *Leptobrachium nigrops* Berry and Hendrickson, 1963, and *L. hendricksoni* Taylor, 1962. Of these, the former three species are endemic to the island (Inger and Stuebing, 1997; Frost, 2011), with *L. gunungense* being restricted to the highlands of Mt. Kinabalu between 1750 and 2200 m a.s.l. (Malkmus, 1996). *L. abbotti* and *L. montanum* occur widely in all regions of the island, in lowland forest and in submontane to montane forest, respectively (Inger et al., 1995; Inger and Stuebing, 1997). *L. montanum* and *L. gunungense* differ substantially in call characteristics (Malkmus, 1996; Malkmus et al., 2002; Matsui, unpublished data), but are very similar morphologically, both without white and black pattern ventrally (Malkmus et al., 2002). In contrast, the belly of *L. abbotti* is heavily marked with a bold black and white mottling, differing from the other two species (Inger and Stuebing, 1997; Fig. 1). Inger et al. (1995) indicated presence in *L. montanum* and absence in *L. abbotti* of a chalky white arc (more exactly, white sclera) on the upper part of eye as another noticeable morphological difference, but this trait can be identified only in live specimens.

Previous molecular studies clarified phylogenetic relationships of Bornean *Leptobrachium* with the taxa from the other localities. Veith et al. (2006) found *L. gunungense* to form a sister taxon to *L. montanum*, which was reported to be a sister taxon to *Leptobrachium hasseltii* Tschudi, 1838 by Zheng et al. (2008).

* Corresponding author. Fax: +81 75 753 6846.
E-mail address: fumi@zoo.zool.kyoto-u.ac.jp (M. Matsui).

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Fig. 1. Color pattern variation in ventral side of Bornean *Leptobrachium*: *L. montanum* from Paramasan, South Kalimantan; *L. abbotti* from Berau, East Kalimantan; and *L. gunungense* from Kinabalu, Sabah. Scale bar = 10 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

However, Rao and Wilkinson (2008) found *L. montanum* to form a monophyletic group with *Leptobrachium smithi* Matsui, Nabhitabhata, and Panha, 1999. These confusions arose from the use of a few specimens from limited ranges, and further clarification by adding more samples from wider areas, especially from the type locality, has been awaited.

More recent studies have revealed close relationships of the Bornean endemic species to Sumatran and Philippine species (Brown et al., 2009; Matsui et al., 2010b; Hamidy and Matsui, 2010). Brown et al. (2009) clarified the presence of three distinct species in the Philippines, one of which (*Leptobrachium lumadorum* Brown, Siler, Diesmos, and Alcala, 2009 from Mindanao) was basal to the clade of other Philippine and Bornean species containing three lineages of unresolved relationships. These lineages were (1) *L. montanum*, (2) *L. abbotti*, and (3) a clade of *L. gunungense* and two sister species from the Philippines (*Leptobrachium tagbanorum* Brown, Siler, Diesmos, and Alcala, 2009 from Palawan and *Leptobrachium mangyanorum* Brown, Siler, Diesmos, and Alcala, 2009 from Mindoro). Matsui et al. (2010b) also found a species from Mindanao to be basal to other taxa from Borneo and Sumatra, but the sister taxon of *L. gunungense* was found to be *L. abbotti*. Furthermore, Matsui et al. (2010b) recovered *L. montanum* to form a paraphyletic group, containing at least two lineages, one of which was basal to the other Bornean and Sumatran taxa.

Clearly, both Brown et al. (2009) and Matsui et al. (2010b) have deficiencies in population sampling. Brown et al.'s (2009) sampling from Borneo was limited to only six samples including two from GenBank data of unknown localities, while Matsui et al. (2010b) had no samples from the Philippines other than Mindanao. Different relationships obtained in the two studies are surely related to this lack of samples, although they commonly revealed that the species from Mindanao (*L. lumadorum*) first diverged from the common ancestor of other species. Nevertheless, an important question arose from these studies, i.e., the place of origin of the two species now found on Mindoro and Palawan and *L. gunungense*. If Brown et al.'s (2009) tree is correct, the common

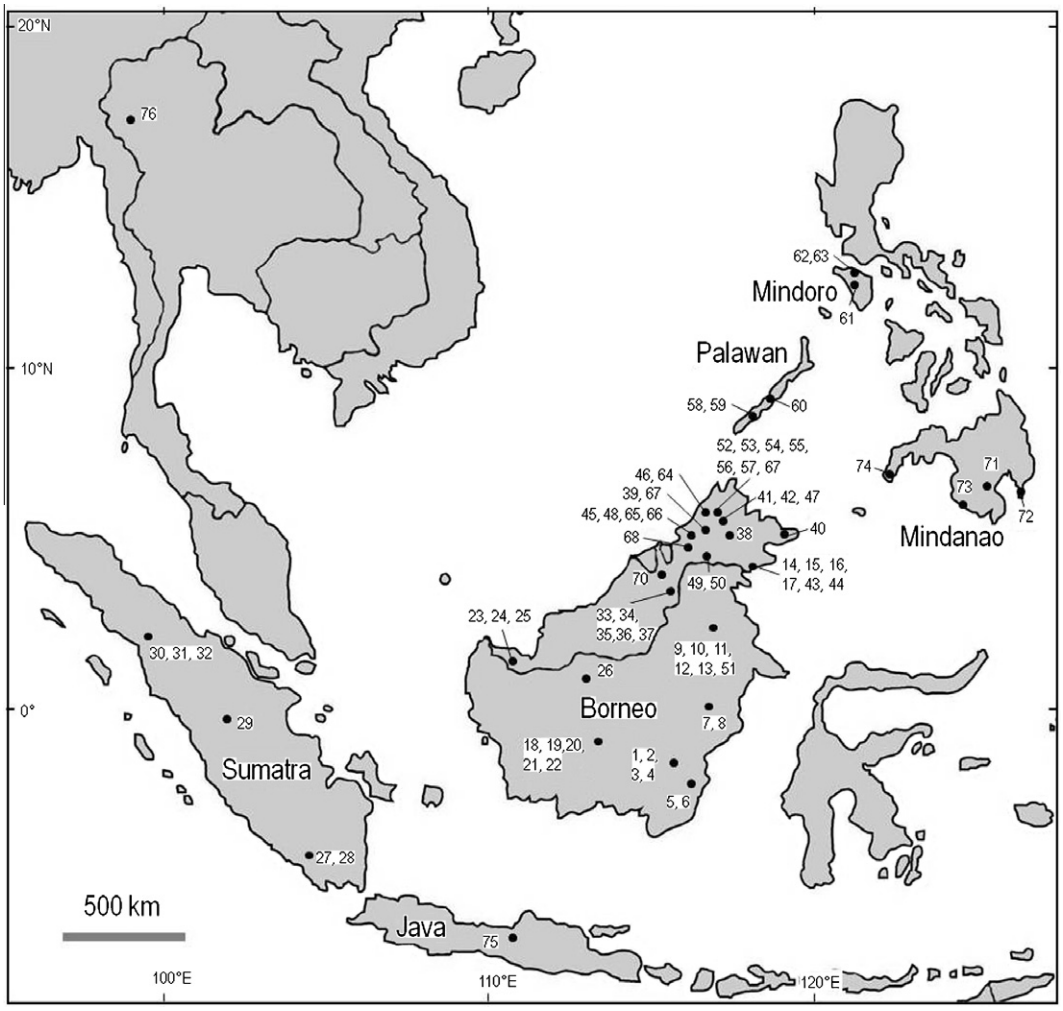

Fig. 2. Map of Southeast Asia showing sampling localities of *Leptobrachium* included in this study. Sample numbers are included in Table 1.