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A previously unrecognized radiation of ranid frogs in Southern Africa revealed by nuclear and mitochondrial DNA sequences

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Abstract

In sub-Saharan Africa, amphibians are represented by a large number of endemic frog genera and species of incompletely clarified phylogenetic relationships. This applies especially to African frogs of the family Ranidae. We provide a molecular phylogenetic hypothesis for ranids, including 11 of the 12 African endemic genera. Analysis of nuclear (*rag-1*, *rag-2*, and *rhodopsin* genes) and mitochondrial markers (12S and 16S ribosomal RNA genes) provide evidence for an endemic clade of African genera of high morphological and ecological diversity thus far assigned to up to five different subfamilies: *Afrana, Cacosternum, Natalobatrachus, Pyxicephalus, Strongylopus*, and *Tomopterna*. This clade has its highest species diversity in southern Africa, suggesting a possible biogeographic connection with the Cape Floral Region. Bayesian estimates of divergence times place the initial diversification of the southern African ranid clade at ~62–85 million years ago, concurrent with the onset of the radiation of Afrotherian mammals. These and other African ranids (*Conraua, Petropedetes, Phrynobatrachus, and Ptychadena*) are placed basally within the Ranoidae with respect to the Eurasian groups, which suggests an African origin for this whole epifamily.

Keywords: Amphibia; Ranidae; Cacosterninae; Petropedetinae; Pyxicephalinae; Raninae; Tomopterninae; Molecular phylogeny; Conservation priorities; Biodiversity hotspot; Africa

1. Introduction

The recent report of the Global Amphibian Assessment project (Stuart et al., 2004) shows that at least a disturbing 42% of amphibian species are experiencing declines, in large part due to still unknown processes. In some cases entire diverse clades of frogs are heavily declining (Lötters et al., 2004). Such non-random extinctions can lead to a severe loss of evolutionary history (Purvis et al., 2000) and a reliable phylogeny of all amphibians is needed to identify them. In several very species-rich cosmopolitan groups of frogs the phylogenetic relationships are still insufficiently known. This lack of a robust phylogenetic hypothesis is especially true for the family Ranidae or True Frogs that contains over 700 species, which are distributed throughout the world. A single genus (*Rana*) is thought to occur on all continents except Antarctica. Yet the phylogenetic relationships among *Rana*, and ranids in general, are largely uncharted (Emerson et al., 2000b). Recent molecular studies have provided important progress in the understanding of ranids and their related groups (Bossuyt and Milinkovitch, 2000; Hoegg et al., 2004; Van der Meijden et al., 2004; Vences et al., 2003b). Some studies have identified India as a reservoir of ancient ranid lineages, and proposed these animals as a model for "Out of India" dispersal of vertebrates (Bossuyt and Milinkovitch, 2001; Roelants et al., 2004). These works demonstrated

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the potential of ranids to decipher general patterns of biogeography and diversification although only a part of the currently recognized ranid diversity has been studied so far. Because most of the endemic African ranid genera are still unstudied from a molecular perspective the biogeographical insights remain incomplete.

Despite recent compelling evidence for the ability for transoceanic dispersal in amphibians (Hedges et al., 1992; Vences et al., 2003b, 2004), there is little doubt that continental drift has had a major influence in shaping their current distribution and phylogeny. The close relationships of the recently discovered *Nasikabatrachus* from India with *Nesomantis* from the Seychelles strikingly demonstrated the importance of the Gondwanan breakup for the vicariance biogeography and hence phylogeny of these basal Neobatrachian frogs (Ranoidei sensu Sokol, 1977). Africa is generally seen as the place of origin for the current distribution of frogs in the superfamily Ranoidea (Biju and Bossuyt, 2003; Feller and Hedges, 1998; Savage, 1973), and one of its subclades, the Arthroleptoidae (Fig. 1), is endemic to this continent (with a few species in Madagascar and on the Seychelles).

Africa is renowned for several endemic radiations such as the Afrotherian mammals (Springer et al., 1997) and the haplochromine cichlid fishes (Verheyen et al., 2003). Africa was united with South America, Australia, Antarctica, India, and Madagascar in the supercontinent Gondwanaland until the end of the late Jurassic. After the breakup of Gondwanaland, Africa remained isolated until it connected with Eurasia. The India–Seychelles–Madagascar plate broke off from Africa 158 to 160 million years ago[1] (mya), and Greater India started to drift northwards across the Indian Ocean about 96–84 mya (Briggs, 2003). The India–Madagascar plate has been suggested as possible biogeographic origin of Asian ranoid subclades (Duellman and Trueb, 1986; Bossuyt and Milinkovitch, 2001), i.e., the Rhacophoridae and at least part of the Ranidae.

Land bridges that connected Africa with Eurasia after its long isolation from other continents allowed Eurasian faunal elements to disperse into Africa, including several ranoid representatives. Species (1) of the dicroglossine genus *Hoplobatrachus*, (2) of the ranine lineage containing the genera/subgenera *Rana* and *Amnirana*, and (3) the rhacophorid genus *Chiromantis* have dispersed into Africa from Eurasia (Kosuch et al., 2001; Vences et al., 2003b). Currently, 21 ranid genera are restricted in their distribution to Africa, most of which are limited to sub-Saharan Africa.

By analyzing nuclear and mitochondrial DNA sequences of representatives of all but one subfamily of ranids we here provide the first inclusive molecular phylogeny of ranid relationships. Our data provide compelling evidence for a deep evolutionary history of many African endemic ranid groups and, unexpectedly, uncover an endemic radiation that includes taxa that had so far been classified into up to five different subfamilies.

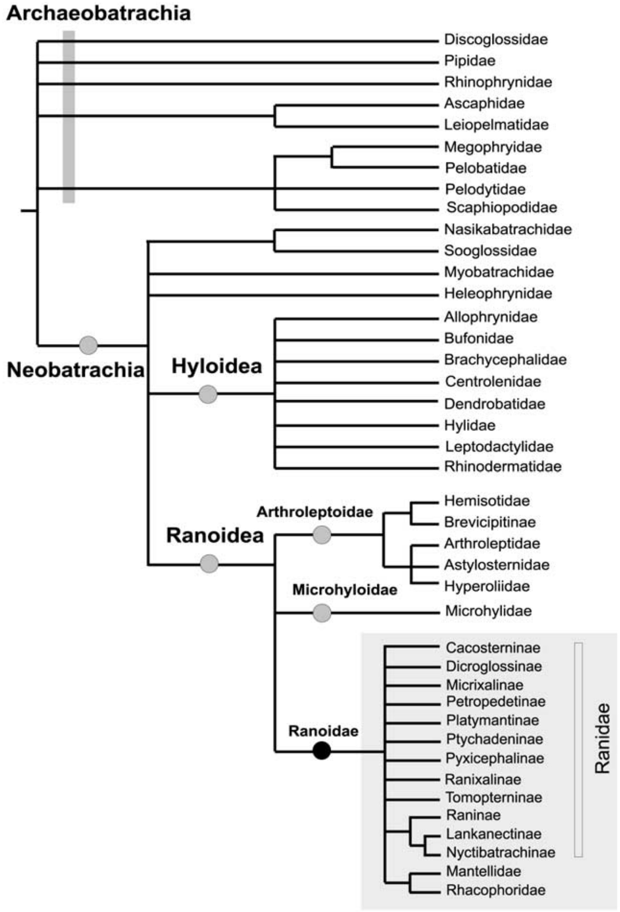

Fig. 1. Schematic representation of the classification of ranids and their phylogenetic position among frogs following Vences and Glaw (2001), with some additions from Dubois (1992) and Blommers-Schlösser (1993), and with modifications from the trees of Biju and Bossuyt (2003), Dubois (2003), Hoegg et al. (2004), Roelants et al. (2004), Van der Meijden et al. (2004), and own unpublished data: the family Ranidae is a paraphyletic assemblage that together with the Mantellidae and Rhacophoridae forms the epifamily Ranoidae. Together with two other epifamilies (the Arthroleptoidae and Microhyloidae) they form the superfamily Ranoidea in the Neobatrachia. The familial scheme used here includes Bombinatoridae in Discoglossidae, and Limnonastidae and Rheobatrachidae in Myobatrachidae.

2. Materials and methods

2.1. Taxonomy and selection of taxa

Duellman and Trueb's (1986) characterization of ranid systematics being 'in a state of chaos' has been heavily quoted but the situation has not much improved

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[1] *Abbreviations used:* mya, million years ago; MP, maximum parsimony; ML, maximum likelihood; BI, Bayesian Inference; NJ, neighbour joining.