

An evolutionary analysis of the vertebrates of Madagascar

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Abstract

Madagascar's vertebrate biodiversity is very unique. However it is still not exactly known how Madagascar got its unique vertebrate fauna. To elucidate this, this study wanted to investigate the spatial origins and overall evolution of the Madagascan vertebrate clades. To do this a maximum likelihood tree was made which included all endemic vertebrate clades and their sister species, from DNA sequences of 29 genes. From this tree could be concluded that most vertebrate clades had their origin in Africa. Only some had an origin that is either South American, Asian or Oceanian. Differences were found in the amount of colonisations per vertebrate group with birds having colonised Madagascar many more times than the other groups. Average species richness per clade differed much between the groups. Amphibian clades had the highest species richness per clade, birds and bats had a low average species richness. There were also differences found in the average number of species per clade depending on continent of origin. Clades with Asian origin were relatively species-rich while clades from Oceania and South America did not contain many species. All in all, this research explains how the different endemic vertebrate clades evolved on the island and where they came from.

Introduction

New species can arise by two alternative biogeographic processes (1). Firstly, they can arise by dispersal, a process in which a species spreads or immigrates from one area to another. In this way two subpopulations are created and they eventually evolve into separate species. An example of dispersal are the Darwin's finches of the Galapagos Islands. The common ancestor of these finches ended up on the Galapagos islands by dispersal from either the Caribbean Islands or South America and radiated on these islands into many different species (2). Dispersal has been important in shaping the biodiversity of oceanic islands (3) and it also played the main role in shaping the biodiversity of the Gondwanan island of New Caledonia (4). Secondly, new species can arise by vicariance, a process in which parts of a population get separated by for example the splitting of continents. Over many generations, they evolve into separate species. There has been an ongoing debate about whether the vertebrate fauna of the island of Madagascar was also mainly shaped by dispersal or if vicariance played a bigger role there (5). Madagascar is a large island which lies south-east from the African continent. The island is special because of its unique fauna. It exhibits high levels of species endemism and biodiversity (6). Also its fauna doesn't look really like that of Africa while the island lies relatively close to this continent (7). Until 160 million years ago, the island was part of the supercontinent Gondwana, which contained South America, Africa, Antarctica, India and Australia. Because of movements of the Earth's crust, Madagascar, together with India, first split from Africa and South America and then from Australia and Antarctica, and began drifting north. 88 million years ago, Madagascar broke away from India and became an isolated island in the Indian Ocean (8). It is known that the fauna of Madagascar in the Cretaceous was a lot different from the contemporary fauna. This changed because of a major meteorite impact at the Cretaceous-Tertiary boundary, which led to the extinction of a large part of the Cretaceous fauna (9). The almost complete lack of Tertiary Madagascan fossils hampers the knowledge on how the current unique biodiversity of Madagascar came into existence (10). However, thanks to the use of molecular time-trees for reconstructing biogeographic patterns together with paleoclimatic and paleogeographic modelling there is now more known about the biogeography of Madagascar (10). Various insect families have dispersed to Madagascar from Africa (11). Furthermore, mammals could have rafted from Africa to Madagascar, because of past ocean currents in the Cenozoic which flowed from Africa to Madagascar (12). To investigate if there is good support for either dispersal or vicariance being the main factor shaping Madagascar's vertebrate fauna Crottini et al. (10) generated a big data set of nearly all native nonflying and nonmarine vertebrates + sister groups to research the timing of Malagasy vertebrate colonisations. They used 2 protein-coding, single copy genes as markers that are present in all gnathostomes and they used multiple cross-validated time constraints in a single time tree to obtain compatible age estimates among clades. Crottini et al. (10) used the newest methods in phylogenetics to make this time tree, like Bayesian inference (13) and the relaxed molecular clock technique which allows the rate of sequence evolution to differ between organisms in a phylogenetic tree (14). The conclusion was that most vertebrate clades have sister groups in Africa and have stem nodes of Cenozoic age, which means that most clades must indeed have dispersed from Africa. Only some of the clades have sister groups in South America and Asia, these groups were significantly older judged by the average ages at which they split from their non-Madagascan sister groups. The dataset of Crottini et al. however still lacked some taxa, particularly bat and bird taxa, so for a lot of Madagascan clades the origins are still not determined. Furthermore, there are still gaps in the knowledge of the overall evolution of

Madagascan vertebrates. It is not known how many colonisations there have been per vertebrate group. It is also unknown if the species richness per clade differs between the vertebrate groups and if spatial origin has an effect on it.

Thus, in my research I extend the Crottini et al. dataset to include more taxa (e.g. birds and bats) and more gene markers. I make sure that all the newly added data are correct by excluding sequences that cause incorrect phylogenetic positions of taxa. After that I make a maximum likelihood tree from the new data which then can help to get a better understanding of the spatial origins and evolution of all Madagascan vertebrates. Specifically, I want to find out: (I) the spatial origins of the Madagascan clades; (II) if there are differences in the number of colonisations between the vertebrate groups; (III) if there are differences in the average species richness per clade between the vertebrate groups and (IV) if there are differences in the number of species per clade depending on continent of origin.

Materials and Methods

The dataset of Crottini et al. initially consisted of 2 genes (RAG1 and BDNF) and 188 taxa. Sequences of new taxa were added for both RAG1 and BDNF. Alignment was done per gene with the help of MAFFT version 7 with default settings (15) which did a rough alignment. Then the gene sequences were further aligned manually. The sequence viewer used was Mesquite version 3.10 with default settings (16). From each gene alignment a distance-based neighbour joining tree with PAUP* under default settings (17) was made to quickly check how the different taxa more or less relate to each other. In this way all the sequences that were wrong could be removed or replaced. Sequences were considered wrong if they caused wrong placements of taxa in the phylogenetic tree. The datasets of 27 new genes were concatenated with the 2 other gene alignments into one super matrix. These datasets had also undergone the alignments and checks. Concatenation was done with SequenceMatrix version 1.8 with default settings (18). The resulting super matrix contained 29 genes (see table 1) and 1733 taxa.

Table 1-The genes included in the super matrix, their corresponding variations in sequence length, the numbers of taxa included for each gene, for each gene it is denoted if it is nuclear or mitochondrial and if it is protein-coding or an RNA gene

Gene	Variation in sequence length in nucleotides (longest-shortest)	# of taxa	Nuclear/Mitochondrial	Protein-coding/RNA gene
12S	1561-940	781	Mitochondrial	RNA gene
16S	734-296	1181	Mitochondrial	RNA gene
16S_2	823-455	329	Mitochondrial	RNA gene
ADORA3	412-356	43	Nuclear	Protein-coding
ADRA2B	1229-218	74	Nuclear	Protein-coding
AR	1689-1230	67	Nuclear	Protein-coding
BDNF	890-505	539	Nuclear	Protein-coding
CMOS	863-611	353	Nuclear	Protein-coding
ENO	908-803	27	Nuclear	Protein-coding
FIBA	701-638	42	Nuclear	Protein-coding
GHR	934-734	72	Nuclear	Protein-coding
H3	333-281	60	Nuclear	Protein-coding
KIAA1239	1720-609	447	Nuclear	Protein-coding
MYC	1394-1196	24	Nuclear	Protein-coding
MYO	726-691	33	Nuclear	Protein-coding
ND1	975-504	108	Mitochondrial	Protein-coding
ND2	1044-336	501	Mitochondrial	Protein-coding
ND4	2498-1960	336	Mitochondrial	Protein-coding
RAG2	1183-439	348	Nuclear	Protein-coding
RAMP	497-482	18	Nuclear	Protein-coding
Rag1	1674-146	817	Nuclear	Protein-coding
SACS	1083-518	378	Nuclear	Protein-coding
SWS	3556-3341	17	Nuclear	Protein-coding
TMO4C4	528-503	50	Nuclear	Protein-coding
VWF11	845-365	42	Nuclear	Protein-coding
VWF28	1282-1044	50	Nuclear	Protein-coding

coi	760-153	956	Mitochondrial	Protein-coding
coii	684-529	85	Mitochondrial	Protein-coding
cytb	1062-324	985	Mitochondrial	Protein-coding

From this super matrix a Maximum Likelihood tree was made to check if there were still any taxa with wrong sequences in the alignment. The ML tree was made with RAXML-HPC2 version 8 (19) on XSEDE in the CIPRES portal (20) with standard settings. *Callorhynchus milii*, a cartilaginous fish, was used as an outgroup. After this, 3 taxa that were at a wrong place in the tree were cast out. The 3 cast out taxa were: *Cheirogaleus crossleyi* A B, *Prionops plumatus* and *Alcedo cristata*. Also, the relationships between the major vertebrate groups in the phylogeny did not exactly correspond to the commonly accepted patterns of vertebrate evolution. For example the lungfish *Protopterus aethiopicus* and the coelacanth *Latimeria menadoensis* were at wrong positions in the tree. Thus a new ML tree was made with a backbone implemented (see Appendix, fig. 1) based on the backbone phylogeny of jawed vertebrates from Irisarri et al. (21). This phylogeny was made with a phylotranscriptomic approach and was found to be robust and strongly supported. In the improved ML tree all the major groups were at the right place. For all taxa in the tree it was determined where they occur by looking at the literature. See figures 1 and 2 for examples of how the origins of the Madagascan clades were determined. In figure 1, the upper Madagascan clade in the tree is the Eupleridae family and it can be seen that this clade is most closely related to *Suricata suricatta* from Africa. Judging by the literature these two groups have a sister group relationship. Thus it can be concluded that the Eupleridae family is of African origin. The lower Madagascan clade, *Suncus madagascariensis*, is in this tree most closely related to *Erinaceus europaeus* from Europe. However these two taxa are not sister species according to the literature. They are not even in the same family. Since *Suncus madagascariensis* has no sister species in this tree its origin cannot be determined. In figure 2 we see two Madagascan clades. The upper one is the Malagasy Scincidae clade which has a sister group relationship with the genus *Chalcides* and so is of African origin. The lower one is the Opluridae clade of which, according to the literature, *Diplolaemus darwinii* is the sister species. This species is from South America and so the Opluridae clade is of South American origin. The average number of species per clade was calculated for each vertebrate group with the sum (*total number of species per group/total number of clades per group*). With the same principal the average species richness per clade was calculated for each continent of origin.

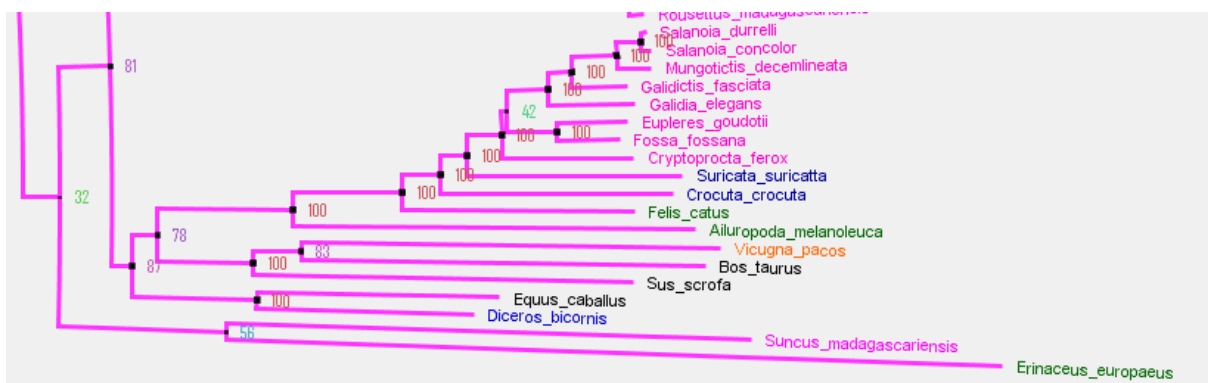


Figure 1-A small part of the vertebrate Maximum Likelihood tree. Bootstrap values shown near nodes. Taxa colours indicate geographic distribution: pink = Madagascar; orange = South America; dark blue = Africa; green = Asia/Oceania/Europe; black = also occurs outside of Madagascar/occurs in multiple continents

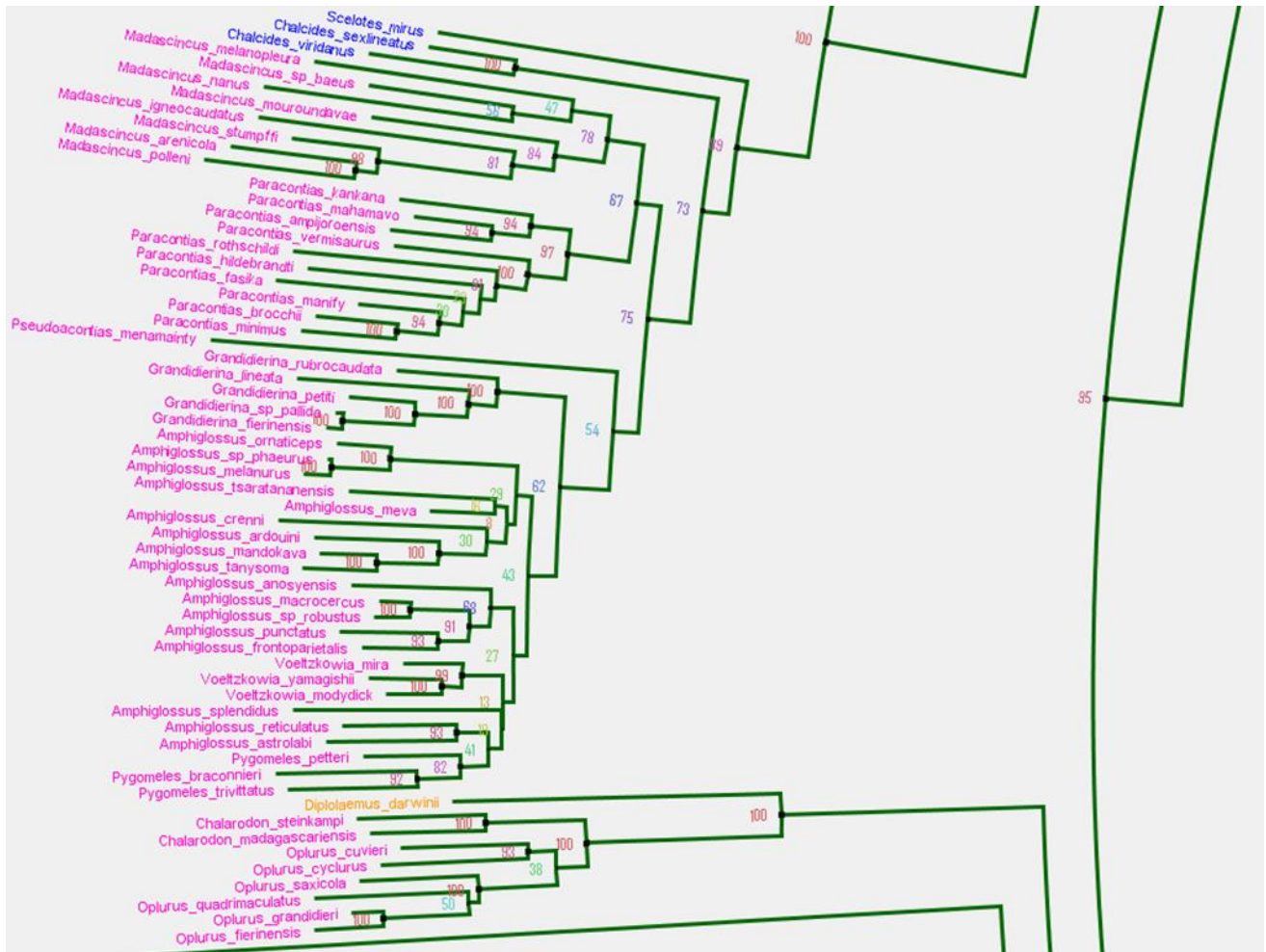


Figure 2-A small part of the vertebrate Maximum Likelihood tree. Bootstrap values shown near nodes. Taxa colours indicate geographic distribution: pink = Madagascar; orange = South America; dark blue = Africa

Results

The finished 29 gene alignments contained 302 taxa on average and had an average maximum sequence length of 1124 nucleotides. In the end, the aligned super matrix, wherein all the gene alignments are included, contains 1730 taxa and is 32588 nucleotides long. There are significant differences in coverage per taxon. From taxa with genetic information of 18 genes and a total sequence length of 19153 nucleotides to taxa with genetic information of only one gene and a total sequence length of 351 nucleotides. The Maximum Likelihood tree (see Appendix, fig. 2) depicts the evolutionary relationships between endemic Malagasy vertebrate clades, their sister species and other vertebrate taxa. Support levels for the deep nodes were generally high. The tree contains 118 endemic clades. The various vertebrate groups in the tree differed from each other in relation to the number of colonisations. The Archosaurs, which include the crocodiles and the birds, have colonised Madagascar most frequently (53 times). The turtles have colonised the island the least amount of times, only two times (see table 2). From 58 clades the origin is certain, most are from Africa. A small part is from Asia, South America or Oceania. From some clades the origin is ambiguous.

Some relationships in the maximum likelihood tree are different than initially expected.:

1. The cichlid species *Paretroplus gymnopreopercularis* is nested within the genus *Ptychochromis* with reasonable support. This indicates that this species may belong to *Ptychochromis*. See figure 3.
2. The monotypic tenrec genus *Limnogale* is nested within the genus *Microgale* with reasonable support.

3. There is reasonable support for the bat genera Mops and Chaerephon being paraphyletic.
4. In this tree two species representing the bat genus Hypsugo are nested within species of the genus Neoromicia with very good support, meaning that the genus Neoromicia is a paraphyletic clade.
5. The rodent genus Macrotarsomys is paraphyletic according to the tree topology. The two Macrotarsomys species are not each other's sister species. Instead, M. bastardi has a sister group relationship with Monticolomys koopmani with very good support.
6. The weaver bird genus Quelea from Africa is nested within species of another weaver genus, Foudia from Madagascar and surrounding islands. The sister group relationship between Quelea and Foudia omissa has reasonable support. This may mean that Foudia is paraphyletic.
7. The bird genus Xanthomixis is paraphyletic. The genus Crossleyia is nested within it with reasonable support.
8. The bird genus Monias is nested within the genus Mesitornis with very good support, which means that Mesitornis is paraphyletic.
9. The bird genus Caprimulgus is paraphyletic with the seabird species Pachyptila desolata nested within this genus with very good support.
10. The monotypic gecko genus Microscalabotes is nested within the genus Lygodactylus with moderate support.



Figure 3-A small part of the vertebrate Maximum Likelihood tree. Bootstrap values shown near nodes. The species Paretroplus gymnopreopercularis is nested within the genus Ptychochromis with reasonable support

The average number of species per colonisation is different between the different vertebrate groups (see figure 3). The archosaurs have the lowest number of species per clade, with an average of 2.1 species per clade. The amphibian clades radiated the most after colonisation, with the amphibian Mantellidae family containing a total of 422 species. There are also differences in the number of species per clade depending on spatial origin. Clades from Asia have radiated far out the most with an average of around 70 species per clade while clades from Oceania and South America have radiated much less (see figure 4).

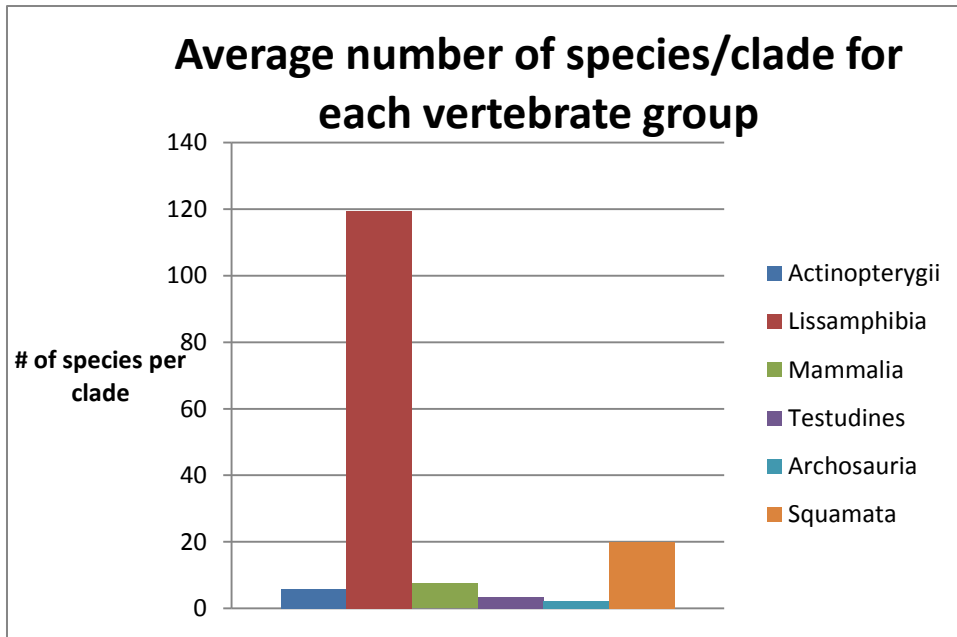


Figure 3-Graph depicting the average number of species after colonisation per vertebrate group

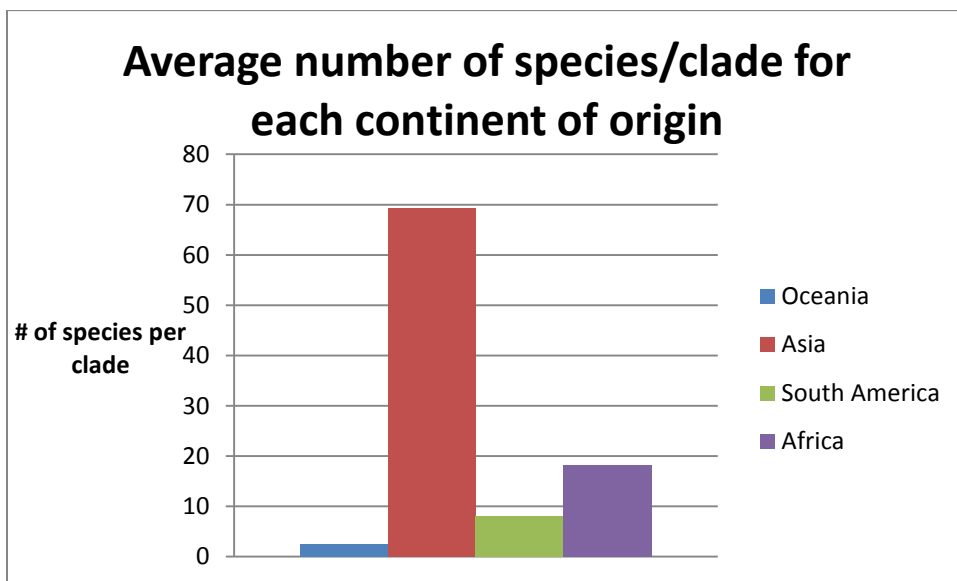


Figure 4- Graph depicting the average number of species after colonisation per continent of origin

Table 2-Endemic Madagascan clades or species with their corresponding origins and species richness

Clade/Species	Origin	Species
Actinopterygii:		
1. Anchariidae	-	1
2. Sauvagella	-	1
3. Ratsirakia	-	1
4. Glossogobius ankarensis	Africa/Asia	1
5. Typhleotris	Oceania	3

6.	Kuhlia sauvagii	-	1
7.	Pachypanchax	Asia	2
8.	Teramulus	-	1
9.	Atherinomorus sp Karimanga	-	1
10.	Bedotiidae	Asia	24
11.	Malagasy Cichlidae	Africa	28
Lissamphibia:			
1.	Heterixalus	Africa	13
2.	Dyscophinae	Asia	4
3.	Cophylinae + Scaphiophryninae	-	154
4.	Ptychadena mascareniensis	Africa	4
5.	Mantellidae	Asia	422
Mammalia:			
1.	Tenrecidae	Africa	32
2.	Suncus madagascariensis	-	1
3.	Eupleridae	Africa	8
4.	Rousettus madagascariensis	-	1
5.	Eidolon dupreanum	Africa	1
6.	Pteropus rufus	-	1
7.	Hipposideros commersoni	-	1
8.	Triaenops auritus	-	1
9.	Triaenops menamena	Africa/Asia	1
10.	Coleura kibomalandy	Africa	1
11.	Emballonura atrata, taivato	-	2
12.	Miniopterus mahafaliensis	-	1
13.	Myzopoda	-	2
14.	Miniopterus manavi, petersoni, aelleni, griffithsi, glenni, majori, griveaudi, sororculus	-	8
15.	Mormopterus jugularis	-	1
16.	Otomops madagascariensis	-	1
17.	Chaerephon jobimena	-	1
18.	Mops leucostigma	Africa/Asia	1
19.	Chaerephon atsinanana	-	1
20.	Chaerephon leucogaster	Africa	1
21.	Myotis goudoti	-	1
22.	Scotophilus marovaza, tandrefana	-	2
23.	Scotophilus robustus	-	1
24.	Hypsugo bemainty	-	1
25.	Malagasy Neoromicia	Africa	3
26.	Nesomynia	Africa	24
27.	Lemuroidea	Africa	104
Testudines:			

1.	Malagasy Testudinidae	Africa	4
2.	Pelomedusoidea	South America/Africa	3
Archosauria:			
1.	Crocodylus niloticus	Africa	1
2.	Aepyornithiformes	-	2
3.	Margaroperdix	-	1
4.	Anas bernieri	-	1
5.	Anas melleri	-	1
6.	Asities	-	4
7.	Coracina cinerea	Africa	1
8.	Dicrurus fortificatus	-	1
9.	Terpsiphone mutata	Africa/Asia	1
10.	Malagasy Vangidae	-	21
11.	Cinnyris notatus, sovimanga	-	2
12.	Motacilla flaviventris	-	1
13.	Lonchura nana	-	1
14.	Foudia madagascariensis & omissa	Africa/Asia	2
15.	Ploceus sakalava, nelicourvi	Africa/Asia	2
16.	Copsychus albospecularis	Asia	1
17.	Saxicola torquata sibilla	Asia/Africa	1
18.	Monticola imerina, bensoni, sharpei	Africa/Asia	3
19.	Mirafra hova	Africa/Asia	1
20.	Zosterops maderaspatanus	-	1
21.	Hypsipetes madagascariensis	Asia	1
22.	Dromaeocercus	Africa/Asia	1
23.	Bernieridae	-	10
24.	Nesillas typica	-	1
25.	Acrocephalus newtoni	-	1
26.	Neomixis	-	3
27.	Cisticola cherinus	Africa	1
28.	Corythornis madagascariensis, vintsiodes	Africa	2
29.	Agapornis canus	Africa	1
30.	Coracopsis	Oceania	2
31.	Brachypteraciidae	-	5
32.	Tyto soumagnei	-	1
33.	Otus rutilus	-	1
34.	Eutriorchis	Africa/Asia	1
35.	Buteo brachypterus	-	1
36.	Haliaeetus vociferoides	Africa	1
37.	Accipiter madagascariensis, francesii, henstii	-	3
38.	Leptosomiformes	-	1
39.	Mesitornithiformes	-	3

40.	Falco newtoni	-	1
41.	Turnix nigricollis	-	1
42.	Charadrius thoracicus	-	1
43.	Pterocles personatus	-	1
44.	Caprimulgus enarratus	-	1
45.	Caprimulgus madagascariensis	-	1
46.	Streptopelia picturata	Africa/Asia	1
47.	Alectroenas madagascariensis	-	1
48.	Treron australis	Asia/Africa	1
49.	Lophotibis	-	1
50.	Canirallus beankaensis, kioloides	Africa	2
51.	Sarothrura insularis	Africa	1
52.	Apus balstoni	-	1
53.	Coua	Africa/Asia	9
Squamata:			
1.	Uroplatus	Africa	20
2.	Malagasy Hemidactylus	Africa	2
3.	Ebenavia+Paroedura	Africa	19
4.	Matoatoa	-	1
5.	Paragehyra	-	4
6.	Blaesodactylus	Africa	5
7.	Geckolepis	-	8
8.	Malagasy Lygodactylus+Microscalabotes	Africa	22
9.	Phelsuma	Africa	41
10.	Zonosaurinae	Africa	18
11.	Malagasy Trachylepis	Africa	11
12.	Malagasy Scincidae	Africa	47
13.	Opluridae	South America	8
14.	Xenotyphlopidae	Asia/Africa	1
15.	Madatylops	Asia/Africa	5
16.	Sanziniinae	-	4
17.	Mimophis	Africa	1
18.	Pseudoxyrhophiinae	Africa	62
19.	Brookesiinae	-	31
20.	Malagasy Chameleoninae	Africa	88

Discussion

The majority of clades of which the origin could unambiguously be determined as having an African sister group, meaning that they originate from the African continent. This finding agrees with previous research in which most of the Malagasy clades also showed an African origin(5,10). From a few taxa could be determined that they are from another continent than Africa. From Oceania there is the cavefish genus Typhleotris and the parrot genus Coracopsis. Originating from South America are the Opluridae, a family of lizards native to Madagascar. Some clades are definitely Asian, these include two fish taxa (Pachypanchax and Bedotiidae), two amphibian taxa (Dyscophinae and Mantellidae) and two bird taxa (Copsychus albospecularis and Hypsipetes madagascariensis). For some other taxa the origin is

ambiguous. For the other half of the taxa the origin could not be determined at all from the phylogenetic tree, because their sister groups were absent in the data set that was used to make the tree. To find out the origins of all Malagasy clades with certainty, genetic information of more related species is needed, preferably from a variety of continents.

The high amount of bird colonisations (52 times) can be explained by their ability to fly. This makes them have a dispersal advantage. Also, they are generally very social animals so when they end up on Madagascar they have a higher chance of being accompanied with others from their species. Thus they then have a higher chance of starting a new population (7). Vertebrate groups with relatively few colonisations like the amphibians (5 times) and the Actinopterygii (11 times) are generally seen as dispersal-disadvantaged (7). However, the ancestors of the turtles could swim and thus were dispersal-advantaged (7), yet the turtles only colonised Madagascar twice.

There is much variation in species richness per clade between vertebrate groups. According to Crottini et al. (10), clade species richness in Madagascar is correlated with clade age and adaptability to rainforest habitats. Bird and bat clades have a particularly low species richness. Almost all birds have colonised Madagascar in Cenozoic times (7) and some of them have colonised the island only very recently (5). Thus the young age of the endemic bird clades could be the reason for their low number of species per clade. The same thing could be said for the bats since it is thought that most bat families only have colonised Madagascar during the last 15 million years (22). The amphibians have an on average high number of species per clade. Especially the Mantellidae and a clade consisting of Cophylinae and Scaphiophryinae are species rich. This could be because both clades have Cretaceous origins and thus have a high clade age (10). Also, all amphibian clades have their highest values of species richness in rainforest (23), meaning that they are able to adapt to rainforest habitats. This too could have affected amphibian species richness in a positive way. There are also differences in speciation between clades of different origins. Clades with an Asian origin have an on average high number of species per clade. It is so high because the Mantellidae family, which consists of 422 species, is from Asia. Clades from South America or Oceania generally have a low number of species per clade.

Some of the relationships found in the ML tree were unexpected. A part of them is supported by the literature: (I) *Limnogale* being nested within *Microgale* agrees with previous research which concluded that *Limnogale* had to be moved to *Microgale* (24); (II) The tree topology of *Mops* and *Chaerephon* corresponds to earlier research in which it was also found that *Mops* and *Chaerephon* aren't monophyletic and that a clade containing *Chaerephon* and *Mops* minus *C. jobimena* is monophyletic (25); (III) An earlier study also confirmed that *Neoromicia* was not monophyletic (26); (IV) *Microscalabotes* being nested within *Lygodactylus* agrees with previous research which synonymised *Microscalabotes* with *Lygodactylus* (27). The possibility of *Foudia* being paraphyletic on the other hand contradicts previous research which showed that the two genera are both monophyletic and are each other's closest relatives (28).

Initially the goal of this research was to use multiple cross-validated time constraints in a single time tree to obtain compatible age estimates among clades. With this time tree conclusions could be made about the temporal origins of the Malagasy clades and so also about if the clades arose by dispersal or vicariance. I tried various programs to make this time tree, such as BEAST, MEGA and MCMCTree, but they all could not handle the dataset. One program was run on a supercomputer and after a whole month the calculations still weren't finished. The cause of the calculation problems was probably the dataset that was used which may have been too big and may have contained too many gaps.

Perspectives

This study shines more light on the evolutionary history of the Madagascan vertebrates. However, not for all clades the continent of origin could be determined. To do this accurately the genetic information of more related species needs to be added to the dataset. Furthermore, to get a more complete picture of the biogeography of Malagasy vertebrates a time tree has to be constructed from the dataset. With a time tree the temporal origins of the clades can be determined. For this a program has to be created that can handle large datasets with many gaps. Only in this way can be found out which clades arose on Madagascar by dispersal and which ones by vicariance. Thus, more research can be done with the current dataset as starting point to further elucidate the origins of Madagascar's vertebrate fauna. Furthermore, more research on the origins and evolution of the vertebrates of other Gondwanan islands

could be performed, since it is lacking right now. Examples of such islands are New Caledonia and New Zealand. It would be interesting to see if comparisons can be made with Madagascar and also what the differences are between the islands.

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Appendix

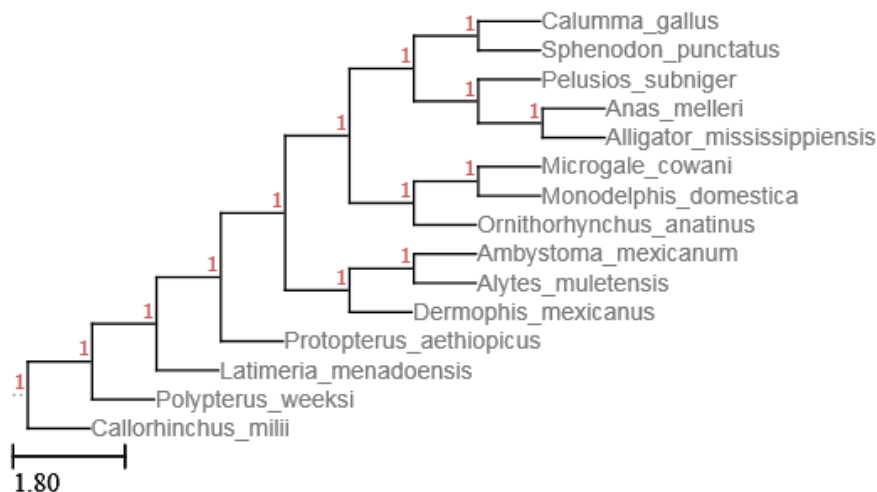


Figure 1-Backbone used in Maximum Likelihood analysis



MLtree_vert.pdf

Figure 2-Veterebrate Maximum Likelihood tree from an analysis of 32588-bp DNA sequences of 29 genes. Bootstrap values shown near nodes. Numbers denote clades as listed in Table 2. Taxa colours indicate geographic distribution: pink = Madagascar; orange = South America; dark blue = Africa; green = Asia/Oceania/Europe; light blue = Comoros/Mascarenes/Seychelles; black = also occurs outside of Madagascar/occurs in multiple continents. Branch colours indicate vertebrate group: grey = Chondrichthyes; olive = Actinopterygii; yellow = Coelacanthiformes/Dipnoi; light green = Lissamphibia; pink = Mammalia; orange = Testudines; purple = Crocodylia; blue = Aves; dark green = Lepidosauria.