



Unexpectedly high levels of lineage diversity in Sundaland puddle frogs (Dicroglossidae: *Occidozyga* Kuhl and van Hasselt, 1822)

Jana M. Flury^{a,h}, Alexander Haas^b, Rafe M. Brown^c, Indraneil Das^d, Pui Yong Min^d, Kueh Boon-Hee^e, Ulrich Scheidt^f, Djoko T. Iskandar^g, André Jankowski^b, Stefan T. Hertwig^{h,i,*}

^a Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany

^b Centrum für Naturkunde, Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

^c Department of Ecology and Evolutionary Biology, Biodiversity Institute, University of Kansas, 1345 Jayhawk Blvd, Dyche Hall, Lawrence, KS 66045, USA

^d Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

^e Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

^f Naturkundemuseum Erfurt, Große Arche 14, 99084 Erfurt, Germany

^g School of Life Sciences and Technology, Institut Teknologi Bandung, Jalan Ganesa 10, Bandung 40132, Indonesia

^h Naturhistorisches Museum der Burgergemeinde Bern, Bernstrasse 15, 3005 Bern, Switzerland

ⁱ University of Bern, Institute of Ecology and Evolution, Baltzerstrasse 6, 3006 Bern, Switzerland

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ABSTRACT

One of the most urgent contemporary tasks for taxonomists and evolutionary biologists is to estimate the number of species on earth. Recording alpha diversity is crucial for protecting biodiversity, especially in areas of elevated species richness, which coincide geographically with increased anthropogenic environmental pressures - the world's so-called biodiversity hotspots. Although the distribution of Puddle frogs of the genus *Occidozyga* in South and Southeast Asia includes five biodiversity hotspots, the available data on phylogeny, species diversity, and biogeography are surprisingly patchy. Samples analyzed in this study were collected throughout Southeast Asia, with a primary focus on Sundaland and the Philippines. A mitochondrial gene region comprising ~ 2000 bp of 12S and 16S rRNA with intervening tRNA Valine and three nuclear loci (*BDNF*, *NTF3*, *POMC*) were analyzed to obtain a robust, time-calibrated phylogenetic hypothesis. We found a surprisingly high level of genetic diversity within *Occidozyga*, based on uncorrected p-distance values corroborated by species delimitation analyses. This extensive genetic diversity revealed 29 evolutionary lineages, defined by the > 5% uncorrected p-distance criterion for the 16S rRNA gene, suggesting that species diversity in this clade of phenotypically homogeneous forms probably has been underestimated. The comparison with results of other anuran groups leads to the assumption that anuran species diversity could still be substantially underestimated in Southeast Asia in general. Many genetically divergent lineages of frogs are phenotypically similar, indicating a tendency towards extensive morphological conservatism. We present a biogeographic reconstruction of the colonization of Sundaland and nearby islands which, together with our temporal framework, suggests that lineage diversification centered on the landmasses of the northern Sunda Shelf. This remarkably genetically structured group of amphibians could represent an exceptional case for future studies of geographical structure and diversification in a widespread anuran clade spanning some of the most pronounced geographical barriers on the planet (e.g., Wallace's Line). Studies considering gene flow, morphology, ecological and bioacoustic data are needed to answer these questions and to test whether observed diversity of Puddle frog lineages warrants taxonomic recognition.

1. Introduction

Our knowledge regarding species diversity (Larsen et al., 2017; Mora et al., 2011; Myers et al., 2000) and underlying evolutionary

processes (de Queiroz 1998; Mayr 1947) continues to expand in many groups of organisms, including “well-studied” terrestrial vertebrates (Cozzuol et al., 2013; Geissmann et al., 2011; Nater et al., 2017; Welton et al., 2010). Over the past 15 years, an average of 155 species

* Corresponding author.

E-mail address: stefan.hertwig@iee.unibe.ch (S.T. Hertwig).

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