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Molecular, morphological and osteological differentiation of a new species of microhylid frog of the genus *Stumpffia* from northwestern Madagascar

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Abstract

We describe a new small-sized microhylid frog of the genus *Stumpffia* and provide a preliminary survey of *Stumpffia* diversity in northern and northwestern Madagascar based on molecular and morphological data. Analysis of 68 previously published and 142 newly generated DNA sequences of a fragment of the mitochondrial 16S rRNA gene revealed a large genetic diversity within *Stumpffia*, with numerous strongly differentiated lineages occurring across Madagascar. The analysis confirmed a clade formed exclusively by *Stumpffia* species occurring in north/northwestern Madagascar and containing almost all species from this area. Based on DNA sequences of the RAG1 gene, we further assess that most of the deep mitochondrial lineages do not share haplotypes in this nuclear marker, even in the few cases of sympatry, which we interpret as confirmation of their status as independent evolutionary lineages. Micro-computed tomography revealed subtle differences in phalangeal formulae among some of these candidate species, providing additional taxonomic characters for future revisions of the genus. The newly described species *Stumpffia analamaina* **sp. nov.** occurs 27 km north-west of Antsohihy and is at present the southernmost confirmed *Stumpffia* of the north/northwestern clade. It is mainly characterized by a snout-vent length of 10–12 mm, advertisement calls consisting of a series of short melodious chirps, manus with four fingers and pes with five toes, with only slight length reduction of first finger and toe. It furthermore does not share haplotypes with any other *Stumpffia* species in 16S or RAG1, and has relatively longer hands and feet compared with other small-sized *Stumpffia* of the north/northwestern clade such as *S. madagascariensis* and *S. pygmaea.* The new species lives in an area of dry forest where it was found in leaf litter along a small stream bordered by degraded gallery vegetation. Due to the limited distributions of almost all species in the north/northwestern *Stumpffia* clade, the rareness of appropriate microhabitat around the type locality of *S. analamaina* **sp. nov.** and the rampant habitat destruction in this part of Madagascar, we propose a threat status of Critically Endangered for this new species.

Key words: Amphibia, Anura, Microhylidae, Cophylinae, *Stumpffia analamaina* **sp. nov.**, Computed Tomography, osteology, molecular phylogeny

Introduction

The fauna of Madagascar is characterized by a remarkable degree of microendemism, that is, species restricted to very small distributional ranges (e.g., Goodman & Benstead 2003; Wilmé *et al.* 2006; Wollenberg *et al.* 2011; Glaw *et al.* 2012). Whether this pattern is unique to Madagascar or is a more general characteristic also of other tropical regions that have been less intensively studied is still unknown (Vences *et al.* 2009). On the other hand, Madagascar's fauna also includes wide-ranging species with little genetic differentiation across their ranges (e.g., for frogs: Rabemananjara *et al.* 2007; Gehring *et al.* 2012). To understand the mechanisms of diversification in this remarkable biota, it is therefore important to identify the factors leading to restricted ranges in some species but not

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