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The radiation of microhylid frogs (Amphibia: Anura) on New Guinea: A mitochondrial phylogeny reveals parallel evolution of morphological and life history traits and disproves the current morphology-based classification

Frank Köhler *, Rainer Günther

Museum für Naturkunde, Humboldt-Universität, Invalidenstr. 43, D-10115 Berlin, Germany

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Abstract

Microhylidae account for the majority of frog species on New Guinea and have evolved an extraordinarily wide range of ecological, behavioural, and morphological traits. Several species are known for their unique paternal care behaviour, which includes guarding of clutches in some and additional froglet transport in other species. We sampled 48 out of 215 New Guinean microhylid species and all but two (*Mantophryne* and *Pherohapsis*) of 18 New Guinean genera and analysed a concatenated data set of partial sequences of the mitochondrial genes 12S and 16S, which comprises 1220 aligned nucleotide positions, in order to infer the phylogenetic relationships within this diverse group of frogs. The trees do provide resolution at shallow, but not at deep branches. Monophyly is rejected for the genera *Callulops*, *Liophryne*, *Austrochaperina*, *Copiula*, and *Cophixalus* as currently recognized. Six clades are well supported: (1) *Hylophorbus* and *Callulops* cf. *robustus*, (2) its sister taxon comprising *Xenorhina*, *Asterophrys turpicola*, and *Callulops* except for *C. cf. robustus*, (3) *Liophryne rhododactyla*, *L. dentata*, *Oxydactyla crassa*, and *Sphenophryne cornuta*, (4) *Copiula* and *Austrochaperina*, (5) *Barygenys exsul*, *Cophixalus* spp., and *Oreophryne*, (6) *Cophixalus sphagnicola*, *Albericus laurini*, and *Choerophryne*. The phylogenies provide evidence for the parallel evolution of parental care modes, life styles, and morphological traits that have thus far been emphasized in recent classifications.

Keywords: Microhylidae; Asterophryinae; Systematics; Parallelism; Paternal care; Life style

1. Introduction

Microhylidae represent one of five families of native New Guinean anurans, but account for the majority of frog species on this landmass and its satellite islands. Current treatments recognize 18 microhylid genera, of which only 4–5 are not endemic in New Guinea (Frost, 2007). This classification rests, however, exclusively on morphological and behavioural characters (Zweifel, 1972, 2000; Burton, 1986; Zweifel et al., 2003, 2005; Menzies, 2006). Because New Guinean microhylids have evolved an extraordinarily wide range of ecological and morphological adaptations in association with various life styles from burrowing in the ground to dwelling in canopy habitats, it remains doubtful if the morphology-based classification truly reflects their phylogenetic relationships. In other anuran groups it was found that morphological characters are particularly prone to homoplasy when they are associated with the possession of distinct life styles that may have been acquired in parallel (Emerson, 1986; Bossuyt and Milinkovitch, 2000). In addition, purely morphology-based classifications of amphibians have frequently been misled by plesiomorphic traits, such as in salamanders (Wiens et al., 2005) or gymn-

* Corresponding author. Fax: +49 30 20938565.
E-mail address: frank.koehler@rz.hu-berlin.de (F. Köhler).

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ophionans (San Mauro et al., 2004). Wake (1991) discussed design limitations in amphibians as one possible reason for this phenomenon.

Australopapuan microhylid frogs are of special interest because they possess unique and derived forms of reproduction. All species develop directly from eggs into four-limbed froglets, skipping the aquatic tadpole stage. Many species deposit their eggs either in holes in the ground, among leaf litter, in funnels of epiphytes, or attach their clutch to leaves (Zweifel, 1972; Bickford, 2002; Günther, 2006). In addition, several species developed parental care. Simpler forms involve guarding of terrestrial or arboreal clutches in species of the genera *Oreophryne*, *Callulops*, *Cophixalus*, *Hylophorbus*, and *Xenorhina* (Simon, 1983; Price, 1992; Johnston and Richards, 1993; Günther, 2006), while in some remarkable cases hatchlings are carried thereafter by their father (Günther et al., 2001; Bickford, 2002, 2004; Günther, 2006). Froglet transport was reported from several species, such as *Oreophryne* cf. *wapoga*, *Sphenophryne cornuta*, *Aphantophryne pansa*, *Liophryne schlaginhaufeni*, *Callulops pullifer*, as recently reviewed by Günther (2006). Our knowledge of the mating behaviour and reproductive strategies of Papuan microhylids is, however, still sketchy and our understanding of the factors that drive evolution of parental care remains unsatisfactory. In addition, hypotheses of the evolution of different behavioural and morphological traits suffer badly from the absence of a well-resolved phylogeny of this neglected group.

Bickford (2004) suggested that microhabitat-specific selection pressures cause the evolution and maintenance of parental care in these frogs. However, we do not attribute the evolution of parental care in the New Guinean Microhylidae to the habitat alone. Froglet transport is known from several New Guinean species that are apparently not closely related to each other. Therefore, we hypothesize that parental care has evolved several times in parallel. This implies that as well as environmental factors, certain intrinsic factors inherent to all or most Australopapuan microhylids are also important. The identity of these factors, however, remains equivocal.

It is the goal of the present study to uncover the phylogenetic relationships among the New Guinean Microhylidae by analysing mtDNA trees that are based on a concatenated data set of partial sequences of the ribosomal genes 12S and 16S. In particular, we want to evaluate the value of certain morphological and behavioural traits with regard to their suitability for the delimitation of taxa and to scrutinize whether the current morphology-based classification also receives support from a molecular perspective. In addition, we address the question of whether certain modes of parental care are indeed randomly distributed across the phylogenetic tree, as suggested by the current systematics, or if the development of particular strategies is perhaps restricted to certain (as yet unrecognized?) lineages. Answering this question will help to understand the evolutionary mechanisms that led to the development of these remarkable behaviours.

2. Materials and methods

2.1. Examined material

The study is based on specimens collected at various localities in the Indonesian part of New Guinea (Papua Province) between 1997 and 2003 by Rainer Günther (details on localities and circumstances in Günther, 2001, 2002; Günther and Richards, 2005; Günther and Knop, 2006). At present the specimens are housed in the Herpetological Collection of the Museum für Naturkunde, Humboldt-Universität, Berlin (ZMB). Additional tissue samples were obtained from Fred Kraus (Bishop Museum, Honolulu) and Stephen Richards (University of Adelaide). This data set of our own sequences was complemented by sequences obtained from GenBank (Table A.1, Appendix). The use of taxonomical names follows the classification suggested by Frost (2007).

2.2. Codens of museum repositories and field codes

ABTC—Australian Biological Tissue Collection, South Australian Museum, Adelaide; AMCC—Ambrose Monell Cryo-Collection, American Museum of Natural History, New York; AMNH—American Museum of Natural History, New York; AMS—Australian Museum, Sydney; ATH—Andrew T. Holycross field series; BPBM—Bishop Museum, Honolulu; CFBH-T—Célio F.B. Haddad tissue collection; CMNH—Cincinnati Museum of Natural History; FK—Fred Kraus collection field numbers; FMNH—Field Museum, Chicago; RdS—Rafael de Sá collection; RG—Rainer Günther collection field numbers; SR—Stephen Richards collection field numbers; TNHC—Texas Natural History Collections, Texas Memorial Museum, Austin; USNM—United States National Museum, Smithsonian Institution, Washington DC; ZMB—Museum für Naturkunde, Humboldt University, Berlin.

2.3. DNA isolation and sequencing

Pieces of muscle tissue taken from specimens in the field were preserved in 75% ethanol. DNA was extracted from tissues that were soaked in water overnight, dried, and macerated in 300 μl lysis buffer containing 10 μl Proteinase K. This solution was incubated for 4 h at 60 °C. Total DNA was extracted by use of a Qiagen DNA extraction kit following the standard protocol for animal tissues. PCR amplifications were conducted in 25 μl volumes containing 1× PCR buffer, 200 μM each dNTP, 2.0 mM $MgCl_2$, 0.5 μM each Primer, 1.25 U of Taq polymerase (Invitek), and approximately 50 ng of DNA.