

Multiple overseas dispersal in amphibians

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Amphibians are thought to be unable to disperse over ocean barriers because they do not tolerate the osmotic stress of salt water. Their distribution patterns have therefore generally been explained by vicariance biogeography. Here, we present compelling evidence for overseas dispersal of frogs in the Indian Ocean region based on the discovery of two endemic species on Mayotte. This island belongs to the Comoro archipelago, which is entirely volcanic and surrounded by sea depths of more than 3500 m. This constitutes the first observation of endemic amphibians on oceanic islands that did not have any past physical contact to other land masses. The two species of frogs had previously been thought to be non-endemic and introduced from Madagascar, but clearly represent new species based on their morphological and genetic differentiation. They belong to the genera *Mantidactylus* and *Boophis* in the family Mantellidae that is otherwise restricted to Madagascar, and are distinguished by morphology and mitochondrial and nuclear DNA sequences from mantellid species occurring in Madagascar. This discovery permits us to update and test molecular clocks for frogs distributed in this region. The new calibrations are in agreement with previous rate estimates and indicate two further Cenozoic transmarine dispersal events that had previously been interpreted as vicariance: hyperoliid frogs from Africa to Madagascar (*Heterixalus*) and from Madagascar to the Seychelles islands (*Tachycnemis*). Our results provide the strongest evidence so far that overseas dispersal of amphibians exists and is no rare exception, although vicariance certainly retains much of its importance in explaining amphibian biogeography.

Keywords: Amphibia; Mantellidae; Madagascar; Comoros; phylogeny; biogeography

1. INTRODUCTION

Amphibians are a key group in historical biogeography because they are often thought to be unable to disperse over saltwater barriers (Duellman & Trueb 1986; Meirte 1999; Bossuyt & Milinkovitch 2001; Inger & Voris 2001; Brown & Guttman 2002). They are well known to be extremely sensitive to osmotic stress and do not survive in salt water, although some species of frogs tolerate or partially inhabit brackish water (Balinsky 1981). Therefore, amphibians are considered to be excellent models for vicariance scenarios as explanation for general biogeographic patterns, and major biogeographic hypotheses have been influenced by the occurrence of endemic amphibians on islands or continents (Duellman & Trueb 1986; Richards & Moore 1996; Worthy *et al.* 1999; Bossuyt & Milinkovitch 2001; Brown & Guttman 2002). One important argument for such interpretations, ever since Darwin (1859), has been that heretoforth no endemic amphibians were known from oceanic islands. By contrast, reptiles are present on many islands and some are known to be excellent over-water dispersers (Censky *et al.* 1998; Arnold 2000; Schoener *et al.* 2001).

Amphibians are widespread on many archipelagos, for instance on the Philippines and in the Sunda, Caribbean

and Pacific regions. Some of these landmasses were probably below sea-level in the past, and their overseas colonization following emergence has been hypothesized (Hedges *et al.* 1992; Hedges 1999). However, all of these islands are made up at least partly by continental fragments for which past land connections cannot be excluded (Heaney 1985; Whitmore 1987; Crother & Guyer 1996). Geological data are seldom sufficiently definitive to ascertain the full submersion of a landmass, and small emerging remains would be sufficient to harbour relict amphibian populations.

A different situation is that of oceanic islands that never had physical contact to other landmasses. These are of complete volcanic origin or were built by coral reefs. They are surrounded by deep waters that make land connections through fluctuating sea levels impossible, and they are mostly too young to assume vanished connections to drifting continents. No endemic amphibian species are known from truly oceanic islands. Some are populated by non-endemic frogs or salamanders, but these are known or assumed to have been introduced. Such has been thought to be the case for Mayotte, an island belonging to the volcanic Comoro archipelago in the Indian Ocean, located between Africa and Madagascar. Mayotte is separated from Madagascar by a geographical distance of 300 km and by sea depths of more than 3600 m, and its origin dates back no further than 10–15 Myr ago (Emerick & Duncan 1982; Nougier *et al.* 1986). The two frog species known from Mayotte are seen as conspecific with taxa

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from Madagascar and of allochthonous origin (Blommers-Schlösser & Blanc 1991; Meirte 1999).

The evolution and biogeography of the highly diverse, but strongly endangered animal diversity of Madagascar and other islands in the western Indian Ocean have been subject to intense debates in recent years (Krause *et al.* 1997; Murphy & Collier 1997; Jansa *et al.* 1999; Bossuyt & Milinkovitch 2001; Farias *et al.* 2001; Meegaskumbura *et al.* 2002; Raxworthy *et al.* 2002). Hypotheses on the time of the origin of these faunas must largely rely on phylogenies of extant taxa because no terrestrial or freshwater fossils are known from the Tertiary period (65–2 Myr ago) of Madagascar (Krause *et al.* 1997). Deep vicariance has often been invoked to explain the origin of Madagascar's endemic vertebrates (Duellman & Trueb 1986; Richards & Moore 1996; Murphy & Collier 1997; Bossuyt & Milinkovitch 2001; Farias *et al.* 2001): their ancestors supposedly evolved in isolation after the breakup of the southern supercontinent Gondwana. During this geological process, Madagascar had been separated from other landmasses in the Jurassic and Cretaceous (Briggs 2003). Recent phylogenies of chameleons and rodents (Jansa *et al.* 1999; Raxworthy *et al.* 2002), however, proposed area cladograms that are not in accordance with the succession of plate tectonic events. Dispersal scenarios therefore seem plausible for these groups but have not been considered for amphibians, which in the western Indian Ocean region are mostly represented by frogs. Caecilians occur on the Seychelles and continental Africa and Asia, whereas salamanders are completely absent. Except for the enigmatic Seychellean sooglossids, all anurans from the Seychelles and Madagascar are included in the superfamily Ranoidea, a highly diverse group of largely unsolved systematics (Duellman & Trueb 1986; Feller & Hedges 1998; Vences & Glaw 2001).

Here, we report on our recent discovery that the Comoro frogs represent previously undescribed species endemic to Mayotte. We use mitochondrial and nuclear DNA sequences to demonstrate the close phylogenetic relationships of these species to the endemic Malagasy radiation of mantellid frogs, thereby providing evidence for their overseas dispersal from Madagascar. Their origin is further used as a new calibration point of a molecular clock. We thereby contribute to the elucidation of processes of amphibian overseas dispersal in the Indian Ocean region and the origin of Madagascar's enigmatic fauna.

2. MATERIAL AND METHODS

(a) Taxon and gene sampling

To understand (i) the distinctness of the Comoro frogs; (ii) their phylogenetic relationships among mantellids; and (iii) their relationships to other frog groups from the Indian Ocean region, we compiled three datasets that differed in the composition of taxa and of DNA fragments.

- (i) We sequenced a fragment of the mitochondrial 16S rRNA gene from all available mantellid species, usually from several individuals and populations, and assembled sequences from over 250 individuals and 120 species. These data will be presented elsewhere.
- (ii) We sequenced fragments of one nuclear (rhodopsin) and

three mitochondrial (12S and 16S rRNA, tRNA_{val}) gene fragments from representatives of all mantellid genera, subgenera and species groups to resolve the relationships within the family; representatives of two other ranoid families (Rhacophoridae: *Polypedates*; Ranidae: *Rana*) were used as the outgroup.

- (iii) We chose members of major clades of ranoid frogs (Bossuyt & Milinkovitch 2000), including previously unstudied African taxa, and of other families that could be informative regarding biogeographic relationships in the Indian Ocean region: to resolve the relationships among these deep clades, we analysed a more comprehensive dataset including two nuclear (rhodopsin, tyrosinase) and four mitochondrial (12S and 16S rRNA, tRNA_{val}, cytochrome *b*) gene fragments. A salamander and representatives of archaic frogs (families Discoglossidae and Pipidae) were used as hierarchical outgroups.

(b) DNA extraction, amplification and sequencing

DNA was extracted from tissue samples preserved in ethanol and sequenced on ABI 3100 and ABI 377 automated sequencers after direct amplification using primers from previous studies (Palumbi *et al.* 1991; Bossuyt & Milinkovitch 2000; Vences *et al.* 2003) or that were developed for this work (sequences in a 5'–3' direction given only for new primers; F, forward primers; R, reverse primers). Cytochrome *b* (up to 1016 bp): CBJ10933 (F); Cytb-a (F); MVZ15L-mod (F)—AAC TWA TGG CCC MCA CMA TMC GWA A; Cytb-c (R); CytbAR-H-mod (R)—TAW ARG GRT CYT CKA CTG GTT G. Tyrosinase (exon 1; 632 bp): Tyr-1b (F); Tyr-1d (F); Tyr-1a (F); Tyr-F40 (F)—AAR GAR TGY TGY CCI GTI TGG; Tyr-Fx3 (F)—ACT GGC CCA YTG THT TYT ACA AC; Tyr-Fx4 (F)—YTG GCC YWY TGT NTT YTA YAA C; Tyr-1g (R); Tyr-1e (R); Tyr-SPA (R)—GAI GAG AAR AAR GAI GCT GGG CT. Rhodopsin (exon 1; 334 bp): Rhod-ma (F)—AAC GGA ACA GAA GGY CC; Rhod-1a (F); Rhod-md (R)—GTA GCG AAG AAR CCT TC; Rhod-1d (R); Rhod-1c (R). 12S rRNA and tRNA_{val} (ca. 700 bp): 12SA-L (F); 12SB-H (R); 16SR3 (R). 16S rRNA (5' fragment; ca. 650 bp): 16S-L3 (F); 16SA-H (R); 16S rRNA (3' fragment; ca. 550 bp): 16SA-L (F); 16SB-H (R). The molecular dataset was complemented by sequences available from GenBank (see <http://www4.ncbi.nlm.nih.gov/>). The 238 new sequences obtained in this study (12S, 55 sequences; 16S, 93; Cyt *b*, 19; Rhod, 52; Tyr, 19) have been deposited in the GenBank database under the accession numbers AY341580–AY341817.

(c) Phylogenetic analysis

Genes were submitted to separate and combined analyses with PAUP* (Swofford 2002) after exclusion of all gapped and hypervariable regions of the rRNA and tRNA genes and the third positions of cytochrome *b*, which are known to be fully saturated at the level of anuran families (Graybeal 1993). Exploratory analyses including these hypervariable sites did not result in relevant differences of cladogram topologies. Maximum likelihood (ML) heuristic searches with 10 random addition sequence replicates were carried out under the tree bisection–reconnection branch-swapping option, after determining the substitution model for each data subset by hierarchical likelihood ratio tests as implemented in MODELTEST, v. 3.06 (Posada & Crandall 1998). For the among-Mantellidae dataset of the concatenated rhodopsin, 12S rRNA, 16S rRNA and

tRNA_{val} sequences (total number of included base pairs: 1875 bp), a general time-reversible (GTR + I + G) substitution model was selected ($-\ln L = 26025.7305$), with empirical base frequencies (freqA = 0.3551; freqC = 0.2199; freqG = 0.1644; freqT = 0.2607) and substitution rates ([A-C] = 3.7878; [A-G] = 10.5790; [A-T] = 5.7768; [C-G] = 1.1797; [C-T] = 29.2325; [G-T] = 1), a proportion of invariable sites of 0.4042 and a gamma distribution shape parameter of 0.6582. For the higher-level relationship dataset of the concatenated rhodopsin, tyrosinase, cytochrome *b*, 12SrRNA, 16S rRNA, and tRNA_{val} sequences (total number of included base pairs: 2625 bp), a Tamura-Nei (TrN + I + G) substitution model was selected ($-\ln L = 30463.0156$), with empirical base frequencies (freqA = 0.3371; freqC = 0.2527; freqG = 0.1497; freqT = 0.2606) and substitution rates ([A-G] = 3.3874; [C-T] = 5.2170; all other rates = 1), a proportion of invariable sites of 0.2851 and a gamma distribution shape parameter of 0.6848.

Non-parametric ML bootstrapping with 100 full heuristic searches was carried out for the two sets of taxa. Analyses using 2000 replicates under maximum parsimony and neighbour-joining methods resulted in identical topologies and similar bootstrap values. Bayesian posterior probabilities were calculated using MrBAYES, v. 2.01 (Huelsenbeck & Ronquist 2001). In accordance with the selection of complex substitution models by MODELTEST (Posada & Crandall 1998), we set the ML parameters to correspond to the the GTR model with site-specific rate variation (some sites invariant and others following a gamma distribution), and proportion of different base categories estimated from the data. We ran a minimum of 300 000 generations, sampling trees every 10 generations. The initial set of generations needed before convergence on stable likelihood values (burnin) was set at 6–7% based on our own empirical evaluation. Competing phylogenetic hypotheses were tested with non-parametric likelihood tests (Shimodaira & Hasegawa 1999). For details of primers, voucher specimens and phylogenetic methods, see electronic Appendices A–E, available on The Royal Society's Publications Web site.

(d) Molecular clock calibrations

The oldest available age of Mayotte (8.7 Myr ago; Nougier *et al.* 1986) provides a calibration of the age of the split between the Comoroan and Malagasy sister species. In addition to the two Comoro–Madagascar splits, we used the divergence between African and South American representatives of the strictly freshwater aquatic family Pipidae at 101 Myr ago (Pitman *et al.* 1993) as a further calibration point. The origin of lissamphibians has been estimated (Kumar & Hedges 1998) at 360 ± 14 Myr ago; an ancient ordinal divergence of the Lissamphibia is therefore possible.

Age estimates were based entirely on nuclear genes to exclude possible influences of saturation in the mitochondrial dataset. We used two separate approaches.

- (i) The ML tree obtained using the complete dataset was submitted to non-parametric rate smoothing, which minimizes ancestor-descendent local rate changes in the absence of rate constancy (Sanderson 1997). Branch lengths had previously been recalculated based on tyrosinase and rhodopsin sequences only. The age of the root (salamander–frog split) was fixed at 370 Myr ago (age of first fossil tetrapods), but alternative searches with the root at 250 Myr ago (age of first frog ancestor: *Triadobatrachus*) were also performed.

- (ii) Age estimates and 95% prediction confidence intervals of major nodes were calculated by regression analysis. For this second approach we used rhodopsin sequences only, because for this gene, data were also available from three frog species pairs that probably arose by vicariance at the end of the Mediterranean salinity crisis in the Messinian (5.3 Myr ago): *Rana cretensis*–*R. cerigensis*, *Alytes dickhillenii*–*A. maurus*, *Pelobates cultripes*–*P. varaldii*.

We consistently assumed ancient ages for the calibration points (old age of the root; oldest age of Comoro islands; assumption of vicariance for the Mediterranean frogs), although more recent dispersals to Mayotte or over the Mediterranean, or a younger age of the root cannot be excluded. Any possible bias of the calibrations will therefore be directed towards an overestimate of the ages of divergence. This is conservative if young ages of divergence are to be demonstrated herein.

3. RESULTS

(a) Discovery and relationships of Comoro frogs

Our intensive surveys on three Comoro islands (Mayotte, Moheli and Grande Comoro) resulted in amphibian findings on Mayotte only. We encountered two distinct frog species that corresponded to those previously recorded as *Mantidactylus granulatus* (Blommers-Schlösser & Blanc 1991; Meirte 1999) and *Boophis tephraeomystax* (Meirte 1999). However, the former differed distinctly in morphology (e.g. smaller size, larger femoral glands, white single versus blackish paired vocal sac) and advertisement calls from *M. granulatus*, while the latter was larger, had a different iris coloration (reddish versus golden-brownish) and a more granular dorsal skin than *B. tephraeomystax*. Despite these differences, the species were clearly assignable to the genera *Mantidactylus* and *Boophis* of the family Mantellidae (Vences & Glaw 2001), based on femoral glands in the *Mantidactylus* males and overall similarity with *B. tephraeomystax* from Madagascar.

Both Comoro frogs, which will be formally described in a forthcoming paper, were common on Mayotte and inhabited secondary habitats, often close to human settlements. A comparison with morphological data from over 5000 voucher specimens of all nominal mantellid species examined by us, and with mitochondrial haplotypes (16S rRNA) of more than 120 described and undescribed mantellids, confirmed that these species have never been found on Madagascar. Minimum pairwise sequence divergence of the Comoro haplotypes as compared with Malagasy species was 5%.

Phylogenetic relationships of the Comoro frogs were revealed by a molecular phylogeny based on 1875 bp of one nuclear and three mitochondrial genes from 47 representatives of all subgenera and species groups of mantellids (figure 1). The Comoro frogs were deeply nested within the Mantellidae. The cladogram placed them as sister species of the Malagasy *M. wittei* and of the *B. doulioti*/*B. tephraeomystax* clades, supported by maximum bootstrap values and Bayesian posterior probabilities (100%) and by non-parametric likelihood ratio tests of alternative topologies (see electronic Appendix F). A comparison of their 16S rRNA haplotypes with those from five populations of *M. wittei*, four populations of *B. tephraeomystax* and five populations of *B. doulioti*, sampled over

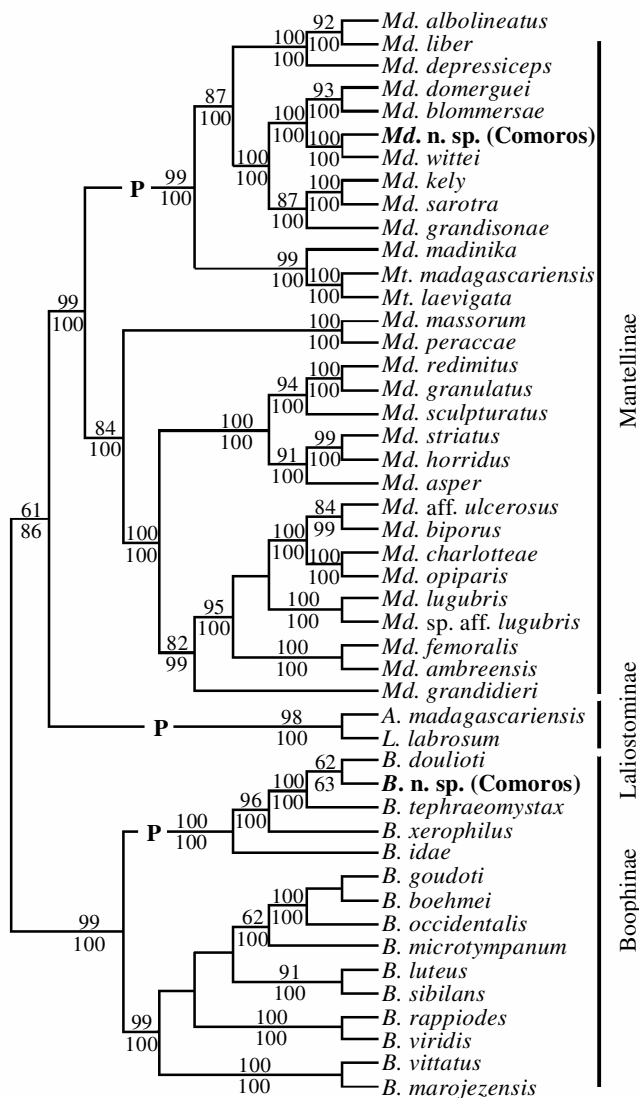


Figure 1. Phylogeny of 47 species of the endemic Malagasy-Comoroan frog family Mantellidae. The tree was obtained by ML analysis of a dataset of 1875 bp of nuclear and mitochondrial genes. Numbers at nodes are results of ML bootstrapping (above, 100 replicates) and Bayesian posterior probabilities (below, 280 000 generations; every tenth generation sampled) in percentages (values below 50% not shown). Genera are abbreviated as follows: *Md*, *Mantidactylus*; *Mt*, *Mantella*; *A*, *Aglyptodactylus*; *L*, *Laliostoma*; *B*, *Boophis*. *Rana temporaria*, *R. temporalis* and *Polypedates cruciger* were used as outgroups. The newly discovered species from Mayotte (Comoros) are printed in bold. The three lineages specialized to reproduction in stagnant water (pond breeders) are marked with a 'P'.

their complete distribution areas (Vences & Glaw 2002), confirmed these relationships. The *Mantidactylus* from Mayotte, furthermore, differed very distinctly from *M. wittei* by advertisement calls, and by a wide array of morphological characters (broader head, longer hands and feet, more strongly enlarged terminal finger disks).

(b) Higher-level relationships and age of Indian Ocean anurans

A phylogeny reconstructed using 2625 bp of two nuclear and four mitochondrial genes (figure 2) corroborated the mantellid clade (Bossuyt & Milinkovitch 2000;

Vences & Glaw 2001), endemic to Madagascar and the Comoros, with five genera that previously (Blommers-Schlösser & Blanc 1991) had been assigned to three different families. The two Seychellean taxa occupied very different positions on the cladogram. *Nesomantis* was the sister group of the two major Neobatrachian lineages, the Hyloidea and Ranoidea (B and C in figure 2; Feller & Hedges 1998), but an alternative position as basal hyloid could not be significantly excluded by likelihood ratio tests. *Tachycnemis* was very closely related to the Malagasy *Heterixalus* in the family Hyperoliidae (Richards & Moore 1996; Vences *et al.* 2003).

The application of non-parametric rate smoothing to branch lengths based on nuclear genes only (figure 2) placed the divergence between the endemic Malagasy taxa (mantellids) and other ranoids into the late Cenozoic. Several relevant splits had much younger ages in the mid- or Late Cenozoic, namely those between Seychellean, Malagasy and African hyperoliids (*Tachycnemis*, *Heterixalus*, *Hyperolius*), and between African and Asian species of the genera *Rana* and *Hoplobatrachus*, and of the family Rhacophoridae (*Chiromantis* and *Polypedates*). These results were corroborated and received statistical significance by the 95% confidence intervals calculated using rhodopsin divergences in a regression analysis (see electronic Appendix G). The resulting rate estimates of 0.03–0.1% rhodopsin divergence lineage⁻¹ Myr⁻¹ corresponded well to a further independent calibration using the synapsid/diapsid split 310 Myr ago (*Homo/Gallus* divergence 0.03% lineage⁻¹ Myr⁻¹) (Kumar & Hedges 1998).

4. DISCUSSION

(a) First evidence for frog overseas dispersal to oceanic islands

The Comoros are entirely volcanic and have never had direct contact to any continental landmass (Nougier *et al.* 1986). Not counting the presently submerged Geyser seamount, Mayotte is the oldest of these islands. Its origin has been estimated at 7.7 ± 1 Myr ago (Nougier *et al.* 1986), while analyses based on the K–Ar method yielded even younger estimates for the oldest shield building volcanism (5.41 ± 0.26 Myr ago; Emerick & Duncan 1982). The lowest historical sea-level in the Indian Ocean (Haq *et al.* 1987) was -145 ± 5 m at 18 400 yr ago (Colonna *et al.* 1996). By analysing British Admiralty nautical charts 2110 and 758 after partially digitalizing and georeferencing them in a geographic information system, we estimate that under these lower sea-levels the Malagasy northwestern coast and Mayotte were still separated by a linear minimum distance of more than 250 km and by ocean depths of more than 3400 m below sea level. Although the Comoros lie on an oceanic ridge, a Cenozoic land connection to Madagascar seems out of the question (Krause *et al.* 1997; Raxworthy *et al.* 2002).

Many new species of frogs have been described from Madagascar in recent years (Vences & Glaw 2001), but most are morphologically close to known species and many were already present in historical collections. Furthermore, these new taxa are usually discovered in primary mid-altitude rainforests. The Comoro frogs inhabit secondary lowland habitats that have been exhaustively surveyed in Madagascar, and the *Mantidactylus* from Mayotte

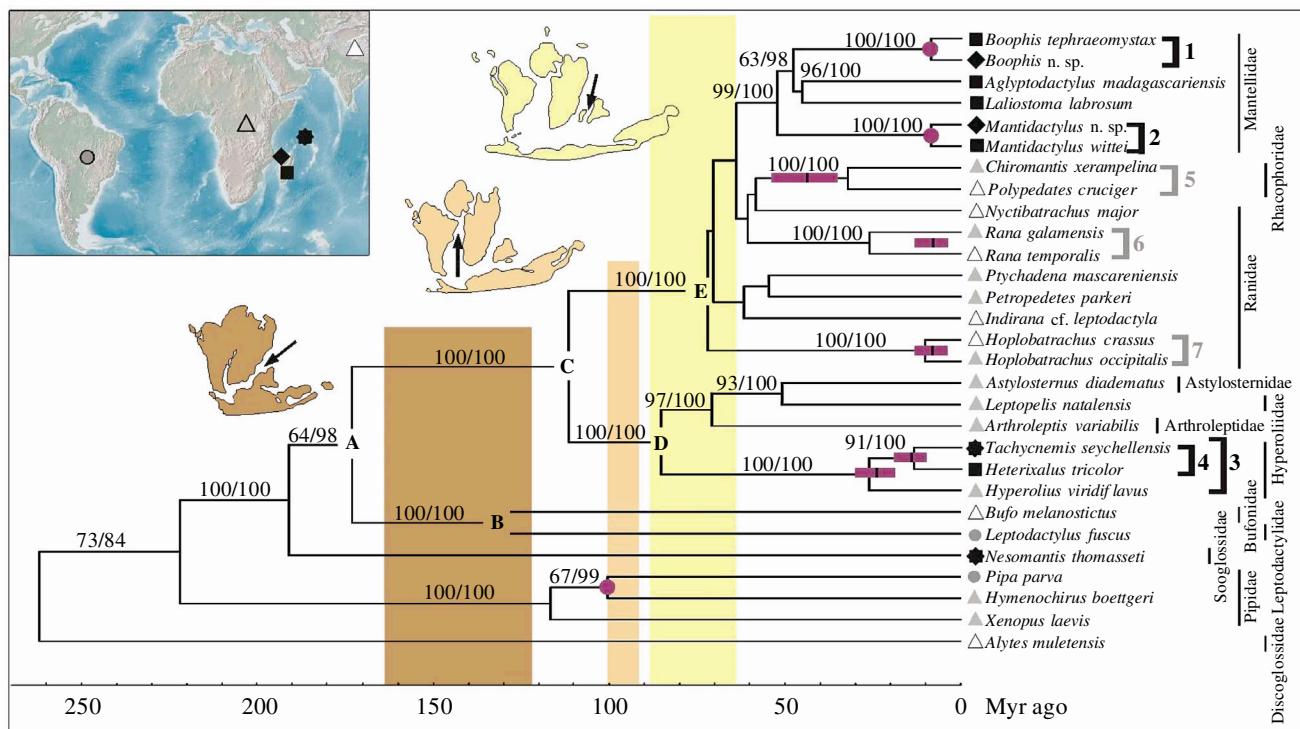


Figure 2. Phylogeny and divergence times of major clades of frogs in the western Indian Ocean region, based on ML analyses of 2625 bp of nuclear and mitochondrial genes. Salamander sequences were used as the outgroup. A phylogram with branch lengths based on nuclear gene divergences only was submitted to non-parametric rate smoothing. The numbers at nodes are results of ML bootstrapping (left, 100 replicates) and Bayesian posterior probabilities (right, 470 000 generations, every tenth generation sampled) in percentages (values below 50% not shown). The mauve horizontal bars show mean and 95% confidence intervals of age estimates of young dispersal events by regression analysis of pairwise divergences of rhodopsin sequences; mauve circles indicate calibrations. The coloured vertical bars encompass the period of separation of Madagascar–Greater India from Africa (Rabinowitz *et al.* 1983), of South America from Africa (Pitman *et al.* 1993) and of Greater India from Madagascar (Storey *et al.* 1995; but see Briggs 2003). Major clades are coded by capital letters: A, Neobatrachia; B, Hyloidea; C, Ranoidea; D, Arthroleptoidei; E, Ranidei. Distribution of taxa on continents is indicated by symbols (see inset map). Cenozoic dispersal events are coded by numbers (black fill, oceanic dispersal): 1, 2, mantellines from Madagascar to Mayotte (Comoros); 3, hyperoliids from Africa to Madagascar; 4, hyperoliids from Madagascar to the Seychelles; 5, rhacophorids from Asia to Africa; 6, 7, ranids from Asia to Africa.

is morphologically distinctive. It is therefore very unlikely that these species also occur on Madagascar but have been so far overlooked. Overseas dispersal remains the only conceivable explanation for the presence of mantellid frogs on Mayotte, and our phylogenetic results demonstrate that two independent dispersal events from Madagascar took place.

Several other examples of putative overseas dispersal in amphibians have been mentioned in the literature. The Philippines harbour a largely endemic frog fauna but were probably entirely submerged during the Oligocene, leaving subsequent dispersal as most probable origin of these amphibians (Heaney 1985; Brown & Lomolino 1998). The same applies to parts of Sulawesi and the Moluccans (Heaney 1985), and endemic frogs are also known from Fiji (Worthy *et al.* 1999). Jamaica, most of Cuba and Puerto Rico were covered by the ocean as well (Buskirk 1985), yet have endemic frog faunas (Hedges 1999). Molecular clock data indicate that most Caribbean islands were colonized by frogs subsequent to their final isolation by the sea (Hedges *et al.* 1992). However, all of these landmasses are fully or partly of continental origin. The Sunda region has been influenced by drifting continental and oceanic material, especially micro-continental blocks, which bear the potential of having shaped much of the

distribution patterns of extant biotas (Whitmore 1987; Moss & Wilson 1998). The Philippines were probably connected to the Asian mainland through land bridges in the Pleistocene (Heaney 1985; Voris 2000). Compelling evidence that Sunda and Philippine amphibians did disperse among islands has been published, but amphibian biogeographers working in these regions usually avoided explicit statements on overseas dispersal (e.g. Inger & Voris 2001; Brown & Guttman 2002). In fact, the complex geological history of these regions makes it difficult to identify unequivocal transmarine dispersal events. Vicariance explanations are also still being put forward to explain the origin of Caribbean (Crother & Guyer 1996) and Pacific amphibians (Worthy *et al.* 1999).

All examples of amphibians on oceanic islands of fully volcanic origin so far refer to non-endemic species of presumed or demonstrated origin by human translocation: *Ptychadena mascareniensis* and *Bufo gutturalis* on Mauritius and Reunion; *Hyla meridionalis* and *Rana perezi* on the Canary archipelago; *Triturus carnifex* on Madeira; *Eleutherodactylus*, *Dendrobates* and *Bufo* species on Hawaii; *Scinax* on Galapagos (Staub 1993; Pleguezuelos 1997; Kraus *et al.* 1999; Snell & Rea 1999). Consequently, our discovery of endemic Comoro frogs is the first instance of endemic amphibian species occurring on fully volcanic and oceanic

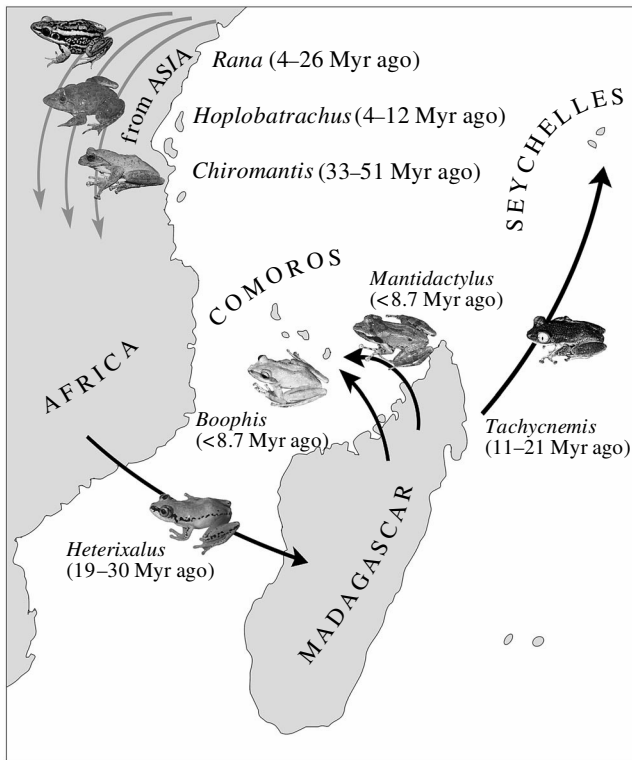


Figure 3. Schematic representation of amphibian dispersal in the western Indian Ocean region, and between Asia and Africa. Age estimate ranges of colonization events are based on combined evidence of regression confidence intervals and non-parametric rate smoothing results, except for the two Comoro colonizations that were used as calibrations. The three Asia–Africa dispersal events (grey arrows) may have benefitted from land connections, while transmarine dispersals must be assumed between Africa, Madagascar, Comoros and the Seychelles.

islands, and thereby the most reliable evidence for overseas dispersal in this vertebrate class.

(b) *Gondwanan versus post-Gondwanan origin of ranoid frogs*

Our molecular clock datings suggest at least two further instances of transmarine frog dispersal in the western Indian Ocean, namely of hyperoliid frogs from Africa to Madagascar (*Heterixalus*), and from Madagascar to the Seychelles (*Tachycnemis*). Genetic divergences between these genera are on the same order of magnitude as those between Comoroan and Malagasy mantellids, and not reconcilable with a Gondwanan vicariance. These dispersal events are estimated to have taken place in the Oligocene and Miocene. In addition, at least three dispersals from Asia to Africa, discussed but not dated by Kosuch *et al.* (2001), also took place in the Tertiary according to our results (figure 3): of rhacophorid treefrogs (*Chiromantis*) in the Eocene, and of *Rana* and *Hoplobatrachus* in the Miocene. However, the latter two taxa probably crossed the Arabian peninsula land connection, whereas the ancestors of *Chiromantis* may have used land bridges in the Tethys sea (Kosuch *et al.* 2001).

Within the Mantellidae, the Comoro frogs are deeply nested in lineages of arboreal or semi-arboreal pond breeders (figure 1), which are adapted to living in unfor-ested areas. The same is true for the Malagasy and

Seychellean hyperoliids (Blommers-Schlösser & Blanc 1991). The tolerance of xeric environments may be a key adaptation enabling anurans to cross ocean barriers, possibly resting on leaves of rafting trees. Ocean currents at present favour a rafting from northwestern Madagascar to Mayotte, whereas in the Early Tertiary they may have temporarily favoured rafting from Africa to Madagascar (Krause *et al.* 1997).

The early radiation of the Ranidei clade (Vences & Glaw 2001), which contains the families Mantellidae (Madagascar–Comoros), Rhacophoridae (mainly Asia) and the paraphyletic Ranidae (figure 2), has been related to their arrival in Asia on the drifting Indian continent (Bossuyt & Milinkovitch 2001), and this fits our molecular age estimates. An alternative scenario linked the major split between ranoid and hyloid Neobatrachians to the separation of Africa and South America (Feller & Hedges 1998), considering the largely Old World–New World disjunction in the diversity centres of both groups. This hypothesis implies that the Ranoidea had not yet radiated at the time of separation of the Madagascar–India continent from Africa. The indication (figure 2) that the Seychellean sooglossids (genus *Nesomantis*) are deeply divergent from other Neobatrachians provides some support for this hypothesis. However, because our sampling of the Hyloidea is limited (two families, Bufonidae and Leptodactylidae only), the divergence estimates associated with this enormous radiation, which contains over 2000 species in 10 families, are tentative only.

Our results do not rule out the possibility that vicariance has played an important role in shaping current amphibian distributions. In fact, there is little doubt that amphibians belong to the lower end of the relative dispersal ability spectrum (Inger & Voris 2001; Brown & Guttman 2002), as also indicated by their absence from most oceanic islands. However, strict assumptions that their distribution has exclusively or almost exclusively been shaped by vicariance and terrestrial dispersal (e.g. Duellman & Trueb 1986; Bossuyt & Milinkovitch 2001) are not warranted according to our results.

(c) *The vertebrate colonization of Madagascar*

According to our results, hyperoliids have colonized Madagascar by overseas dispersal subsequent to its separation from the African mainland. In the absence of a convincing phylogenetic resolution among ranids (clade E in figure 2), similar origins can not yet be excluded for other Malagasy frog lineages, such as the Mantellidae. This is in agreement with the fact that Madagascar almost exclusively harbours relatively modern lineages of non-marine vertebrates (Vences *et al.* 2001). Only four to five of these have a fossil record dating back into the Mesozoic (see electronic Appendix H), and the available paleontological data from the Malagasy Latest Cretaceous suggest a biotic change in deep time (Krause *et al.* 1997, 1999). We surveyed and re-assessed literature data of 20 vertebrate clades with representatives in Madagascar and of reliably known phylogenetic relationships (see electronic Appendix H). Groups originating by Gondwanan vicariance would be expected to show biogeographic affinities to India, which was last connected to Madagascar (Rabinowitz *et al.* 1983; Pitman *et al.* 1993; Storey *et al.* 1995; Briggs 2003). However, the closest relatives of the

Malagasy taxa occur in Africa in 12 cases and in India–Asia in only three cases. The area cladograms of only three out of 20 groups coincide with the succession of plate tectonic events. The Malagasy fauna therefore contains an important component that originated by dispersal from Africa, possibly using currently submerged islands as stepping stones (Krause *et al.* 1997; McCall 1997). Multiple additional dispersal events from Madagascar to the Comoros and Seychelles, and partly back to Africa, subsequently obscured this original biogeographic signal (Jansa *et al.* 1999; Raxworthy *et al.* 2002). This contributed to the current diversity in origins among terrestrial and freshwater vertebrates in the Indian Ocean region.

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