

Genetic diversity and phylogenetic relationships of *Mabuya* spp. (Squamata: Scincidae) from western Indian Ocean islands

Sara Rocha^{1,2,3,*}, Miguel A. Carretero^{1,2}, D. James Harris^{1,2}

Abstract. Western Indian Ocean islands (excluding Madagascar) are inhabited by at least five species of the lizard genus *Mabuya*, for which diversity and inter-island phylogenetic relationships are still unclear, particularly concerning island populations of *M. maculilabris*, *M. comorensis* and *M. striata*. With a comprehensive sampling across the Comoros archipelago and the islands of Zanzibar and Pemba (Tanzania) we use molecular data and previously published sequences of these species to describe genetic variation across their insular range and explore the possible distinction of insular populations of the different species, contributing to the clarification of their status and knowledge of their colonization patterns. We describe patterns of cryptic diversity within *M. comorensis*, revealing a West to East colonization of the Comoros archipelago and clarify the status of Malagasy populations of this species. Cryptic variation is also observed within *M. maculilabris* and *M. striata*, and the status of several continental and island populations discussed. *M. sechellensis* is shown to be paraphyletic relative to *M. wrightii*. Available data for all Afro-Malagasy *Mabuya* reveals additional possible instances of paraphyly within some species but does not allow for further resolution of basal relationships within this group, pointing to rapid and simultaneous divergences within this group and the need for multiple independent markers to recover the history of this group.

Keywords: Africa, Comoros, cryptic diversity, island colonisation, *Mabuya*, Madagascar, Seychelles, *Trachylepis*, Western Indian Ocean islands.

Introduction

Five species of the genus *Mabuya* (Squamata: Scincidae) are known to occur across the western Indian Ocean archipelagos of the Comoros, the Seychelles, Zanzibar and Pemba (following Spawls et al., 2002; Carretero, Rocha and Harris, 2005; Gerlach, 2007) with several others inhabiting exclusively the island of Madagascar (Glaw and Vences, 2004). It has been hypothesised that the Malagasy species have multiple origins, and molecular data does not contradict this (Mausfeld et al., 2000; but see Whiting et al., 2006). However, little is known concerning the variation and inter-island relationships within the Comoran species *M. comoren-*

sis, on the relationship of Comoran populations of *M. striata* with its continental conspecifics or on the Zanzibar and Pemba populations of both *M. striata* and *M. maculilabris*. The Seychelles archipelago is also inhabited by two endemic species, *M. wrightii* and *M. sechellensis*, that are known to belong to the Afro-Malagasy clade of *Mabuya* (Carranza et al., 2001; Jesus, Harris and Brehm, 2005), now usually referred to as *Trachylepis* (although see Jesus, Harris and Brehm, 2005 and Whiting et al., 2006 for arguments against the use of this nomenclature) but their precise phylogenetic relationships are not known.

Mabuya maculilabris has an impressive distribution encompassing Cameroon and the Gulf of Guinea islands of S. Tomé and Príncipe in West Africa, to Mozambique, Tanzania and the offshore islands of Mozambique, Europa, Zanzibar and Pemba in East Africa (Biodiversity occurrence data accessed through GBIF Data Portal, www.gbif.net, 2009-08-12). Possible intraspecific variation has long been recognized within this species, and led to the definition of the “*Mabuya maculilabris*-group” (sensu

1 - CIBIO-UP, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus Agrário de Vairão, 4485-661 Vairão, Portugal

2 - Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, FC4, 4169-007, Porto, Portugal

3 - Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, Vigo 36310, Spain

* Corresponding author; e-mail: sara.rocha@mail.icav.up.pt

Broadley, 1974). Although several taxa were assigned to this group its composition remained unstable (see Mausfeld-Lafdhya et al., 2004). Mausfeld-Lafdhya et al. (2004) used molecular data to explore genetic variation within this group and reported two significantly distinct clades defined by Western and Eastern African specimens, respectively, but the use of a very reduced dataset (a few samples from Tanzania and Cameroon) inhibited the clarification of the status of the several described species and subspecies, mainly of most island populations such as *M. maculilabris casuarinae* (from Mozambique island); *M. ssp. infralineata* (Boettger, 1913; Broadley, 1974; Brygoo, 1981) (from Europa island) and *M. m. albotaeiniata* (from Pemba island, Tanzania). By comparing their sequences from Tanzania with one sequence from Carranza et al., (2001), from a specimen from Mozambique, these authors further hypothesized on another cryptic species within this group, but further exploration was not performed. Also Jesus, Harris and Brehm (2005) argued that *M. maculilabris* is most probably a species complex, as at least five lineages could be identified using a 12s rRNA gene dataset. However, again, sampling was limited, and only three east African published sequences were used (*M. comorensis* from Madagascar, *M. maculilabris casuarinae* and *M. maculilabris maculilabris* from Mozambique).

Mabuya comorensis also has an unstable taxonomic history. It is mostly confined to island populations, from the Comoros archipelago, but some specimens from Tanzania and Kenya were described as *M. comorensis* and *M. maculilabris comorensis* (Loveridge, 1929), indicating an unclear distinction between *M. maculilabris* and *M. comorensis*, and a close relationship, recently confirmed by molecular data (Mausfeld-Lafdhya et al., 2004). Variation has also been recognised within *M. comorensis*, with Peters (1854) describing *Euprepes comorensis* from Anjouan, Comoros, and naming a new species from Grand Comoro *E. angasjanus* (Peters, 1882), although it was considered a variant of

M. comorensis. To date, relationships of specimens from the Comoros were never investigated using molecular data. Used in earlier phylogenetic analyses was instead an individual from Nosy Tanikely, a small offshore island in Northwest Madagascar, where this species is known to occur. The relationship between this and the Comoran forms is also of interest.

Mabuya striata is another species of the Afro-Malagasy clade with an impressively wide distribution, from Congo and Namibia in West Africa to Tanzania, Kenya, and Mozambique in East Africa (Biodiversity occurrence data accessed through GBIF Data Portal, www.gbif.net, 2009-08-12) and with several island populations (Zanzibar, Pemba, Anjouan – Comoros) whose status and possible distinction from continental forms was also never assessed using molecular data.

We sampled across the archipelago of the Comoros (*Mabuya comorensis* and *M. striata*) and the islands of Zanzibar and Pemba (*M. striata* and *M. maculilabris*) and also from the Tanzanian coast (*M. maculilabris*) and analysed these samples together with published sequences of *M. maculilabris* from the West African islands of the Gulf of Guinea and from Mozambique (East Africa) and of continental *M. striata*. A sample from *Mabuya* spp. from Europa Island was also obtained. We thus aimed to describe genetic variation across the (eastern African) insular range of these species and explore the possible distinction of insular populations of the different species, contributing to the clarification of their status and the knowledge of their colonization patterns. Furthermore, we included both Seychelles' species in a comprehensive phylogenetic analysis together with all available sequences of the "Afro-Malagasy" clade to re-examine their phylogenetic relationships.

Material and methods

Specimens used in this study and respective locations can be found in table 1. Sampling was carried out across the

Table 1. Specimens used in this study, respective locations (detailed GPS coordinates for Comoran samples can be found in Carretero et al., 2005) and Genbank accession nos.

Species	Individual	Location	Genbank accession nos.	
			Cyt- <i>b</i>	16s
<i>M. comorensis</i>	MY1	Dzaoui (airport road), Mayotte, Comoros	HM192681	HM192751
<i>M. comorensis</i>	MY12	Chirongui, Mayotte, Comoros	HM192682	HM192750
<i>M. comorensis</i>	MY20	Kani-Kéli, Mayotte, Comoros	HM192683	
<i>M. comorensis</i>	MY25	M'zouazia, Mayotte, Comoros	HM192684	
<i>M. comorensis</i>	MY37	Bouéni (road to), Mayotte, Comoros	HM192685	HM192749
<i>M. comorensis</i>	MY49	Ouangani, Mayotte, Comoros	HM192686	
<i>M. comorensis</i>	MY51	Mangajou, Mayotte, Comoros	HM192687	HM192748
<i>M. comorensis</i>	MY55	Sada, Mayotte, Comoros	HM192688	
<i>M. comorensis</i>	MY59	Bambo Est, Mayotte, Comoros	HM192689	
<i>M. comorensis</i>	MY61	Bandrélé, Mayotte, Comoros	HM192690	
<i>M. comorensis</i>	MY81	Trévani, Mayotte, Comoros	HM192691	
<i>M. comorensis</i>	AJ3	Moutsamoudou, Anjouan, Comoros	HM192692	HM192752
<i>M. comorensis</i>	AJ7	Bazimini, Anjouan, Comoros	HM192693	
<i>M. comorensis</i>	AJ14	Mboüeladougou, Anjouan, Comoros	HM192694	
<i>M. comorensis</i>	AJ17	Adda-Douéni, Anjouan, Comoros	HM192695	
<i>M. comorensis</i>	AJ18	Foret de Moya, Anjouan, Comoros	HM192696	HM192753
<i>M. comorensis</i>	AJ22	Pomoni, Anjouan, Comoros	HM192697	
<i>M. comorensis</i>	AJ25	Dindi, Anjouan, Comoros	HM192698	
<i>M. comorensis</i>	AJ26	Mchakojou, Anjouan, Comoros	HM192699	
<i>M. comorensis</i>	AJ29	Haiko, Anjouan, Comoros	HM192700	
<i>M. comorensis</i>	AJ33	Koni-Djodjo, Anjouan, Comoros	HM192701	
<i>M. comorensis</i>	MH7	Fomboni, Moheli, Comoros	HM192702	HM192758
<i>M. comorensis</i>	MH11	Badjo, Moheli, Comoros	HM192703	HM192756
<i>M. comorensis</i>	MH20	Gnombéni, Moheli, Comoros	HM192704	
<i>M. comorensis</i>	MH21	Ouanani, Moheli, Comoros	HM192705	
<i>M. comorensis</i>	MH23	Ouhoni, Moheli, Comoros	HM192706	HM192757
<i>M. comorensis</i>	MH25	Sambia, Moheli, Comoros	HM192707	
<i>M. comorensis</i>	MH35	Mbouerani, Moheli, Comoros	HM192708	
<i>M. comorensis</i>	MH37	Mbatsé, Moheli, Comoros	HM192709	
<i>M. comorensis</i>	GC5	Moroni, Grand Comoro, Comoros	HM192712	HM192754
<i>M. comorensis</i>	GC16	N of Moroni airport, Grand Comoro, Comoros	HM192713	
<i>M. comorensis</i>	GC18	Mouadja, Grand Comoro, Comoros	HM192714	
<i>M. comorensis</i>	GC36	Mouadzaza, Grand Comoro, Comoros	HM192715	
<i>M. comorensis</i>	GC42	Mbambani, Grand Comoro, Comoros	HM192716	HM192755
<i>M. comorensis</i>	GC47	Bandanadji, Grand Comoro, Comoros	HM192717	
<i>M. comorensis</i>	E	Moroni, Grand Comoro, Comoros	HM192710	
<i>M. comorensis</i>	G	Itzandra, Grand Comoro, Comoros	HM192711	
<i>M. comorensis</i>		Nosy Tanikely, Madagascar		AY153565
<i>M. maculilabris albotaeniata</i>	PB4	Mkoani, Pemba Island, Tanzania	HM192718	
<i>M. maculilabris albotaeniata</i>	PB8	Chake, Pemba Island, Tanzania	HM192719	
<i>M. maculilabris albotaeniata</i>	PB10	East Chake, Pemba Island, Tanzania	HM192720	
<i>M. maculilabris albotaeniata</i>	PB12	East Chake, Pemba Island, Tanzania	HM192721	
<i>M. maculilabris albotaeniata</i>	PB18	Jondeni, Pemba Island, Tanzania	HM192722	
<i>M. maculilabris albotaeniata</i>	PB20	Wete, Pemba Island, Tanzania	HM192723	
<i>M. maculilabris albotaeniata</i>	PB22	Wete, Pemba Island, Tanzania	HM192724	
<i>M. maculilabris albotaeniata</i>	PB23	Konde, Pemba Island, Tanzania	HM192725	
<i>M. maculilabris albotaeniata</i>	PB27	Ngezi, Pemba Island, Tanzania	HM192726	
<i>M. maculilabris albotaeniata</i>	PB29	Airport, Pemba Island, Tanzania	HM192727	
<i>M. maculilabris maculilabris</i>		Mozambique	AF280269	
<i>M. maculilabris casuarinae</i>			AF280270	AY151474
<i>M. (maculilabris) infralineata</i>	Mab4Eur	Europa Island	HM192728	
<i>M. maculilabris maculilabris</i>		East Africa		AY070356

Table 1. (Continued).

Species	Individual	Location	Genbank accession nos.	
			Cyt- <i>b</i>	16s
<i>M. maculilabris maculilabris</i>	TZ10	Dar Es Salaam, Tanzania	HM192729	
<i>M. maculilabris maculilabris</i>	TZ11	Dar Es Salaam, Tanzania	HM192730	
<i>M. maculilabris maculilabris</i>	TZ16	Tanga, Tanzania	HM192731	
<i>M. maculilabris maculilabris</i>	TZ36	Lushoto, Tanzania	HM192732	
<i>M. maculilabris maculilabris</i>	Z20	Zanzibar Island, Tanzania	HM192733	
<i>M. maculilabris maculilabris</i>	Z36	Zanzibar Island, Tanzania	HM192734	
<i>M. maculilabris maculilabris</i>		S. Tomé Island, West Africa	AY997770	
<i>M. maculilabris maculilabris</i>		Rolas, S. Tomé Is., West Africa	AY997769	
<i>M. maculilabris maculilabris</i>	599	Terra Velha, Principe	HM192735	
<i>M. striata</i>	PB1	Mkoani, Pemba Island, Tanzania	HM192736	
<i>M. striata</i>	Z18	Cwaka bay, Zanzibar Island, Tanzania	HM192737	
<i>M. striata</i>	Z29	Kiwengwa, Zanzibar Island, Tanzania	HM192738	
<i>M. striata</i>	TZ5	Dar Es Salaam, Tanzania	HM192739	
<i>M. striata</i>	TZ9	Dar Es Salaam, Tanzania	HM192741	
<i>M. striata</i>	AJ28	Haiko, Anjouan, Comoros	HM192740	
<i>M. striata</i>		Namibia	AY217812	
<i>M. margaritifera</i>		Probably Tanzania (pet trade)	AF153591	
<i>M. margaritifera</i>		Mozambique	AF280268	
<i>M. hoeschi</i>		Namibia	AY217809	
<i>M. varia</i>	TZ25	Lushoto, Tanzania	HM192742	
<i>M. varia</i>	TZ33	Lushoto, Tanzania	HM192743	
<i>M. gravenhorstii</i>		Madagascar	DQ239123	
<i>M. madagascariensis</i>		Madagascar	DQ239124	
<i>M. elegans</i>		Madagascar	DQ239122	
<i>M. perrotetii</i>		Ghana	DQ239146	
<i>M. capensis</i>		South Africa	DQ239178	
<i>M. occidentalis</i>		South Africa	DQ239182	
<i>M. quinquetaeniata</i>		unknown	DQ239183	
<i>M. quinquetaeniata</i>		unknown	DQ239115	
<i>M. quinquetaeniata</i>		Ghana	DQ239148	
<i>M. quinquetaeniata</i>		unknown	EU443143	
<i>M. quinquetaeniata</i>		unknown	AF153593	
<i>M. socotrana</i>		Socotra Island	AF280273	
<i>M. acutilabris</i>		Namibia	DQ239181	
<i>M. sechellensis</i>	MA2	Mahé Island, Seychelles	HM192744	
<i>M. sechellensis</i>	27CUR	Curieuse Island, Seychelles	HM192745	
<i>M. wrightii</i>	15SP	Saint Pierre Island, Seychelles	HM192746	
<i>M. boettgeri</i>		Madagascar	DQ239120	
<i>M. vato</i>		Madagascar	DQ239125	
<i>M. aureopunctata</i>		Madagascar	DQ239119	
<i>M. dumasi</i>		Madagascar	DQ239121	
<i>M. homalocephala</i>		South Africa	DQ239116	
<i>M. atlantica</i>		Fernando de Noronha, Brazil	DQ239155	
<i>M. affinis</i>		unknown	AF153587	
<i>M. ozorri</i>	625	Annobon Island, West Africa	HM192747	
<i>M. multifasciata</i>		Lao PDR, Asia	DQ239138	

western Indian Ocean islands of Mayotte, Anjouan, Moheli and Grand Comoro (Comoros), Europa, Zanzibar and Pemba and the Seychelles, and a few locations on the Tanzanian coast (fig. 1). From the Genbank database (17-07-09) all the available sequences of *Mabuya* spp. currently attributed to the Afro-Malagasy clade (sensu Mausfeld et al., 2002; Bauer, 2003) except *M. vittata* (see Jesus, Harris and

Brehm, 2005 for a justification) were downloaded, amounting to 20 species, out of the ca. 70 that supposedly belong to this clade (assessed through the TIGR reptile database, Uetz, 2009, under *Trachylepis*).

From the tissues collected, genomic DNA from each individual was extracted using a standard high-salt (Sambrook, Fritsh and Maniatis, 1989) or phenol-chloroform

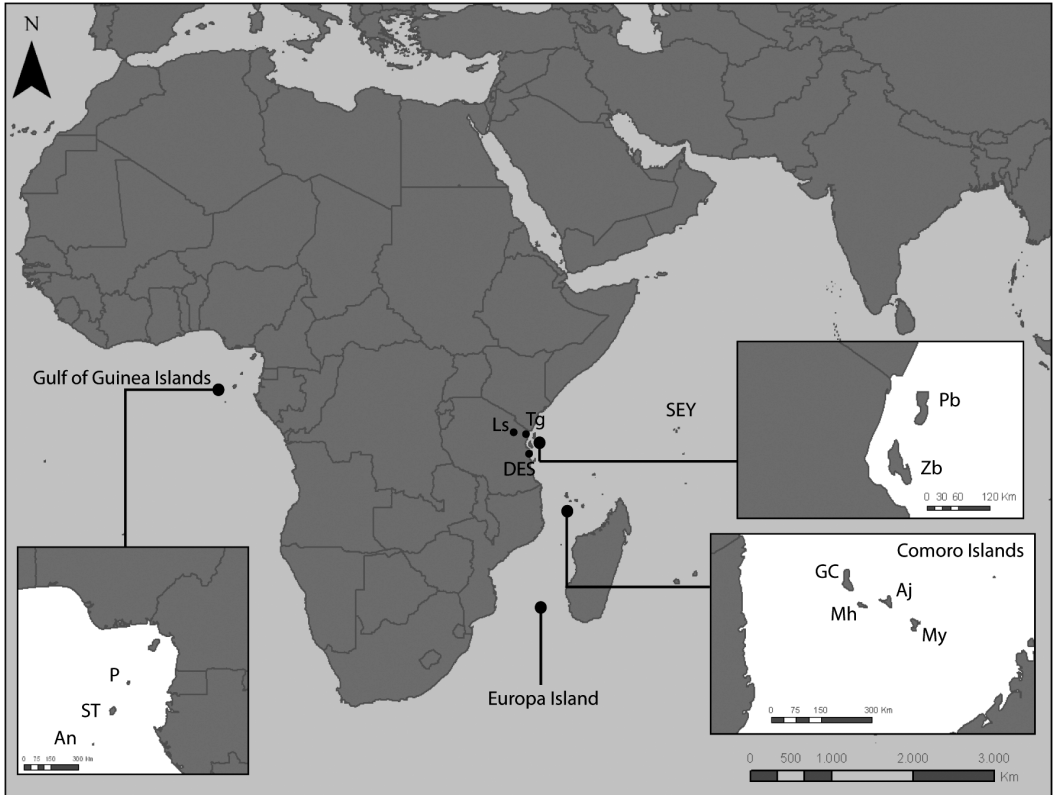


Figure 1. Map showing main sampling regions for *M. maculilabris* and *M. comorensis*. P: Príncipe; ST: São Tomé; An: Annobon (Gulf of Guinea Islands); Tg: Tanga; Ls: Lushoto; DES: Dar Es Salaam; Zb: Zanzibar; Pb: Pemba (Tanzania); GC: Grand Comoro; Mh: Moheli; Aj: Anjouan; My: Mayotte (Comoros); SEY: Seychelles. See table 1 for detailed locations.

(Taggart et al., 1992) protocol. A fragment with approximately 700 bp of the cytochrome *b* (*Cyt-b*) mitochondrial gene was amplified via PCR for most individuals with forward primer CB1 (5'-CCATCCAACATCTCAGCATGATGAAA-3'), modified from the original CBL14841 from Kocher et al. (1989), and reverse CB3H (Palumbi et al., 1991). For some individuals for which amplification was difficult, forward primers designed specifically for *M. sechellensis* and *M. wrightii* were used (CBMabF: 5'-TCTCAGCATGATGAAAYTTTGG-3') and CBMabPLF3 (5'-AGCATGATGAAATTTGGTCC-3') and successfully amplified an internal region of approximately 650 bp. PCR reaction conditions are given in Rocha et al. (2009), and sequences were determined by a commercial facility (Macrogen, Seoul, Korea). In the same way, 16s rRNA gene sequences were obtained for a subset of *M. comorensis* individuals (2 to 4 individuals from each island), using primers 16sA-L and 16sB-H (Palumbi et al., 1991) and standard conditions. Sequences have been deposited in GenBank under accession nos. HM192681-HM192755.

Cytochrome-*b* sequences obtained were aligned using BioEdit (Hall, 1999) with remaining Afro-Malagasy *Mabuya* spp. from Genbank (alignment was unambiguous) and with *M. multifasciata*, which was used as outgroup, as it is clearly phylogenetically distant from Afro-Malagasy

species, belonging instead to the Asian clade (Mausfeld et al., 2002). Only a subset of *M. comorensis* samples (2-3 individuals from each island) was used for the phylogenetic analysis and identical haplotypes from any species were removed, resulting in a 58 taxa (and 623 bp) dataset. The appropriate models of evolution were determined through jModeltest (Guindon and Gascuel, 2003; Posada, 2008), both for the complete dataset and for the 1st, 2nd and 3rd codon positions independently, using the AICc criteria (following Posada and Buckley, 2004), and were used to run both Maximum-Likelihood (ML) and Bayesian Analysis using respectively PhyML 3.0 (Guindon and Gascuel, 2003) and MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Support for ML analysis was assessed through 1000 bootstrap replicates also performed in PhyML. Bayesian analyses (BI) were run for 11 million generations and stationarity and convergence of independent runs were checked using AWTY (Nylander et al., 2008). Analyses were performed both considering a single model for the whole dataset or an independent model for each codon position (partitioned Bayesian analysis, sensu Brandley, Schmitz and Reeder, 2005) and Bayes Factor (BF) used to identify the most appropriate partitioning strategy. Substitution model parameters were always unlinked across partitions, while topology and branch lengths were linked across partitions.

Maximum-parsimony (MP) analysis was carried out through a heuristic search (10 replicates) using TBR branch-swapping algorithm and support for nodes estimated by 1000 bootstrap replicates.

Within *M. comorensis*, in order to visualize the diversity and relationships between the haplotypes within each island, statistical parsimony (Templeton, Crandall and Sing, 1992), implemented in the program TCSv1.2.1 (Clement, Posada and Crandall, 2000) was used and the 95% parsimony connection limit applied. The obtained 16s rRNA gene sequences of *M. comorensis* were used for comparison with the published sequence from a Malagasy individual. For this they were aligned in MAFFT ver.6 under the G-INS-I strategy (Kato et al., 2005) together with the published sequence of the Malagasy *M. comorensis* (Mausfeld et al., 2002) and of *M. m. casuarinae* (Carranza et al., 2001). Only Maximum-likelihood analysis was performed in this dataset, using the same methodology as above.

Results

The final dataset for phylogenetic analysis (cyt-*b*) consisted of 58 taxa and a 623 bp alignment, from which 293 sites were variable and 274 were parsimony informative. Judging from the Bayes factor ($2\ln BF = 722$, see Kass and Raftery, 1995) the tree obtained when allowing codon positions to have independent models was identified as the preferred inference (Bayesian) of phylogenetic relationships within this group. Maximum-likelihood and MP analysis recovered similar topologies (not shown) and support values were also largely concordant (fig. 2). Haplotype networks are represented in fig. 3.

M. comorensis is a monophyletic group, with specimens from Grand Comoro, Moheli and Mayotte/Anjouan defining also well-supported clades, harbouring considerable levels of diversity (figs 2 and 3). Anjouan and Mayotte populations of *M. comorensis* are not recovered as reciprocally monophyletic in any of the phylogenetic analyses, although it's clear from the haplotype network (fig. 3) that each island harbours an exclusive group of haplotypes, separated by a minimum of 6 mutational steps. The close relationship of *M. comorensis* with *M. maculilabris* is also confirmed, with *M. comorensis* making *M. maculilabris* paraphyletic. Both *M. maculilabris casuarinae* (Mozambique Island,

Mozambique) and *M. comorensis* from Madagascar (Nosy Tanikely) cluster within the Moheli clade of *M. comorensis* (for *M. comorensis* from Madagascar we compared the published 16s sequence with 16s sequences of Comoran specimens – fig. 2, right). Four additional lineages within *M. maculilabris* are evident from our analysis, with some of them harbouring considerable diversity. *Mabuya m. infralineata* from Europa Island clusters with a sample from coastal Mozambique (2.4% diff.) and both define a well-supported clade together with individuals from the island of Pemba, Tanzania. Individuals from the Gulf of Guinea islands of S. Tomé and Príncipe did not form a monophyletic group. The fourth *M. maculilabris* lineage observed comprised individuals from continental Tanzania and from the island of Zanzibar, with some variation observed between Northern (TZ16; TZ36) and southern (TZ10; TZ11) Tanzania and Zanzibar.

Species from the Seychelles Islands formed a monophyletic lineage but *M. sechellensis* revealed to be paraphyletic relative to *M. wrightii*. Their phylogenetic affinities are uncertain, as basal relationships within this whole clade, in general, are unresolved. This analysis further revealed some additional possible instances of paraphyly (involving *M. quinquetaeniata*; *M. margaritifera* and *M. striata* – fig. 2). Within *M. striata*, deep differentiation (and possible paraphyly) was found between a Western African sample (Namibia) and remaining specimens from Eastern Africa and Indian Ocean Islands (Tanzania, Zanzibar, Pemba and Anjouan). Little or no differentiation however was found within this later group (fig. 2). Contrary to most recent previous estimates (Whiting et al., 2006), Malagasy *Mabuya* spp. formed two well-supported but unrelated clades, both in ML, BI and MP analysis.

Discussion

Mabuya comorensis was found to be a highly diverse clade, with each of the Comoro islands

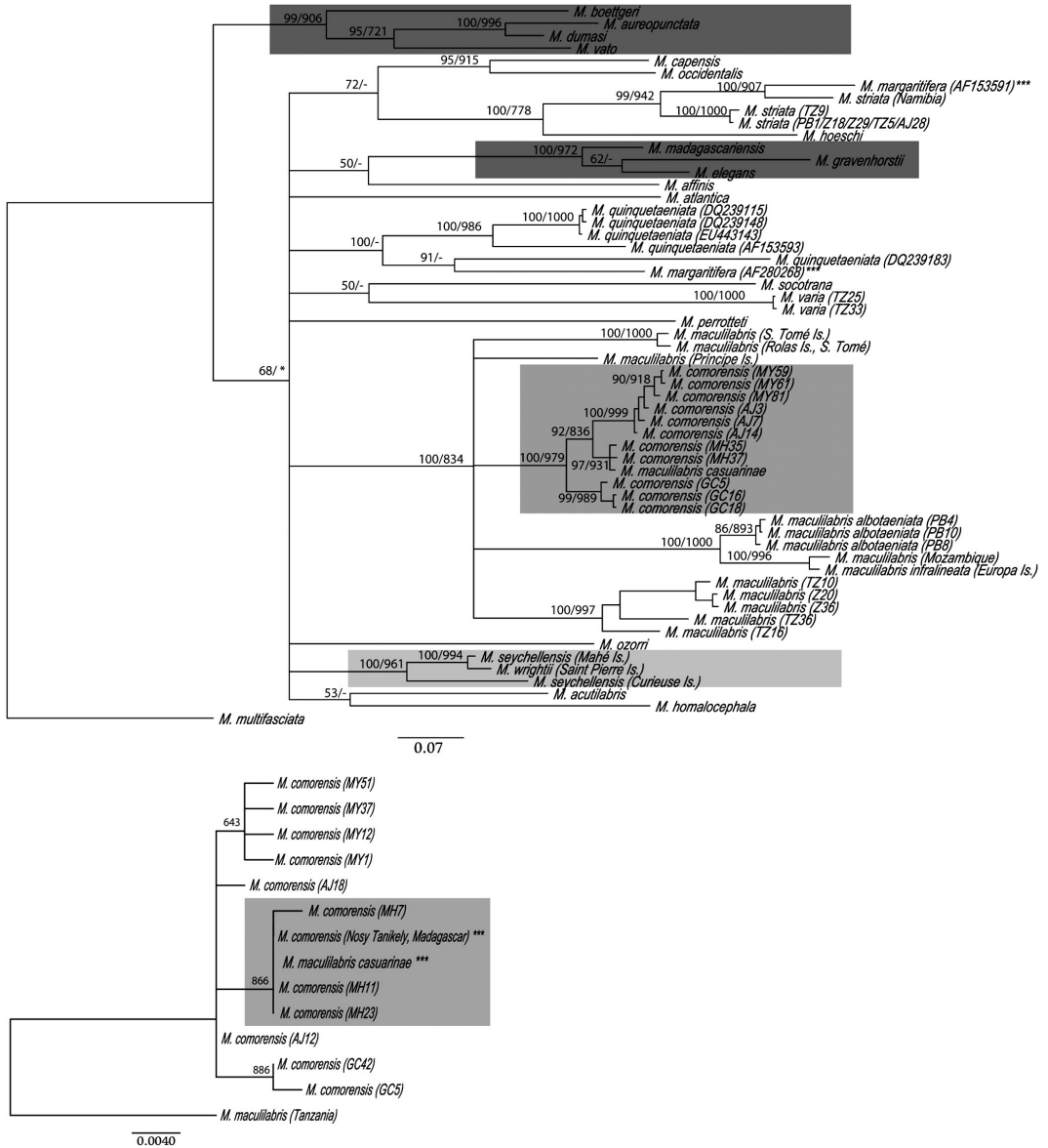


Figure 2. Bayesian 50% majority-rule consensus phylogenetic tree from partitioned analysis. Posterior probabilities (PP) and ML bootstrap (BS) values (PP/BS) are given above branches when higher than 0.5/500 (- represents BS values below 500 and an * signs nodes not recovered in the ML and MP analysis). MP bootstrap values above 500 are given below the branches (or in 3rd place). Relevant clades are highlighted: Malagasy groups (dark grey); *M. comorensis* and the Seychelles Islands clade (lighter greys). Individuals of *M. margaritifera* (paraphyletic) are signed with ***. On the right: ML tree of the 16s rRNA gene data for the subset of *M. comorensis* individuals. BS support values (higher than 500) are given above branches. Clade from Moheli is highlighted and individuals from Madagascar and *M. maculilabris casuarinae* signed with ***.

(Grand Comoro, Moheli, Anjouan and Mayotte) harbouring an exclusive haplotype lineage. Although further sampling is still needed to determine its putative sister group along the East African coast, the well supported tree topol-

ogy for the Comoros samples clearly suggests a colonisation from West to East within the archipelago. Differentiation between lineages is not particularly high (1% – between Anjouan and Mayotte – to 7.4% between Mayotte and

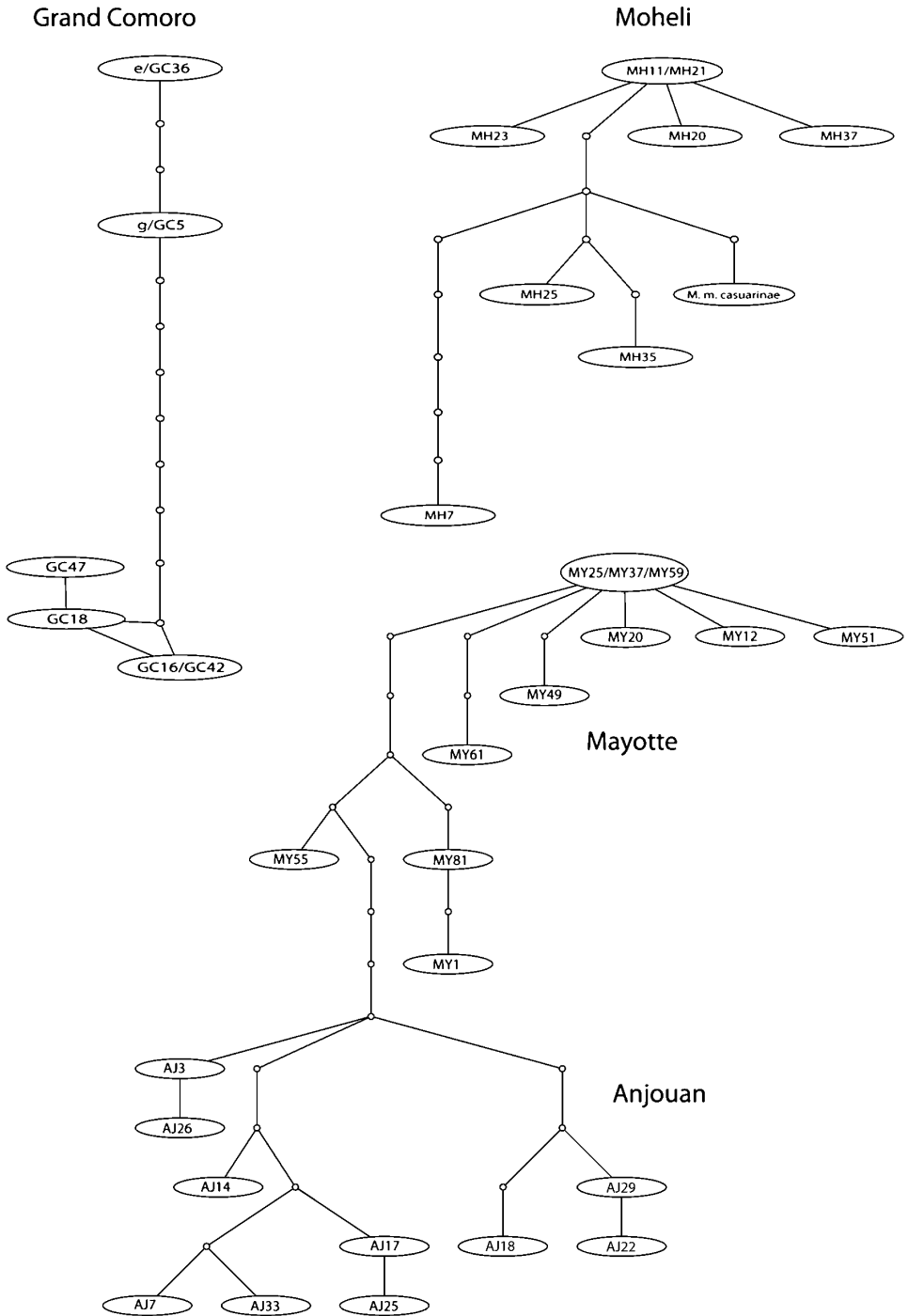


Figure 3. Haplotype networks representing variation within *M. comorensis*. Oval size is proportional to haplotype frequency (sample code is given within each and respective locations can be found on table 1). Each branch represents one substitution and each open circle a missing haplotype. Each group of haplotypes (3) is given as defined by the 95% parsimony connection limit.

Grand Comoro; number of base substitutions per site from net average between groups of sequences) when compared to the 13.5% average distance between species within genera reported by Harris (2002), but it is higher or at the same level of the one seen between many sister species of Cape Verde *Mabuya* for this same fragment (0.033 to 0.107, range of average net distances between species, from Carranza et al., 2001). Given their geographical isolation, it is probable that these populations do not have the opportunity to interbreed and that each mtDNA lineage defines an independent evolutionary unit. Though sampling is limited, the diversity within each *M. comorensis* lineage does not appear to be correlated with island age, as all islands seem to harbour similar levels of haplotype diversity (see haplotype networks), despite their significant age differences – Mayotte, 10–15 Myr; Anjouan, 11.5 Myr; Moheli, c. 5 Myr and Grand Comoro 0.5 Myr (Montaggioni and Nougier, 1981; Emerick and Duncan, 1982; Nougier et al., 1986). *Mabuya comorensis* from Madagascar clearly clusters within the Moheli Island clade, without differentiation relative to Moheli individuals, suggesting a very recent origin of this species in Madagascar probably due to human introduction. A similar situation occurs with *M. maculilabris casuarinae*. Broadley (1974) had already stated its similarity to *M. comorensis* when he described this subspecies (Mausfeld-Lafdhya et al., 2004) from Casuarina Island off the coast of North Mozambique. Using molecular data, Jesus, Harris and Brehm (2005) confirmed its lack of differentiation relative to *M. comorensis* (from Madagascar). Here, using the faster-evolving mtDNA marker cytochrome-*b* and a comprehensive sampling of *M. comorensis*, this “sub-species” is clearly placed within the Moheli island clade of *M. comorensis*, and, again, supports the hypothesis of a human-aided introduction, or at least very recent dispersal. This should definitely lead to a taxonomic reclassification of this “species” in order to better reflect its phylogenetic position.

More remarkably, *M. maculilabris* harbours multiple highly divergent lineages. In addition to the already described (Mausfeld-Lafdhya et al., 2004) eastern and western African clades and the multiple western African lineages (Jesus, Harris and Brehm, 2005), further cryptic lineages are present within East Africa. Individuals from Pemba Island, Tanzania (*M. maculilabris albotaeniata*), form a well-supported clade, closely related to *M. maculilabris infralineata* from Europa Island and a continental sample from Mozambique. Deeply divergent from these, another well-supported clade is defined by samples from Zanzibar and Tanzania, within which, furthermore, slight differentiation is observed between individuals from central (Dar Es Salaam) and northern (Tanga, Lushoto) Tanzania. Considering the high number of lineages observed and the limited range of this species sampled, this is certainly a species-complex that probably harbours yet more lineages within both continental East and West Africa. In particular, specimens assigned to the originally described *Euprepes anchietae*, *Euprepes notabilis* (Angola), *Mabuia Boulangeri* (Makonde Plateau, Tanzania), as well as to *Mabuia maculilabris major* and its several varieties from D.R. Congo, Tanzania and Uganda described by Sternfeld in 1912 (see Mausfeld-Lafdhya et al., 2004, appendix 2), were never to our knowledge included in a molecular phylogeny. The status of both western (Jesus, Harris and Brehm, 2005) and eastern (this study) insular populations is, on the contrary, now quite well known.

Mabuya striata demonstrated both similarities and differences from the phylogeographic patterns recovered in *M. maculilabris*. In common, there is also a deep East-West Africa divergence (in this case *M. striata* may even be paraphyletic), with samples from Tanzania and the Western Indian Ocean having a 12% *p*-distance from the individual from Namibia available on Genbank. Nevertheless, no differentiation was observed across the insular range of this species: individuals from the Co-

moros (Anjouan), Zanzibar, Pemba, and Tanzania shared the same or very closely related haplotypes, pointing to a very recent spread of this species across these Western Indian Ocean Islands.

Concerning the Seychelles species, *M. sechellensis* and *M. wrightii*, their monophyly (as a lineage) is still corroborated, but no evident close relationship to other species of this Afro-Malagasy clade was recovered. Contrary to a previous hypothesis (Peters 1882 in Gerlach, 2005), they do not appear to be closely related to *M. maculilabris*. The paraphyletic status of *M. sechellensis* is noteworthy and warrants further investigation on the intraspecific structure of both these species.

The inclusion of all available Afro-Malagasy clade sequences revealed two additional instances of paraphyly, both involving *M. margaritifera* with (1) *M. quinquetaeniata* and (2) *M. striata* that should be further investigated. They also support the (at least) two colonizations of Madagascar by this genus, with two well-supported clades defined by *M. mada-gascariensis*, *M. elegans* and *M. gravenhorsti* (1) and *M. boettgeri*, *M. vato*, *M. aureopunctata* and *M. dumasi* (2), this latter one recovered, in the Bayesian analysis, as basal to the entire Afro-Malagasy group (although with low PP). This result is not in agreement with the Malagasy monophyletic clade recovered by Whiting et al. (2006) using considerably more data (~5000 bp, of mitochondrial and nuclear genes), but we included, however, a higher number of African species in the analysis (17 contra 11). Considering that more than 70 species are estimated to belong to this clade, the question of the single or multiple colonization of Madagascar must still remain open pending a more comprehensive sampling effort.

The overall tree topology points to rapid and simultaneous divergences within this group and to the need of multiple independent markers to recover the order of the branching events within this apparently rapid radiation.

Acknowledgements. This research was partially supported by grants from Fundação para a Ciência e Tecnologia (FCT) SFRH/BD/1745/2004 (to SR), POCTI/BSE/46647/2000 and PTDC/BIA-BDE/65745/2006. We are grateful to Darren Peck for the sample from Europa Island and to José Jesus for providing unpublished sequences of *M. maculilabris* from Gulf of Guinea Islands. We further acknowledge Dr. Denis Matatiken and Wilna Accouche (at the Ministry of Environment and Natural Resources, Seychelles) and the Seychelles Bureau of Standards for the permit to obtain samples from the Seychelles.

References

- Bauer, A. (2003): On the identity of *Lacerta punctata* Linnaeus 1758, the type species of the genus *Euprepis* Wagner 1830, and the generic assignment of Afro-Malagasy skinks. *Afr. J. Herpetol.* **52**: 1-7.
- Boettger, O. (1913): Reptilien und Amphibien von Madagaskar, den Inseln und dem Festland Ostafrikas (Sammlung Voeltzkow 1889-1895 und 1903-1905). In: Reise in Ostafrika in den Jahren 1903-1905 mit Mitteln der Hermann und Elise geb. Heckmann Wentzel-Stiftung ausgeführt von Professor Dr. Alfred Voeltzkow, Band III. Systematische Arbeiten Heft IV, p. 269-375. Voeltzkow, A., Ed., Wissenschaftliche Ergebnisse, Stuttgart.
- Brandley, M.C., Schmitz, A., Reeder, T.W. (2005): Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. *Syst. Biol.* **54**: 373-390.
- Broadley, D.J. (1974): A review of the *Mabuya striata* complex in South-Eastern Africa (Sauria: Scincidae). Occasional Papers of the National Museums and Monuments of Rhodesia **B6**: 45-79.
- Brygoo, É.R. (1981): Systématique des Lézards Scincidés de la région malgache. VIII. Les *Mabuya* des îles de l'Océan Indien occidental: Comores, Europa, Séchelles. *Bulletin du Museum National d'Histoire Naturelle (Sér. 4)* **3**: 911-930.
- Carranza, S., Arnold, E.N., Mateo, J.A., Lopez-Jurado, L.F. (2001): Parallel gigantism and complex colonization patterns in the Cape Verde scincid lizards *Mabuya* and *Macrosцинus* (Reptilia: Scincidae) revealed by mitochondrial DNA sequences. *Proc. Biol. Sci.* **268**: 1595-1603.
- Carretero, M.A., Rocha, S., Harris, D.J. (2005): Recent observations of reptiles in the Comoro islands (Western Indian Ocean). *Herp. Bull.* **91**: 19-28.
- Clement, M., Posada, D., Crandall, K.A. (2000): TCS: A computer program to estimate gene genealogies. *Mol. Ecol.* **9**: 1657-1659.
- Emerick, C.M., Duncan, R.A. (1982): Age progressive volcanism in the Comores Archipelago, western Indian Ocean and implications for Somali plate tectonics. *Earth Planet Sci. Lett.* **60**: 415-428.
- Gerlach, J. (2005): Inter-island variation and taxonomy of Seychelles *Trachylepis*. *Afr. J. Herpetol.* **54**: 31-42.

- Gerlach, J. (2007): Terrestrial and Freshwater Vertebrates of the Seychelles Islands. Backhuys Publishers, Leiden, The Netherlands.
- Glaw, F., Vences, M. (2007): A Field Guide to the Amphibians and Reptiles of Madagascar, 3rd Edition. Vences & Glaw, Köln.
- Guindon, S., Gascuel, O. (2003): A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* **52**: 696-704.
- Hall, T.A. (1999): Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **41**: 95-98.
- Harris, D.J. (2002): Reassessment of comparative genetic distance in reptiles from the mitochondrial cytochrome b gene. *Herpetol. J.* **12**: 85-86.
- Huelsenbeck, J.P., Ronquist, F. (2001): MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754-755.
- Jesús, J., Harris, D.J., Brehm, A. (2005): Phylogeography of *Mabuya maculilabris* (Reptilia) from São Tomé island (gulf of Guinea) inferred from mtDNA sequences. *Mol. Phylogenet. Evol.* **37**: 503-510.
- Kass, R.E., Raftery, A.E. (1995): Bayes factors. *J. Amer. Statist. Assoc.* **90**: 773-795.
- Katoh, K., Kuma, K., Miyata, T., Toh, H. (2005): Improvement in the accuracy of multiple sequence alignment program MAFFT. *Genome Inform.* **16**: 22-33.
- Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C. (1989): Dynamics of mitochondrial-DNA evolution in animals – amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86**: 6196-6200.
- Loveridge, A. (1929): East African reptiles and amphibians in the United States National Museum. *Bull. United States Natl. Mus.* **151**: 1-129.
- Mausfeld, P., Vences, M., Schmitz, A., Veith, M. (2000): First data on the molecular phylogeography of scincid lizards of the genus *Mabuya*. *Mol. Phylogenet. Evol.* **17**: 11-14.
- Mausfeld, P., Schmitz, A., Böhme, W., Misofa, A., Vrcibradic, D., Rocha, C.D.F. (2002): Phylogenetic affinities of *Mabuya atlantica* Schmidt, 1945, endemic to the Atlantic Ocean archipelago of Fernando de Noronha (Brazil): Necessity of partitioning the genus *Mabuya* Fitzinger, 1826 (Scincidae: Lygosominae). *Zool. Anz.* **241**: 281-293.
- Mausfeld-Lafdhiya, P., Schmitz, A., Ineich, I., Chirio, L. (2004): Genetic variation in two African *Euprepis* species (Reptilia, Scincidae), based on maximum-likelihood and Bayesian analyses: Taxonomic and biogeographic conclusions. *Bonner Zoologische Beiträge* **52**: 159-177.
- Montaggioni, L., Nougier, J. (1981): Les enclaves de roches détritiques dans les volcans d'Anjouan (Archipel des Comores): origine et interprétation dans le cadre de l'évolution du canal de Mozambique. *Bull. Soc. Géol. France* **23**: 595-601.
- Nougier, J., Cantagrel, J.M., Karche, J.P. (1986): The Comores Archipelago in the western Indian Ocean: volcanology, geochronology, and geodynamic setting. *J. Afr. Earth Sci.* **5**: 135-145.
- Nylander, J.A., Wilgenbusch, J.C., Warren, D.L., Swofford, D.L. (2008): AWTY (are we there yet?): A system for graphical exploration of mcmc convergence in Bayesian phylogenetics. *Bioinformatics* **24**: 581-583.
- Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G. (1991): The simple fool's guide to pcr, version 2.0. Privately published, Hawaii.
- Peters, W.C.S. (1854): Diagnosen neuer Batrachier, welche zusammen mit der früher (24. Juli und 17. August) gegebenen Übersicht der Schlangen und Eidechsen mitgeteilt werden. Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königlich-Preussischen Akademie der Wissenschaften zu Berlin **1854**: 614-628.
- Peters, W.C.S. (1882): Zoologie III. Amphibien. Naturwissenschaftliche Reise nach Mossambique auf Befehl seiner Majestät des Königs Friedrich Wilhelm IV. In den Jahren 1842 bis 1848 ausgeführt von Wilhelm C.H. Peters. G. Reimer, Berlin.
- Posada, D. (2008): jModeltest: Phylogenetic model averaging. *Mol. Biol. Evol.* **25**: 1253-1256.
- Posada, D., Buckley, T.R. (2004): Model selection and model averaging in phylogenetics: Advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Syst. Biol.* **53**: 793-808.
- Rocha, S., Vences, M., Glaw, F., Posada, D., Harris, D.J. (2009): Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma*. *Mol. Phylogenet. Evol.* **52**: 530-537.
- Sambrook, J., Fritsch, E.F., Maniatis, T. (1989): Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, New York.
- Spawls, S., Howell, K., Drewes, R., Ashe, J. (2002): A Field Guide to the Reptiles of East Africa. Academic Press, San Diego.
- Sternfeld, R. (1912): Reptilia. In: Ergebnisse der Deutschen Zentral-Afrika-Expedition 1907-1908. Klindkhart & Biermann, Leipzig.
- Taggart, J.B., Hynes, R.A., Prodoul, P.A., Ferguson, A. (1992): A simplified protocol for routine total DNA isolation from salmonid fishes. *J. Fish Biol.* **40**: 963-965.
- Templeton, A.R., Crandall, K.A., Sing, C.F. (1992): A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619-633.
- Uetz, P. (2009): The reptile database. Available at <http://www.reptile-database.org/>. Accessed in August 2009.
- Whiting, A.S., Sites, J.W.S., Pellegrino, K.C.M., Rodrigues, M.T. (2006): Comparing alignment methods for inferring the history of the new world lizard genus *Mabuya* (Squamata: Scincidae). *Mol. Phylogenet. Evol.* **38**: 719-730.