



Origin of Madagascar's extant fauna: a perspective from amphibians, reptiles and other non-flying vertebrates

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ABSTRACT

The origins of the highly endemic and partly very diverse fauna and flora of Madagascar were longtime unsolved and are still strongly disputed, although dispersalists are recently prevailing over advocates of Gondwanan vicariance. Madagascar has been separated from other continents and continental fragments since the Mesozoic, and the assumption of vicariance origins requires very old ages of the lineages involved. Herein I review the recent progress of knowledge on the molecular phylogenetic relationships of the amphibians, reptiles, and other non-flying vertebrates of Madagascar. Of 17 Malagasy clades for which relationships seem to be resolved with sufficient and unanimous support, the largest proportion have their sister-group in Africa, and very few show a general area cladogram consistent with the succession of events in the fragmentation of the supercontinent Gondwana. A survey of pairwise sequence divergences between 25 Malagasy lineages and their non-Malagasy relatives in the 16S and 12S rRNA genes resulted in values that in most cases were distinctly below the saturation plateaus of these genes. Multiple calibrations based on 34 largely independent data points indicate that such saturation would be strongly expected in the case of ages corresponding to the geographical isolation of Madagascar in the Jurassic and Cretaceous. A Cenozoic age for most Malagasy lineages is therefore most likely. Analysis of phylogenetic patterns of some taxa provides indications for a scenario in which the ancestors of the Malagasy clades first arrived by transmarine dispersal from Africa at the Malagasy west coast, and in a second step a subset of them underwent species-rich radiations into the rainforests.

KEY WORDS: Madagascar - Historical biogeography - Adaptive radiation - Molecular phylogeny.

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INTRODUCTION

Madagascar, the fourth largest island of the world, is characterized by a remarkable degree of endemism of its fauna and flora. Taking into account the summary of Glaw & Vences (2000) and subsequent taxonomic changes, almost 205 nominal species of amphibians and 358 reptiles are known from Madagascar, with degrees of species-level endemism of 99% and 93%. The diversity of freshwater fishes amounts to 143 species of which 70% are endemic (De Rham & Nourissat, 2002, Sparks & Stiassny, 2004;). From 100 to 102 native mammals (except bats) are known, with an endemism of 98-100% (Goodman *et al.*, 2003, and own data). However, these total numbers of species diversity must be seen as preliminary. Ongoing taxonomic work in Madagascar has led to the discovery of new species at an unprecedented pace in all of these four orders. The use of molecular techniques will lead to a very steep increase in the recognition of species, especially among Malagasy amphibians. The general picture so far indicates a high species diversity of Malagasy amphibians and reptiles, a mammal diversity comparable with that of other regions, and a relatively low diversity of freshwater fishes, especially at the family level.

The biogeographic origin of these taxa has been much disputed, but recent progress in assembling molecular phylogenies has led to a large improvement of the available data basis. It is now clear that many Malagasy taxa belong to species-rich endemic radiations, and in many cases the sister taxa of the Malagasy lineages have been identified. In several cases, also information on the phylogenetic pattern within the radiations has become available. In the present paper I provide a review of progress in knowledge on the phylogeny and origins of Madagascar's vertebrates. Additionally I will present a comprehensive though tentative molecular clock analysis based on mitochondrial genes for ribosomal RNA.

GEOLOGICAL FRAMEWORK

Madagascar has been isolated from other landmasses since ancient times. Together with Africa, India, Australia, Antarctica and South America, it formed the southern supercontinent Gondwana which, during the Mesozoic, broke apart into its present fragments. The separation of Madagascar from Africa was one of the earliest events (at 165-121 mya; Barron *et al.*, 1981; Rabinowitz *et al.*, 1983; Storey, 1995), followed by the separation of Africa from South America at 101-86 mya (Pitman *et al.*, 1991). For a long period, Madagascar probably formed a separate Madagascar-India-Seychelles continent (but see Briggs 2003a) until, at 88-63 mya, India and the Seychelles drifted northwards (Storey *et al.*, 1995). India and the Seychelles separated at ca. 65 mya (Plummer & Belle 1995; Gnos *et al.* 1997). India collided with the Asian plate not later than 57

mya (Beck *et al.*, 1995). During the Cretaceous, a connection of Madagascar to the Antarctica through the now largely submerged Kerguelen plateau was possible (Sampson *et al.*, 1998; Marks & Tikku, 2001), but afterwards it is likely that no further land connection existed. Hypotheses of Cenozoic connections through now submerged seamounts in the Davie fracture ridge (McCall, 1997) are strongly disputed (Krause *et al.*, 1999). However, these seamounts - like the volcanic Comoro islands which do not date back further than the Miocene (Battistini, 1996) - may have constituted stepping stones between Madagascar and the African mainland (Krause *et al.*, 1999).

Paleoclimatically, the working hypotheses proposed by Wells (2003) indicate a temperate to subtropical-dry climate during much of the Mesozoic of Madagascar, with a rather arid period in the Paleocene. The dry vegetation in Madagascar would be of older age than the humid forest; the origin of the eastern rainforest belt may have taken place in the Oligocene (Wells, 2003).

PALEONTOLOGICAL FINDINGS

Paleontologically, Madagascar has been a rich source of fossil vertebrate remains from the Mesozoic and Late Paleozoic (Flynn & Wyss, 2003). Important findings from the Permian and Triassic include dinosaurs (Flynn *et al.*, 1999), diapsid reptiles with gliding and aquatic adaptations (see Carroll, 1988), and a representative of the lineage ancestral to frogs, *Triadobatrachus* (Rage & Rocek, 1989). A particularly informative fauna has been recovered from the Latest Cretaceous, including gondwanatherian and marsupial mammals (Krause *et al.*, 1997b; Krause, 2001, 2003), primitive birds (Forster *et al.*, 1996, 1998), dinosaurs (Sampson *et al.*, 1998, 2001), crocodyliforms (Buckley *et al.*, 2000), mastoid snakes (Scanlon & Lee, 2000), archaic actinopterygian fishes (Gottfried & Krause, 1998) and frogs (Asher & Krause, 1998). No terrestrial and freshwater fossils are known from the Tertiary of Madagascar due to the lack of suited deposits. For the Pleistocene, subfossil deposits draw the picture of a fauna relatively similar to the extant one, with some spectacular megafaunal elements (giant lemurs; pigmy hippos) and a few unique taxa such as the elephant birds, e.g., *Aepyornis*, and the enigmatic mammal *Plesio-rycteropus* (Krause *et al.*, 1997) that went extinct upon the arrival of humans at Madagascar (Burney *et al.*, 2003).

MOLECULAR PHYLOGENIES AND ORIGIN OF MALAGASY CLADES

Cichlid fishes

These fishes belong to the best studied Malagasy ichthyofaunal elements (see De Rham & Nourissat, 2002; Sparks & Stiassny, 2004). According to molecular data of Farias *et al.* (1999, 2000), the Malagasy genera

(all endemic) are among the most basal cichlids. Together with the Indian cichlids (genus *Etroplus*), they form the sister group of the species-rich clade including Neotropical and African taxa both of which are monophyletic groups as well. The Malagasy *Paretroplus* is the sister group of *Etroplus*. Cichlid fossils are known since the Eocene (Murray, 2001).

Aplocheiloid fishes

The molecular phylogeny of aplocheiloid killifishes shows a pattern similar to that of cichlids (Murphy & Collier, 1997). The Neotropical and African clades are sisters to each other, together forming the sister group of the Indian-Malagasy clade. Within the latter, the single species occurring on the Seychelles (*Pachypanchax playfairii*) is sister to the two Malagasy *Pachypanchax* rather than to the Indian *Aplocheilus*.

Other fishes

Stiassny & de Pinna (1994) pointed to the fact that several Malagasy freshwater fishes are phylogenetically basal within their respective lineages. Beside cichlids and aplocheiloids, this is also true for catfishes (genus *Ancharius*) and atherinomorphs (family Bedotiidae). The bedotiids are probably related to the Australasian rainbowfishes (Dyer & Chernoff, 1996), but this relationship is still to be confirmed by molecular data. Among the remaining freshwater fishes from Madagascar, the relationships of the killifish *Pantanodon mada-gascariensis* to its supposed relative *P. stublmanni* from brackish waters in East Africa, and the relationships of the mugilid *Agonostomus telfairii* (from Madagascar, Comoros and Mascarenes) to its supposed Neotropical relative *A. monticola* (see Stiassny & Harrison, 2000) remain to be tested with molecular datasets. In any case, it must be stressed that these groups, as also cichlids and aplocheiloids, belong to higher clades for which the known fossil ages are much younger than the mid-Mesozoic separation of Africa and Madagascar (Lundberg, 1993; Patterson, 1993; Briggs, 2003b).

Mantellid frogs

Molecular data (Richards & Moore, 1998; Vences *et al.*, 2000; Bossuyt & Milinkovitch, 2000) have shown that several Malagasy genera previously thought to belong to two non-endemic families (Rhacophoridae, Ranidae) in fact form a single endemic clade which today is considered as Mantellidae (Vences & Glaw, 2001): *Aglyptodactylus*, *Boophis*, *Laliostoma* (previously *Tomopterna*), *Mantella* and *Mantidactylus*. Richards & Moore (1998) and Bossuyt & Milinkovitch (2000, 2001) assumed that the mainly Asian treefrogs of the Rhacophoridae are the sister-group of mantellids. However, this grouping did not always receive high statistical support (Bossuyt & Milinkovitch 2000; Vences *et al.*, 2003; Hoegg *et al.*, 2004) and I therefore do not yet consider it as sufficiently corroborated (though likely).

Microhylid frogs

Beside the Mantellidae, this is the second most numerous families of frogs in Madagascar. However, phylogenetic data on their relationships and origins are so far rudimentary. Three subfamilies of microhylids are known from Madagascar, the Cophylinae, Scaphiophryninae and Dyscophinae. The Cophylinae are almost certainly a monophyletic radiation (Vences *et al.*, 2002b), while so far it is uncertain whether the scaphiophrynine genera *Scaphiophryne* and *Paradoxophyla* are actually closely related. Also the sister taxa of the Malagasy lineages are uncertain; *Dyscophus* is considered to form the family Dyscophinae together with the Asian *Calluella*, but molecular data so far could not clarify this relationship (Vences M., unpublished data).

Hyperoliid frogs

This family is mostly African, except for the Seychellian genus *Tachycnemis* and the Malagasy *Heterixalus*. Molecular results (Richards & Moore, 1996; Vences *et al.*, 2003a, b) provided very strong support for a sister-group relationship of these two genera; the clade formed by them is nested among African genera, with *Afixalus* being most likely their sister group.

Ranid frogs

Two species considered to belong to this family are known from Madagascar. Malagasy specimens of *Hoplobatrachus tigerinus* show no relevant genetic differentiation from Indian populations and therefore have almost certainly been introduced (Kosuch *et al.*, 2001). This contrasts with the situation in *Ptychadena mascareniensis*. This species is the most common frog in Madagascar and is also widespread in Africa. Own data (Vences *et al.*, 2004) have shown a remarkable molecular variability of Malagasy population and the absence of the Malagasy haplotypes from Africa. This indicates that the Malagasy populations belong to a native species, but final conclusions await analysis of sequences from Southeast African populations.

Crocodyles

The Malagasy populations undoubtedly belong to the Nile crocodile, *Crocodylus niloticus*. Samples from a crocodile farm in Antananarivo had the same 16S rRNA haplotype as East African populations (Mausfeld P. & Böhme W., personal communication), but nothing is known about the supposed variant '*robustus*' that historically had been described from the Malagasy highlands.

Pelomedusine turtles

Populations from Madagascar are considered to be conspecific with the three African species *Pelomedusa subrufa*, *Pelusios castanoides* and *Pelusios subniger* (see Glaw & Vences, 1994). No molecular confirmation has so far been published, but relationships of the Malagasy populations to African species have never been doubted.

Podocnemine turtles

The endemic Malagasy genus *Erymnochelys* (with one species, *E. madagascariensis*) is one of three examples from the reptiles in which a Malagasy clade has well-corroborated phylogenetic relationships to a Neotropical clade. Morphological and molecular data agree that *Erymnochelys* is related to South American podocnemines, more particular that it is the sister group of the genus *Podocnemis* (Georges *et al.*, 1998; Noonan, 2001). Podocnemine fossils, however, are also known from Africa, indicating that the group had a more widespread distribution until recently (Noonan, 2001).

Land tortoises

The extant Malagasy land tortoises of the genera *Astrochelys* and *Pyxis* belong into a clade that also contains Indian Ocean giant tortoises, genera *Aldabrachelys* and *Cylindraspis* (sometimes also classified as *Dipsochelys*); the sister group of this entire Indian Ocean clade is formed by African species of the genus *Geochelone*, more particularly by *G. pardalis* (Caccone *et al.*, 1999, Austin & Arnold, 2001, Palkovacs *et al.*, 2002).

Iguanas

While Africa and Asia is populated by the acrodont lizard family Agamidae, the Neotropics, some Pacific islands, and Madagascar harbour representatives of the pleurodont family Iguanidae. The Malagasy genera *Oplurus* and *Chalarodon* (subfamily Oplurinae) are a monophyletic group that is nested among South American species, although their exact relationships have not been fully clarified (Titus & Frost, 1996; Schulte *et al.*, 1998; Frost *et al.*, 2001). Iguana fossils are absent from Africa but have been found in Laurasia; a colonization of Madagascar passing the Indian subcontinent in the Early Cenozoic has therefore been proposed (Rage, 1996).

Chameleons

These bizarre lizards clearly belong to the acrodont lineage and are therefore closely related to the predominantly Asian and African agamas. Chameleons are known from Africa, Madagascar and the Seychelles, and a single species (closely related to European and North African species) is also distributed in India and Sri Lanka. Madagascar harbours three genera: dwarf chameleons of the genus *Brookesia* (endemic to Madagascar) and large arboreal chameleons, genera *Calumma* (also present on the Seychelles) and *Furcifer* (also present on the Comoros and Mascarenes, the latter probably due to introduction). A recent scenario proposed that chameleons colonized Africa out of Madagascar in several instances (Raxworthy *et al.*, 2001), but a closer look at the area cladogram shows that this conclusion relies on some particular nodes that are not sufficiently corroborated by the molecular data alone (see also Townsend & Larson, 2002). It seems clear, however, that more than one dispersal event must be invoked to explain the presence of the various groups in Madagascar and Africa.

Lygosomine skinks

In Madagascar, lygosomine lizards are represented by the genus *Mabuya* which was considered to be cosmopolitan until its recent partition (Mausfeld *et al.*, 2002). The Malagasy species clearly belong into the African clade (Mausfeld *et al.*, 2000; Honda *et al.*, 2003). This genus seems to be the sister-group of the South American taxa, with the Asian taxa being the most basal clade and the sister group of the lineage containing African-Malagasy, South American and Caboverdian species. Although first indications seemed to indicate that the two species groups of Malagasy species originated independently from African taxa (Mausfeld *et al.*, 2000), more extensive molecular data rather seem to support the monophyly of a Malagasy lineage (Mausfeld *et al.*, 2002; Honda *et al.*, 2003).

Scincine skinks

A relatively large number of endemic scincine genera and species are known from Madagascar, but very little is known on their phylogenetic relationships. Molecular data of Schmitz A. (personal communication) and co-workers provide some indications of the inter-relationships among Malagasy scincine lizards, but their closest non-Malagasy relatives have not yet been identified with sufficient reliability.

Gerrhosaurids

The endemic Malagasy genera *Zonosaurus* and *Trabeloptychus* clearly belong to this otherwise exclusively African lizard family, as corroborated by molecular and karyological data (Odierna *et al.*, 2002).

Geckos

African representatives of this lizard family were studied with immunological methods by Joger (1985). Bauer (1990) and Kluge & Nussbaum (1995) concluded for most Malagasy gecko genera that they belong to a radiation also including African taxa, but a conclusive test of this hypothesis with molecular markers is still lacking.

Pseudoxyrhophiine colubrid snakes

The phylogenetic relationship of the colubrid snakes of Madagascar has long been unsolved. Zaher (2000) placed most Malagasy genera, together with *Dityopphis* from Socotra island, into one separate subfamily Pseudoxyrhophiinae. This arrangement was confirmed by nuclear and mitochondrial DNA data (Nagy *et al.*, 2003). Within caenophidian snakes, this subfamily clearly is nested within a clade of mainly African colubrids (Vidal & Hedges, 2002).

Psammophiine colubrid snakes

Besides the pseudoxyrhophiines, a second clade of colubrid snakes in Madagascar is represented by the genus *Mimophis*. According to Nagy *et al.* (2003) and

Vidal & Hedges (2002), this genus belongs to the African-Asian subfamily Psammophiinae.

Boine snakes

Like iguanas, boas occur in South America, Madagascar and the Pacific, but are absent in Africa and Asia, where giant snakes are instead represented by pythons (Pythoninae). According to Vences *et al.* (2001b), the Malagasy genera *Sanzinia* and *Acrantophis* form a monophyletic clade that is the sister group of a clade composed of the South American genera. Rage (1996) pointed to the fact that boine fossils are not known from Africa but from Laurasia, hypothesizing a colonization of Madagascar from Laurasia via the Indian subcontinent. Rage (2002) identified fossil remains from the Paleocene as belonging to the extant South American genus *Coralus*, which would imply a diversification of boines (including the Malagasy clade) already at that early stage.

Typhlopoid snakes

These small, largely fossorial snakes have not been comprehensively studied using molecular data sets. One parthenogenetic species, *Ramphotyphlops braminus*, is very widespread and its origin in Madagascar may have been due to human translocation. All other species are endemic.

Ratite birds

The flightless elephant birds, family Aepyornithidae, are among the most prominent representatives of Madagascar's now extinct megafauna. Based on partial mitochondrial sequences, Cooper *et al.* (2001) place the species into a lineage containing the Australian/New Zealand species cassowary, emu and kiwi, this whole assemblage being the sister group of the African ostrich. All these species were the sister group of a group containing the extinct New Zealand moas and the South American rhea was the most basal ratite, supported by complete mitochondrial sequences.

Carnivores

Malagasy carnivores are a small radiation containing civet-like, mongoose-like and cat-like taxa. Their phylogenetic relationships were uncertain for a long time. Yoder *et al.* (2003) provided strong molecular evidence for the monophyly of the clade containing all Malagasy taxa. The hierarchical sister groups of this clade are the mostly African herpestids (mongooses) and hyaenas.

Tenrecs

This group of insectivores, which contains hedgehog-like, shrew-like, mole-like and even semiaquatic species, has recently been demonstrated to form a spectacular example of convergence. Its representatives belong to a basal eutherian clade that is largely restricted to Africa, the Afrotheria. This arrangement is based on very strong evidence from nuclear and mitochondrial

genes (e.g., Murphy *et al.*, 2001). Molecular data also demonstrated that the Malagasy tenrecs are probably one monophyletic group, and that their closest relatives are the African otter shrews, Potamogalinae (Douady *et al.*, 2002; Olson & Goodman, 2003).

Nesomyine rodents

The phylogenetic relationships and biogeographic relationships of Malagasy rodents have not yet been satisfyingly clarified. Data from mitochondrial 12S rRNA and nuclear genes (Dubois *et al.*, 1996; Michaux *et al.*, 2001) support a topology that places Malagasy taxa (the endemic subfamily Nesomyinae) nested among mainly African genera, but only few Malagasy taxa have been included in these studies. In contrast, a very taxon-rich analysis based on cytochrome *b* sequences supported non-monophyly of the nesomyines and their origin from Asia, followed by several Out-of-Madagascar dispersals to Africa (Jansa *et al.*, 1999). However, the statistical support for this topology was extremely low.

Lemurs

This is certainly the most prominent radiation of Malagasy vertebrates. Molecular data demonstrate that lemurs are a monophyletic group endemic to Madagascar (Yoder *et al.*, 1996; Stanger-Hall & Cunningham, 1998) that is sister to a clade containing lorises and galagos (Yoder *et al.*, 2001).

Other mammals

Beside the four endemic radiations listed above, a number of other extant or recently extinct mammal lineages are known from Madagascar. The extinct *Plesioxycteropus* might have been related to the African armadillo, and the likely extinct pygmy hippo almost certainly originated by recent colonization from Africa. The shrew *Suncus murinus* has probably been introduced by humans, while the status of the dwarf shrew *S. madagascariensis* is unclarified. Molecular data have shown that the (almost certainly introduced) house mouse populations of Madagascar originated from the Arabian peninsula (Duplantier *et al.*, 2002). The status of the wild boar (*Potamochoerus larvatus*) is also uncertain; it is considered to occur in two (endemic) subspecies in Madagascar, but on the other hand its origin is thought to be due to introduction (Garbutt, 1999).

Summarizing the data presented above (Fig. 1), there are currently 17 lineages in which the sister-group of a Malagasy clade has been identified with sufficient reliability in a molecular analysis. Counting smaller Indian Ocean islands as part of the Malagasy region, in 11 of these cases, the sister-group of the Malagasy clade is predominantly or exclusively distributed in Africa (hyperoliid frogs, crocodiles, land tortoises, two lineages of chameleons, lygosomine scincs, gerrhosaurids, two lineages of colubrid snakes, carnivores, tenrecs). In three cases, the sister-group is distributed in South America

(podocnemine turtles, boas, iguanas). In only two groups, the sister-group of the Malagasy clade is undoubtedly distributed in South Asia (cichlid and aplocheiloid fishes), and in one group, it occurs in Australia and New Zealand (ratite birds). Counting the number of cases in which the area cladograms of these groups agree with the succession of events leading to fragmentation of Gondwana, it applies only to three groups: aplocheiloid and cichlid fishes, and to a limited extent to hyperoliid frogs.

PATTERNS OF MALAGASY VERTEBRATE RADIATIONS

The data summarized above impressively demonstrate the power of molecular phylogenetic analyses to elucidate phylogenetic relationships in the presence of morphological convergences. In mantellid frogs (Richards & Moore, 1998; Vences *et al.*, 2000; Bossuyt & Milinkovitch, 2000), pseudoxyrhophiine colubrid snakes (Nagy *et al.*, 2003), lemurs and carnivores (Yoder *et al.*, 1996, 2003), the Malagasy species turned out to belong to single monophyletic clades that underwent spectacular morphological diversification, strikingly paralleling that

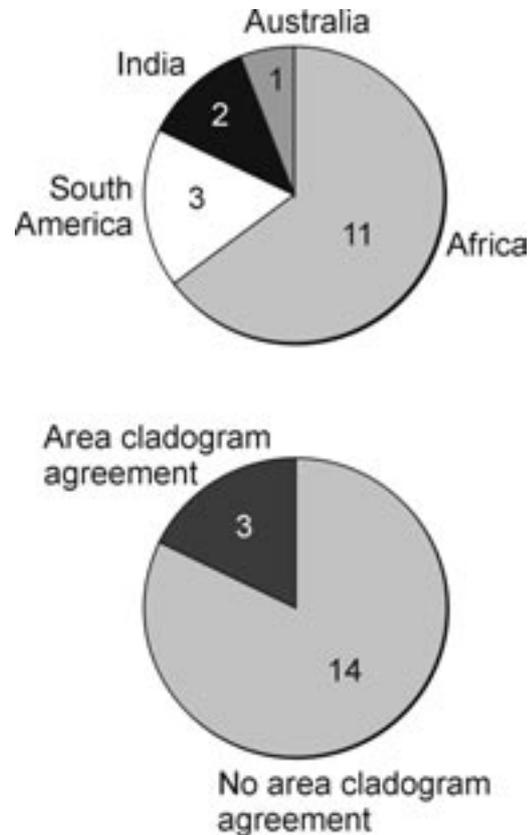


Fig. 1 - Pie charts for 17 clades of Malagasy vertebrates in which the phylogeny is well assessed by molecular data sets as discussed in the text, indicating the main distribution of their non-Malagasy sister clades (above) and the agreement of the area cladograms derived from the phylogenies with the succession of events in the course of fragmentation of Gondwana (below).

in other non-Malagasy groups. The same might be true for scincine lizards, tenrecs (Douady *et al.*, 2002), nesomyine rodents (Dubois *et al.*, 1996), and others, in which a phylogenetic consensus is still to be achieved. It has also been demonstrated for a group of flying vertebrates, the vanga birds (Yamagishi *et al.*, 2001). In other lineages such as chameleons (Raxworthy *et al.*, 2002; Townsend & Larson, 2002) and mycesaline butterflies (Torres *et al.*, 2001), independent colonisations by various subclades, from Madagascar to Africa or vice-versa, seem to have taken place.

A common pattern of the Malagasy vertebrate clades is that the species-rich assemblages have their centres of diversity in the rainforests of the east of Madagascar rather than in the dry west. Subclades in several groups also seem to be less species-rich in the west (Vences *et al.*, 2000, 2002a). However, it is quite relevant that there is almost no major lineage exclusive to the east whereas several (mostly species-poor) lineages are largely restricted to the west (Vences *et al.*, 2000): iguanas, podocnemine turtles, land tortoises, maybe the extinct elephant birds. Phylogenetically basal taxa in several groups seem to occur in the west rather than the east (Vences *et al.*, 2000, 2002a, b).

MOLECULAR CLOCK DATA

For each of the Malagasy vertebrate clades, I attempted to find molecular clock calibrations for the 12S and

16S rRNA genes from groups that are phylogenetically as close as possible. From the literature, I compiled a number of phylogenetic splits that were suited for molecular clock calibration; 16S (preferably) or 12S rRNA sequences were then obtained from GenBank or by own lab work. The assumed ages of these splits were based on fossil knowledge (mammals), age of lakes in which intralacustrine radiations took place (in cichlid fishes), connection of Mediterranean islands via sea-level fluctuations (in Aegean water frogs and Sardo-Corsican salamanders), volcanic age of islands (Canary island geckos, lacertid and scincid lizards), and ancient vicariations (Australia-South America connection in chelid turtles). Phylogeny and age estimate information was extracted from Irwin *et al.* (1991), Mayer & Bischoff (1991), Allard *et al.* (1992), Caccone *et al.* (1994), Frye & Hedges (1995), Battistini (1996), Beerli *et al.* (1996), Gonzalez *et al.* (1996), McCune (1997), Rassmann (1997), Bischoff (1998), Brown & Pestano (1998), Schmincke (1998), Seddon *et al.* (1998), Pesole *et al.* (1999), Carranza *et al.* (2000), Widmer *et al.* (2000), Vences *et al.* (2001a) and Verheyen *et al.* (2003). Phylogenetic splits and ages used for calibration are listed in Appendix I.

Figs 2 and 3 show the results of the molecular clock calibrations using the total 16S pairwise sequence divergences and the transversion divergences. Because transversions accumulate much more slowly than transitions, they should show fewer signals of saturation. Indeed, in Fig. 2, the oldest calibration (chelid turtles; 65 mya) seems to be on a saturation plateau. However, for this particular

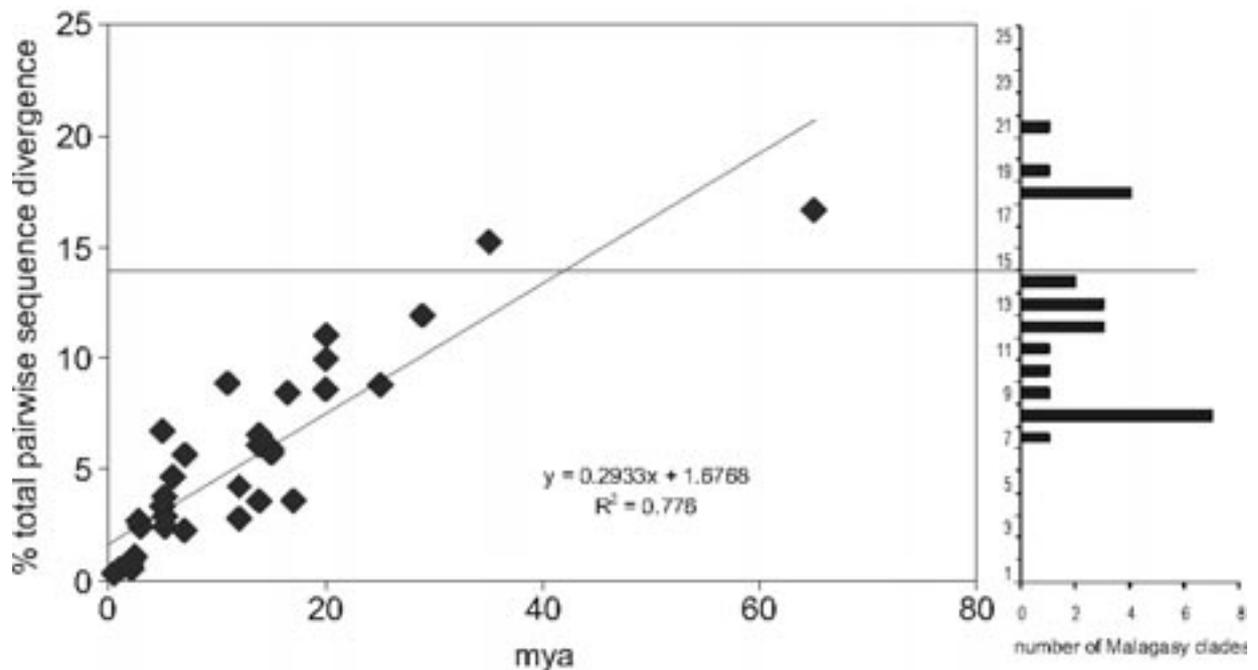


Fig. 2 - Scatterplot and regression line of total pairwise sequence divergences (in percent) of the 16S rRNA gene against estimated age in million years (mya) of vertebrate lineages used for molecular clock calibration (Appendix I). The bars in the inset figure to the right indicate the distribution of pairwise divergences between related Malagasy and non-Malagasy taxa (see Appendix II). The dotted horizontal line marks 14% divergence which corresponds to a gap in the distribution of values for Malagasy clades, and below which the calibration data show no signs of saturation.

calibration, 65 mya is a very conservative age estimate, and an alternative of 42 mya (Seddon *et al.*, 1998) would be perfectly in line with the other calibration points.

The pairwise sequence divergences of the Malagasy clades to their non-Malagasy sister-groups (and other related non-Malagasy taxa), as represented by the bar insets in Figs. 2 and 3, in large part are below the potential saturation plateau values. From the calibration scatterplots, there is no indication that saturation could play a relevant role below a total divergence of 14% or a transversion divergence below 6%; yet, of the 25 divergences tested in total, 19 and 17, respectively, are below these threshold values. There also is no distinct correlation between the geographical distribution of the sister group of the respective Malagasy clade and the genetic divergences (Fig. 4). This indicates that, irrespective of the biogeographic origin of the clades of Malagasy amphibians, reptiles and other vertebrates, they apparently colonized Madagascar in most cases later than 65 mya, thus posterior to the Cretaceous, and almost certainly after the separation of Madagascar from Africa. There is little doubt that any lineage of such old age (165-121 mya) should have reached the saturation plateau of the 16S gene between 15 and 20% total divergence (Ortí & Meyer, 1997). The only consistent alternative explanation of the low 16S rRNA divergences encountered would be to postulate a heavily reduced molecular substitution rate, either in the Malagasy taxa as compared to their sister groups, or in all lineages

present in Madagascar as compared to those used for calibration. I used the program PHYLTEST (Kumar, 1996) to perform relative rate tests (Takezaki *et al.*, 1995), using both Jukes-Cantor distances and distances based on transversions only. The results (not shown) showed no consistent pattern of Malagasy taxa having a particularly slow molecular rate, and the rate of the lineages used for calibration was in most cases not significantly faster than in the Malagasy clades. Also, a closer look at the taxa that show divergences above the saturation plateaus (Appendix II), reveals that several of them (tenrecs, lemurs and aplocheiloid fishes) belong to higher inclusive clades that are very unlikely to have existed in the Jurassic according to paleontological findings (Carroll, 1988; Patterson, 1993). This line of evidence perfectly agrees with the conclusions of Krause *et al.* (1997), Vences *et al.* (2001a), Raxworthy *et al.* (2001) and Yoder *et al.* (2003), who concluded that transoceanic dispersal, mostly between Africa and Madagascar, was the most important factor for the origin of many Malagasy vertebrate.

The molecular clock calculations presented here must be taken very cautiously, considering the severe qualifications and numerous pitfalls of this method (e.g., Hillis *et al.*, 1996) and the uncertainty about the substitution model applying to the various taxa. Indeed, the results herein are just a first screening of the genetic divergences of Malagasy taxa, and a rough placement of the values obtained onto a relative timescale. However, sev-

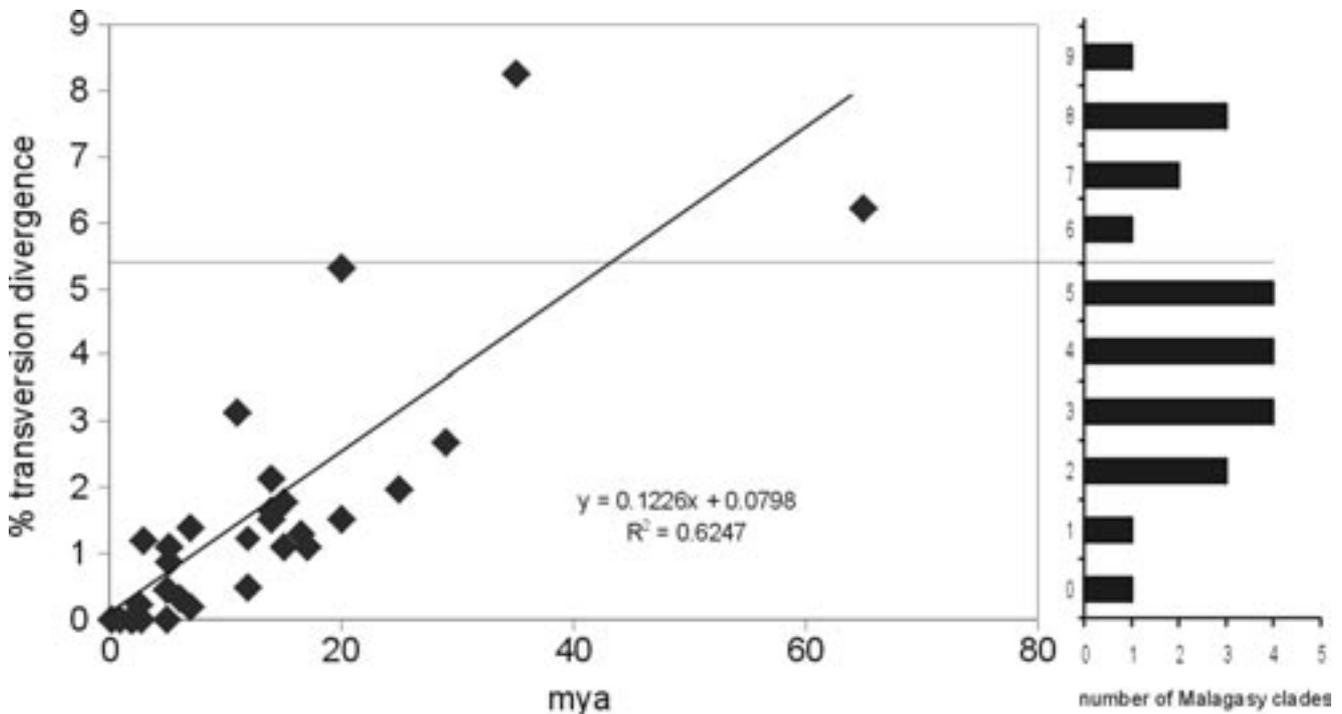


Fig. 3 - Scatterplot and regression line of pairwise transversion sequence divergences (in percent) of the 16S rRNA gene against estimated age in million years (mya) of vertebrate lineages used for molecular clock calibration (Appendix I). The bars in the inset figure to the right indicate the distribution of pairwise divergences between related Malagasy and non-Malagasy taxa (see Appendix II). The dotted horizontal line marks 5-6% divergence which corresponds to a gap in the distribution of values for Malagasy clades, and below which the calibration data show no signs of saturation.

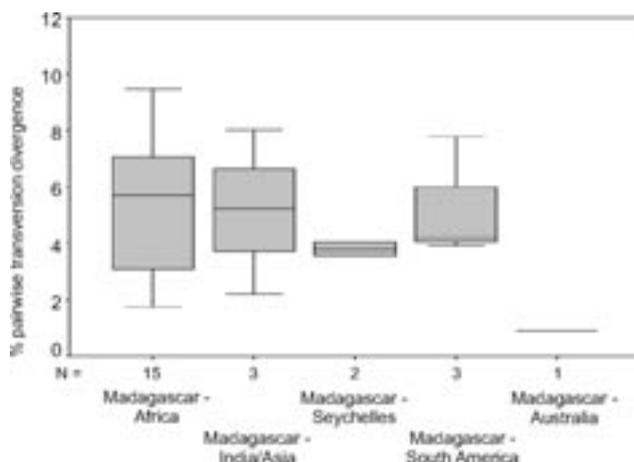


Fig. 4 - Boxplot of pairwise transversion divergences of the 16S rRNA gene between related Malagasy and non-Malagasy taxa, grouped by the general distribution of the non-Malagasy species.

eral factors can be used to advocate that the indications for Cenozoic ages of the Malagasy vertebrates are no artefact: on one hand, all our calibrations were based on the most conservative time estimates available, and molecular clocks inherently tend to overestimate rather than underestimate ages (Rodríguez-Telles *et al.*, 2002). Additionally, even though errors in single calibrations and estimates may occur, the probability of an error simultaneously affecting all values is low. The overall picture is therefore unlikely to change with the application of more thorough molecular clock methods.

CONCLUSIONS

Comparing recent research on the biogeography of the fauna of landmasses in the Indian Ocean, a conflict between two lines of evidence becomes apparent. On one hand, it seems clear that India and Sri Lanka are very important centres of endemism, and that some lineages of plants (Conti *et al.*, 2002) and animals (amphibians: Bossuyt & Milinkovitch, 2001; Gower *et al.*, 2002) radiated out of India into other tropical regions of Asia. It seems to be very appealing to relate this pattern to ancestral taxa that arrived in Asia on the drifting Indian plate (but see Briggs 2003). Relationships between Indian and Malagasy taxa, which have to be assumed in this case, are indeed likely for dinosaurs (Sampson *et al.*, 1998), archaic, extinct mammals (Krause *et al.*, 1997b), and sooglossid/ nasikabatrachid frogs (Biju & Bossuyt, 2003). On the other hand, the expected biogeographic patterns (close relationships between Indian and Malagasy taxa) did not turn up in thorough molecular phylogenies, except for those of aplocheiloid and cichlid fishes. In contrast, dispersal scenarios from Africa have been favoured to explain the origin of Malagasy taxa. Moreover, the molecular clock datings agree with palaeontology to indicate a post-Gondwanan age

of the Malagasy vertebrate clades (Vences *et al.*, 2001a; Raxworthy *et al.*, 2001; Yoder *et al.*, 2003; this study). It will be a promising line of research to investigate these contradictions and to integrate them into a comprehensive scenario of historical biogeography of this region.

For Madagascar, from the available data it seems most likely that the original Gondwanan fauna went largely extinct in the Mesozoic, and was replaced by new radiations from ancestors arriving via transmarine dispersal (Krause 2003), a process for which Krause *et al.* (1997) coined the term 'biotic change in deep time'. The new colonizers obviously arrived in most cases at the Malagasy west coast, which is characterized by arid climatic environments. To survive, these species had to be adapted to arid conditions. In a first step they spread over western Madagascar and probably the highland savannahs. In a second step, some taxa succeeded to adapt to rainforest environments and underwent species-rich radiations (Vences *et al.*, 2002a). In groups which colonized Madagascar in the Paleocene and Eocene, or which were survivors from Gondwanan times, the hypothetical origin of the eastern rainforest belt in the Oligocene (Wells, 2003) may have been the trigger for their major diversification. To understand which key innovations were relevant during these radiations, and why some lineages were more successful in rainforest colonization and diversification in Madagascar, remains a major challenge for future studies.

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APPENDIX I

Molecular clock calibrations used in this study. Behind each pair or group of taxa, the assumed age of separation in million years, total pairwise divergence including indels (except Cichlidae: without indels), and transversion divergence are given in parentheses. Data refer to homologous fragments of the 16S rRNA gene, except some squamate calibrations (*Gallotia*, *Tarentola*, *Chalcides*) which are based on a fragment of the 12S rRNA gene which, according to own data, has a slightly slower substitution rate. By regression of 78 16S and 12S data pairs from fishes, turtles, frogs and mammals, I found the 12S substitution rate to be 91% of that of the 16S fragment; 12S data in the following were corrected correspondingly to make them comparable with the 16S data.

Fishes (Cichlidae): Within Ectodini [Tanganyika] (12 mya; 4.23%; 1.23%); within Lamprologini [Tanganyika] (12 mya; 2.80%; 0.50%); within Mbuna [Malawi] (2 mya; 0.60%; 0.00%); within non-Mbuna [Malawi] (2 mya; 0.27%; 0.00%); within Barombi Mbo clade (1 mya; 0.30%; 0.00%); within Victoria haplochromines (0.2 mya; 0.00%; 0.00%). Amphibians: *Dendrobates sylvaticus* - *D. speciosus*/*D. pumilio* (3 mya; 2.42%; 1.2%); *Rana ridibunda* - *R. cretensis* (5.2 mya; 2.39%; 0.87%); *R. bedriagae* - *R. cerigensis* (1.8 mya; 0.44%; 0.00%); *R. bedriagae* - *R. cretensis* (5.2 mya; 2.83%; 1.09%); *R. cretensis* - *R. cerigensis* (5.2 mya; 3.79%; 1.09%); *Euproctus asper* - *E. platycephalus*/*E. montanus* (29 mya; 11.90%; 2.70%); *E. montanus* - *E. platycephalus* (15 mya; 5.90%; 1.10%). Turtles: *Chelus/Hydromedusa* - *Pseudemydura/Elseya* (65 mya; 16.67%; 6.21%). Squamates: *Gallotia stehlini* - *G. atlantica*, *G. galloti*, *G. simonyi* (14 mya; 6.50%; 1.52% [12S corr.]); *G. atlantica* - *G. galloti*/*G. simonyi* (11 mya; 8.85%; 3.14% [12S corr.]); *G. galloti* - *G. simonyi* (7 mya; 5.65%; 1.40% [12S corr.]); *Tarentola gomerensis* - *T. delalandi* (15 mya; 5.69%; 1.77% [12S corr.]); *T. boettgeri* - *T. delalandi* (20 mya; 10.99%; 5.30% [12S corr.]); *T. boettgeri* - *T. gomerensis* (20 mya; 9.96%; 5.30% [12S corr.]); *Chalcides sexlineatus* - *C. viridanus* (14 mya; 6.09%; 2.14% [12S corr.]); *Mabuya comorensis* - *M. maculilabris* (14 mya; 3.55%; 1.64% [12S corr.]). Primates: *Homo* - *Pan* (6 mya; 4.68%; 0.32%); *Pan paniscus* - *P. troglodytes* (2.5 mya 1.11%; 0.22%). Rodents: *Rattus norvegicus* - *Mus musculus* (35 mya; 15.20%; 8.26%). Ungulates: *Equus caballus* - *E. asinus* (7 mya; 2.20%; 0.20%); *Balaenoptera musculus* - *B. physalus* (5 mya; 3.36%; 0.00%); *Cervus unicolor* - *Muntiacus reevesii* (17 mya; 3.57%; 1.09%); *Gazella thomsoni* - *Madoqua kirki* (16.5 mya; 8.46%; 1.31%); *Bos taurus* - *Capra/Ovis/Antilocapra* (20 mya; 8.61%; 1.53%); *Capra hircus* - *Ovis aries* (5 mya; 6.68%; 0.44%); *Bos taurus* - *Cervus/Muntiacus* (25 mya; 8.81%; 1.96%). Carnivores: *Phoca vitulina* - *Halichoerus grypus* (2.7 mya; 2.65%; 0.00%).

APPENDIX II

Pairwise divergence between Malagasy taxa and their non-Malagasy sister groups (total pairwise divergence including indels and transversion divergence). Data refer to homologous fragments of the 16S rRNA gene, except for ratite, tenrec and rodent data which are corrected values based on a fragment of the 12S rRNA gene (see legend to appendix I).

Cichlid fishes: African clade - Malagasy/ Indian clade (11.53%; 3.14%); *Paretroplus*, Madagascar - *Etiopplus*, India (7.81%; 2.2%). Aplocheiloid fishes: African clade - Malagasy clade (17.76%; 8.43%); *Aplocheilus*, India - *Pachypanchax*, Madagascar (13.32%; 5.24%); *Pachypanchax*, Seychelles - *Pachypanchax*, Madagascar (9.55%; 4.03%). Mantellid frogs: Rhacophoridae. Mantellidae (18.52%; 8.03%). Hyperoliid frogs: *Afrixalus*, Africa - *Heterixalus*, Madagascar (12.92%; 5.86%); *Heterixalus*, Madagascar - *Tachycnemis*, Seychelles (7.49%; 3.56%). Podocnemine turtles. *Podocnemis* - *Erymnochelys* (12.82%; 7.79%). Land tortoises: *Geochelone pardalis* - *Pxyxis/Asterochelys* (7.72%; 1.74%). Iguanas: *Oplurus cuvieri* - *Phymaturus somuncurensis* (13.86%; 3.93%). Chameleons: *Calumma billeni* - *Bradypodion* sp. (12.81%; 4.47%); *Brookesia peyrierasi* - *Rhampholeon breviceaudatus* (17.13%; 8.44%). Lygosomine skinks: *Mabuya elegans* - *M. quinqueteniata* (6.87%; 3.04%). Scincine skinks: *Chalcides ocellatus* - *Pygomeles braconneri* (8.74%; 4.04%). Gerrhosaurids: *Zonosaurus madagascariensis* - *Gerrhosaurus nigrolineatus* (17.86%; 6.40%). Pseudoxyrhophiine colubrid snakes: *Elaphe quatuorlineata* - *Liopholidophis lateralis* (11.03%; 5.70%). Psammophiine colubrid snakes: *Mimophis mahfablensis* - *Psammophis condanarus* (10.13%; 5.82%). Boine snakes: *Sanzinia/Acrantophis* - *Boa/Epicrates/Eunectes* (7.91%; 4.21%). Ratite birds: *Mullerornis* - *Dromaius* (7.6%; 0.9%). Carnivores: *Galidia elegans* - *Crocota crocuta* (7.75%; 2.64%). Tenrecs: *Tenrec/Echinops* - *Micropotamogale* (20.8%; 7.7%). Nesomyine rodents: *Macrotarsomys/Nesomys* - *Cricetomys* (11.70%; 2.06%). Lemurs: *Galago* - *Lemur/Hapalemur/Microcebus/Propithecus/Daubentonia* (17.51%; 9.48%).