Vertebrate time-tree elucidates the biogeographic pattern of a major biotic change around the K–T boundary in Madagascar

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The geographic and temporal origins of Madagascar's biota have long been in the center of debate. We reconstructed a time-tree including nearly all native nonflying and nonmarine vertebrate clades present on the island, from DNA sequences of two single-copy protein-coding nuclear genes (*BDNF* and *RAG1*) and a set of congruent time constraints. Reconstructions calculated with autocorrelated or independent substitution rates over clades agreed in placing the origins of the 31 included clades in Cretaceous to Cenozoic times. The two clades with sister groups in South America were the oldest, followed by those of a putative Asian ancestry that were significantly older than the prevalent clades of African ancestry. No colonizations from Asia occurred after the Eocene, suggesting that dispersal and vicariance of Asian/Indian groups were favored over a comparatively short period during, and shortly after, the separation of India and Madagascar. Species richness of clades correlates with their age but those clades that have a large proportion of species diversity in rainforests are significantly more species-rich. This finding suggests an underlying pattern of continuous speciation through time in Madagascar's vertebrates, with accelerated episodes of adaptive diversification in those clades that succeeded radiating into the rainforests.

Cretaceous-Tertiary | historical biogeography | lineage diversification | rainforest adaptation | overseas dispersal

Madagascar's unique biodiversity has attracted the interest of evolutionary biologists and biogeographers for a long time. This island was part of the Gondwana supercontinent. As a part of Indo-Madagascar, it separated from Africa 160–130 Mya. The breakup of Indo-Madagascar and northwards drifting of India and the Seychelles started 88 Mya, leaving Madagascar isolated in the Indian Ocean and without subaerial connection to any other landmass for the last 65–80 Myr (1).

The isolation of Madagascar coincided with the end of the Cretaceous, a period of global mass extinction and biotic turnover, probably linked to a major meteorite impact marking the Cretaceous–Tertiary (K–T) boundary at 65.5 Mya (2). In Madagascar, the different composition of the Late Cretaceous vs. the extant vertebrate fauna led to the hypothesis of a major biotic change in deep time (3). The Cretaceous fauna included lungfishes, gars, nonranoid giant frogs, dinosaurs, and marsupial and gondwanatherian mammals (3–8), whereas the extant vertebrate fauna is composed of mainly percomorph freshwater fishes, ranoid frogs, modern squamate reptiles, lemurs, rodents, carnivores, afrotherian mammals, bats, and numerous families of birds (9).

Reconstructing the temporal pattern of this striking biotic turnover is hampered by the almost complete lack of post-Cretaceous and pre-Pleistocene terrestrial fossil deposits. This fossil gap presents difficulties in understanding how and when the extant clades of vertebrates colonized the island, and how their subsequent diversification took place. In recent times, explicit paleogeographic and paleoclimatic modeling associated with the reconstruction of molecular time-trees have started to resolve the biogeography of Madagascar, previously characterized as one of the greatest mysteries of natural history (10, 11). For numerous Malagasy clades of amphibians, squamates, and mammals, sister-group relationships to African taxa and a Cenozoic age are now well established, suggesting a predominance of Out-of-Africa overseas dispersal favored by oceanic paleocurrents (11–15). However, the exact timing of colonizations, as well as their possible clustering in particular periods, remain unstudied for many vertebrate clades and are contentious for others (11), largely because of the use of different molecular markers and time constraints. In addition, the closest relatives of several other taxa are found in South America or Asia (16, 17). The temporal pattern of vertebrate colonization of Madagascar from these different source continents has not been comprehensively studied to date.

Analyses of molecular data have led to great progress in understanding the timing of vertebrate diversification (18, 19). Here, we generated a comprehensive dataset that uses the same molecular markers and time constraints for nearly all terrestrial and freshwater vertebrate clades occurring on Madagascar and their sister taxa. The selected genes, *BDNF* (brain-derived neurotrophic factor) and *RAG1* (recombination activating gene 1), are single-copy, protein-coding, and universal among gnathostomes. We use multiple cross-validated time constraints in a single time-tree to obtain compatible age estimates across clades (20). Those estimates allow us to assess general biogeographic patterns of Madagascar's colonization by vertebrates, for which we test whether: (*i*) the majority of extant vertebrate clades colonized Madagascar during or after the K–T boundary, as suggested by the fossil record (7); (*ii*) colonizations followed different temporal patterns depending on the source continent; (*iii*) species richness of clades are related to their age in Madagascar; and (*iv*) clade diversification was influenced by habitat type.

Results

Vertebrate Time-Trees Based on Congruent Time Constraints. Phylogenetic analyses of the combined 1,747 base pairs of *RAG1* and *BDNF* for 188 taxa representing nonflying and nonmarine Malagasy vertebrate clades, their known non-Malagasy sister-groups, and a set of other vertebrate taxa, recovered most of the generally accepted deep and shallow relationships among vertebrates (Fig. 1). Time-tree reconstruction with 43 selected time constraints was based on two approaches that either allow substitution rates to vary independently over clades (ICR) or in an autocorrelated fashion (ACR) (*Materials and Methods*). The extremes of the 95% credibility intervals (CrIs) from the two approaches were merged into a single composite CrI (21).

A high congruence among most of an initial set of 48 time constraints was obtained in three cross-validation approaches using ACR, here named CV1–CV3 (see *SI Appendix* for details). In CV1 we assessed statistically the effect of removing single constraints on the overall differences between the constraint ages and molecular age estimates. None of the initial time constraints were highly incongruent, as their removal did not lead to a significant decrease in the variance of the s parameter in one-tailed *F*-tests (22). In CV2 we performed separate analyses after excluding one constraint in turn, and manually assessed whether the CrI estimated for the respective node was congruent with the original (excluded) constraint. This analysis flagged only three of our initial 48 constraints as incongruent. In CV3 we performed separate analyses after exclusion, in turn, of all but one constraint. The great majority of time-trees recovered correctly most constraints within CrIs. A preferred time-tree was then calculated after exclusion of five constraints, including the three that were most incongruent in CV2 (*SI Appendix*).

Times and Patterns of the Vertebrate Colonizations of Madagascar. Crown divergence times were not available for all taxa and cannot be obtained for monospecific clades, such as podocnemidid turtles. We thus summarized stem divergence times for the 31 Malagasy clades analyzed (Fig. 1). Although the colonization patterns reconstructed by ACR and ICR at first seem rather different (Fig. 2 and *SI Appendix*), the ages per clade are correlated (nonparametric correlation, Spearman's $R = 0.664$, $P < 0.001$) and concordant regarding the general temporal framework of colonization and differences among source regions. The two analyses agree in a predominant Cenozoic age of origin of the extant Malagasy vertebrates, with ages of 25 (ACR) and 23 (ICR) of 31 clades reconstructed at ≤65.5 Mya (Table 1). The composite CrIs exceeded 125 Mya only in three cases (podocnemidid and testudinid turtles, and tenrecs), confirming that the majority of extant vertebrates must have colonized Madagascar after its separation from Africa at 130 Mya. Additionally, the analyses display clade-age differences depending on their geographic origin. In both analyses, the two clades with South American origins, iguanids and podocnemidid turtles, have Mesozoic stem divergences. In addition, there is a trend for clades with assumed Asian or Asian/African ancestry being older than those originating from Africa: 60 Mya (77–41 Mya) vs. 43 Mya (79–4 Mya) in ACR analyses, 66 Mya (92–39 Mya) vs. 38 Mya (101–1 Mya) in ICR; this result is significant for the ICR estimations (*U* test: $Z = 2.88$, $P = 0.003$), and nearly so for the ACR estimations ($Z = 1.73$; $P = 0.08$). No colonization of unambiguous Asian origin was recovered as younger than 39 Mya by any analysis.

Prevailing ocean currents were periodically favoring rafting from Africa to Madagascar in the Early Cenozoic, but not after a reconfiguration of surface flows in the mid-Miocene, 20–15 Mya (10). Nevertheless, our analyses suggest three arrivals from Africa after the 15-Mya tipping point: ptychadenid frogs, crocodiles, and *Hemidactylus* geckos (Fig. 2A and Table 1). All of the 11 out-of-Madagascar dispersals are relatively young (36–3 Mya) and peak after the Oligocene (Fig. 2B). The dispersal of the ancestors of day geckos to the Mascarene Islands located east of Madagascar occurred at 22 Mya, and dispersals to the continental Seychelles, and to Asia and Africa occurred in the Oligocene and Eocene (36–28 Mya). Dispersals to the Comoro islands west of Madagascar were recovered by both analyses post-Oligocene, in agreement with favorable surface currents in this period, a pattern confirmed by the cross-validations also for those Comoro-Malagasy splits used as age constraints in the main analyses.

Clade Age and Rainforest Habitat Influence Species Richness. Besides the extraordinary degree of endemism at higher taxonomic levels, Madagascar's biota is characterized by a high, although incompletely known, species richness, and by a high proportion of range-restricted species that are microendemic to particular areas of the island (9). This pattern might also be typical for other tropical regions, but Madagascar is a particularly well-suited model region to determine the underlying patterns of biotic diversification (23).

A debated biological question is whether species richness is primarily influenced by clade longevity or diversification rate (24, 25). Among Malagasy vertebrates we expect species richness to be correlated with clade age if their diversification took place following a stochastic process of phylogenetic clade accumulation through time. In contrast, if many of the clades underwent rapid adaptive radiations after reaching the island, we expect this correlation to be weak or absent. Using nonparametric rank correlation analyses, stem age and species richness are significantly correlated based on ICR ages (Spearman's $R = 0.477$; $P = 0.007$) and nearly so based on ACR ($R = 0.316$; $P = 0.084$) (Fig. 2 C and D).

We defined a number of covariables likely to influence either the diversification process or the species-area relationship of clades (*Materials and Methods*). An ANCOVA model supports that species numbers increase with ACR clade age, as well as with external fertilization, parental care, terrestrial habits, and occurrence in rainforest, the latter variable being the most significant predictor (*SI Appendix*, Table S8).

Madagascar is renowned for its high diversity of biomes, ranging from subarid shrublands to humid rainforests (9). Vertebrate clades are unevenly distributed over these biomes, with some clades predominantly found in humid and subhumid forests and others in dry forests and open habitats. The six most species-rich clades in our analysis with >50 species (mantellid and microhylid frogs, pseudoxyrhophiine snakes, chameleons, skinks, and lemurs) are rather variable in age (96–20 Mya) but all have a majority or an important proportion of their species diversity living in rainforests (*SI Appendix*, Table S7). Among all clades, those with the majority (>75%) of species in rainforests have significantly higher species richness than those distributed in both habitats, and the lowest species richness is found in clades specializing to dry and open biomes (Fig. 2F). These three groups of clades do not differ significantly in clade age (Fig. 2E) but most of the species-rich rainforest clades colonized Madagascar at the onset of the Eocene, just before the putative spread of Madagascar's rainforests (26).

Discussion

This comprehensive assessment of Malagasy vertebrate ages and colonization patterns robustly reconstructs the origins of these organisms in Cretaceous and Cenozoic times. Because we included all clades in a single time-tree and cross-validated all time constraints, we can exclude biases, which could arise if incongruent constraints are used in independent single-clade analyses. Even taking into account the conservative composite CrIs, there are only three estimates >125 Mya. This finding strongly supports a predominant origin of Madagascar's extant vertebrates after Madagascar separated from Africa at 160–130 Mya (15). Most of these clades have African sister groups,
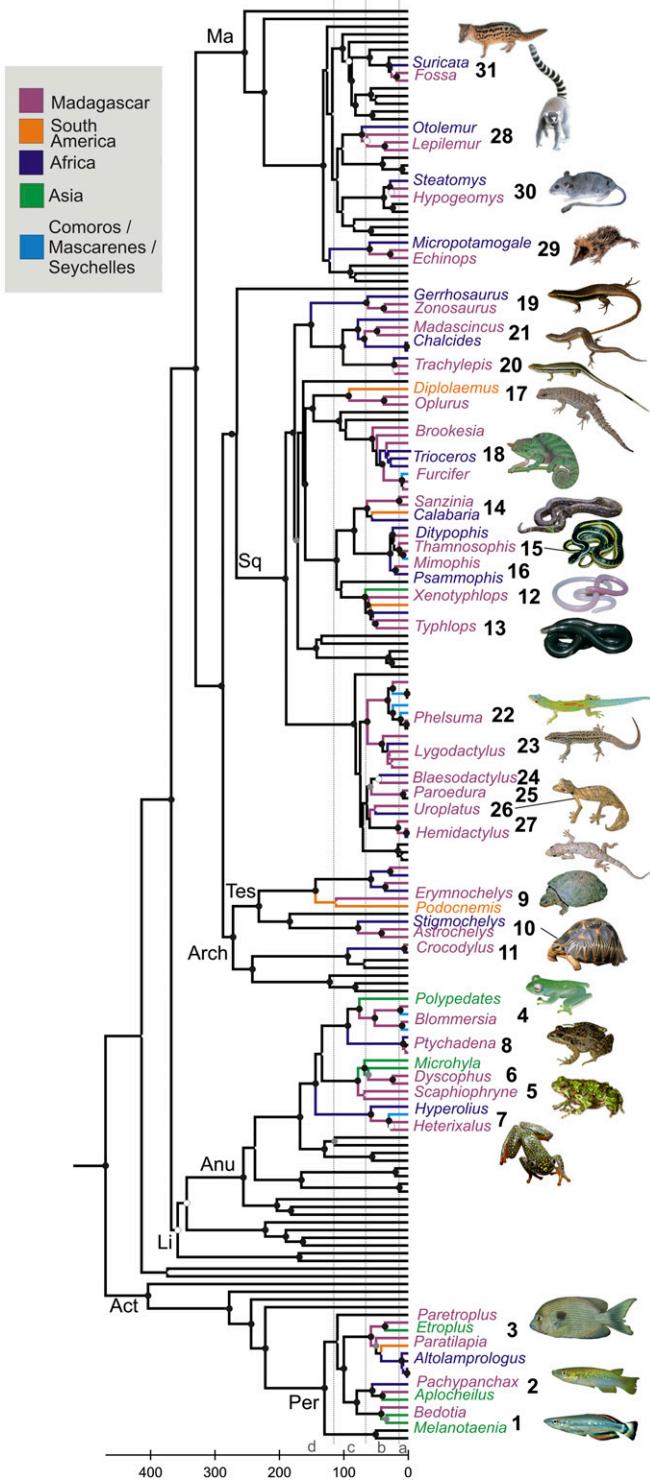
Fig. 1. ACR vertebrate time-tree based on a 50% majority rule consensus tree from a Bayesian analysis of 1,747-bp DNA sequences of the *RAG1* and *BDNF* genes. Black dots at nodes indicate Bayesian support >0.98, gray dots 0.95–0.98, and white dotted nodes were topology-constrained before the analysis. Colors indicate geographic distribution of taxa and numbers denote clades as listed in Table 1. Only representative taxa of the clades occurring in Madagascar and their sister groups are named (see *SI Appendix* for tree with names of all terminals); *Inset* pictures show one representative species for most of the numbered Malagasy clades. Major clade abbreviations: Act, Actinopterygii; Anu, Anura; Arch, Archosauria; Li, Lissamphibia; Ma, Mammalia; Per, Percomorpha; Sq, Squamata; Tes, Testudines. Vertical gray dotted lines separate four main time intervals, at bottom: (a) <15 Mya,

confirming a predominance of overseas dispersal from Africa (11), in agreement with clade age estimates previously published for 17 Malagasy clades (15). ACR ages are in 12 clades on average 19% (0–43%) older than the published data and in five clades on average 23% (6–29%) younger (*SI Appendix*, Table S6). ICR ages are more strongly deviating: eight clades are on average 23% (8–39%) younger, and nine clades on average 35% (2–54%) older. Several of the ICR ages are rather unrealistic, given previous assessments, such as an age of tenrecs as old as 103 Mya and of tortoises as young as 16 Mya. We therefore consider the ACR results to be more reliable. In general, we acknowledge that any single-point estimate in our data might be subject to changes as novel methods, better time constraints, or more comprehensive molecular datasets become available. However, we expect changes not to exceed the composite CrIs obtained here and, rather, to support even younger ages given our conservative selection of age constraints.

Only three literature ages fall outside of the ACR CrIs: cichlids and xenotyphlopids, which were younger, and hyperoliid frogs, which were older in our analyses. Among the biogeographically most-relevant deviations of our data from previous analyses (27, 28) are the younger ages of cichlids and of the other two fish clades. The ACR and ICR analyses agreed in placing the origin of these fishes into the Latest Cretaceous or Early Cenozoic, thus supporting dispersal hypotheses for their origin (29). The origin of Madagascar boas was estimated in the Paleocene–Eocene rather than Cretaceous, suggesting dispersal from Africa rather than from Antarctica, as suggested before (17).

Our results can be directly compared with an explicit model for stem divergence ages for Malagasy vertebrates (11) assuming 80% dispersal and 20% vicariance, which predicts a clear peak at the time of Indo-Madagascar breakup at 88–60 Mya. This finding is in perfect agreement with the results of the ACR analysis (Fig. 2*A*), whereas the ICR ages suggest a more recent Cenozoic peak and a more regular spacing of ages in the Mesozoic (*SI Appendix*). Divergences >90 Mya are rare in our analyses but are predicted, albeit at low prevalence, by the model. This discordance confirms paleontological evidence for a massive biotic change around the K–T boundary. It also suggests that this biotic change affected all vertebrates, including small frogs and lizards, for which paleontological evidence is typically scarce.

Only a few taxa are missing from our analysis. Apart from flying vertebrates (bats and birds), these include a genus of cyprinodontiform fishes (*Pantanodon*), with one representative in Africa, some nonprimary freshwater fish clades, three gecko clades (*Geckolepis*, *Matoatoa*, and *Paragehyra*) with uncertain phylogenetic relationships, and *Cryptoblepharus* shoreline lizards, which dispersed recently from the Australasian region. Three extinct Pliocene-Pleistocene clades are the nonflying *Aepyornis* related to the Australian and New Zealand flightless birds, pygmy hippos with clear relationships to Africa, and the Malagasy aardvark *Plesiorycteropus*, which might belong into the Afrotheria. Most of the bird and bat lineages, as well as hippos and aardvarks, presumably arrived in Madagascar between the Oligocene and present (15), and their inclusion would further reinforce the pattern of Cenozoic origins of most of Madagascar's extant vertebrates. However, bats and birds are independent from ocean currents and thus include numerous post-Oligocene arrivals from Asia (15).

In ACR and ICR analyses, the largest proportion of divergence times at 88–60 Mya corresponds to groups with Asian or

corresponding to the period where surface currents did not favor dispersal from Africa; (b) 66–16 Mya, when sea currents favored overseas rafting from Africa; (c) 120–66 Mya, when overland dispersal or short-distance rafting from Asia was possible; (d) >121 Mya, when overland dispersal from Africa and Antarctica might have been possible (15).
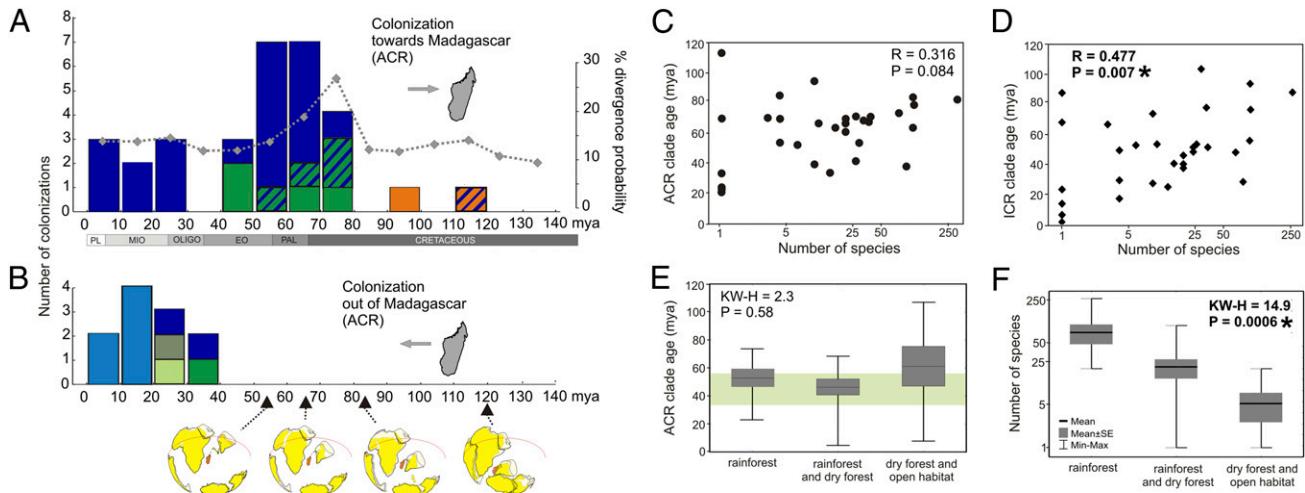
Fig. 2. Age distribution of Malagasy vertebrate clades and species-richness correlates. (*A*) Histogram of ACR stem divergence ages for vertebrate clades occurring in Madagascar derived from the time-tree in Fig. 1, color-coded depending on the distribution of their sister group. Rhomboids connected by dotted line show the probability of clade origins derived from a model assuming 80% dispersal and 20% vicariance (11). (*B*) ACR age histogram for clades of assumed out-of-Madagascar dispersal. *Inset* maps (1) show reconstructions of continental shorelines. (*C* and *D*) Scatterplots of species richness (logarithmic scale) of endemic Madagascar clades vs. stem age of the ACR and ICR analyses, respectively. (*E* and *F*) Plots of species richness (logarithmic scale) and stem age of endemic Madagascar clades with highest species diversity distribued in rainforest (>75% of species), in rainforest as well as dry forest, or predominantly (>75%) in dry forest (and deforested areas within humid bioclimates). The light green bar in *E* marks the Eocene period during which the origin of Madagascar's rainforest has been hypothesized (26).

ambiguously Asian/African ancestry, fitting the expectations of Indo-Madagascar vicariance. However, the Malagasy mantellid and dyscophine frogs are phylogenetically deeply nested in widespread Asian taxa. This finding suggests at least occasional ancient dispersal from India to Madagascar (30), probably facilitated by discontinuous land bridges and stepping-stones after the connection of India with the Asian plate (1). No colonizations from Asia are estimated after the Eocene, which suggests that colonizations from Asia became severed after India had reached its current position, with a large open-sea distance to Madagascar (15).

The two clades with clear South American relationships among extant taxa, iguanas and podocnemidid turtles, are by far oldest in the ACR analyses and were among the oldest seven in the ICR analysis, confirming previous estimates (17). However, our data indicate that these clades might even be older than previously thought, and could have reached Madagascar in the Early Cretaceous. This theory would be in agreement with recent paleogeographic reconstructions (31, 32) that do not support the previously hypothesized Late Cretaceous connections of Madagascar and South America via Antarctica and the Kerguelen/ Gunnerus ridges (16). The third group for which such a connection has been previously invoked, Madagascar boas, turned out to be distinctly younger, which agrees with their recently discovered phylogenetic relationship to the African *Calabaria* (17). Clades of African ancestry were rather evenly spaced over the Cenozoic, and a few of them even in the period 15–0 Mya, coinciding with an unfavorable pattern of surface currents (10).

The equilibrium model of island biogeography predicts a positive area/species diversity relationship because of balanced rates of colonization and extinction (33). Because Madagascar's biota evolved largely in isolation, with a limited number of colonizations, the assembly of its extant species richness has been mainly a consequence of speciation processes rather than immigration. In such situations, a nonequilibrium model of diversity can be applied and species richness is expected to mainly be influenced by variation in net diversification rates or clade age (34). Our analyses are congruent in supporting clade age (time since colonization) as one predictor of species richness of Madagascar's vertebrates. This

analysis suggests, in many but not all clades, a rather regular net diversification rate with time, but the rather low correlation coefficients suggest an influence also of other drivers of species richness. In our analysis, the relative time lags between (*i*) the separation of the Malagasy clade from its non-Malagasy sister group and (*ii*) the first speciation events within Madagascar decreases with clade species richness (*SI Appendix*). This decrease could be explained by species-poor clades having been more strongly affected by past extinctions, also at deep levels, but we hypothesize it indicates lower diversification rates of these clades instead.

One factor explaining such differences in diversification rate might be the ability of a clade to adapt to rainforest conditions. Although the subarid and arid biomes of Madagascar span over wide environmental gradients in altitude, moisture, and soil composition, which especially for squamate reptiles offer numerous opportunities for specialization, mainly species-poor clades are the ones predominantly living in these habitats. In fact, among the clades with fewer than 20 species none has its center of diversity in rainforests. The species-poor clades specialized to dry conditions are either old Gondwanan relicts (e.g., podocnemidid turtles, iguanas), or very recent colonizers. The vast majority of such young clades that arrived in the Miocene or later (e.g., *Ptychadena*, *Crocodylus*, *Hemidactylus*, *Trachylepis*, *Mimophis*), contain no or very few strict rainforest specialists but are composed of species adapted to dry conditions or generalists surviving in open landscapes. This finding might reflect plesiomorphic traits favorable for overseas dispersal, and for survival after arriving at Madagascar's dry west coast after a transoceanic rafting from Africa. The ability of a clade to adapt to rainforest might have been influenced by intrinsic morphological or physiological constraints, or by ecological interactions with other organisms: either in terms of predator–prey relationships (e.g., the diversity of pseudoxyrhophiine snakes might be influenced by the earlier diversification of frogs and lizards, which constitute their main prey), or competition (e.g., psammophiine snakes might not have radiated into rainforests because these were already occupied by the earlier pseudoxyrhophiine snake radiation).

In conclusion, clade species richness in Madagascar is influenced by clade age but also by their adaptability to rainforest

EVOLUTION

Table 1. Vertebrate clades endemic to Madagascar and their estimated stem-based age and species richness

Clade	ACR stem age	ICR stem age	Species
Actinopterygii			
1 Bedotiidae – As	42 (24-68)	51 (26–82)	26
2 Aplocheilidae – As	41 (24–65)	53 (28–82)	6
3 Cichlidae – Af	58 (38–86)	76 (46–103)	33
Lissamphibia			
4 Mantellidae – As	76 (50–108)	87 (55–122)	266
5 Microhylidae: Cophylinae/ Scaphiophryninae– As/Af	77 (49–114)	92 (63–123)	95
6 Microhylidae: Dyscophinae – As	62 (37–94)	65 (38–95)	3
7 Hyperoliidae – Af	57 (30–94)	53 (25–85)	10
8 Ptychadenidae – Af	8 (2–20)	13 (4–24)	1
Archosauria			
9 Podocnemididae – SA/Af	112 (73–159)	87 (65–111)	1
10 Testudinidae – Af	79 (33–134)	16 (6–30)	4
11 Crocodylidae – Af	5 (0–20)	1 (0–2)	1
Squamata			
12 Xenotyphlopidae – As/Af	61 (42–82)	66 (43–90)	1
13 Typhlopidae – As/Af	54 (36–74)	39 (21–59)	15
14 Boidae – Af	61 (48–75)	47 (22–74)	4
15 Lamprophiidae: Pseudoxyrhophiinae – Af	24 (14–37)	28 (15–41)	80
16 Lamprophiidae: Psammophiinae – Af	19 (10–31)	22 (10–37)	1
17 Opluridae – SA	90 (62–120)	72 (32–117)	9
18 Chamaeleonidae – Af	54 (34–77)	54 (34–75)	94
19 Gerrhosauridae – Af	61 (32–96)	37 (17–63)	19
20 Scincidae: *Trachylepis* – Af	19 (9–37)	24 (10–39)	13
21 Scincidae: Scincinae – Af	65 (39–96)	47 (23–70)	67
22 Gekkonidae: *Phelsuma* – Af	62 (39–91)	49 (34–65)	34
23 Gekkonidae: *Lygodactylus* – Af	62 (39–91)	49 (34–65)	24
24 Gekkonidae: *Blaesodactylus* – Af	42 (23–68)	27 (10–45)	4
25 Gekkonidae: *Paroedura* – Af	57 (34–86)	43 (27–60)	19
26 Gekkonidae: *Uroplatus* – Af	51 (29–78)	38 (20–58)	19
27 Gekkonidae: *Hemidactylus* – Af	4 (1–10)	6 (1–11)	1
Mammalia			
28 Strepsirhini (lemurs) – As/Af	71 (51–94)	73 (46–103)	96
29 Tenrecidae – Af	60 (40–84)	101 (62–141)	29
30 Muridae: Nesomyinae – Af	28 (17–42)	47 (26–71)	24
31 Eupleridae – Af	26 (16–38)	26 (14–39)	9

Clade numbers as in Fig. 1; Af, Africa; As, Asia; SA, South America. Ages are given in Mya.

habitats. In contrast to purely vicariant speciation scenarios that have typically been invoked in Madagascar (23), these results suggest a role for adaptive speciation during at least some episodes of the evolutionary history of species-rich clades, but possibly less so for species-poor clades. We predict that future exploration of this and other evolutionary hypothesis in the Madagascar model system will much benefit from the comprehensive temporal framework provided herein.

Materials and Methods

Total genomic DNA was extracted using standard protocols and a combination of various degenerated primers were used to amplify overlapping fragments for a total length of 535 aa of the *RAG1* gene (amino acid positions 467–1001 in human RAG1) and a fragment corresponding to 221 aa of the *BDNF* gene (see *SI Appendix* for details). Chromatographs were checked and sequences were aligned using CodonCode Aligner (v. 3.7.1, Codon Code). The alignment of newly determined sequences was complemented with sequences retrieved from GenBank (*SI Appendix,* Table S2). For *BDNF* the software Gblocks (35) was used to delete highly divergent regions, which were either not unambiguously aligned or saturated by multiple substitutions. Additionally, all positions with gaps in both genes were excluded from the analyses. The final concatenated alignment was 1,747 bp long.

We conducted partitioned Bayesian inference searches based on the concatenated dataset in MrBayes 3.1.2 (36) with two partitions: first-plus-second positions, and third positions, grouped for both genes. The partition scheme was selected based on a Bayes factor analysis (*SI Appendix*). Both

partitions were assigned to a general time-reversible substitution model with estimated γ-shaped distribution and proportion of invariable sites, as suggested by MrModeltest (37). We performed four independent runs of 20 million generations sampling trees every 1,000 generations. The first four million generations were discarded based on empirical evaluation of convergence (see *SI Appendix* for methods used). A number of nodes were constrained according to well-established knowledge on vertebrate phylogeny (Fig. 1 and *SI Appendix*).

We selected 48 age constraints across the vertebrate tree, of which 43 were used for the final analysis (*SI Appendix*), with a preference for ample, conservative estimates rather than narrow upper and lower constraints or point estimates because fossil uncertainties are prone to lead to pseudoaccuracy. Constraints are coded C1–C48 (missing numbers refer to excluded constraints; details and references, and rationale for exclusion in *SI Appendix*). Slashes represent the phylogenetic split of one clade from the other; when no slash is given, the age is of the split of the respective clade from their unspecified sister group. C1, diapsids/synapsids, 338–288 Mya; C2, lungfishes/tetrapods, 419–408 Mya; C3, archosaurs/lepidosaurs, 299.8–259.7 Mya; C4, birds/crocodiles, 250–235 Mya; C5, alligators/caimans, 71–66 Mya; C6, *Pelomedusa/ Pelusios* turtles, >25 Mya; C7, *Erymnochelys/Podocnemis* turtles, >65 Mya; C8, Cryptodira/Pleurodira turtles, >210 Mya; C9, podocnemidid/pelomedusid turtles, >100 Mya; C10, sea turtles, >110 Mya; C11, turtles, >220 Mya; C12, Sphenodon/squamates, >228 Mya; C13, geckos, >55 Mya; C14, amphisbaenian/lacertid lizards, > 64 Mya; C17, Booidea/Caenophidia snakes, >75 Mya; C18, Comoran *Furcifer* chameleons, <15 Mya; C20, Canary geckos *Tarentola boettgeri/delalandi*, <14 Mya; C21, Comoran gecko *Phelsuma nigristriata*, <15 Mya; C22, Comoran *Phelsuma comorensis*, <15 Mya; C23, Mascarene

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Phelsuma inexpectata/ornata, <2.1 Mya; C24, Canary skinks *Chalcides sexli-neatus/viridanus*, <14 Mya; C25, Caniformia/Feliformia, 63.8–42.8 Mya; C26, hippomorph/ceratomorph Perissodactyla, 58–54 Mya; C28, Lagomorpha, 61.5–48.6 Mya; C30, Cetartiodactyla, 65–55 Mya; C31, Spanish/Moroccan *Dis-coglossus* frogs, >5.3 Mya; C32, Spanish/Moroccan *Alytes* frogs, >5.3 Mya; C33, Comoran *Blommersia* frogs, <15 Mya; C34, Comoran *Boophis* frogs, <15 Mya; C35, frog/salamander, >230 Mya; C36, *Calyptocephalella* frogs, >53 Mya; C37, pelomedusine/pelodryadine treefrogs, >42 Mya; C38, African/American pipid frogs, >86 Mya; C39, cryptobranchid/hynobiid salamanders, >161 Mya; C40, pipid frogs, >140 Mya; C41, discoglossid frogs, >167 Mya; C42, Lake Tanga-nyika cichlid fishes, <12 Mya; C43, Lake Malawi cichlids, <2 Mya; C44, cichlids, >45 Mya; C45, Actinopterygii, >392 Mya; C46, Tetraodontiformes, >98 Mya; C47, Ostariophysi, >146 Mya; C48, Elopomorpha, >151 Mya.

Time-trees were reconstructed with two different relaxed-clock methods with uncorrelated and correlated substitution rates, respectively, over clades (here named ICR and ACR), using the computer programs BEAST (38) and Multidivtime (39). For methodological details of analyses, see *SI Appendix*. Age estimates for stem-based nodes (separation of Malagasy clades from their closest non-Malagasy sister group) as well as stem-based ages of as-sumed out-of-Madagascar dispersal events were extracted from the time-tree results. Composite 95% CrIs were compiled by combining CrIs from ICR and ACR analyses which in simulation studies (20) cover the true time in >97% of the estimated times.

Species richness of endemic Madagascar clades was compiled from recent publications (complete species list in *SI Appendix*). Linear models (ANCOVAs) were performed in R (40) on species richness as dependent and a series of factors that might influence species richness as independent variables: clade age, rainforest occurrence, body size, age to maturity, endothermy, trophic position, internal fertilization, parental care, and aquatic vs. terrestrial habits. Factors were deleted sequentially from the full model based on the Akaike Information Criterion.

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EVOLUTION

Supplementary Information Appendix

A. Crottini, O. Madsen, C. Poux, A. Strauß, D.R. Vieites, M. Vences:
A vertebrate timetree elucidates the biogeographic pattern of a major biotic change around the K-T boundary in Madagascar

Extended Methods

Total genomic DNA was extracted using proteinase K (10 mg/ml) digestion followed by a standard salt-extraction protocol (S1) or from ethanol-preserved tissue following the protocol of the Wizard SV Genomic DNA Purification System (Promega) (S2). A combination of degenerated primers was used to amplify overlapping DNA fragments corresponding to 535 aminoacids (AA) of the Recombination Activating Gene 1 (RAG1) gene (AA 467–1001 in human); a fragment of 221 AA of the Brain-derived Neurotrophic Factor (BDNF) gene was amplified using one primer pair, except for the lungfish for which several primer combinations were needed. All primers used are listed in Table S1.

For non-mammalian samples standard Polymerase chain reactions were performed in a final volume of 11 µl and using 0.3 µl each of 10 pmol primer, 0.25 µl of total dNTP 10 mM (Promega), 0.08 µl of 5 U/ml GoTaq, and 2.5 µl 5 GoTaq Reaction Buffer (Promega). PCR conditions were as follows. BDNF: 94°C for 120 s, followed by 39 cycles of 94°C (20 s), 57°C (45 s), 72°C (120 s), and a final extension phase of 72°C (600 s). RAG1: 94°C (120 s) followed by 45 cycles of 94°C (20 s), 50–53°C (50 s), 72°C (180 s), and a final extension of 72°C (600 s). For mammalian BDNF the PCR reactions were preformed in a final volume of 50 µl on 50 to 200 ng of DNA with the Expand DNA system (Roche) using the following program: 120 s at 94 ºC; 30 to 35 cycles of 15 s at 94 ºC, 60 s at 54–58 ºC, and 90 s at 72 ºC; and a final step of 120–600 s at 72°C. DMSO (1.3% to 2.5%) and/or Betaine (1 M) was added for some samples. For RAG1 in mammals, the PCR reactions were preformed in a final volume of 40 µl on 50 to 200 ng of DNA with the HotStarTaq Plus Master Mix Kit (Qiagen) using the following program: 300 s at 95ºC; 40 cycles of 30 s at 94ºC, 50 s at 54ºC and 90 s at 72ºC; and a final step of 600 s at 77°C. Q solution x5 was added for all samples. PCR products were purified from a 1% agarose gel, using GFX PCR DNA & Gel Band Purification Kit (Amersham Biosciences).

PCR products were resolved on automated sequencers (Applied Biosystems: ABI 3130XL or 3730). Newly determined sequences are deposited in Genbank (see Table S2). Chromatographs were checked and sequences were edited and aligned using CodonCode Aligner (v. 3.7.1, Codon Code Corporation). Besides newly determined sequences, some DNA sequences were retrieved from Genbank and in some instances concatenated chimera sequences of different species were compiled from GenBank. For a complete list of taxa, voucher numbers and Genbank accession numbers used in this study see Table S2.

The software Gblocks (S3) was used to delete highly divergent regions that could either not being unambiguously aligned or that were saturated by multiple substitutions. The complete coding region of the BDNF contains (across all vertebrates) three conserved functional motifs (the starting motif, the subtilisin binding site, the glycation and furin site) and one highly conserved Nerve Growth Factor (NGF) domain. The excluded region corresponded to the entire portion of the three functional motifs at the 5' end of the gene. This exclusion was mostly based on difficulties to align Chondrichthyes and Actinopterygii with all the other vertebrates. Additionally all positions with gaps in both genes were excluded from the analyses.

In order to obtain a topology congruent with the most recent phylogenetic hypotheses and reduce computing time, the following constraints were used in the Bayesian phylogenetic analyses: (a) Chiroptera were constrained in basal position to the Carnivora, Cetartiodactyla and Perissodactyla; (b) Daubentoniidae, Lemuridae and Cheirogaleidae were constrained as monophyletic; (c) Nesomyinae were contrained as monophyletic; (d) *Blaesodactylus antongilensis* and *Homopholis walbergii* were constrained as sister species; (e) the Malagasy *Mabuya aureopunctata* and *M. gravenhorstii* were contrained as monophyletic; (f) *Heterixalus tricolor* and *Heterixalus madagascariensis* were constrained as sister species; (g) all amphibians were constrained as monophyletic; (h) Anura and Caudata were constrained as monophyletic with Gymnophiona in basal position.

A Bayes factor analysis (Table S3) was conducted to select the most appropriate partition scheme for Bayesian phylogenetic analysis, and based on the results, we chose the scheme with two partitions (1st plus 2nd positions, and 3rd positions, grouped for both genes).

Convergence and mixing of chains in the Bayesian phylogenetic analyses were assessed by examining output files with the AWTY (Are we There Yet) graphical exploration software (S4). This tool confirmed that (i) split frequencies among runs were strongly correlated, (ii) there was no obvious trend in cumulative split frequencies

of a series of 40 randomly selected splits, (iii) topological differences between trees sampled by independent runs stabilized after ca. 1.5 million generations. Hence, these indicators did not find any indication of poor convergence in our phylogenetic analysis.

Two different approaches were conducted to calculate a timetree from our molecular data:

(i) Bayesian molecular dating (S5) was applied using the Multidivtime program package (S6). This method uses a probabilistic model to quantify changes of substitution rates over time. It relaxes the molecular clock by allowing continuous autocorrelation of substitution rates among the branches of the phylogenetic tree. This approach estimates rates accurately (S7), without requiring the root of the tree to be fixed at a particular date but estimates its age starting from a prior value (split of Chondrichthyes from their sister group, 528 mya). We used default settings of Multidivtime as recommended (S8) except for the following parameters: rtrate = 0.000779, rtratesd = 0.000390, rttm = 528.0, rttmsd = 100.0, bigtime = 600.0; except rtrate and rtratesd all in million years. The concatenated sequence data set was partitioned as for the Bayesian phylogenetic analyses (1st plus 2nd positions, and 3rd positions, grouped for both genes) and branch lengths calculated under the F84+gamma model of sequence evolution, which is the most complex model available in Multidivtime. However, different parameter-rich substitution models generally produce branch lengths that are highly correlated (S9). The use of F84 instead of the GTR+I+G model should therefore not significantly alter age estimates. Initially Markov chain Monte Carlo (MCMC) analyses including all 48 time constraints were run four times: twice for ten million generations with a "burn in" of half million generations ('long runs') and twice for 1000000 generations with a "burn in" of 100000 generations ('short runs') with chains sampled every 100 generations in all analyses. The difference in calculated node age, mutual and between long and short runs, were less than 1% for all nodes, leading us to apply 'short runs' in all subsequent analyses. We performed multiple independent runs as cross-validation of age constraints (see Results) and calculated the final timetree after exclusion of C15, C16, C19, C27, and C29, again with several independent runs of one million generations plus one run with ten million generations, and with less than 1% differences among all nodes except one (with a max. difference of 3.8%).

(ii) Additionally, reconstruction of phylogenetic relationships and molecular dating were also conducted by BEAST v1.6.0 (S10). The 50% majority-rule consensus tree obtained with MrBayes and rendered ultrametric using Multidivtime, was used as a starting tree for independent BEAST runs and a lognormal uncorrelated relaxed clock model was specified. We included two speciation process models: birth–death (S11) and pure birth (S12) as they were shown to yield different age estimates (S11). We ran analyses either fixing the topology of the starting tree or letting BEAST searching for the best tree (still constraining the monophyly of the nodes under interest). The Bayes factor as implemented in Tracer 1.5 (a part of the BEAST package) was used to select the best-fitting model under the smoothed marginal likelihood estimate and with 1000 bootstrap replicates (S13). Four different analyses with 8 independent runs of 10 million generations each were undertaken by sampling every 10000th generations. Tracer 1.5 was used to check for convergence of the model likelihood and parameters between each run until reaching stationarity. Results were considered reliable once the effective sampling size (ESSs) of all parameters was above 100. The resulting log and tree files were then combined using LogCombiner. In order to check whether our data were informative, we ran analyses sampling only from the priors distribution.

To understand whether clade age influences clade species richness we transformed (S14) data of species richness and body size (snout-vent length, SVL) to fit requirements of linear analysis. A linear model (ANCOVA) was performed on species richness as dependent and a series of factors that might influence species richness as independent variables: clade age, rainforest occurrence, body size, endothermy, trophic position, internal vs. external fertilization, parental care, and aquatic vs. terrestrial habitat. Factors were deleted sequentially from the full model based on the Akaike Information Criterion, AIC (S15) until the minimum adequate model was reached. Due to restricted sample size, interactions were not included in the full model. Analyses were performed in R 2.9.2 (S16) including the package car (S17).

Extended and Additional Results

Cross-validation of time constraints indicated only three of our initial 48 constraints as somewhat incongruent with the remaining set of calibrations (Table S5): C19 - the colonization of the younger volcanic islands by lizards (*Gallotia*) at <14 mya; C29 - the origin of primates at 66–56 mya; and C27 - the split of panungulates at 65–54 mya. For the two mammal constraints (primates and panungulates), our data indicated substantially older ages than suggested by fossil estimates. For primates, this agrees with recent publications (S18, S19) that place the origin of crown primates at about 87 mya. Similarly, the panungulate constraint has been criticized as possibly being too young (S20) and reconstructed to be at 78 mya (S21). Therefore, the paleontological

constraints typically used for the most recent common ancestor (MRCA) of primates and for the MRCA of paenungulates appear to represent underestimates of the actual divergence times of these groups. This suggests that the earliest fossils found up to now are far from representing the earliest paenungulate and primate and that older fossils belonging to this groups remain to be found, and supports our decision to exclude these constraints. Calibrations C15 and C16 (split of acrodont iguanians and split of iguanians based on the fossil *Bharatagama*) were excluded because of doubts on fossil identity, after ascertaining that their inclusion (which results in somewhat older ages of most squamate nodes) does not alter the statistiocal significance of the influence of clade age on species richness. .

One conspicuous pattern in the data set is the presence of very long branches at the basis of some species-poor and old Malagasy clades. In 20 clades for which stem and crown ages are repesented in our data set, we substracted crown from stem age to obtain the stem-crown age gap in cladogenesis, here abbreviated SCG. The obtained SCG values are significantly correlated with stem age (but not with species numbers) in the two analyses (ACR: R=0.652; P=0.0018; ICR: R=0.665, P=0.0014). This may indicate that the programs stretch basal branches to become artificially longer in old clades. To correct for such a possible influence of oversmoothing, we calculated a relative SCG, dividing SCG by stem age. These relative SCG values were significantly negatively correlated with species richness (non-parametric correlations; ACR: Spearman's R= −0.639, P=0.0024; ICR: R= −0.660, P=0.0015) (Fig. S3) but not with clade age, suggesting that groups in which diversification started soon after colonization are the most species rich ones.

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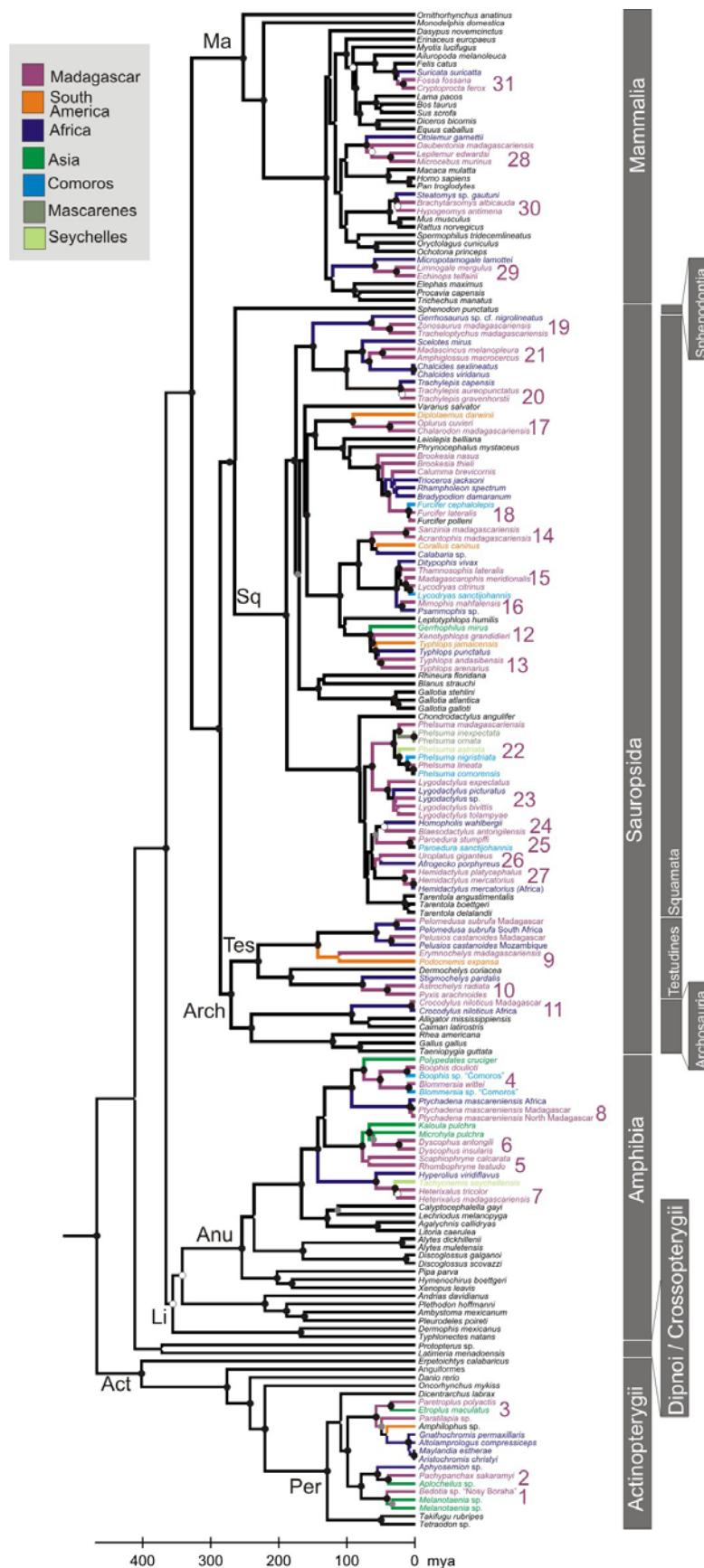
Figure S1. Vertebrate timetree calculated based on a 50% majority rule consensus tree from a Bayesian analysis of 1747 bp DNA sequences of the RAG1 and BDNF genes (as in Fig. 1, showing names of all taxa included). Black dots at nodes indicate Bayesian support >0.98, grey dots 0.95–0.98, and white dotted nodes were constrained before the analysis. Numbers denote clades as listed in Table 1 and Table S6.

6
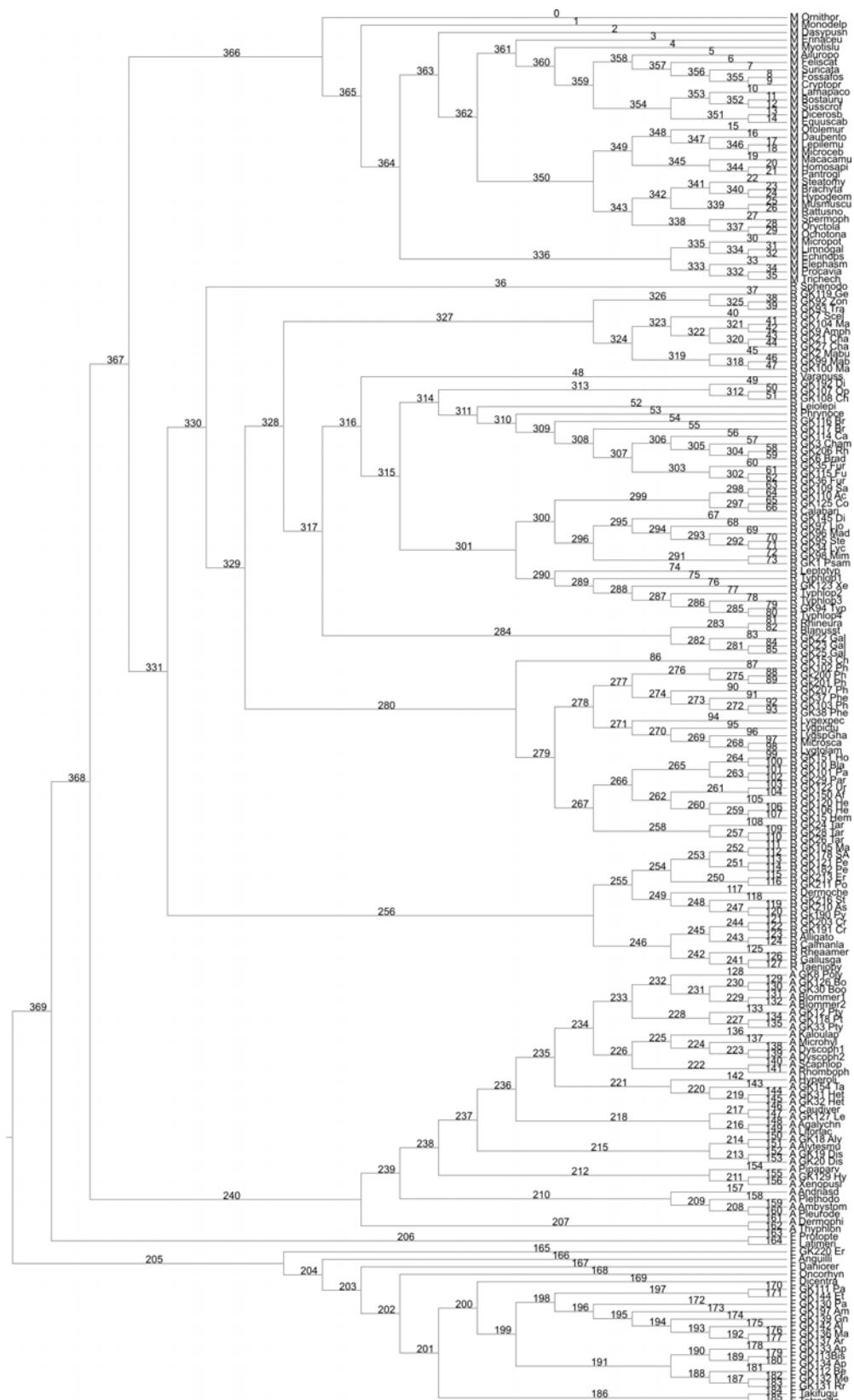
Figure S2. Ultrametric tree obtained by ACR analysis (using Multidivtime), showing node numbers (compare with Table S4 for placement of age constraints).

7
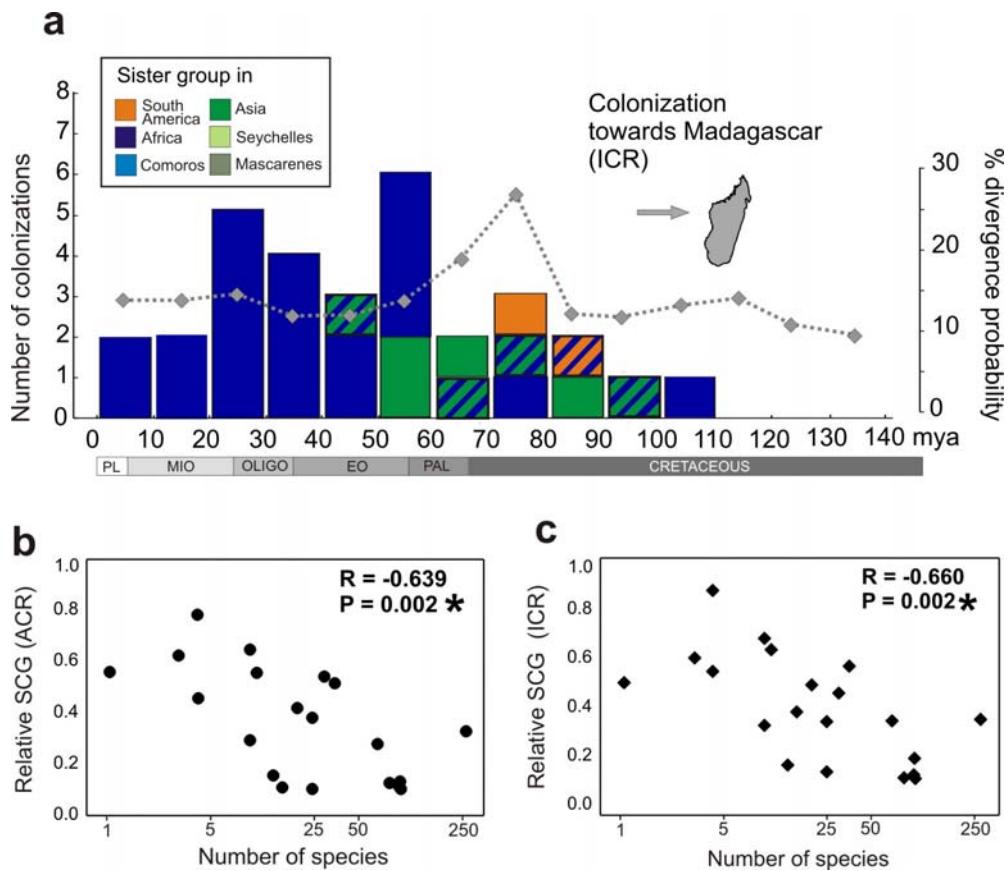
Figure S3. (a) Histogram of ICR stem divergence age distribution of Malagasy vertebrate clades, derived from the timetree in Fig. 1 of the main paper, color-coded depending on the distribution of their sister group. Rhomboids connected by dotted line in upper figure show the probability of clade origins derived from a model assuming 80% dispersal and 20% vicariance (S22). (b/c) Scatterplots of species richness of endemic Madagascar clades vs. relative SCG values (stem minus crown age, divided by stem age for correction of oversmoothing), separately for ACR and ICR analyses. Test statistics refer to Spearman's R in non-parametric correlations, asterisks indicate significance.

Table S1. Primers used to amplify and sequence the fragments of the RAG1 and BDNF genes used in this study.

Gene	Primer name	Sequence (5' - 3')	Source
BDNF	BDNF_DRV_F1	ACCATCCTTTTCCTKACTATGG	S23
BDNF	BDNF_DRV_R1	CTATCTTCCCCTTTTAATGGTC	S23
BDNF	BDNF_DRV_F2	ACTATGGTTATTTCATAC	New
BDNF	BDNF_DRV_R3	GTGCATACACACGAAGTGTC	New
BDNF	BDNF_30	GTCAAGTGCCTTTGGAG	New
BDNF	BDNF_50	GACATGTCCACKGCAGTC	New
RAG1	Amp_F1	ACAGGATATGATGARAAGCTTGT	S24
RAG1	Amp_R1	AACTACGCTGCATTKCCAATRTCACA	S24
RAG1	Amp_R2	GGTGYTTYAACACATCTTCCATYTCRTA	S24
RAG1	Mart_R6	GTGTAGAGCCARTGRTGYTT	S24
RAG1	RAG1_DRV_UCF	GGGMGGCAGATCTTYCAGCC	New
RAG1	RAG1_DRV_UCR	TTGGACTGCCTGGCATTCAT	New
RAG1	Mart_FL1	AGCTGGAGYCARTAYCAYAARATG	S24
RAG1	Amp_F2	ACNGGNMGICARATCTTYCARCC	S24

RAG1	RAG1_DRV_LygoF	ATAACAGGAAGGCAGATTTTCCAG	New
RAG1	RAG1_DRV_LygoR	CTTGGACTGCCTGGCATTCAT	New
RAG1	RAG1_FOR1	CARTAYCAYAAGATGTACMGGAC	New
RAG1	RAG1_FOR2	CAYAAGATGTACMGGACNGT	New
RAG1	RAG1_REV1	GACTGYCTGGCRTTCATYTTBCG	New
RAG1	RAG1_REV2	TTGGACTGCCTGGCRTTCAT	New
RAG1	RAG1_REV3	TRTGRGYRTTCATAAACYTYTG	New
RAG1	RAG1_51	GCNGARAGRGARGCYATGAA	New
RAG1	RAG1_31	TAGCCYCCCAKCTCNAG	New
RAG1	RAG1ForB	AACTGTGYTGCAAGCCVYTG	New (Internal)
RAG1	RAG1ForC	CTCCGVAARAAGATGAAYCTVAA	New (Internal)
RAG1	RAG1RevB	ACAGTCTCYTKGGTCATNAGC	New (Internal)
RAG1	RAG1RevC	ATGCCTCCCAKCTCMAGC	New (Internal)

Table S2. Species included in this study and Genbank accession numbers of the respective RAG1 and BDNF sequences. Taxa are listed according to their appearance in the tree. For species belonging to an endemic Malagasy clade, the clade number (as in Table 1 of main paper) is given. For direct sister groups of endemic Malagasy clades, the clade number with the notion sg for "sister group" is given. * indicate sequences extracted from whole-genome shortgun sequences.

Genus	Species	Clade no.	RAG1	BDNF
Suricata	*suricatta*	sg 31	JQ073171	JQ073048
Fossa	*fossana*	31	JQ073172	JQ073049
Cryptoprocta	*ferox*	31	JQ073173	JQ073050
Otolemur	*garnettii*	sg 28	AAQR03002574.1*	AAQR03002289.1*
Daubentonia	*madagascariensis*	28	JQ073174	JQ073051
Lepilemur	*edwardsi*	28	JQ073175	JQ073052
Microcebus	*murinus*	28	ABDC01016729.1*/ABDC01016728.1*	EU142161
Steatomys	sp. "gautuni"	sg 30	JQ073176	JQ073053
Brachytarsomys	*albicauda*	30	JQ073177	JQ073054
Hypogeomys	*antimena*	30	JQ073178	JQ073055
Micropotamogale	*lamottei*	sg 29	JQ073179	JQ073056
Limnogale	*mergulus*	29	JQ073180	JQ073057
Echinops	*telfairii*	29	JQ073181	AAIY01688610.1*
Lama	spp.		*L. glama* AF305953	*L. pacos* JQ073058
Bos	*taurus*		DAAA02041329.1*	DAAA02041147.1*
Sus	*scrofa*		AB091392	NM214259
Diceros	*bicornis*		JQ073182	JQ073059
Equus	*caballus*		NC009155	AB264324
Ailuropoda	*melanoleuca*		ACTA01092121.1*	U56638*
Felis	*catus*		ACBE01349034.1*	ACBE01347057.1*
Homo	*sapiens*		NM000448	NM170735
Oryctolagus	*cuniculus*		M77666	AAGW02037408.1*
Ochotona	*princeps*		JQ073183	JQ073060
Elephas	*maximus*		EF551560/AY125021	JQ073061
Procavia	*capensis*		ABRQ01060068.1*	JQ073062
Trichechus	*manatus*		JQ073184	JQ073063
Monodelphis	*domestica*		U51897	AAFR03017856.1*
Ornithorhynchus	*anatinus*		AAPN01039734.1	AAPN01345083.1*
Dasypus	*novemcinctus*		AAGV020408147.1*	AAGV020138133.1*
Myotis	*lucifugus*		AAPE02039491.1*	AAPE02023582.1*
Pan	*troglodytes*		AACZ03078426.1*	AACZ03081690.1*
Erinaceus	*europaeus*		AANN01829829.1*	AANN01683139.1*

Macaca	*mulatta*		NW001100721.1*	AANU01216369.1*
Mus	*musculus*		AEKR01051132.1*	AEKR01039813.1*
Rattus	*norvegicus*		AABR05104208.1*	X67108
Spermophilus	*tridecemlineatus*		AAQQ01493562.1*	AAQQ01153161.1*
Zonosaurus	*madagascariensis*	19	JQ073185	JQ073064
Tracheloptychus	*madagascariensis*	19	JQ073186	JQ073065
Gerrhosaurus	sp. cf. nigrolineatus	sg 19	JQ073187	JQ073066
Oplurus	*cuvieri*	17	JQ073188	JQ073067
Chalarodon	*madagascariensis*	17	JQ073189	JQ073068
Diplolaemus	*darwinii*	sg 17	JQ073190	JQ073069
Sanzinia	*madagascariensisv olontany*	14	JQ073191	JQ073070
Acrantophis	*madagascariensis*	14	JQ073192	JQ073071
Corallus	*caninus*		JQ073193	JQ073072
Calabaria	*reinhardtii*	sg 14	EU402839/ AY487391	EU402631
Madascincus	*melanopleura*	21	JQ073194	JQ073073
Amphiglossus	*macrocercus*	21	JQ073195	JQ073074
Scelotes	*mirus*	sg 21	JQ073196	JQ073075
Lycodryas	*citrinus*	15	JQ073197	JQ073076
Madagascarophis	*meridionalis*	15	JQ073198	JQ073077
Liopholidophis	*lateralis*	15	JQ073199	JQ073078
Ditypophis	*vivax*	sg 15	JQ073200	JQ073079
Lycodryas	*sanctijohannis*	15	JQ073201	JQ073080
Mimophis	*mahfalensis*	16	JQ073202	JQ073081
Psammophis	sp. (Namibia)	sg 16	JQ073203	JQ073082
Trachylepis	*aureopunctatus*	20	JQ073204	JQ073083
Trachylepis	*gravenhorstii*	20	JQ073205	JQ073084
Trachylepis	*capensis*	sg 20	JQ073206	JQ073085
Calumma	*brevicornis*	18	JQ073207	JQ073086
Furcifer	*lateralis*	18	JQ073208	JQ073087
Brookesia	*nasus*	18	JQ073209	JQ073088
Brookesia	*thieli*	18	JQ073210	JQ073089
Trioceros	*jacksoni*	(18)	JQ073211	JQ073090
Rhampholeon	*spectrum*	(18)	JQ073212	JQ073091
Bradypodion	*damaranum*	(18)	JQ073213	JQ073092
Furcifer	*cephalolepis*	(18)	JQ073214	JQ073093
Furcifer	*polleni*	(18)	JQ073215	JQ073094
Pelomedusa	*subrufa*		JQ073216	JQ073095
Pelomedusa	*subrufa*		JQ073217	JQ073096
Pelusios	*castanoides* (Mozambique)		JQ073218	JQ073097
Pelusios	*castanoides* (Madagascar)		JQ073219	JQ073098
Erymnochelys	*madagascariensis*	9	JQ073220	JQ073099
Podocnemis	*expansa*	sg 9	JQ073221	JQ073100
Astrochelys	*radiata*	10	JQ073222	JQ073101
Pyxis	*arachnoides*	10	JQ073223	JQ073102
Stigmochelys	*pardalis*	sg 10	JQ073224	JQ073103
Phelsuma	*madagascariensis*	22	JQ073225	JQ073104
Phelsuma	*lineata*	22	JQ073226	JQ073105
Phelsuma	*astriata*	(22)	JQ073227	JQ073106
Phelsuma	*nigristriata*	(22)	JQ073228	JQ073107
Phelsuma	*comorensis*	(22)	JQ073229	JQ073108
Phelsuma	*inexpectata*	(22)	JQ073230	JQ073109
Phelsuma	*ornata*	(22)	JQ073231	JQ073110
Lygodactylus	*bivittis*	23	JQ073232	JQ073111
Lygodactylus	*expectatus*	23	JQ073233	JQ073112
Lygodactylus	*tolampyae*	23	JQ073234	JQ073113
Lygodactylus	*picturatus*	(23)	JQ073235	JQ073114
Lygodactylus	sp.	(23)	JQ073236	JQ073115

Blaesodactylus	*antongilensis*	24	JQ073238	JQ073117
Homopholis	*wahlbergii*	sg 24	JQ073237	JQ073116
Paroedura	*stumpffi*	25	JQ073239	JQ073118
Uroplatus	*giganteus*	26	JQ073240	JQ073119
Afrogecko	*porphyreus*	sg 25/26	JQ073241	JQ073120
Chondrodactylus	*angulifer*		JQ073242	JQ073121
Paroedura	*sanctijohannis*	(25)	JQ073243	JQ073122
Hemidactylus	*mercatorius*	27	JQ073244	JQ073123
Hemidactylus	*platycephalus*		JQ073246	JQ073125
Hemidactylus	*mabouia*	sg 27	JQ073245	JQ073124
Crocodylus	*niloticus*	11	JQ073247	JQ073126
Crocodylus	*niloticus*	sg 11	JQ073248	JQ073127
Typhlops	*andasibensis*	13	JQ073249	GU902453
Xenotyphlops	*grandidieri*	12	JQ073250	GU902457
Typhlops	*arenarius*	13	GU902699	GU902455
Typhlops	*jamaicensis*		EU402866	EU402664
Leptotyphlops	*humilis*		EU402851	EU402648
Afrotyphlops	*punctatus*		GU902645	GU902395
Gerrhophilus	*mirus*		GU902644	GU902394
Chalcides	*sexlineatus*		JQ073251	JQ073128
Chalcides	*viridanus*		JQ073252	JQ073129
Gallotia	*atlantica*		JQ073253	JQ073130
Gallotia	*galloti*		JQ073254	JQ073131
Gallotia	*stehlini*		JQ073255	JQ073132
Tarentola	*angustimentalis*		JQ073256	JQ073133
Tarentola	*boettgeri*		JQ073257	JQ073134
Tarentola	*delalandii*		JQ073258	JQ073135
Sphenodon	*punctatus*		AY662576	GU457846
Phrynocephalus	spp.		*P. raddei* AY662586	*P. mystaceus* DQ340735
Leiolepis	*belliana*		FJ356734	AY987965
Gallus	*gallus*		NM001031188	NM001031616
Dermochelys	*coriacea*		FJ039918	FJ039908
Taeniopygia	*guttata*		XM002199682	DQ086496
Rhea	*americana*		DQ881836	EU737942
Alligator	*mississippiensis*		AF143724	EU275888
Caiman	*latirostris*		AY239167	EF646298
Blanus	*strauchi*		AY444050	FJ441847
Rhineura	*floridana*		AY662618	GU457878
Varanus	spp.		*V. salvator* EU402828/ *V. griseus* AY662608	EU402618
Boophis	*doulioti*	4	JQ073259	JQ073138
Blommersia	*wittei*	4	AY323774	EF396018
Boophis	sp. "Comoros"	4	JQ073260	JQ073139
Blommersia	sp. "Comoros"	4	AY323775	EF396017
Polypedates	*cruciger*	sg 4	JQ073261	JQ073140
Dyscophus	*antongili*	6	EF396084	EF396005
Dyscophus	*insularis*	6	EF396083	EF396006
Kaloula	*pulchra*	sg 5/6	EF396091	EF396015
Scaphiophryne	*calcarata*	5	EF396106	EF396032
Rhombophryne	*testudo*	5	EF396105	EF396031
Microhyla	*pulchra*	sg 5/6	EF396093	EF396021
Heterixalus	*tricolor*	7	JQ073262	JQ073141
Heterixalus	*madagascariensis*	7	JQ073263	JQ073142
Tachycnemis	*seychellensis*	(7)	JQ073264	JQ073143
Hyperolius	*viridiflavus*	sg 7	AY323769	EF396013
Ptychadena	*mascareniensis*	8	JQ073265	JQ073144
Ptychadena	*mascareniensis*	8	JQ073266	JQ073145
Ptychadena	*mascareniensis*	sg 8	JQ073267	JQ073146
Alytes	*dickhillenii*		JQ073268	JQ073147

Alytes	*muletensis*		AY323755	EF407510
Discoglossus	*galganoi jeanneae*		JQ073269	JQ073148
Discoglossus	*scovazzi*		JQ073270	JQ073149
Calyptocephalella	*gayi*		AY583337	JQ073150
Lechriodus	*melanopyga*		JQ073271	JQ073151
Hymenochirus	*boettgeri*		JQ073272	JQ073152
Pipa	*parva*		AY323761	EF407512
Xenopus	*leavis*		L19324	BC082887
Agalychnis	*callidryas*		AY323765	EF407508
Litoria	*caerulea*		AY323767	EF407509
Plethodon	*hoffmanni*		EU275801	EU275883
Andrias	*davidianus*		AY650142	EU275889
Ambystoma	*mexicanum*		AY323752	EF195175
Pleurodeles	*poireti*		EU275787	EF453368
Dermophis	*mexicanus*		JQ073273	JQ073153
Typhlonectes	*natans*		JQ073274	JQ073154
Paretroplus	*polyactis*	3	JQ073275	JQ073155
Paratilapia	sp.	3	JQ073276	JQ073156
Etroplus	*maculatus*	3	JQ073277	JQ073157
Maylandia	*estherae*		JQ073278	JQ073158
Aristochromis	*christyi*		JQ073279	JQ073159
Gnathochromis	*permaxillaris*		JQ073280	JQ073160
Altolamprologus	*compressiceps*		JQ073281	JQ073161
Amphilophus	sp.		JQ073282	JQ073162
Pachypanchax	*sakaramyi*	2	JQ073283	JQ073163
Aphyosemion	sp.		JQ073284	JQ073164
Aplocheilus	sp.	sg 2	JQ073285	JQ073165
Bedotia	sp. "Nosy Boraha"	1	JQ073286	JQ073166
Melanotaenia	sp.	sg 1	JQ073287	JQ073167
Melanotaenia	sp.	sg 1	JQ073288	JQ073168
Anguillidae (*Ophichthus/Anguilla*)			*Ophichthus gomesii* AY430203	*Anguilla anguilla* AY762996
Takifugu	*rubripes*		AF108420	CAAB01000551
Tetraodon	*nigroviridis*		CAAE01014715.1	CAAE01009551.1
Danio	*rerio*		NM131389	BC066399
Dicentrarchus	*labrax*		EF095651	FJ711591
Oncorhynchus	*mykiss*		U15663	GU108576
Erpetoichthys	*calabaricus*		JQ073291	MISSING
Protopterus	ssp.		*P.dolloi* AY442928	*P.* sp. EU275856
Latimeria	*menadoensis*		JQ073290	JQ073170
Squalus	*acantias*		JQ073289	JQ073169
Callorhinchus	*milii*		AAVX01004067.1*	AAVX01645327*

Table S3. Bayes factors. 2ln Bayes factors results of comparisons of all four partitioning strategies with the concatenated dataset. Bayes factors were calculated from estimated harmonic means of likelihoods by the sump command in MrBayes, and subsequently the partition strategy P3 was chosen for the final analysis.

Partition	N partitions	Partition identity	Harmonic mean	P3	P1	P2
P4	2	BDNF gene; RAG1 gene	−203384.91	−5060.38	−3971.34	−3426.76
P2	6	BDNF codon positions; RAG1 codon positions	−201671.53	−1633.62	−544.58	-
P1	1	All data combined	−201399.24	−1089.04	-	
P3	2	1st and 2nd positions of both BDNF and RAG1; 3rd position of both BDNF and RAG1	−200854.72	-		

Table S4. Detailed list and descriptions of age constraints used for calculation of timetrees, with references for each constraint. Clade numbers as described in Materials and Methods of main paper. Calibrations C15 and C16 (marked with an asterisk) were excluded because of doubts on fossil identity; C19, C27 and C29 (marked with two asterisks) were excluded because of their poor performance in cross-validation tests.

Constraintnumber	Major taxon	Kind of constraint	Description	Constraint age	Reference
C43	Actinopterygii	island / lake	Oldest age of Lake Malawi cichlids	<2	S25
C42	Actinopterygii	island / lake	Oldest age of Lake Tanganyika cichlids	<12	S25
C44	Actinopterygii	fossil	split of cichlids from other Percomorpha (oldest fossils: Eocene cichlid)	>45	S26
C46	Actinopterygii	fossil	Split of Tetraodontiformes from other percomorphs [earliest fossil tetraodontiform from the Cenomanian (Cretaceous)]	>98	S27
C47	Actinopterygii	fossil	Split of Ostariophysi (*Danio*) from other fishes (earliest stem ostaryophysan: Tischlingerichthys from Tithonian (Jurassic))	>146	S27
C48	Actinopterygii	fossil	Split of Elopomorpha = *Anguilla* from other fishes (stem-elopomorph *Elopsomolos*)in the the Kimmeridgian (Jurassic)	>151	S27
C45	Actinopterygii	fossil	Minimum age of Actinopterygiaknown from the Givetian/Eifelian boundary (earliest stem Actinopteri)	>392	S27
C39	Lissamphibia	fossil	Minimum age of split of hynobiid and cryptobranchid salamanders (oldest fossil: cryptobranchid record)	>161	S28
C38	Lissamphibia	vicariance	Minimum age of the separation among African and South American pipid frogs (continental separation of Africa and South America)	>86	S28
C31	Lissamphibia	vicariance	Split of Spanish/Moroccan *Discoglossus* at end of Messinian salinity crisis	>5.3	S29
C32	Lissamphibia	vicariance	Split of Spanish/Moroccan *Alytes* at end of Messinian salinity crisis	>5.3	S29
C37	Lissamphibia	vicariance	Minimum age of the split between *Agalychnis* and *Litoria* (last connection between Australia and South America	>42	S28
C36	Lissamphibia	fossil	Minimum age of *Calyptocephalella* (oldest fossils) = minimum age of the split between *Calyptocephalella* and *Lechriodus* (fossil records of *Calyptocephalella*).	>53	S28
C33	Lissamphibia	island / lake	Maximum age of the split between Malagasy/Comoran *Blommersia* earlier than rise of Mayotte (volcanic origin of the oldest Comoro island Mayotte)	<15	S30
C34	Lissamphibia	island / lake	Split of Malagasy/Comoran *Boophis* earlier than rise of Mayotte	<15	S30
C41	Lissamphibia	fossil	The oldest fossils attributed to the Discoglossidae (*Eodiscoglossus oxoniensis*) split Discoglossids other frogs	>167	S31
C40	Lissamphibia	fossil	Minimum age of the split of pipid frogs from their sister group (records of Mesozoic fossil pipids, oldest fossil)	>140	S28
C35	Lissamphibia	fossil	Minimum age of frog-salamander split (fossil record of frog ancestor *Triadobatrachus*)	>230	S28
C5	Archosauria	fossil	Alligator-Caiman split	66–71	S32

C4	Archosauria	fossil	Split between birds and crocodiles	235–250.4*	S32
C10	Archosauria	fossil	Split of sea turtles from their sister group (oldest sea turtle fossil *Santanachelys*)	>110	S33
C7	Archosauria	fossil	*Erymnochelys – Podocnemis* split (fossil of *Erymnochelys*)	>65	S34
C6	Archosauria	fossil	*Pelomedusa – Pelusios* split	>25	S34
C9	Archosauria		Split between Podocnemididae – Pelomedusidae	>100	S34
C8	Archosauria	fossil	Split between Cryptodira and Pleurodira	>210	S34
C11	Archosauria	fossil	Split of turtles from other reptiles (oldest turtle fossil, *Odontochelys*)	>220	S35
C20	Squamata	island / lake	Canary island colonization by geckos (*Tarentola boettgeri – T. delalandi*) – age emergence of island Gran Canaria	<14	S36
C22	Squamata	island / lake	Split of *Phelsuma comorensis* from Malagasy *Phelsuma* – oldest age of Mayotte	<15	S30
C21	Squamata	island / lake	Split of *Phelsuma nigristriata* from Malagasy *Phelsuma* – oldest age of Mayotte	<15	S30
C23	Squamata	island / lake	Split between *Phelsuma inexpectata – P. ornata* (max age of Reunion colonized by inexpectata from the older Mauritius)	<2.1	S30
C19**	Squamata	island / lake	Canary island colonization by lizards (*Gallotia stehlini – G.atlantica*) – age emergenceof island Gran Canaria	<14	S36
C14	Squamata	fossil	Amphisbaenian – lacertid split (fossil rhineurid)	> 64	S37
C17	Squamata	fossil	Split of Booidea from Caenophidia (earlist booid fossil)	>75	S34
C18	Squamata	island / lake	Split of Comoran *Furcifer* from Malagasy taxa (max. age of Mayotte)	<15	S30
C15*	Squamata	fossil	Split of acrodont iguanians	>165	S34
C16*	Squamata	fossil	Split of iguanians (fossil of *Bharatagama*, Middle Jurassic) from other lizards	>180	S34
C24	Squamata	island / lake	Canary island colonization by lizards (*Chalcides sexlineatus – C.viridanus*) – age of island Gran Canaria	<14	S36
C13	Squamata	fossil	Split of geckos from other squamates (oldest fossil gecko eggs)	>55	S38
C12	Squamata	fossil	*Sphenodon* – squamate split (earliest rhynchocephalian)	>228	S37
C3	Squamata	fossil	Split between archosaurs and lepidosaurs	259.7–299.8*	S32
C27**	Mammalia	fossil	Split Paenungulata	54–65	S2
C28	Mammalia	fossil	Split Lagomorpha	48.6–61.5	S39
C29**	Mammalia	fossil	Split Primates	55.5–65.8	S39
C26	Mammalia	fossil	Split between horse – rhino	54–58	S2
C30	Mammalia	fossil	Split Cetartiodactyla	55–65	S2
C25	Mammalia	fossil	Split between Caniformis – Feliformis	42.8–63.8	S40
C1	Tetrapoda	fossil	Split between diapsids and synapsids	338–288	S41
C2	Sarcopterygii	fossil	Split between lungfish and tetrapods	408–419	S32

Table S5. Results of cross validation procedures (CV2 and CV3; CV1 was an automated procedure described in the main text) carried out on the set of age constraints as detailed in Table S4. Constraint numbers as in Table S4 and main paper. Two different series of cross validations were carried out: in CV2 all constraints except one were in turn excluded, while in CV3, one constraint was in turn excluded. The first three columns show by how many CV2 analyses a certain constraint was recovered, considering either only the absolute age estimate, or the standard error (SE), or the credibility intervals (CrIs). The next three columns show for CV2 how many other constraints were recovered by the analysis based on each single constraint, based on absolute estimates, SE or CI. The last three columns show for CV3 whether the excluded constraint was recovered by the analysis, based on absolute age estimate or considering SE or CI. Red colour marks particularly poor performance values for a constraint in being recovered or recovering other constraints in CV2, or negative results in CV3.

Constraint number	CV2 Recovered	CV2 Recovered (SE)	CV2 Recovered (CrI)	CV2 Recovers	CV2 Recovers (SE)	CV2 Recovers (CrI)	CV3 Recovered	CV3 Recovered (SD)	CV3 Recovered (CrI)
C43	47	47	47	27 (29)	42 (43)	45	Yes	Yes	Yes
C42	9 (15)	47	47	29	42 (43)	45 (46)	Almost	Yes	Yes
C44	47	47	47	26 (28)	41 (43)	45	Yes	Yes	Yes
C46	45	47	47	25 (27)	40	45	Yes	Yes	Yes
C47	47	47	47	26 (28)	42 (43)	45	Yes	Yes	Yes
C48	47	47	47	26 (28)	41 (42)	45	Yes	Yes	Yes
C45	3	39	43	26 (27)	40	42	No	No	No
C39	47	47	47	26 (28)	41 (42)	45	Yes	Yes	Yes
C38	47	47	47	26 (28)	42 (43)	45	Yes	Yes	Yes
C31	47	47	47	25 (28)	42	45	Yes	Yes	Yes
C32	47	47	47	26 (28)	41 (43)	45	Yes	Yes	Yes
C37	46	47	47	25 (27)	41	45	Yes	Yes	Yes
C36	47	47	47	26 (28)	42 (43)	45	Yes	Yes	Yes
C33	2 (4)	47	47	30 (34)	41 (43)	46	Yes	Yes	Yes
C34	1	47	47	30 (33)	40 (41)	46	Almost	Yes	Yes
C41	47	47	47	24 (27)	42 (43)	45	Yes	Yes	Yes
C40	47	47	47	26 (27)	42 (43)	45	Yes	Yes	Yes
C35	47	47	47	26 (27)	42 (43)	45	Yes	Yes	Yes
C5	2 (9)	46	46	29 (31)	42 (43)	45	No	Yes	Yes
C4	9 (15)	41	45	29 (31)	39 (41)	45	Almost	Yes	Yes
C10	47	47	47	26 (28)	42 (43)	45	Yes	Yes	Yes
C7	47	47	47	26 (28)	42 (43)	45	Yes	Yes	Yes
C6	47	47	47	26 (28)	42	45	Yes	Yes	Yes
C9	46	47	47	26 (28)	41 (42)	45	Yes	Yes	Yes
C8	42	45 (46)	47	25 (27)	39 (40)	44	Yes	Yes	Yes

C11	45	47	47	26 (28)	41	45	Yes	Yes	Yes
C20	38 (44)	47	47	29 (30)	41 (42)	46	Yes	Yes	Yes
C22	47	47	47	26 (29)	42 (43)	46	Yes	Yes	Yes
C21	1 (4)	45 (47)	47	30 (33)	41	46	No	Yes	Yes
C23	1	47	47	29 (31)	41	46	No	Yes	Yes
C19	0	8 (32)	44 (45)	33 (35)	40	45	No	No	No
C14	47	47	47	26 (29)	42 (43)	45	Yes	Yes	Yes
C17	0	3 (4)	41 (44)	29 (30)	38	42 (44)	No	Yes	Yes
C18	47	47	47	30 (31)	40 (42)	45 (46)	Yes	Yes	Yes
C15	2	32	42 (43)	28 (29)	37 (38)	40	Yes	Yes	Yes
C16	2	30 (32)	41 (42)	28 (29)	37 (38)	40	Almost	Yes	Yes
C24	47	47	47	27 (31)	42 (43)	45	Yes	Yes	Yes
C13	47	47	47	26 (29)	42 (43)	45 (46)	Yes	Yes	Yes
C12	45 (46)	45 (46)	47	25 (28)	40	45	Yes	Yes	Yes
C3	6 (7)	42	45	31 (32)	39 (40)	42 (43)	Yes	Yes	Yes
C27	1	2	29 (32)	27 (29)	37 (38)	43	No	Yes	Yes
C28	1	3	40	29 (32)	38	43 (46)	Yes	Yes	Yes
C29	0	0	1 (2)	24 (25)	36	40	No	No	No
C26	3 (8)	43 (44)	46	29 (30)	41 (42)	44	No	No	Almost
C30	12 (36)	44	46	28 (30)	41 (42)	44	No	No	Almost
C25	2	27 (36)	47	28 (31)	41 (42)	46	Yes	Yes	Yes
C1	4	40	42 (43)	28 (32)	40	41	Yes	Yes	Yes
C2	1 (2)	38	43	32 (34)	40	44	No	Yes	Yes

Table S6. List of endemic Malagasy clades and their recovered stem and crown ages using the ACR approach (with Multidivtime software), as well as non-Malagasy clades for which an out-of-Madagascar dispersal is assumed. For node numbers, see Figure S2.

Clade number	Number of species	Taxonomic name	Description	Distribution of sister group	Node number -stem	Stem-age (mya)	Stem age in literature (mya)	Reference	Percent difference to literature	Node number - crown	Crown-age (mya)	Crown / Stem age ratio (percent)	Stem minus Crown age
1	26	Bedotiidae	Bedotiid fishes	Asia	188	41.9	n.a.		n.a.	n.a.	n.a.	n.a.	n.a.
2	6	Aplocheilidae	Malagasy species of *Pachypanchax*	Asia	189	40.6	n.a.		n.a.	n.a.	n.a.	n.a.	n.a.
3	33	Cichlidae	Malagasy clade of cichlid fishes	Africa	198	58.1	96	S27	40% younger	n.a.	n.a.	n.a.	n.a.
4	266	Mantellidae	Mantellid frogs	Asia	232	75.7	61	S42	20% older	231	51.2	67.6	24.5
5	95	Microhylidae: Scaphiophryninae and Cophylinae	Scaphiophrynine + Cophyline frogs	Asia/Africa	226	77.0	66	S42	14% older	222	66.9	86.9	10.1
6	3	Microhylidae: Dyscophinae	Dyscophine frogs	Asia	224	61.5	55	S42	11% older	223	22.8	37.1	38.7
7	10	Hyperoliidae	Malagasy hyperoliid frogs (*Heterixalus*)	Africa	221	57.1	25	S43	56% older	219	25.0	43.8	32.1
8	1	Ptychadenidae	Malagasy grass frog (*Ptychadena mascareniensis*)	Africa	228	7.8	n.a.		n.a.	227	3.4	43.6	4.4
9	1	Podocnemididae	Rere turtle (*Erymnochelys*)	SouthAmerica/Africa	250	111.6	76	S34	32% older	n.a.	n.a.	n.a.	n.a.
10	4	Testudinidae	Malagasy genera of land tortoises	Africa	248	78.5	n.a.		n.a.	247	42.6	54.3	35.9
11	1	Crocodylidae	Malagasy populations of nile crocodile	Africa	244	5.4	n.a.		n.a.	n.a.	n.a.	n.a.	n.a.
12	1	Xenotyphlopidae	Xenotyphlopid blindsnakes	Africa/Asia	288	60.7	97	S44	37% younger	n.a.	n.a.	n.a.	n.a.
13	15	Typhlopidae	Malagasy clade of typhlopid blindsnakes	Africa/Asia	286	53.7	ca. 65	S44	17% younger	285	48.0	89.4	5.7
14	4	Boidae	Malagasy genera of giant snakes	Africa	299	60.8	77	S34	21% younger	298	12.5	20.6	48.3
15	80	Lamprophiidae: Pseudoxyrhophiinae	Pseudoxyhophiine snakes	Africa	295	23.8	31	S45	23% younger	294	20.8	87.4	3.0
16	1	Lamprophiidae: Psammophiinae	Malagasy psammophiine snake (*Mimophis*)	Africa	291	18.7	13	S45	30% older	n.a.	n.a.	n.a.	n.a.
17	9	Opluridae	Malagasy iguanas	SouthAmerica	313	90.0	90	S34	0% older/younger	312	31.5	35.0	58.5
18	94	Chamaeleonidae	Chameleons	Africa	309	53.7	ca. 60	S46	10% younger	308	47.7	88.8	6.0
19	19	Gerrhosauridae	Malagasy gerrhosaurid lizards	Africa	326	60.5	n.a.		n.a.	325	35.0	57.9	25.5

No	n	Taxon	Description	Region									
20	13	Scincidae: *Trachylepis*	Malagasy species of *Trachylepis* skinks	Africa	319	19.1	n.a.		n.a.	318	16.1	84.3	3.0
21	67	Scincidae: Scincinae	Malagasy linages of scincine skinks	Africa	322	65.1	n.a.		n.a.	321	47.0	72.2	18.1
22	34	Gekkonidae: Phelsuma	*Phelsuma* day geckos	Africa	278	62.3	n.a.		n.a.	277	29.7	47.7	32.6
23	24	Gekkonidae: *Lygodactylus*	*Lygodactylus* day geckos	Africa	278	62.3	n.a.		n.a.	271	38.4	61.6	23.9
24	4	Gekkonidae: *Blaesodactylus*	*Blaesodactylus* geckos	Africa	264	42.3	n.a.		n.a.	n.a.	n.a.	n.a.	n.a.
25	19	Gekkonidae: *Paroedura*	*Paroedura* geckos	Africa	265	57.0	n.a.		n.a.	n.a.	n.a.	n.a.	n.a.
26	19	Gekkonidae: *Uroplatus*	*Uroplatus* leaftail geckos	Africa	261	50.7	n.a.		n.a.	n.a.	n.a.	n.a.	n.a.
27	1	Gekkonidae: *Hemidactylus*	Malagasy populations of house gecko (*H. mercatorius*)	Africa	259	4.1	n.a.		n.a.	n.a.	n.a.	n.a.	n.a.
28	96	Strepsirhini	Lemurs	Africa/Asia	348	71.2	60	S2	16% older	347	63.7	89.5	7.5
29	29	Tenrecidae	Tenrecs	Africa	335	59.7	42 / 47	S2 / S47	21% older (BMC)	334	27.1	45.4	32.6
30	24	Muridae: Nesomyinae	Endemic Malagasy subfamily of rodents	Africa	341	27.9	24	S2	14% older	340	25.0	89.6	2.9
31	9	Eupleridae	Endemic Malagasy family of carnivores	Africa	356	25.8	26	S2	0%	355	18.2	70.5	7.6

Taxon	Species	Region		
Cichlidae: Dispersal to India/Asia	*Etroplus*	Asia	197	35.7
Hyperoliidae: Dispersal to Seychelles	*Tachycnemis*	Seychelles	220	28.1
Phelsuma: Dispersal to Comoros 1	Comoran *Phelsuma* species	Comoros	273	11.1
Phelsuma: Dispersal to Comoros 2	Comoran *Phelsuma* species	Comoros	272	3.4
Phelsuma: Dispersal to Mascarenes	Mascarene radiation of *Phelsuma*	Mascarenes	276	21.5
Lygodactylus: Dispersal to Africa	African *Lygodactylus* day geckos	Africa	269	28.1
Paroedura: Dispersal to Comoros	*Paroedura sanctijohannis*	Comoros	263	6.6
Chameleons: Dispersal to Comoros	Comoran species of *Furcifer*	Africa	306	33.3
Boophis: Dispersal to Comoros	*Boophis* sp. "Comoros"	Comoros	292	6.8
Blommersia: Dispersal to Comoros	*Blommersia* sp. "Comoros"	Comoros	229	10.2
Lycodryas: Dispersal to Comoros	*Lycodryas sanctijohannis*	Comoros	230	11.2

Table S7. List of endemic Malagasy clades, their ecological, physiological and behavioral traits used as covariables to analyse the influence of clade age on species richness, and their occurrence "1" predominantly in rainforest (>75% of species), "2" in similar proportions in rainforest and dry forest / open areas, or 3 predominantly (>75% of species) in dry forest or in open areas within humid or subhumid regions.

Clade number	Taxonomic name	Aquatic or water-dependent 1 / non water-dependent2	Maximum Body Mass (cm SVL)	Ectotherm 1, Endotherm 2	Trophic position 1 herbivorous, 2 omnivorous/insectivorous, 3 carnivores feeding mainly on vertebrates	Internal fertilization 1 no, 2 yes	Parental care in numerous or all species 1 no, 2 yes	Generation time (years; estimated)	Rainforest / dry forest occurrence
1	Bedotiidae	1	20	1	2	1	1	1	1
2	Aplocheilidae	1	8	1	2	1	1	1	2
3	Cichlidae	1	40	1	2	1	2	2	2
4	Mantellidae	1	12	1	2	1	2	1	1
5	Microhylidae: Scaphiophryninae and Cophylinae	1	6	1	2	1	2	1	1
6	Microhylidae: Dyscophinae	1	10.5	1	2	1	1	2	2
7	Hyperoliidae	1	4	1	2	1	1	1	2
8	Ptychadenidae	1	5.5	1	2	1	1	1	3
9	Podocnemididae	1	41	1	1	2	1	20	3
10	Testudinidae	2	45	1	2	2	1	10	3
11	Crocodylidae	1	600	1	3	2	2	10	2
12	Xenotyphlopidae	2	26.3	1	2	2	1	1	3
13	Typhlopidae	2	60	1	2	2	1	1	2
14	Boidae	2	320	1	3	2	1	3	2
15	Lamprophiidae: Pseudoxyrhophiinae	2	170	1	3	2	1	2	1
16	Lamprophiidae: Psammophiinae	2	100	1	3	2	1	2	3
17	Opluridae	2	39	1	2	2	1	2	3
18	Chamaeleonidae	2	29.5	1	2	2	1	1	1
19	Gerrhosauridae	2	24.5	1	2	2	1	2	2
20	Scincidae: *Trachylepis*	2	11.2	1	2	2	1	1	2
21	Scincidae: Scincinae	2	22.6	1	2	2	1	1	2
22	Gekkonidae: Phelsuma	2	13.5	1	2	2	1	1	1

23	Gekkonidae: *Lygodactylus*	2	3.8	1	2	2	1	1	2
24	Gekkonidae: *Blaesodactylus*	2	13.2	1	2	2	1	2	2
25	Gekkonidae: *Paroedura*	2	10.7	1	2	2	1	1	3
26	Gekkonidae: *Uroplatus*	2	19.8	1	2	2	1	2	1
27	Gekkonidae: *Hemidactylus*	2	5.6	1	2	2	1	1	2
28	Strepsirhini	2	72	2	2	2	2	2	2
29	Tenrecidae	2	39	2	2	2	2	1	1
30	Muridae: Nesomyinae	2	35	2	2	2	2	1	1
31	Eupleridae	2	80	2	3	2	2	2	2

Table S8. Significance of different factors for clade species richness. All factors used for ANCOVA are listed. Analysis included either clade age estimates retrieved from ACR or ICR analyses. If marked with „deleted", the respective factor was deleted during model simplification based on AIC. The lower part of the table shows the results of Tukey's honest significance test on the variable "rainforest occurrence" in the minimum adequate model. See Table 7 for categories and original data of all factors.

	ACR	ICR
model	$R^2=0.74$; $F_{7,23}=9.32$, $P=<0.001$	$R^2=0.69$; $F_{7,23}=7.19$, $P=<0.001$
clade age	**0.002**	**0.019**
rainforest occurrence	**<0.001**	**<0.001**
body size	deleted	deleted
age to maturity	deleted	deleted
endothermy	0.211	0.124
trophic position	deleted	deleted
internal vs. external fertilization	**0.013**	0.069
parental care	**0.041**	0.092
aquatic vs. terrestrial habitat	**0.003**	**0.008**

	ACR	ICR
level	P value after adjustment for multiple comparison	P value after adjustment for multiple comparison
2-1	**0.002**	**0.038**
3-1	**<0.001**	**<0.001**
3-2	**0.004**	**0.017**

Appendix. Complete list of species and confirmed candidate species, CCS (S48), of native non-flying terrestrial and freshwater vertebrates of Madagascar. Species are listed separately for endemic clades, and brief discussions are included of the monophyly, characteristics and sister groups of the respective clades. Note that the clades might contain additional species; only those clade members occurring in Madagascar are listed. Clades not included in the list and/or in the analysis are: *Cryptoblepharus* skinks, *Pelusios* and *Pelomedusa* turtles (possibly introduced to Madagascar, therefore included in the timetree but not in further analyses), *Geckolepis, Matoatoa* and *Paragehyra* geckos, *Pantanodon* fishes, and numerous other fishes occurring in freshwater but being non-primary freshwater clades. Taxonomic authority is given for all nominal species reptiles and amphibians (not compiled for fishes and mammals). Names of candidate species set in quotation marks and not in italics are used as conditional names in the sense of the International Code of Zoological Nomenclature. Undescribed candidate species are given either with the name to which they were referred in the mentioned publications, or with the field or museum number of a voucher specimen that can serve for future reference (DRV, David R. Vieites field number; FAZC, Franco Andreone Zoological Collection; FGZC, Frank Glaw Zoological Collection; MRSN, Museo Regionale di Scienze Naturali di Torino; ZCMV; Zoological Collection Miguel Vences; ZMA, Zoological Museum Amsterdam; ZSM, Zoologische taatssammlung München).

Clade 1: Bedotiidae (26 species)
Clade description: The Bedotiidae are an endemic clade populating lotic waters on Madagascar's east coast. Their monophyly and sister-group relationship to the Australasian rainbowfishes (Melanotaeniidae) has been assessed by analysis of an extensive molecular data set (S49).
Bedotia geayi; *Bedotia madagascariensis*; *Bedotia longianalis*; *Bedotia marojejy*; *Bedotia masoala*; *Bedotia tricolor*; *Bedotia vondrozo*; *Bedotia* sp. "bemarivo"; *Bedotia* sp. "betampona"; *Bedotia* sp. "garassa"; *Bedotia* sp. "lazana"; *Bedotia* sp. "mahanara"; *Bedotia* sp. "manombo"; *Bedotia* sp. "marosivy"; *Bedotia* sp. "nosivolo"; *Bedotia* sp. "ranomafana"; *Bedotia* sp. "sambava"; *Rheocles alaotrensis*; *Rheocles derhami*; *Rheocles lateralis*; *Rheocles pellegrini*; *Rheocles sikorae*; *Rheocles wrightae*; *Rheocles* sp. "ambatovy"; *Rheocles* sp. "andapa"; *Rheocles* sp. "rianila"

Clade 2: Aplocheilidae (6 species)
Clade description: The Aplocheilidae are small killifish containing the South Asian genus *Aplocheilus*, and the genus *Pachypanchax* that occurs in western and northern Madagascar and includes one species from the Seychelles (not studied in our phylogeny). Sister-group relationships among these two genera have been assessed by molecular data (S50).
Pachypanchax sakaramyi; *Pachypanchax omalonotus*; *Pachypanchax arnoulti*; *Pachypanchax patriciae*; *Pachypanchax sparksorum*; *Pachypanchax varatraza*

Clade 3: Cichlidae (33 species)
Clade description: Cichlid fishes are a species-rich monophyletic group of rather typical western Gondwana distribution, with numerous species in the Neotropics, Africa, and Madagascar, and one species-poor genus (*Etroplus*) in South Asia. Molecular phylogenetic reconstructions to date (e.g., S51, S52; and the tree reconstructed herein)

place the cichlids from Madagascar and India paraphyletically at the basis of the tree: *Etroplus* (India) and *Paretroplus* (Africa) form a monophyletic group, and the remaining cichlid genera from Madagascar (ptychochromines) form a second basal clade among the Cichlidae. Given this basal position of the Madagascar cichlids we here conservatively reconstructed the basal node of all cichlids as representing the origin of the taxa from Madagascar, and assume dispersal to India of the ancestors of *Etroplus*.
Katria katria; *Oxylapia polli*; *Paratilapia polleni*; *Paratilapia bleekeri; Paratilapia* sp. "all black"; *Paratilapia* sp. "fony"; *Paratilapia* sp. "ihotry"; *Ptychochromis grandidieri*; *Ptychochromis oligacanthus*; *Ptychochromis* sp. "black saroy"; *Ptychochromis* sp. "green garaka"; *Ptychochromis* sp. "inornatus"; *Ptychochromis* sp. "kotro/onilahy"; *Ptychochromis* sp. "mipentina"; *Ptychochromis* sp. "nossibeensis"; *Ptychochromoides betsileanus*; *Ptychochromoides vondrozo*; *Ptychochromoides* sp. "itasy"; *Paretroplus damii*; *Paretroplus kieneri*; *Paretroplus maculatus*; *Paretroplus maromandia*; *Paretroplus menarambo*; *Paretroplus nourissati*; *Paretroplus petiti*; *Paretroplus polyactis*; *Paretroplus* sp. "ankarafantsika"; *Paretroplus* sp. "bambabe"; *Paretroplus* sp. "dridrimena"; *Paretroplus* sp. "lac parinadrina"; *Paretroplus* sp. "sofia"; *Paretroplus* sp. "tsimoly"; *Paretroplus* sp. "ventitry"

Clade 4: Mantellidae (266 species)
Clade description: The Mantellidae are a species rich clade of frogs restricted to Madagascar and (with two undescribed endemic species) the island of Mayotte, Comoros. Mantellids are deeply phylogenetically nested within Asian clades of the cosmopolitan ranoid frogs, and are sister to the Asian Rhacophoridae (S42, S43, S53, S54). This indicates an origin of the Mantellidae from Asia/India.
Aglyptodactylus laticeps Glaw, Vences & Böhme; *Aglyptodactylus madagascariensis* (Duméril); *Aglyptodactylus securifer* Glaw, Vences & Böhme;

Aglyptodactylus sp. 2 CCS (S48); *Aglyptodactylus* sp. 3 CCS (S48); *Blommersia angolafa* Andreone, Rosa, Noel, Crottini, Vences & Raxworthy; *Blommersia blommersae* (Guibé); *Blommersia dejongi* Vences, Köhler, Pabijan & Glaw; *Blommersia domerguei* (Guibé); *Blommersia galani* Vences, Köhler, Pabijan & Glaw; *Blommersia grandisonae* (Guibé); *Blommersia kely* (Glaw & Vences); *Blommersia sarotra* (Glaw & Vences); *Blommersia* sp. 11 CCS (S48); *Blommersia* sp. 2 CCS (S48); *Blommersia* sp. 5 CCS (S48); *Blommersia* sp. 7 CCS (S48); *Blommersia* sp. 8 CCS (S48); *Blommersia* sp. 9 CCS (S48); *Blommersia wittei* (Guibé); *Boehmantis microtympanum* (Angel); *Boophis albilabris* (Boulenger); *Boophis albipunctatus* Glaw & Thiesmeier; *Boophis andohahela* Andreone, Nincheri & Piazza; *Boophis andrangoloaka* (Ahl); *Boophis andreonei* Glaw & Vences; *Boophis anjanaharibeensis* Andreone; *Boophis ankaratra* Andreone; *Boophis arcanus* Glaw, Köhler, De la Riva, Vieites & Vences; *Boophis axelmeyeri* Vences, Andreone & Vieites; *Boophis baetkei* Köhler, Glaw & Vences; *Boophis blommersae* Glaw & Vences; *Boophis boehmei* Glaw & Vences; *Boophis bottae* Vences & Glaw; *Boophis brachychir* (Boettger); *Boophis burgeri* Glaw & Vences; *Boophis calcaratus* Vallan, Vences & Glaw; *Boophis doulioti* (Angel); *Boophis elenae* Andreone; *Boophis englaenderi* Glaw & Vences; *Boophis entingae* Glaw, Köhler, De la Riva, Vieites & Vences; *Boophis erythrodactylus* (Guibé); *Boophis feonnyala* Glaw, Vences, Andreone & Vallan; *Boophis goudotii* Tschudi; *Boophis guibei* (McCarthy); *Boophis haematopus* Glaw, Vences, Andreone & Vallan; *Boophis haingana* Glaw, Köhler, De la Riva, Vieites & Vences; *Boophis idae* (Steindachner); *Boophis jaegeri* Glaw & Vences; *Boophis laurenti* Guibé; *Boophis liami* Vallan, Vences & Glaw; *Boophis lichenoides* Vallan, Glaw, Andreone & Cadle; *Boophis lilianae* Köhler, Glaw & Vences; *Boophis luciae* Glaw, Köhler, De la Riva, Vieites & Vences; *Boophis luteus* (Boulenger); *Boophis madagascariensis* (Peters); *Boophis majori* (Boulenger); *Boophis mandraka* Blommers-Schlösser; *Boophis marojezensis* Glaw & Vences; *Boophis miadana* Glaw, Köhler, De la Riva, Vieites & Vences; *Boophis microtympanum* (Boettger); *Boophis miniatus* (Mocquard); *Boophis obscurus* Boettger; *Boophis occidentalis* Glaw & Vences; *Boophis opisthodon* (Boulenger); *Boophis pauliani* (Guibé); *Boophis periegetes* Cadle; *Boophis picturatus* Glaw, Vences, Andreone & Vallan; *Boophis piperatus* Glaw, Köhler, De la Riva, Vieites & Vences; *Boophis praedictus* Glaw, Köhler, De la Riva, Vieites & Vences; *Boophis pyrrhus* Glaw, Vences, Andreone & Vallan; *Boophis quasiboehmei* Vences, Köhler, Crottini & Glaw; *Boophis rappiodes* (Ahl); *Boophis reticulatus* Blommers-Schlösser; *Boophis rhodoscelis* (Boulenger); *Boophis roseipalmatus* Glaw, Köhler, De la Riva, Vieites & Vences;

Boophis rufioculis Glaw & Vences; *Boophis sambirano* Vences & Glaw; *Boophis sandrae* Glaw, Köhler, De la Riva, Vieites & Vences; *Boophis schuboeae* Glaw & Vences; *Boophis septentrionalis* Glaw & Vences; *Boophis sibilans* Glaw & Thiesmeier; *Boophis solomaso* Vallan, Vences & Glaw; *Boophis* sp. 15 CCS (S48); *Boophis* sp. 18 CCS (S48); *Boophis* sp. 28 CCS (S48); *Boophis* sp. 33 CCS (S48); *Boophis* sp. 35 CCS (S48); *Boophis* sp. 8 CCS (S48); *Boophis spinophis* Glaw, Köhler, De la Riva, Vieites & Vences; *Boophis tampoka* Köhler, Glaw & Vences; *Boophis tasymena* Vences & Glaw; *Boophis tephraeomystax* (Duméril); *Boophis tsilomaro* Vences, Andreone, Glos & Glaw; *Boophis ulftunni* Wollenberg, Andreone, Glaw & Vences; *Boophis viridis* Blommers-Schlösser; *Boophis vittatus* Glaw, Vences, Andreone & Vallan; *Boophis williamsi* (Guibé); *Boophis xerophilus* Glaw & Vences; *Gephyromantis ambohitra* (Vences & Glaw); *Gephyromantis asper* (Boulenger); *Gephyromantis atsingy* Crottini, Glaw, Casiraghi, Jenkins, Mercurio, Randrianantoandro, Randrianirina & Andreone; *Gephyromantis azzurrae* Mercurio & Andreone; *Gephyromantis blanci* Guibé; *Gephyromantis boulengeri* Methuen; *Gephyromantis cornutus* (Glaw & Vences); *Gephyromantis corvus* (Glaw & Vences); *Gephyromantis decaryi* Angel; *Gephyromantis eiselti* Guibé; *Gephyromantis enki* (Glaw & Vences); *Gephyromantis granulatus* (Boettger); *Gephyromantis horridus* (Boettger); *Gephyromantis klemmeri* Guibé; *Gephyromantis leucocephalus* Angel; *Gephyromantis leucomaculatus* (Guibé); *Gephyromantis luteus* (Methuen & Hewitt); *Gephyromantis malagasius* (Methuen & Hewitt); *Gephyromantis moseri* (Glaw & Vences); *Gephyromantis plicifer* (Boulenger); *Gephyromantis pseudoasper* (Guibé); *Gephyromantis redimitus* (Boulenger); *Gephyromantis rivicola* (Vences, Glaw & Andreone); *Gephyromantis runewsweeki* Vences & De la Riva; *Gephyromantis salegy* (Andreone, Aprea, Vences & Odierna); *Gephyromantis schilfi* (Glaw & Vences); *Gephyromantis sculpturatus* (Ahl); *Gephyromantis silvanus* (Vences, Glaw & Andreone); *Gephyromantis* sp. 1 CCS (S48); *Gephyromantis* sp. 11 CCS (S48); *Gephyromantis* sp. 13 CCS (S48); *Gephyromantis* sp. 17 CCS (S48); *Gephyromantis* sp. 22 CCS (S48); *Gephyromantis* sp. 24 CCS (S48); *Gephyromantis* sp. 25 CCS (S48); *Gephyromantis* sp. 26 CCS (S48); *Gephyromantis* sp. 27 CCS (S48); *Gephyromantis* sp. 4 CCS (S48); *Gephyromantis* sp. 5 CCS (S48); *Gephyromantis* sp. 6 CCS (S48); *Gephyromantis* sp. 7 CCS (S48); *Gephyromantis* sp. 8 CCS (S48); *Gephyromantis* sp. 9 CCS (S48); *Gephyromantis spinifer* (Blommers-Schlösser & Blanc); *Gephyromantis striatus* (Vences, Glaw, Andreone, Jesu & Schimmenti); *Gephyromantis tandroka* (Glaw & Vences); *Gephyromantis thelenae* (Glaw & Vences); *Gephyromantis*

tschenki (Glaw & Vences); *Gephyromantis ventrimaculatus* (Angel); *Gephyromantis webbi* (Grandison); *Gephyromantis zavona* (Vences, Andreone, Glaw & Randrianirina); *Guibemantis albolineatus* (Blommers-Schlösser & Blanc); *Guibemantis bicalcaratus* (Boettger); *Guibemantis depressiceps* (Boulenger); *Guibemantis flavobrunneus* (Blommers-Schlösser); *Guibemantis kathrinae* (Glaw, Vences & Gossmann); *Guibemantis liber* (Peracca); *Guibemantis pulcher* (Boulenger); *Guibemantis punctatus* (Blommers-Schlösser); *Guibemantis* sp. 10 CCS (S48); *Guibemantis* sp. 14 CCS (S48); *Guibemantis* sp. 19 CCS (S48); *Guibemantis* sp. 20 CCS (S48); *Guibemantis* sp. 3 CCS (S48); *Guibemantis* sp. 5 CCS (S48); *Guibemantis* sp. 6 CCS (S48); *Guibemantis* sp. 9 CCS (S48); *Guibemantis timidus* (Vences & Glaw); *Guibemantis tornieri* (Ahl); *Laliostoma labrosum* (Cope); *Mantella aurantiaca* Mocquard; *Mantella baroni* Boulenger; *Mantella bernhardi* Vences, Glaw, Peyrieras, Böhme & Busse; *Mantella betsileo* (Grandidier); *Mantella cowani* Boulenger; *Mantella crocea* Pintak & Böhme; *Mantella ebenaui* (Boettger); *Mantella expectata* Busse & Böhme; *Mantella haraldmeieri* Busse; *Mantella laevigata* Methuen & Hewitt; *Mantella madagascariensis* (Grandidier); *Mantella manery* Vences, Glaw & Böhme; *Mantella milotympanum* Staniszewski; *Mantella nigricans* Guibé; *Mantella pulchra* Parker; *Mantella* sp. 1 CCS (S48); *Mantella viridis* Pintak & Böhme; *Mantidactylus aerumnalis* (Peracca); *Mantidactylus albofrenatus* (Müller); *Mantidactylus alutus* (Peracca); *Mantidactylus ambohimitombi* Boulenger; *Mantidactylus ambreensis* Mocquard; *Mantidactylus argenteus* Methuen; *Mantidactylus bellyi* Mocquard; *Mantidactylus betsileanus* (Boulenger); *Mantidactylus biporus* (Boulenger); *Mantidactylus bourgati* Guibé; *Mantidactylus brevipalmatus* Ahl; *Mantidactylus charlotteae* Vences & Glaw; *Mantidactylus cowanii* (Boulenger); *Mantidactylus curtus* (Boulenger); *Mantidactylus delormei* Angel; *Mantidactylus femoralis* (Boulenger); *Mantidactylus grandidieri* Mocquard; *Mantidactylus guttulatus* (Boulenger); *Mantidactylus lugubris* (Duméril); *Mantidactylus madecassus* (Millot & Guibé); *Mantidactylus majori* Boulenger; *Mantidactylus melanopleura* (Mocquard); *Mantidactylus mocquardi* Angel; *Mantidactylus noralottae* Mercurio & Andreone; *Mantidactylus opiparis* (Peracca); *Mantidactylus pauliani* Guibé; *Mantidactylus* sp. 1 CCS (S48); *Mantidactylus* sp. 13 CCS (S48); *Mantidactylus* sp. 14 CCS (S48); *Mantidactylus* sp. 17 CCS (S48); *Mantidactylus* sp. 18 CCS (S48); *Mantidactylus* sp. 19 CCS (S48); *Mantidactylus* sp. 20 CCS (S48); *Mantidactylus* sp. 21 CCS (S48); *Mantidactylus* sp. 22 CCS (S48); *Mantidactylus* sp. 23 CCS (S48); *Mantidactylus* sp. 24 CCS (S48); *Mantidactylus* sp. 26 CCS (S48); *Mantidactylus* sp. 27 CCS (S48); *Mantidactylus* sp. 28 CCS (S48); *Mantidactylus* sp. 29 CCS (S48); *Mantidactylus* sp. 3 CCS (S48);

Mantidactylus sp. 30 CCS (S48); *Mantidactylus* sp. 32 CCS (S48); *Mantidactylus* sp. 33 CCS (S48); *Mantidactylus* sp. 35 CCS (S48); *Mantidactylus* sp. 36 CCS (S48); *Mantidactylus* sp. 40 CCS (S48); *Mantidactylus* sp. 41 CCS (S48); *Mantidactylus* sp. 44 CCS (S48); *Mantidactylus* sp. 45 CCS (S48); *Mantidactylus* sp. 46 CCS (S48); *Mantidactylus* sp. 47 CCS (S48); *Mantidactylus* sp. 48 CCS (S48); *Mantidactylus* sp. 49 CCS (S48); *Mantidactylus* sp. 52 CCS (S48); *Mantidactylus* sp. 57 CCS (S48); *Mantidactylus* sp. 59 CCS (S48); *Mantidactylus* sp. 7 CCS (S48); *Mantidactylus tricinctus* (Guibé); *Mantidactylus ulcerosus* (Boettger); *Mantidactylus zipperi* Vences & Glaw; *Mantidactylus zolitschka* Glaw & Vences; *Spinomantis aglavei* (Methuen & Hewitt); *Spinomantis bertini* (Guibé); *Spinomantis brunae* (Andreone, Glaw, Vences & Vallan); *Spinomantis elegans* (Guibé); *Spinomantis fimbriatus* (Glaw & Vences); *Spinomantis guibei* (Blommers-Schlösser); *Spinomantis massi* (Glaw & Vences); *Spinomantis microtis* (Guibé); *Spinomantis nussbaumi* Cramer, Rabibisoa & Raxworthy; *Spinomantis peraccae* (Boulenger); *Spinomantis phantasticus* (Glaw & Vences); *Spinomantis* sp. 5 CCS (S48); *Spinomantis* sp. 6 CCS (S48); *Spinomantis* sp. 8 CCS (S48); *Spinomantis tavaratra* Cramer, Rabibisoa & Raxworthy; *Tsingymantis antitra* Glaw, Hoegg & Vences; *Wakea madinika* (Vences, Andreone, Glaw & Mattioli)

Clade 5: Microhylidae: Cophylinae + Scaphiophryninae (95 species)
Clade description: The Microhylidae is a cosmopolitan group of largely unresolved basal relationships (S42, S53). Two endemic microhylid subfamilies from Madagascar, the Scaphiophryninae and Cophylinae, are reciprocally monophyletic and together form a clade (S47). The exact relationships of this clade to other microhylids are uncertain, and we therefore here consider they originated from either Asia or Africa. *Scaphiophryne boribory* Vences, Raxworthy, Nussbaum & Glaw; *Scaphiophryne brevis* (Boulenger); *Scaphiophryne calcarata* (Mocquard); *Scaphiophryne gottlebei* Busse & Böhme; *Scaphiophryne madagascariensis* (Boulenger); *Scaphiophryne marmorata* Boulenger; *Scaphiophryne menabensis* Glos, Glaw & Vences; *Scaphiophryne spinosa* Steindachner; *Paradoxophyla palmata* (Guibé); *Paradoxophyla tiarano* Andreone, Aprea, Odierna & Vences; *Anodonthyla boulengeri* Müller; *Anodonthyla emilei* Vences, Glaw, Köhler & Wollenberg; *Anodonthyla jeanbai* Vences, Glaw, Köhler & Wollenberg; *Anodonthyla montana* Angel; *Anodonthyla moramora* Glaw & Vences; *Anodonthyla nigrigularis* Glaw & Vences; *Anodonthyla pollicaris* (Boettger); *Anodonthyla rouxae* Guibé; *Anodonthyla theoi* Vences, Glaw, Köhler & Wollenberg; *Anodonthyla vallani* Vences, Glaw, Köhler & Wollenberg; *Anondonthyla*

hutchisoni Fenolio, Walvoord, Stout, Randrianirina
& Andreone; *Cophyla berara* Vences, Andreone &
Glaw; *Cophyla occultans* (Glaw & Vences);
Cophyla phyllodactyla Boettger; *Madecassophryne
truebae* Guibé; *Platypelis alticola* (Guibé);
Platypelis barbouri Noble; *Platypelis cowani*
Boulenger; *Platypelis grandis* (Boulenger);
Platypelis mavomavo Andreone, Fenolio &
Walvoord; *Platypelis milloti* Guibé; *Platypelis
pollicaris* Boulenger; *Platypelis tetra* Andreone,
Fenolio & Walvoord; *Platypelis tsaratananaensis*
Guibé; *Platypelis tuberifera* (Methuen);
Plethodontohyla bipunctata (Guibé);
Plethodontohyla brevipes Boulenger;
Plethodontohyla fonetana Glaw, Köhler, Bora,
Rabibisoa, Ramilijaona & Vences; *Plethodontohyla
guentheri* Glaw & Vences; *Plethodontohyla
inguinalis* Boulenger; *Plethodontohyla mihanika*
Vences, Raxworthy, Nussbaum & Glaw;
Plethodontohyla notosticta (Günther);
Plethodontohyla ocellata Noble & Parker;
Plethodontohyla tuberata (Peters); *Rhombophryne
alluaudi* (Mocquard); *Rhombophryne coronata*
(Vences & Glaw); *Rhombophryne coudreaui*
Angel; *Rhombophryne guentherpetersi* (Guibé);
Rhombophryne laevipes (Mocquard);
Rhombophryne mangabensis Glaw, Köhler &
Vences; *Rhombophryne matavy* D'Cruze, Köhler,
Vences & Glaw; *Rhombophryne minuta* (Guibé);
Rhombophryne serratopalpebrosa (Guibé);
Rhombophryne testudo Boettger; *Stumpffia be*
Köhler, Vences, D'Cruze & Glaw; *Stumpffia
gimmeli* Glaw & Vences; *Stumpffia grandis* Guibé;
Stumpffia hara Köhler, Vences, D'Cruze & Glaw;
Stumpffia helenae Vallan; *Stumpffia
madagascariensis* Mocquard; *Stumpffia megsoni*
Köhler, Vences, D'Cruze & Glaw; *Stumpffia
psologlossa* Boettger; *Stumpffia pygmaea* Vences &
Glaw; *Stumpffia roseifemoralis* Guibé; *Stumpffia
staffordi* Köhler, Vences, D'Cruze & Glaw;
Stumpffia tetradactyla Vences & Glaw; *Stumpffia
tridactyla* Guibé; *Cophyla* sp. 1 "Ambre" (S80: p.
135, plate 5d); *Stumpffia* sp. 1 CCS (S48);
Stumpffia sp. 6 CCS (S48); *Stumpffia* sp. 8 CCS
(S48); *Stumpffia* sp. 9 CCS (S48); *Stumpffia* sp. 10
CCS (S48); *Stumpffia* sp. 11 CCS (S48); *Stumpffia*
sp. 13 CCS (S48); *Stumpffia* sp. 14 CCS (S48);
Stumpffia sp. 15 CCS (S48); *Stumpffia* sp. 16 CCS
(S48); *Stumpffia* sp. 18 CCS (S48); *Stumpffia* sp. 19
CCS (S48); *Stumpffia* sp. *"Tsaratanana"* (ZCMV
12372); *Scaphiophryne* sp. 1 CCS (S48);
Scaphiophryne sp. 2 *"Marotondrano"* CCS (S48);
Rhombophryne sp. 2 CCS (S48); *Rhombophryne* sp.
6 CCS (S48); *Rhombophryne* sp. 8 CCS (S48);
Rhombophryne sp. 9 CCS (S48); *Rhombophryne* sp.
10 *"Tsaratanana"* (ZCMV 12404); *Plethodontohyla*
sp. 1 CCS (S48); *Plethodontohyla* sp. 3 CCS (S48);
Platypelis sp. 4 CCS (S48); *Platypelis* sp. 6 CCS
(S48); *Platypelis* sp. 7 CCS (S48); *Platypelis* sp. 8
CCS (S48); *Platypelis* sp. 10 CCS (S48)

Clade 6: Microhylidae: Dyscophinae (3 species)
Clade description: The dyscophines are the third
endemic subfamily of microhylids in Madagascar.
This species poor clade is clearly phylogenetically
nested among various Asian microhylid clades
(S42, S53) and an origin from Asia is therefore here
assumed.
Dyscophus insularis Grandidier; *Dyscophus
antongilii* (Grandidier); *Dyscophus guineti*
Grandidier

Clade 7: Hyperoliidae (10 species)
Clade description: Hyperoliids are a clade of
predominantly African distribution, with one genus
(Heterixalus) in Madagascar and the monotypic
Tachycnemis on the Seychelles. *Heterixalus* is
closely related to *Tachycnemis*, and the monophyly
of *Heterixalus* relative to *Tachycnemis* is not fully
supported but also not contradicted by the
molecular data available to date (S55). The
Heterixalus-Tachycnemis clade isphylogenetically
nested within hyperoliids and related to the African
Afrixalus and *Hyperolius* (S43, S56).
Heterixalus alboguttatus (Boulenger); *Heterixalus
andrakata* Glaw & Vences; *Heterixalus betsileo*
(Grandidier); *Heterixalus boettgeri* (Mocquard);
Heterixalus carbonei Vences, Glaw, Jesu &
Schimmenti; *Heterixalus luteostriatus* (Andersson);
Heterixalus madagascariensis (Duméril & Bibron);
Heterixalus punctatus Glaw & Vences; *Heterixalus
rutenbergi* (Boettger); *Heterixalus tricolor*
(Boettger)

Clade 8: Ptychadenidae (1 species)
Clade description:The family Ptychadenidae is an
exclusively African and Malagasy clade of ranoid
frogs, until recently included in the family Ranidae
but deeply divergent from other ranids. *Ptychadena*
is a species-rich genus distributed over most of
Africa. One species, *P. mascareniensis*, was
described from the Mascarene islands and thought
to occur on the Mascarenes, Seychelles,
Madagascar, and Africa, and possibly being non-
native in Madagascar. Molecular evidence suggests
that the species is highly genetically diverse in
Madagascar, with several deep clades, and thus
must have colonized the island naturally and long
before human colonization. From Madagascar it has
been introduced to the Mascarenes and Seychelles.
African populations thought to belong to this
species are genetically highly divergent and
probably belong to several distinct species (S57,
S58).
Ptychadena mascareniensis (Duméril & Bibron)

Clade 9: Podocnemididae (1 species)
Clade description: The single podocnemidid turtle
in Madagascar belongs to the monotypic, endemic
genus *Erymnochelys* and is related to the South
American genera *Podocnemis* and *Peltocephalus*
(S34, S59). Although fossils attributed to the

Podocnemididae also have been recovered from Africa and Europe we here follow recent literature that assumed a land connection via Antarctica to explain their presence in Madagascar, and thus assume South America as their geographic origin. Note that we do not consider here other Malagasy terrapins (*Pelomedusa* and *Podocnemis*) of the Pelomedusidae as current data point to their possible introduction by humans from Africa.
Erymnochelys madagascariensis (Grandidier)

Clade 10: Testudinidae (4 species)
Clade description: Of the five species of tortoises in Madagascar, one (*Kinyxis belliana*) is widespread in Africa and might be non-native in Madagascar. The other four species form a monophyletic clade endemic to the island. To this clade also belong the giant tortoises of the Seychelles, and based on an ancient DNA sequence probably also two extinct species of Madagascar giant tortoises. This clade in turn is sister to a clade containing various African genera of tortoises (S60, S61).
Astrochelys (Angonoka) yniphora (Vaillant); *Astrochelys (Astrochelys) radiata* (Shaw); *Pyxis arachnoides arachnoides* Bell; *Pyxis planicauda* (Grandidier)

Clade 11: Crocodylidae (1 species)
Clade description: Malagasy populations of the Nile crocodile are genetically differentiated and show some similarities to East African populations, based on a microsatellite analysis (S62).
Crocodylus niloticus Laurenti

Clade 12: Xenotyphlopidae (1 species)
Clade description: This recently described family of blindsnakes contains a single species endemic to Madagascar. It is sister to a species rich clade distributed in Africa, Asia, Australia, Madagascar, and South America (mainly Caribbean). The origin of the Neotropical taxa is considered to be due to dispersal. The basalmost blindsnake clade is Asian (S44).
Xenotyphlops grandidieri (Mocquard); Note: *X. mocquardi* considered as synonym)

Clade 13: Typhlopidae (15 species)
Clade description: Malagasy blindsnakes (except *Xenotyphlops*) as far as known belong to a monophyletic group that forms a trichotomy with one Eurasian/Australian clade and one African/Neotropical clade (S44). We here assume the origin of Malagasy *Typhlops* to be ambiguous, African or Asian.
Typhlops arenarius (Grandidier); *Typhlops boettgeri* Boulenger; *Typhlops decorsei* Mocquard; *Typhlops domerguei* Roux-Estève; *Typhlops madagascariensis* Boettger; *Typhlops microcephalus* Werner; *Typhlops mucronatus* Boettger; *Typhlops ocularis* Parker; *Typhlops rajeryi* Renoult & Raselimanana; *Typhlops reuteri*

Boettger; *Typhlops* sp. 1 (ZCMV 5540); *Typhlops* sp. 2 (ZCMV 1913); *Typhlops* sp. 3 (FGZC 1724); *Typhlops* sp. 3 (ZSM 871/2010); *Typhlops* sp. 4 (MRSN-FAZC 11125).

Clade 14: Boidae (4 species)
Clade description: Malagasy giant snakes are a species-poor group of disputed relationships. They were long considered to be related to the Neotropical boas, but recent molecular analyses (S34, S63) have shown they are a clade sister to the genus *Calabaria* from Africa. We therefore here reconstruct their origin as African rather than South American.
Acrantophis dumerili Jan; *Acrantophis madagascariensis* (Duméril & Bibron); *Sanzinia madagascariensis* (Duméril & Bibron); *Sanzinia volontany* Vences & Glaw; Note: *S.* volontany here provisionally ranked as full species.

Clade 15: Lamprophiidae: Pseudoxyrhophiinae (80 species)
Clade description: Malagasy snakes in the subfamily Pseudoxyrhophiinae were long included in the family Colubridae which has recently been partitioned, and are now seen by some authors as part of the Lamprophiidae. Malagasy pseudoxyrhophiines form a species-rich clade containing all but one of Madagascar's advanced (caenophidian) snakes. This clade is sister to the Socotran genus *Ditypophis* (S45), and several other African snake genera are probably related to pseudoxyrhophiines as well, such as slug-eaters (*Duberria*) and *Amplorhinus* (S64).
Alluaudina bellyi Mocquard; *Alluaudina mocquardi* Angel; *Brygophis coulangesi* (Domergue); *Compsophis albiventris* Mocquard; *Compsophis boulengeri* (Peracca); *Compsophis fatsibe* (Mercurio & Andreone); *Compsophis infralineatus* (Günther); *Compsophis laphystius* (Cadle); *Compsophis vinckei* (Domergue); *Compsophis zeny* (Cadle); *Dromicodryas bernieri* (Duméril & Bibron); *Dromicodryas quadrilineatus* (Duméril & Bibron); *Exallodontophis albignaci* (Domergue); *Heteroliodon fohy* Glaw, Vences & Nussbaum; *Heteroliodon lava* Nussbaum & Raxworthy; *Heteroliodon occipitalis* (Boulenger); *Ithycyphus blanci* Domergue; *Ithycyphus goudoti* (Schlegel); *Ithycyphus miniatus* (Schlegel); *Ithycyphus oursi* Domergue; *Ithycyphus perineti* Domergue; *Langaha alluaudi* Mocquard; *Langaha madagascariensis* Bonnaterre; *Langaha pseudoalluaudi* Domergue; *Leioheterodon geayi* Mocquard; *Leioheterodon madagascariensis* Duméril & Bibron; *Leioheterodon modestus* (Günther); *Liophidium apperti* Domergue; *Liophidium chabaudi* Domergue; *Liophidium maintikibo* Franzen, Jones, Raselimanana, Nagy, D'Cruze, Glaw & Vences; *Liophidium pattoni* Vieites, Ratsoavina, Randrianiaina, Nagy, Glaw & Vences; *Liophidium rhodogaster* (Schlegel); *Liophidium therezieni* Domergue; *Liophidium*

torquatum (Boulenger); *Liophidium trilineatum* Boulenger; *Liophidium vaillanti* (Mocquard); *Liopholidophis dimorphus* Glaw, Nagy, Franzen & Vences; *Liopholidophis dolicocercus* (Peracca); *Liopholidophis grandidieri* Mocquard; *Liopholidophis rhadinaea* Cadle; *Liopholidophis sexlineatus* (Günther); *Liopholidophis varius* (Fischer); *Lycodryas carleti* (Domergue); *Lycodryas citrinus* (Domergue); *Lycodryas gaimardii* (Schlegel); *Lycodryas granuliceps* (Boettger); *Lycodryas guentheri* (Boulenger); *Lycodryas inopinae* (Domergue); *Lycodryas inornatus* (Boulenger); *Lycodryas pseudogranuliceps* (Domergue); *Madagascarophis colubrinus colubrinus* (Schlegel); *Madagascarophis meridionalis* Domergue; *Madagascarophis ocellatus* Domergue; *Micropisthodon ochraceus* Mocquard; *Pararhadinaea melanogaster melanogaster* (Boettger); *Parastenophis betsileanus* (Günther); *Phisalixella arctifasciata* (Duméril & Bibron); *Phisalixella iarakaensis* (Domergue); *Phisalixella tulearensis* (Domergue); *Phisalixella variabilis* (Boulenger); *Pseudoxyrhopus ambreensis* Mocquard; *Pseudoxyrhopus analabe* Nussbaum, Andreone & Raxworthy; *Pseudoxyrhopus ankafinaensis* Raxworthy & Nussbaum; *Pseudoxyrhopus heterurus* (Jan); *Pseudoxyrhopus imerinae* (Günther); *Pseudoxyrhopus kely* Raxworthy & Nussbaum; *Pseudoxyrhopus microps* Günther; *Pseudoxyrhopus oblectator* Cadle; *Pseudoxyrhopus quinquelineatus* (Günther); *Pseudoxyrhopus sokosoko* Raxworthy & Nussbaum; *Pseudoxyrhopus tritaeniatus* Mocquard; *Thamnosophis epistibes* (Cadle); *Thamnosophis infrasignatus* (Günther); *Thamnosophis lateralis* (Duméril & Bibron); *Thamnosophis martae* (Glaw, Franzen & Vences); *Thamnosophis mavotenda* Glaw, Nagy, Köhler, Franzen & Vences; *Thamnosophis stumpffi* (Boettger); *Madagascarophis* sp. (from Montagne des Francais); *Liopholidophis* sp. 1 (from Andasibe); *Liopholidophis* sp. 2 (from Marojejy)

Clade 16: Lamprophiidae: Psammophiinae (1 species)
Clade description: The genus *Mimophis* is endemic to Madagascar and contains a single species. *Mimophis* is phylogenetically nested within the mainly African subfamily Psammophiinae (S45, S64).
Mimophis mahfalensis (Grandidier)

Clade 17: Opluridae (9 species)
Clade description: The iguanas of Madagascar are currently often considered as own family Opluridae. This clade is composed of two genera which are phylogenetically nested among Neotropical iguanas (S34).
Chalarodon madagascariensis Peters; *Oplurus cuvieri* (Gray); *Oplurus cyclurus* (Merrem); *Oplurus fierinensis* Grandidier; *Oplurus grandidieri* Mocquard; *Oplurus quadrimaculatus* Duméril; *Oplurus saxicola* Grandidier; *Chalarodon* sp. "Esomony" (FGZC 2330); *Oplurus* sp. aff. *saxicola* "Esomony" (FGZC 2551).

Clade 18: Chamaeleonidae (94 species)
Clade description: Chameleons are a complex clade for the assessment of biogeographic relationships due to numerous dispersal events and unclarified relationships among many major clades (S46, S65). Chameleons occur in Madagascar and Africa, with single species on India and the Seychelles which however are not related to each other but represent independent dispersals out of Africa (S46, S65). We here use the initial diversification between chameleons from Madagascar (*Brookesia*) and other chameleons (Africa), to assess their age of origin in Madagascar. An alternative would have been to score their separation from agamas and (based on the distribution of agamas) an ambiguous origin from Africa or Asia.
Brookesia ambreensis Raxworthy & Nussbaum; *Brookesia antakarana* Raxworthy & Nussbaum; *Brookesia bekolosy* Raxworthy & Nussbaum; *Brookesia betschi* Brygoo, Blanc & Domergue; *Brookesia bonsi* Ramanantsoa; *Brookesia brygooi* Raxworthy & Nussbaum; *Brookesia decaryi* Angel; *Brookesia dentata* Mocquard; *Brookesia ebenaui* (Boettger); *Brookesia exarmata* Schimmenti & Jesu; *Brookesia griveaudi* Brygoo, Blanc & Domergue; *Brookesia karchei* Brygoo, Blanc & Domergue; *Brookesia lambertoni* Brygoo & Domergue; *Brookesia lineata* Raxworthy & Nussbaum; *Brookesia lolontany* Raxworthy & Nussbaum; *Brookesia minima* Boettger; *Brookesia nasus nasus* Boulenger; *Brookesia perarmata* (Angel); *Brookesia peyrierasi* Brygoo, Blanc & Domergue; *Brookesia ramanantsoai* Brygoo & Domergue; *Brookesia stumpffi* Boettger; *Brookesia superciliaris* (Kuhl); *Brookesia therezieni* Brygoo & Domergue; *Brookesia thieli* Brygoo & Domergue; *Brookesia tuberculata* Mocquard; *Brookesia vadoni* Brygoo & Domergue; *Brookesia valerieae* Raxworthy; *Calumma amber* Raxworthy & Nussbaum; *Calumma ambreense* (Ramanantsoa); *Calumma andringitraense* (Brygoo, Blanc & Domergue); *Calumma boettgeri* (Boulenger); *Calumma brevicorne* (Günther); *Calumma capuroni* (Brygoo, Blanc & Domergue); *Calumma crypticum* Raxworthy & Nussbaum; *Calumma cucullatum* (Gray); *Calumma fallax* (Mocquard); *Calumma furcifer* (Vaillant & Grandidier); *Calumma gallus* (Günther); *Calumma gastrotaenia* (Boulenger); *Calumma glawi* Böhme; *Calumma globifer* (Günther); *Calumma guibei* (Hillenius); *Calumma guillaumeti* (Brygoo, Blanc & Domergue); *Calumma hafahafa* Raxworthy & Nussbaum; *Calumma hilleniusi* (Brygoo, Blanc & Domergue); *Calumma jejy* Raxworthy & Nussbaum; *Calumma malthe* (Günther); *Calumma marojezense* (Brygoo, Blanc & Domergue); *Calumma nasutum* (Duméril & Bibron); *Calumma*

oshaughnessyi (Günther); *Calumma parsonii parsonii* (Cuvier); *Calumma peltierorum* Raxworthy & Nussbaum; *Calumma peyrierasi* Brygoo & Domergue; *Calumma tarzan* Gehring, Pabijan, Ratsoavina, Köhler, Vences & Glaw; *Calumma tsaratananense* (Brygoo & Domergue); *Calumma tsycorne* Raxworthy & Nussbaum; *Calumma vatosoa* Andreone, Mattioli, Jesu & Randrianirina; *Calumma vencesi* Andreone, Mattioli, Jesu & Randrianirina; *Furcifer angeli* (Brygoo & Domergue); *Furcifer antimena* (Grandidier); *Furcifer balteatus* (Duméril & Bibron); *Furcifer belalandaensis* (Brygoo & Domergue); *Furcifer bifidus* (Brongniart); *Furcifer campani* (Grandidier); *Furcifer labordi* (Grandidier); *Furcifer lateralis* (Gray); *Furcifer minor* (Günther); *Furcifer nicosiai* Jesu, Mattioli & Schimmenti; *Furcifer oustaleti* (Mocquard); *Furcifer pardalis* (Cuvier); *Furcifer petteri* (Brygoo & Domergue); *Furcifer rhinoceratus* (Gray); *Furcifer timoni* Glaw, Köhler & Vences; *Furcifer tuzetae* (Brygoo, Bourgat & Domergue); *Furcifer verrucosus verrucosus* (Cuvier); *Furcifer willsii* (Günther); *Brookesia* sp. 1 "desperata" (Foret d'Ambre; ZSM 2170/2007); *Brookesia* sp. 2 "micra" (Nosy Hara; ZSM 2181/2007); *Brookesia* sp. 3 "confidens" (Ankarana; ZSM 2150/2007) ; *Brookesia* sp. 4 "tristis" (M. des Francais; ZSM 1704/2004); *Brookesia* sp. 5 "brunoi" (ZCMV 12784); *Calumma* sp. 1 "vohibola" (ZSM 645/2009); *Calumma* sp. 2 aff. boettgeri "Ambre" (FGZC 1019); *Calumma* sp. 3 aff. *fallax*-gallus "Ambohitsara" (ZCMV 303); *Calumma* sp. 4 aff. *gallus "*Ambatofotsy*"* (FGZC 2692); *Calumma* sp. 5 aff. *gallus* "South" (ZCMV 8642); *Calumma* sp. 6 aff. *nasutum* "Andohahela 2" (FGZC 2400); *Calumma* sp. 7 aff. *nasutum* "Anjozorobe-Andohahela" (FGZC 2508); *Calumma* sp. 8 aff. *nasutum* "Vohidrazana-Makira" (FGZC 2692); *Calumma* sp. 9 aff. *nasutum* (Sahafina); *Calumma* sp. 10 aff. *malthe* "North"(DRV 6125); *Calumma* sp. 11 aff. *guibei* "Bemanevika" (DRV 6393); *Calumma* sp. 12 aff. *guibei* (ZCMV 12307); *Calumma* sp. 13 aff. *vencesi* "Makira" (ZCMV 11275).

Clade 19: Gerrhosauridae (19 species)
Clade description: The plated lizards are a family exclusively occurring in Africa and Madagascar. Although no comprehensive molecular phylogeny of the family has been published, the available data clearly support the monophyly of Malagasy gerrhosaurids (S66, S67).
Tracheloptychus madagascariensis Peters; *Tracheloptychus petersi* Grandidier; *Zonosaurus aeneus* (Grandidier); *Zonosaurus anelanelany* Raselimanana, Raxworthy & Nussbaum; *Zonosaurus bemaraha* Raselimanana, Raxworthy & Nussbaum; *Zonosaurus boettgeri* Steindachner; *Zonosaurus brygooi* Lang & Böhme; *Zonosaurus haraldmeieri* Brygoo & Böhme; *Zonosaurus karsteni* (Grandidier); *Zonosaurus laticaudatus* (Grandidier); *Zonosaurus madagascariensis* (Gray); *Zonosaurus maramaintso* Raselimanana, Nussbaum & Raxworthy; *Zonosaurus maximus* Boulenger; *Zonosaurus ornatus* (Gray); *Zonosaurus quadrilineatus* (Grandidier); *Zonosaurus rufipes* (Boettger); *Zonosaurus subunicolor* (Boettger); *Zonosaurus trilineatus* Angel; *Zonosaurus tsingy* Raselimanana, Raxworthy & Nussbaum

Clade 20: Scincidae: *Trachylepis* (13 species)
Clade description: The genus *Trachylepis* includes "lygosomine" scincid lizards from Africa and Madagascar that previously had been included in a near-cosmopolitan genus *Mabuya*. Although early studies failed to resolve Malagasy *Trachylepis* as monophyletic, in recent molecular phylogenies the Malagasy taxa (except the possibly introduced *T. comorensis*) form a clade that is phylogenetically nested among African *Trachylepis* (S68).
Trachylepis aureopunctata (Grandidier); *Trachylepis boettgeri* (Boulenger); *Trachylepis dumasi* (Nussbaum & Raxworthy); *Trachylepis elegans* (Peters); *Trachylepis gravenhorstii* (Duméril & Bibron); *Trachylepis lavarambo* (Nussbaum & Raxworthy); *Trachylepis madagascariensis* (Mocquard); *Trachylepis nancycoutuae* (Nussbaum & Raxworthy); *Trachylepis tandrefana* (Nussbaum, Raxworthy & Ramanamanjato); *Trachylepis tavaratra* (Ramanamanjato, Nussbaum & Raxworthy); *Trachylepis vato* (Nussbaum & Raxworthy); *Trachylepis vezo* Ramanamanjato, Nussbaum & Raxworthy; *Trachylepis volamenaloha* (Nussbaum, Raxworthy & Ramanamanjato)

Clade 21: Scincidae: Scincinae (67 species)
Clade description: The scincid lizards from Madagascar are a morphologically diverse and species-rich group that forms a clade which clearly is related to other African scincines (S69).
Amphiglossus alluaudi (Brygoo); *Amphiglossus andranovahensis* (Angel); *Amphiglossus anosyensis* Raxworthy & Nussbaum; *Amphiglossus ardouini* (Mocquard); *Amphiglossus astrolabi* Duméril & Bibron; *Amphiglossus crenni* (Mocquard); *Amphiglossus decaryi* (Angel); *Amphiglossus elongatus* (Angel); *Amphiglossus frontoparietalis* (Boulenger); *Amphiglossus gastrostictus* (O'Shaugnessy); *Amphiglossus macrocercus* (Günther); *Amphiglossus mandady* Andreone & Greer; *Amphiglossus mandokava* Raxworthy & Nussbaum; *Amphiglossus melanurus* (Günther); *Amphiglossus meva* Miralles et al.; *Amphiglossus ornaticeps* (Boulenger); *Amphiglossus poecilopus* (Barbour & Loveridge); *Amphiglossus praeornatus* (Angel); *Amphiglossus punctatus* Raxworthy & Nussbaum; *Amphiglossus reticulatus* (Kaudern); *Amphiglossus spilostichus* Andreone & Greer; *Amphiglossus splendidus* (Grandidier); *Amphiglossus stylus* Andreone & Greer; *Amphiglossus tanysoma* Andreone & Greer; *Amphiglossus tsaratananensis* (Brygoo); *Androngo*

trivittatus trivittatus (Boulenger); *Cryptoblepharus boutonii cognatus* (Boettger); *Madascincus ankodabensis* (Angel); *Madascincus arenicola* Miralles et al.; *Madascincus igneocaudatus* (Grandidier); *Madascincus intermedius* (Boettger); *Madascincus macrolepis* (Boulenger); *Madascincus melanopleura* (Günther); *Madascincus minutus* (Raxworthy & Nussbaum); *Madascincus mouroundavae* (Grandidier); *Madascincus nanus* (Andreone & Greer); *Madascincus polleni* (Grandidier); *Madascincus stumpffi* (Boettger); *Paracontias brocchii* Mocquard; *Paracontias fasika* Köhler, Vences, Erbacher & Glaw; *Paracontias hafa* Andreone & Greer; *Paracontias hildebrandti* (Peters); *Paracontias holomelas* (Günther); *Paracontias kankana* Köhler, Vieites, Glaw, Kaffenberger & Vences; *Paracontias manify* Andreone & Greer; *Paracontias milloti* Angel; *Paracontias minimus* (Mocquard); *Paracontias rothschildi* Mocquard; *Paracontias tsararano* Andreone & Greer; *Paracontias vermisauru* Miralles et al.; *Pseudoacontias angelorum* Nussbaum & Raxworthy; *Pseudoacontias madagascariensis* Bocage; *Pseudoacontias menamainty* Andreone & Greer; *Pseudoacontias unicolor* Sakata & Hikida; *Pygomeles braconnieri* Grandidier; *Pygomeles petteri* Pasteur & Paulian; *Sirenoscincus yamagishii* Sakata & Hikida; *Voeltzkowia (Grandidierina) fierinensis* (Grandidier); *Voeltzkowia (Grandidierina) petiti* (Angel); *Voeltzkowia (Voeltzkowia) lineata* (Mocquard); *Voeltzkowia (Voeltzkowia) mira* Boettger; *Voeltzkowia (Voeltzkowia) rubrocaudata* (Grandidier); *Amphiglossus* sp. 1 "robustus" (S80); *Madascincus* sp. 2 "baeus" (S80); *Madascincus* sp. 1 "vitreus" (S80); *Voeltzkowia* sp. 1 "pallida" (S80); *Madascincus* sp. 3. aff. *intermedius* (FGZC 474).

Clade 22: Gekkonidae: *Phelsuma* (34 species)
Clade description: Day geckos of the genus *Phelsuma* are a clade that diversified in Madagascar, and from here multiple colonizations of other islands of the Indian Ocean took place. Available phylogenetic information is ambiguous regarding the sister group of *Phelsuma*, but it forms a monophyletic group together with *Lygodactylus* and *Rhoptropella*, a monotypic genus from Africa (S70, S71). We therefore assume African origins for *Phelsuma* and use the *Lygodactylus-Phelsuma* split as a proxy for the timing of the separation among these three genera. If *Rhoptropella* is indeed the sister group of *Phelsuma* as suggested by a recent analysis (S71), then the actual origin of *Phelsuma* probably is slightly younger than reconstructed by us which however should not have any relevant influence on our conclusions.
Phelsuma abbotti chekei Börner & Minuth; *Phelsuma antanosy* Raxworthy & Nussbaum; *Phelsuma barbouri* Loveridge; *Phelsuma berghofi* Krüger; *Phelsuma borai* Glaw, Köhler & Vences; *Phelsuma breviceps* Boettger; *Phelsuma dorsivittata* Mertens; *Phelsuma dubia* (Boettger);

Phelsuma flavigularis Mertens; *Phelsuma gouldi* Crottini, Gehring, Glaw, Harris, Lima & Vences; *Phelsuma grandis* Gray; *Phelsuma guttata* Kaudern; *Phelsuma hielscheri* Rösler, Obst & Seipp; *Phelsuma hoeschi* Berghof & Trautmann; *Phelsuma kely* Schönecker, Bach & Glaw; *Phelsuma klemmeri* Seipp; *Phelsuma kochi* Mertens; *Phelsuma laticauda laticauda* (Boettger); *Phelsuma lineata lineata* Gray; *Phelsuma madagascariensis* Gray; *Phelsuma malamakibo* Nussbaum, Raxworthy, Raselimanana & Ramanamanjato; *Phelsuma masohoala* Raxworthy & Nussbaum; *Phelsuma modesta modesta* Mertens; *Phelsuma mutabilis* (Grandidier); *Phelsuma parva* Meier; *Phelsuma pronki* Seipp; *Phelsuma pusilla pusilla* Mertens; *Phelsuma quadriocellata quadriocellata* (Peters); *Phelsuma ravenala* Raxworthy, Ingram, Rabibisoa & Pearson; *Phelsuma roesleri* Glaw, Gehring, Köhler, Franzen & Vences; *Phelsuma seippi* Meier; *Phelsuma serraticauda* Mertens; *Phelsuma standingi* Methuen & Hewitt; *Phelsuma vanheygeni* Lerner.

Clade 23: Gekkonidae: *Lygodactylus* (24 species)
Clade description: The dwarf geckos of Madagascar are a paraphyletic group, with African and South American taxa nested within the Malagasy clade (S72). Similar to *Phelsuma*, we assume the current phylogenetic trichotomy of *Phelsuma*, *Lygodactylus*, and the African Rhoptropella to be indicative of an African origin of these geckos, and use the separation of *Phelsuma* and *Lygodactylus* as a proxy for the colonization of Madagascar. This scenario assumes diversification of *Lygodactylus* in Madagascar, and colonization of Africa from there. An alternative scenario is an origin of *Lygodactylus* and *Phelsuma* from a common ancestor in Madagascar, but without more reliable phylogenetic data, this scenario appears to be too speculative.
Lygodactylus arnoulti Pasteur; *Lygodactylus bivittis* (Peters); *Lygodactylus blancae* Pasteur; *Lygodactylus blanci* Pasteur; *Lygodactylus decaryi* Angel; *Lygodactylus expectatus* Pasteur & Blanc; *Lygodactylus guibei* Pasteur; *Lygodactylus heterurus heterurus* Boettger; *Lygodactylus intermedius* Pasteur; *Lygodactylus klemmeri* Pasteur; *Lygodactylus madagascariensis madagascariensis* (Boettger); *Lygodactylus miops* Günther; *Lygodactylus mirabilis* (Pasteur); *Lygodactylus montanus* Pasteur; *Lygodactylus ornatus* Pasteur; *Lygodactylus pauliani* Pasteur & Blanc; *Lygodactylus pictus* (Peters); *Lygodactylus rarus* Pasteur & Blanc; *Lygodactylus roavolana* Puente, Glaw, Vieites & Vences; *Lygodactylus tolampyae* (Grandidier); *Lygodactylus tuberosus* Mertens; *Lygodactylus verticillatus* Mocquard; *Lygodactylus* sp. 1 aff. *pictus* (ZMA 19595); *Lygodactylus* sp. 2 aff. *madagascariensis* (ZSM 783/2001)

Clade 24: Gekkonidae: *Blaesodactylus* (4 species)

Clade description: Malagasy species of velvet geckos form a clade that corresponds to the endemic genus *Blaesodactylus*. Their sister group is the African genus *Homopholis* (S73). *Blaesodactylus antongilensis* Böhme & Meier; *Blaesodactylus boivini* (Duméril); *Blaesodactylus sakalava* (Grandidier); *Blaesodactylus ambonihazo* Bauer et al.

Clade 25: Gekkonidae: *Paroedura* (19 species)
Clade description: *Paroedura* geckos are a clade endemic to Madagascar and the Comoros (S71, S74). Sister to *Paroedura* is probably the endemic Malagay genus *Ebenavia* with 2 species which however is here not included due to remaining phylogenetic uncertainties. The *Paroedura* clade is nested among African geckos (S71)
Paroedura androyensis (Grandidier); *Paroedura bastardi* (Mocquard); *Paroedura gracilis* (Boulenger); *Paroedura homalorhina* (Angel); *Paroedura ibityensis* Rösler & Krüger; *Paroedura karstophila* Nussbaum & Raxworthy; *Paroedura lohatsara* Glaw, Vences & Schmidt; *Paroedura maingoka* Nussbaum & Raxworthy; *Paroedura masobe* Nussbaum & Raxworthy; *Paroedura oviceps* (Boettger); *Paroedura picta* (Peters); *Paroedura stumpffi* (Boettger); *Paroedura tanjaka* Nussbaum & Raxworthy; *Paroedura vahiny* Nussbaum & Raxworthy; *Paroedura vazimba* Nussbaum & Raxworthy; *Paroedura* sp. 1 aff. *homalorhina* (ZSM 1530/2008); *Paroedura* sp. 2 Nosy Hara (ZSM 2189/2007); *Paroedura* sp. 3 Ankarafantsika (ZSM 484/2001); *Paroedura* sp. 4 aff. *tanjaka* Bemaraha (ZSM 128/2006).

Clade 26: Gekkonidae: *Uroplatus* (19 species)
Clade description: Leaf tail geckos, genus *Uroplatus*, form a clade endemic to Madagascar (S75). They are phylogenetically nested among other African geckos (S71).
Uroplatus alluaudi Mocquard; *Uroplatus ebenaui* Boettger; *Uroplatus fimbriatus* (Schneider); *Uroplatus finiavana* Ratsoavina, Louis, Crottini, Randrianiaina, Glaw & Vences; *Uroplatus giganteus* Glaw, Kosuch, Henkel, Sound & Böhme; *Uroplatus guentheri* Mocquard; *Uroplatus henkeli* Böhme & Ibisch; *Uroplatus lineatus* (Duméril & Bibron); *Uroplatus malahelo* Nussbaum & Raxworthy; *Uroplatus malama* Nussbaum & Raxworthy; *Uroplatus phantasticus* Boulenger; *Uroplatus pietschmanni* Böhle & Schönecker; *Uroplatus sameiti* Böhme & Ibisch; *Uroplatus sikorae* Boettger; *Uroplatus* sp. 1 aff. *ebenaui* (ZCMV 12280); *Uroplatus* sp. 2 aff. *ebenaui* Tsaratanana (ZCMV 12388); *Uroplatus* sp. 3 aff. *ebenaui* Tsaratanana (DRV 6280); *Uroplatus* sp. 4 aff. *henkeli* Bemaraha (ZSM 113/2006); *Uroplatus* sp. 5 aff. *sikorae* (ZSM 264/2004).

Clade 27: Gekkonidae: Hemidactylus (1 species)
Clade description: Of the three species of *Hemidactylus* occurring in Madagascar, one is genetically diverse (S76) and its mitochondrial haplotypes segregate biogeographically in a pattern agreeing with other reptiles (S77), which indicates its native status on the island. The Malagasy populations are related to conspecific populations from the African mainland.
Hemidactylus mercatorius Gray

Clade 28: Strepsirhini (Lemurs) (96 species)
Clade description: Lemurs are a well-known and species-rich component of Maagascar's fauna. Molecular data clearly support monophyly of all Malagasy lemurs (e.g., S2, S78) and their sister-group relationship with a clade composed of loris and galagos. Although galagos occur in Africa only, loris are also found in Asia, an we therefore here give the origin of the lemur colonization of Madagascar as ambiguous African or Asian.
Microcebus murinus; Microcebus griseorufus; Microcebus berthae; Microcebus myoxinus; Microcebus ravelobensis; Microcebus bongolavensis; Microcebus danfossi; Microcebus margotmarshae; Microcebus sambiranensis; Microcebus mamiratra; Microcebus tavaratra; Microcebus arnholdi; Microcebus rufus; Microcebus jollyae; Microcebus lehilahytsara; Microcebus simmonsi; Microcebus macarthurii; Microcebus mittermeieri; Mirza coquereli; Mirza zaza; Allocebus trichotis; Cheirogaleus medius; Cheirogaleus sibreei; Cheirogaleus major; Cheirogaleus crossleyi; Cheirogaleus minusculus; Phaner furcifer; Phaner pallescens; Phaner parienti; Phaner electromontis; Lepilemur mustelinus; Lepilemur betsileo; Lepilemur microdon; Lepilemur jamesorum; Lepilemur wrightae; Lepilemur fleuretae; Lepilemur hollandorum; Lepilemur seali; Lepilemur scottorum; Lepilemur milanoii; Lepilemur ankaranesis; Lepilemur septentrionalis; Lepilemur dorsalis; Lepilemur tymerlachsonorum; Lepilemur mittermeieri; Lepilemur sahamalazensis; Lepilemur grewcockorum; Lepilemur otto; Lepilemur edwardsi; Lepilemur aeeclis; Lepilemur ahmansonorum; Lepilemur randrianasoloi; Lepilemur ruficaudatus; Lepilemur hubbardorum; Lepilemur petteri; Lepilemur leucopus; Hapalemur griseus; Hapalemur meridionalis; Hapalemur occidentalis; Hapalemur alaotrensis; Hapalemur aureus; Prolemur simus; Lemur catta; Eulemur fulvus; Eulemur rufus; Eulemur rufifrons; Eulemur albifrons; Eulemur sanfordi; Eulemur cinereiceps; Eulemur collaris; Eulemur macaco; Eulemur flavifrons; Eulemur coronatus; Eulemur rubriventer; Varecia variegata; Varecia rubra; Avahi laniger; Avahi mooreorum; Avahi peyrierasi; Avahi betsileo; Avahi ramanantsoavanai; Avahi meridionalis; Avahi occidentalis; Avahi cleesei; Avahi unicolor; Propithecus verreauxi; Propithecus deckenii; Propithecus coronatus; Propithecus coquereli; Propithecus tattersalli; Propithecus diadema; Propithecus edwardsi; Propithecus

candidus; Propithecus perrieri; Indri indri; Daubentonia madagascariensis

Clade 29: Tenrecidae (29 species)
Clade description: Monophyly of all current genera of Malagasy tenrecs relative to all other extant afrotherian groups is well supported by molecular data (S47, S2). Their sister group are the exclusively African potamogalines (otter shrews). *Geogale aurita; Limnogale mergulus; Microgale brevicaudata; Microgale cowani; Microgale dobsoni; Microgale drouhardi; Microgale dryas; Microgale fotsifotsy; Microgale gracilis; Microgale gymnorhyncha; Microgale longicaudata; Microgale monticola; Microgale nasoloi; Microgale parvula; Microgale principula; Microgale pusilla; Microgale soricoides; Microgale taiva; Microgale talazaci; Microgale thomasi; Microgale jobihely; Microgale jenkinsae; Oryzorictes hova; Oryzorictes tetradactylus; Echinops telfairii; Hemicentetes semispinosus; Hemicentetes nigriceps; Setifer setosus; Tenrec ecaudatus*

Clade 30: Muridae: Nesomyinae (24 species)
Clade description: Recent molecular data are unambiguous in supporting the monophyly of Malagasy rodents (S2), which are included all in the subfamily Nesomyinae. Those genera not yet included in multi-locus phylogenetic assessments are known to be closely related to other genera from Madagascar (S79). Although muroid relationships cannot yet be considered to be fully clarified, the available data are unambiguous in suggesting that an African clade of taxa is sister to nesomyines (S2).
Brachytarsomys albicauda; Brachytarsomys villosa; Brachytarsomys ramirohitra; Brachytarsomys betsiloensis; Eliurus ellermani; Eliurus grandidieri; Eliurus majori; Eliurus minor; Eliurus myoxinus; Eliurus penicillatus; Eliurus petteri; Eliurus tanala; Eliurus webbi; Gymnuromys roberti; Hypogeomys antimena; Macrotarsomys bastardi; Macrotarsomys ingens; Monticolomys/Macrotarsomys koopmani; Monticolomys/Macrotarsomys petteri; Nesomys audeberti; Nesomys lambertoni; Nesomys rufus; Voalavo gymnocaudatus; Voalavo antsahabensis

Clade 31: Eupleridae (9 species)
Clade description: Although they have historically been classified in different families, molecular data clearly suggest that all Malagasy carnivores form a monophyletic group (currently considered as endemic family Eupleridae) and that the predominantly African herpestids are their sister group (S2, S78).
Galidia elegans; Galidictes fasciata; Galidictis grandidieri; Mungotictis decemlineata; Salanoia concolor; Salanoia durrelli; Cryptoprocta ferox; Eupleres goudotii; Fossa fossana