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Phylogeny and phylogeography of *Mantophryne* (Anura: Microhylidae) reveals cryptic diversity in New Guinea

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ABSTRACT

New Guinea is one of five high biodiversity wilderness areas, and frog diversity is exceptionally large, with more than 400 species described to date. The microhylid frog genus *Mantophryne* is endemic to New Guinea and consists of four species, three of which have narrow geographic distributions and a fourth, *M. lateralis*, with a broad range that spans the eastern half of the island. Here, we sequence 104 *Mantophryne* samples for three mitochondrial and three nuclear loci to reconstruct the first phylogeny of the genus and to examine spatial patterns of diversity within *M. lateralis*. Results indicate that the wide-ranging *M. lateralis* is composed of at least nine geographically separated and well-supported lineages that represent putative species. Biogeographic analysis suggests that *Mantophryne* evolved on the eastern Papuan peninsula with subsequent dispersal westward, as well as overwater dispersal events to the Louisiade and D'Entrecasteaux archipelagos.

1. Introduction

New Guinea is the world's largest and highest tropical island and is one of the most biodiverse regions on the planet (Beehler, 1993; Dinerstein and Wikramanayake, 1993; Myers et al., 2000). The complex geological history of New Guinea has played a major role in the high level of biodiversity, especially in reptiles and amphibians (Heads, 2002). Relatively recent tectonic activity, including island accretion, montane uplift, and volcanism, have created a complex landscape with extreme habitat heterogeneity and topographic relief along a steep elevational gradient from sea-level to over 5000 m. Accretion of the Inner and Outer Melanesian Island Arcs onto the Australian plate formed the central mountain range approximately 5–10 million years ago (Pigram and Davies, 1987; Abbott et al., 1994; Allison, 1996; Hall, 1997; Tregoning et al., 1999; Heads, 2002).

The frog diversity on New Guinea represents approximately 8% of global diversity, despite accounting for only about 0.6% of global land area. However, frog biodiversity is extremely underestimated; species accumulation curves demonstrate that the true number on New Guinea is likely double the current 410 described species (Allison, 1996; Austin et al., 2008; Allison et al., 2010). If this prediction is correct, it would mean that New Guinea would represent an astonishing biogeographic focal center of frog diversity. The family Microhylidae is the most diverse group of frogs in New Guinea with over 218 described species representing 53% of New Guinean amphibian diversity, almost all species being endemic (Allison et al., 2010).

Based predominantly on call structure and subtle differences in morphology, Günther recently described six new species from the previously monotypic microhylid *Hylophorbus*, showing that the widespread "*H. rufescens*" is actually composed of multiple geographically separated species (Günther, 2001). However, only a handful of populations in the westernmost portion of the range were examined and *Hylophorbus* also extends into the eastern part of New Guinea. Another microhylid frog with a similarly broad range is *Mantophryne lateralis*, which occurs throughout the lowlands of eastern New Guinea (Boulenger, 1897; Zweifel, 1972; Burton, 1986; Menzies, 2006). This broad range is concordant with *Hylophorbus* and with many other amphibian species and makes *M. lateralis* a good candidate for examining patterns of genetic diversity in eastern New Guinea. *Mantophryne* currently includes only three other species, all with restricted geographic ranges (Fig. 1): *M. axanthogaster* (Sudest Island) (Kraus and Allison, 2009); *M. infulata* (Arau and the Huon Peninsula) (Zweifel, 1972; Burton, 1986); and *M. louisiadensis* (Rossel Island) (Parker, 1934; Zweifel, 1972; Burton, 1986).

Here we use data from multiple loci to reconstruct the phylogeny of the genus *Mantophryne* in order to examine spatial and topological patterns of diversity. In addition, we conduct a biogeographic analysis using ancestral reconstructions to decipher areas

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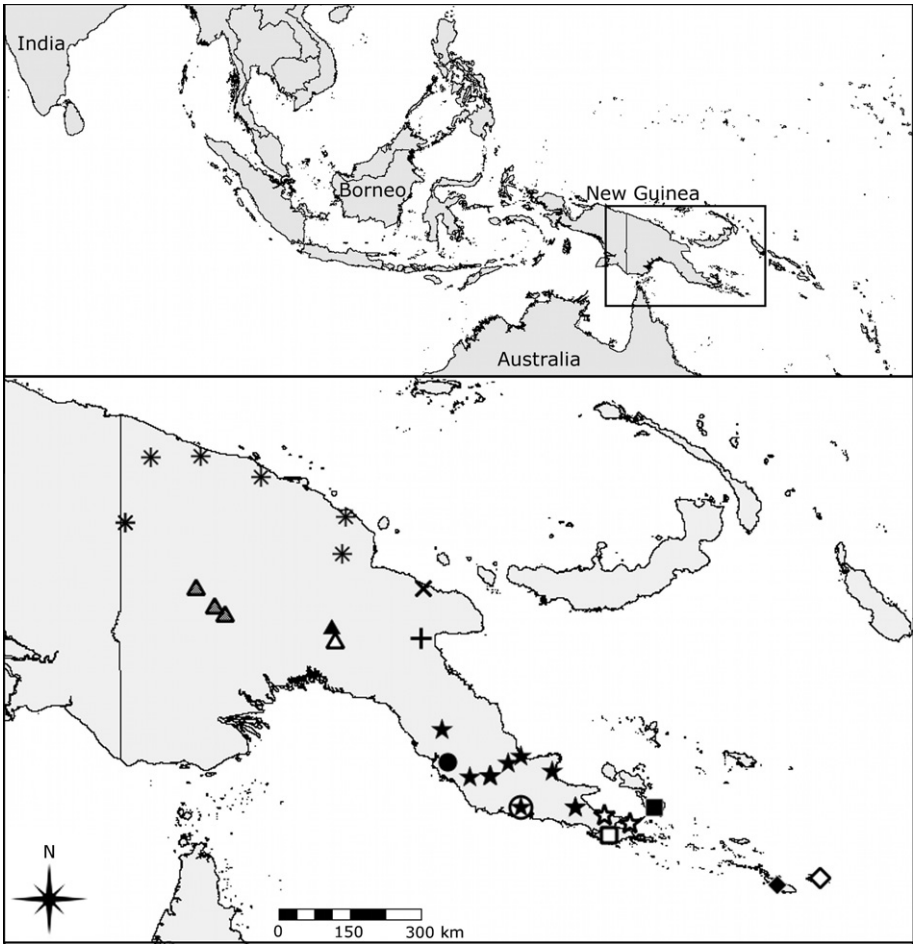

Fig. 1. Distribution map of all putative *Mantophryne* putative species. Symbols correspond to those in Fig. 3. Two species occur in Amau, indicated by an open circle and a closed star.

of origin and patterns of dispersal. Our analyses, while confined to a single genus, may provide a template for examining cryptic diversity in other frogs in New Guinea.

2. Materials and methods

2.1. Sampling

A total of 104 ingroup samples were examined, representing all four described species of *Mantophryne*: 94 samples spanning the distribution of *M. lateralis*, four *M. louisiadensis*, five *M. axanthogaster*, and one *M. infulata* (Fig. 1). To test the monophyly of *Mantophryne*, we also included 24 samples representing four described and multiple undescribed species of *Hylophorbus*, one sample of the monotypic genus *Pherohapsis* (*P. menziesi*) and eight samples of a putative new species of *Mantophryne* from Amau Village, Central Province, Papua New Guinea. Based on higher-level asterophryine phylogenetics (Köhler and Günther, 2008), we also included outgroup samples of the genera *Austrochaperina, Callulops*, *Choerophryne*, and *Sphenophryne*. Voucher numbers and collection localities are provided in Supplementary Appendix 1.

2.2. DNA isolation, amplification, and sequencing

Whole genomic DNA was extracted from liver tissue using the Qiagen DNeasy Blood and Tissue Kit (Valencia, California, USA). Three mitochondrial gene regions (*12S*, *16S*, and *cytochrome b*) and three nuclear loci (*Tyrosinase, c-myc* exon 2, and *c-myc* exon 3) were selected based on their utility in previous studies that included microhylid frogs and sequenced using previously published primers (Table 1) (Richards and Moore, 1996; Bossuyt and Milinkovitch, 2000; Wiens et al., 2005; Köhler and Günther, 2008). Target gene regions were amplified and as in Austin et al. (2010a,b) and purified as in Austin et al. (2011). Purified amplicons were then cycle sequenced in both directions with BigDye v. 3.1 Terminator Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using previously published protocols and sequenced on an ABI 3100 automated capillary sequencer (Austin et al., 2010a,b).

Complementary strands were assembled and visually edited in Sequencher v4.7 (Gene Codes Corp., Ann Arbor, MI, USA) and aligned in ClustalX2 (Larkin et al., 2007). The number of variable sites for each locus was computed using Mega 5.0 (Tamura et al., 2011). All sequences were deposited in GenBank; accession numbers are available in the Supplementary Appendix 1.

2.3. Phylogenetic analysis

To estimate phylogenetic relationships, Bayesian inference, maximum likelihood (ML), and maximum parsimony (MP) were implemented for individual loci as well as for a concatenated dataset of all six loci. Maximum parsimony analyses were conducted in PAUP* ver.4.0b10 (Swofford, 2003) using PAUPrat (Sikes and Lewis, 2001) to implement the parsimony ratchet (Nixon, 1999). Fifty replicates of 5000 ratchet iterations and 20% character perturbation