



Deciphering patterns of transoceanic dispersal: the evolutionary origin and biogeography of coastal lizards (*Cryptoblepharus*) in the Western Indian Ocean region

Sara Rocha^{1,2}, Miguel A. Carretero¹, Miguel Vences³, Frank Glaw⁴ and D. James Harris^{1,2*}

¹Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO/UP), ICETA, Campus Agrário de Vairão, Vila do Conde, Portugal, ²Departamento de Zoologia-Antropologia, Faculdade de Ciências da Universidade do Porto, Porto, Portugal, ³Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, University of Amsterdam, Amsterdam, The Netherlands and ⁴Zoologische Staatssammlung, München, Germany

ABSTRACT

Aim *Cryptoblepharus* is a genus of small arboreal or rock-dwelling scincid lizards, widespread through the Indo-Pacific and Australian regions, with a disjunct outlier in the Malagasy region. The taxonomy within this genus is controversial, with different authors ranking the different forms (now some 36) at various levels, from different species to subspecies of a single species, *Cryptoblepharus boutonii*. We investigated the biogeography and genetic differentiation of the *Cryptoblepharus* from the Western Indian Ocean region, in order to understand their origin and history.

Location Western Indian Ocean region.

Methods We analysed sequences of mitochondrial DNA (partial 12s and 16s rRNA genes, 766 bp) from 48 specimens collected in Madagascar, Mauritius, the four Comoros islands and East Africa, and also in New Caledonia, representing the Australo-Pacific unit of the distribution.

Results Pairwise sequence divergences of *c.* 3.1% were found between the New Caledonian forms and the ones from the Western Indian Ocean. Two clades were identified in Madagascar, probably corresponding to the recognized forms *cognatus* and *voeltzkowi*, and two clades were identified in the Comoro islands, where each island population formed a distinct haplotype clade. The East African samples form a monophyletic unit, with some variation existing between Pemba, Zanzibar and continental Tanzania populations. Individuals from Mauritius form a divergent group, more related to populations from Moheli and Grand Comore (Comoros islands) than to the others.

Main conclusions The level of divergence between the populations from the Western Indian Ocean and Australian regions and the geographic coherence of the variation within the Western Indian Ocean group are concordant with the hypothesis of a colonization of this region by a natural transoceanic dispersal (from Australia or Indonesia). The group then may have diversified in Madagascar, from where it separately colonized the East African coast, the Comoros islands (twice), and Mauritius. The genetic divergence found is congruent with the known morphological variation, but its degree is much lower than typically seen between distinct species of reptiles.

Keywords

Africa, Comoros, *Cryptoblepharus*, island colonization, Madagascar, Mauritius, Scincidae, Squamata, transoceanic dispersal.

*Correspondence: D. James Harris, Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO/UP), ICETA, Campus Agrário de Vairão, 4485-661 Vila do Conde, Portugal.
E-mail: james@mail.icav.up.pt

INTRODUCTION

Cryptoblepharus is a pan-Pacific genus of *c.* 36 morphologically similar skinks, formerly considered as subspecies of a single variable species, *Cryptoblepharus boutonii* (Mertens, 1934; Greer, 1974). These lizards occur in two disjunct areas: (1) the eastern end of the Indo-Australian archipelago, Australia and Oceania; and (2) islands of the far Western Indian Ocean and adjacent parts of the African coast (Fig. 1a, adapted from Greer, 1974 and Branch, 1988).

Some attempts have been made to unveil the origin and biogeographical history of *Cryptoblepharus*. Mertens (1931) suggested that an ancestral form evolved in Southeast Asia and migrated to Australia, where the genus evolved and diversified, and, by passive means of dispersal, radiated to its present distribution in the Australian and Indo-Pacific regions. Greer (1974), analysing the intergeneric relationships of *Cryptoblepharus*, suggested that the most primitive *Cryptoblepharus* could be derived from *Emoia*, another genus with a wide distribution in the Pacific region and another obvious excellent transmarine disperser. Neither of these authors, however, offered any explanatory hypothesis for the disjunct Western Indian Ocean distribution. Biogeographical theory indicates three mechanisms by which *Cryptoblepharus* could have achieved its disjunct, widespread distribution: the evolution of an ancestral form at a time when the regions were connected; natural long-distance transoceanic dispersal over a long period of time; or recent human-mediated transportation. Judging from other studies of skinks (Carranza & Arnold, 2003), *Cryptoblepharus* is not sufficiently ancient for a Gondwanan origin, and the recognized morphological variation within the genus supports the pre-human occurrence of *Cryptoblepharus* across its distribution range. It is therefore likely that *Cryptoblepharus* has had a long period of evolution and has naturally dispersed to large and small islands, rafting on driftwood or vegetation mats, with some islands possibly being used as 'stepping stones' to colonize others, a pattern already identified in archipelagos

such as Hawaii (Gillespie, 2002, 2004) and the Canary islands (Thorpe *et al.*, 1994; Brown & Pestano, 1998). In fact, many natural attributes of *Cryptoblepharus*, such as low metabolic requirements, ectothermy, frequent occurrence on marine beaches and adaptation to habitats devoid of fresh water could facilitate the crossing of open seas (e.g. Fricke, 1970). These oceanic dispersals could have been as extensive as the *c.* 6000 km that separates the Indo-Australian and the Western Indian Ocean regions, and may explain the colonization of Madagascar, East Africa and the surrounding islands by *Cryptoblepharus*. In fact, recent evidence has led to a resurrection of the dispersal hypothesis in historical biogeography, indicating that such dispersal events may have been more common than previously thought by vicariance biogeographers (de Queiroz, 2005).

In the last extensive review of this genus in the Western Indian Ocean, Brygoo (1986) recognized 13 forms, with allopatric distributions (Fig. 1b). With the exception of elevating the Europa island form, *C. b. bitaeniata*, to a specific status, and proposing the treatment of *C. b. mayottensis* (from Mayotte, Comoros) and *C. b. mohelicus* (from Moheli, Comoros) as varieties of the subspecies *C. b. gloriosus* (from Glorioso island), he maintained the subspecific treatment given to the forms by previous authors. P. Horner (unpublished data), in a multivariate analysis of morphological data, identified 13 *Cryptoblepharus* taxa from the Western Indian Ocean region, 12 of which were distinguished by two or more statistically significant morphological differences. He considered all 13 taxa to be separate 'species' (P. Horner, pers. comm.).

The complexity in assigning a taxonomic status to these forms comes from their long-appreciated distinctive colour patterns together with their distribution: there is a good deal of variation between the forms (Mertens, 1931), but since most of them occur on separate islands, their true status is beyond the test of sympatry (Greer, 1974). Thus it is unclear if there exists one polytypic species or several distinct species, a problem that can now be evaluated using a molecular approach.

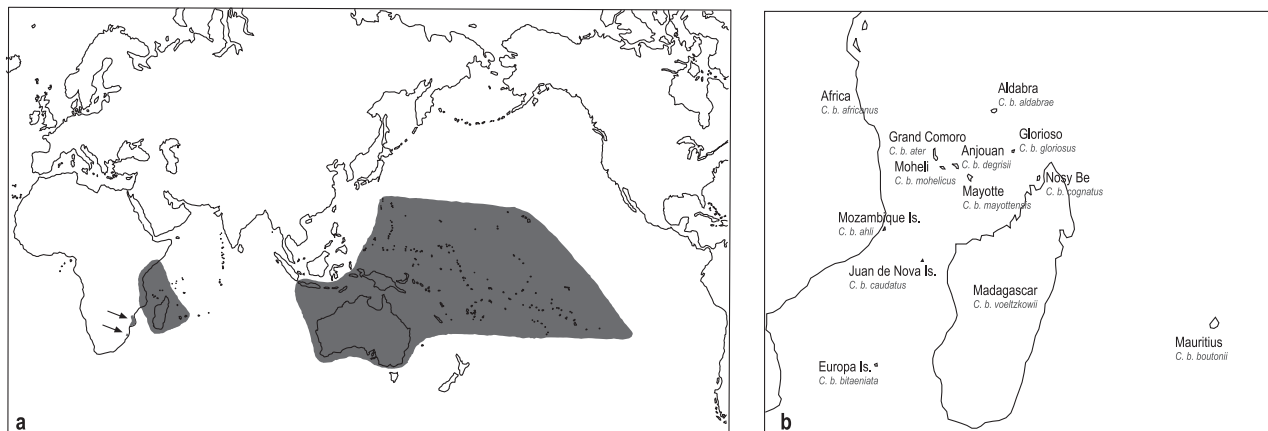


Figure 1 (a) Distribution of the genus *Cryptoblepharus* (adapted from Greer, 1974 and Branch, 1988). (b) Distribution of the Western Indian Ocean subspecies (from Brygoo, 1986).

Here we examine *Cryptoblepharus* from the Western Indian Ocean region, including eight recognized forms from Madagascar (two), the Comoros archipelago (four), East Africa (one), and Mauritius Island (one). *Cryptoblepharus novocaledonicus*, from New Caledonia, was also included in the analysis. On the basis of mitochondrial DNA sequences we elucidate the phylogenetic relationships among these forms and address possible historical vicariant and dispersal patterns that may have shaped their current distribution.

MATERIALS AND METHODS

Tissue samples (tail tips) were collected in various localities (see Table 1 and Fig. 2) across Madagascar, Mauritius, the four major islands of the Comoros archipelago, the East African coast (mainland Tanzania, Zanzibar and Pemba islands) and New Caledonia and preserved in 98% ethanol. Total genomic DNA was extracted using standard high-salt protocols (Sambrook *et al.*, 1989), and fragments of the 12s and 16s rRNA genes were amplified using universal primers (12Sa and 12Sb from Kocher *et al.*, 1989 and 16Sar-L and 16Sbr-H from Palumbi *et al.*, 1991) and following Harris *et al.* (1998). The PCR products were sequenced in an automated DNA sequencer (ABI PRISM 310) following the manufacturer's instructions. GenBank accession numbers for new sequences are DQ118039–DQ118080. For one individual from Madagascar, sequences of both genes were already available on GenBank (Schmitz *et al.*, 2005). Sequences were aligned manually using BIOEDIT (Hall, 1999), and genes were combined, resulting in a 766-bp fragment. Within the *Cryptoblepharus* group the alignment was unambiguous as only single indels were included. The Lygosominae skinks *Leiopisma telfairi* (Carranza & Arnold, 2003) and *Emoia cyanura* (Whiting *et al.*, 2003) were included as outgroups. A short hypervariable region (15 bp) was removed for the analysis with these outgroups.

Maximum likelihood (ML), maximum parsimony (MP) and Bayesian analyses were performed. For the ML and Bayesian analysis, the model of nucleotide substitution that best fits our data set was selected using Modeltest 3.06 PPC (Posada & Crandall, 1998), under the Akaike information criterion (following Posada & Buckley, 2004). For the combined data set, the best-fitting model was the General Time Reversible (GTR + I + G), with base frequencies and substitution rates estimated from the data, a proportion of invariable sites of 0.6713, and a gamma distribution shape parameter of 0.7343. The software PHYLML (available at <http://www.lirmm.fr/w3ifa/MAAS/>), which implements an algorithm that adjusts tree topology and branch lengths simultaneously (Guindon & Gascuel, 2003), departing from an initial tree constructed using BIONJ (Gascuel, 1997), was used to perform the ML analysis, under the chosen model, with parameters estimated and optimized along the analysis and 1000 bootstraps. Bayesian analysis was performed with MrBayes v.3.0 (Huelsenbeck & Ronquist, 2001), using both the combined data, and

data partition by gene, applying an appropriate model for each gene (also selected using Modeltest and the Akaike information criterion). These were the TVM + G for 12s and GTR + I for 16s.

The application of these models resulted in a distorted tree topology, however, probably as a result of the high distance of the outgroups compared with all *Cryptoblepharus*. The estimated proportion of invariable sites was very low (67% for the combined fragment, and 80% for the 16s only), causing the estimate of topology to be incorrectly rooted (see results). Thus, we applied the model to both combined and partitioned-by-gene Bayesian analysis, excluding the proportion of invariable sites (*I*) parameter. Parameters were estimated as part of the analysis with four Markov chains incrementally heated with the default heating values. All analyses started with randomly generated trees and ran for 2×10^6 generations, saving one tree in every 100 generations. The log-likelihood values of the sample points were plotted against the generation time and all the trees prior to reaching stationarity were discarded, making sure that burn-in samples were not retained. Combining the remaining trees, a 50% majority rule consensus tree was generated. The frequency of any particular clade of the consensus tree represents the posterior probability of that clade (Huelsenbeck & Ronquist, 2001). Maximum parsimony (MP) analysis was also carried out, in PAUP* 4.0b10 (Swofford, 2002), using heuristic searches involving tree bisection and reconnection (TBR) branch swapping, with 100 replicates. Gaps were considered as a fifth state and all characters were weighted equally. Robustness of these trees was assessed by bootstrap analysis (Felsenstein, 1985) involving 1000 pseudo-replications.

Because the level of divergence within the *Cryptoblepharus* sequences was low, a median-joining network (Bandelt *et al.*, 1999) was constructed using NETWORK software (Fluxus Engineering, Suffolk, UK) for these sequences only. Networks of interconnected haplotypes represent the evolutionary relationships and gene genealogies within species better than the bifurcating patterns usually recovered by methods of phylogenetic inference (Posada & Crandall, 2001).

RESULTS

We obtained a total of 48 *Cryptoblepharus* sequences for both the 12s and 16s gene fragments. Maximum parsimony analysis recovered 16 equally most parsimonious trees (180 steps; consistency index 0.872; retention index 0.910). The strict consensus tree of the MP tree topologies was identical to the ML tree, although less resolved. In the Bayesian analysis of data, both considering one model for the combined fragments (GTR + G + I) and one independent model for each gene (TVM + G for 12s and GTR + I for 16s), the tree topology was clearly distorted, with the outgroup rooting the tree with haplotypes from Grand Comoro, an estimate not concordant with previous analysis nor with the network topology (Fig. 2). When the estimate of invariable sites was removed, the resulting Bayesian analysis obtained a tree topology

Table 1 List of samples used for analysis, geographic locations, and GenBank accession numbers for 12s and 16s

Species	Specimen	Locality	Island; country	Accession numbers
<i>C. b. cognatus</i>	2000/MB11	Ambolobozaleki, south Diego	Madagascar	DQ118039/DQ118060
<i>C. b. cognatus</i>	2000/MB56	Nosy Fanihy	Madagascar	DQ118040/DQ118061
<i>C. b. cognatus</i>	2000/MB32	Nosy Fanihy	Madagascar	DQ118040/DQ118061
<i>C. b. cognatus</i>	2000/MC5	Nosy Fanihy	Madagascar	DQ118040/DQ118061
<i>C. b. cognatus</i>	2000/MC9	Nosy Sakatia	Madagascar	DQ118041/DQ118062
<i>C. b. cognatus</i>	2000/MB61	Nosy Sakatia	Madagascar	DQ118041/DQ118062
<i>C. b. cognatus</i>	2000/MB43	Nosy Be	Madagascar	DQ118042/DQ118063
<i>C. b. voeltzkowi</i>	2000/M520	Ifaty, SW Madagascar	Madagascar	DQ118052/DQ118073
<i>C. b. voeltzkowi</i>	FGZC78	Lebanona Beach, Tolagnaro	Madagascar	DQ118053/DQ118074
<i>C. b. voeltzkowi</i>	FGZC79	Lebanona Beach, Tolagnaro	Madagascar	DQ118053/DQ118074
<i>C. b. voeltzkowi</i>	FGZC80	Lebanona Beach, Tolagnaro	Madagascar	DQ118053/DQ118074
<i>C. b. voeltzkowi</i>	FGZC84	Lebanona Beach, Tolagnaro	Madagascar	DQ118053/DQ118074
<i>C. b. voeltzkowi</i>	FGZC87	Lebanona Beach, Tolagnaro	Madagascar	DQ118053/DQ118074
<i>C. b. voeltzkowi</i>	CbvE25	St. Augustin, near Ianantsony	Madagascar	AY308336/AY308219
<i>C. b. mayottensis</i>	MY68	Mamoutzou, harbour	Mayotte; Comoros	DQ118043/DQ118064
<i>C. b. mayottensis</i>	MY69	Mamoutzou, harbour	Mayotte; Comoros	DQ118042/DQ118063
<i>C. b. mayottensis</i>	MY70	Mamoutzou, harbour	Mayotte; Comoros	DQ118044/DQ118065
<i>C. b. degrisii</i>	AJ4	Moutsamoudu	Anjouan; Comoros	DQ118047/DQ118068
<i>C. b. degrisii</i>	AJ6	Moutsamoudu	Anjouan; Comoros	DQ118046/DQ118067
<i>C. b. degrisii</i>	AJ20	Moya	Anjouan; Comoros	DQ118045/DQ118066
<i>C. b. degrisii</i>	AJ21	Moya	Anjouan; Comoros	DQ118045/DQ118066
<i>C. b. mohelicus</i>	MH16	Fomboni, harbour	Moheli; Comoros	DQ118054/DQ118075
<i>C. b. mohelicus</i>	MH17	Fomboni, harbour	Moheli; Comoros	DQ118054/DQ118075
<i>C. b. mohelicus</i>	MH18	Djayézi	Moheli; Comoros	DQ118054/DQ118075
<i>C. b. ater</i>	GC59	Moroni, harbour	Grand Comore; Comoros	DQ118056/DQ118077
<i>C. b. ater</i>	GC60	Moroni, harbour	Grand Comore; Comoros	DQ118056/DQ118077
<i>C. b. ater</i>	GC61	Moroni, harbour	Grand Comore; Comoros	DQ118056/DQ118077
<i>C. b. ater</i>	GCh	Gouni, Sandini	Grand Comore; Comoros	DQ118056/DQ118077
<i>C. b. ater</i>	GCI	Gouni, Sandini	Grand Comore; Comoros	DQ118056/DQ118077
<i>C. b. ater</i>	F893	Chomoni beach	Grand Comore; Comoros	DQ118055/DQ118076
<i>C. b. boutonii</i>	Maur	Gabriel island	Mauritius, Mascarenes	AF280116/AY151445
<i>C. b. boutonii</i>	Ma623	Ille de la Passe	Mauritius, Mascarenes	DQ118057/DQ118078
<i>C. b. boutonii</i>	Ma624	Ille de la Passe	Mauritius, Mascarenes	DQ118057/DQ118078
<i>C. b. africanus</i>	Z6	Stone town,	W Zanzibar, Tanzania	DQ118050/DQ118071
<i>C. b. africanus</i>	Z7	Stone town	W Zanzibar, Tanzania	DQ118050/DQ118071
<i>C. b. africanus</i>	Z8	Stone town	W Zanzibar, Tanzania	DQ118050/DQ118071
<i>C. b. africanus</i>	Z9	Stone town	W Zanzibar, Tanzania	DQ118050/DQ118071
<i>C. b. africanus</i>	Z32	Kiwengwa	E Zanzibar, Tanzania	DQ118051/DQ118072
<i>C. b. africanus</i>	Z45	Mena Bay	SW Zanzibar, Tanzania	DQ118051/DQ118072
<i>C. b. africanus</i>	Z46	Mena Bay	SW Zanzibar, Tanzania	DQ118051/DQ118072
<i>C. b. africanus</i>	PB13	East Chake	Pemba, Tanzania	DQ118048/DQ118069
<i>C. b. africanus</i>	PB14	East Chake	Pemba, Tanzania	DQ118048/DQ118069
<i>C. b. africanus</i>	PB16	Jondeni	SW Pemba, Tanzania	DQ118049/DQ118070
<i>C. b. africanus</i>	PB28	Ngezi	N Pemba, Tanzania	DQ118049/DQ118070
<i>C. b. africanus</i>	TZ46	DarEsSallam, Msanani penins.	continental Tanzania	DQ118050/DQ118071
<i>C. b. africanus</i>	TZ47	DarEsSallam, Msanani penins.	continental Tanzania	DQ118050/DQ118071
<i>C. novocaledonicus</i>	AMB7210	Isle of Pines	New Caledonia	DQ118059/DQ118080
<i>C. novocaledonicus</i>	AMB8050	Loyalty island	New Caledonia	DQ118058/DQ118079
<i>Leiopisma telfairi</i>		Round Island	Mauritius	AF280122/AY151450
<i>Emoia cyanura</i>		Vitilevu, Sigacota	Fiji	AY218018/AY217968

concordant with the previous ones. The ML tree, with ML and MP bootstrap values and Bayesian posterior probabilities of data partitioned analysis, is represented in Fig. 3.

Cryptoblepharus from the Western Indian Ocean region form a monophyletic unit with c. 3.1% pairwise divergence

(average between group uncorrected *P*-distance) in relation to *C. novocaledonicus* (more than 20 mutational steps). Among taxa from the Western Indian Ocean region, pairwise sequence divergences ranged from 0% to 2.4%. A detailed analysis of the distribution of the haplotypes within

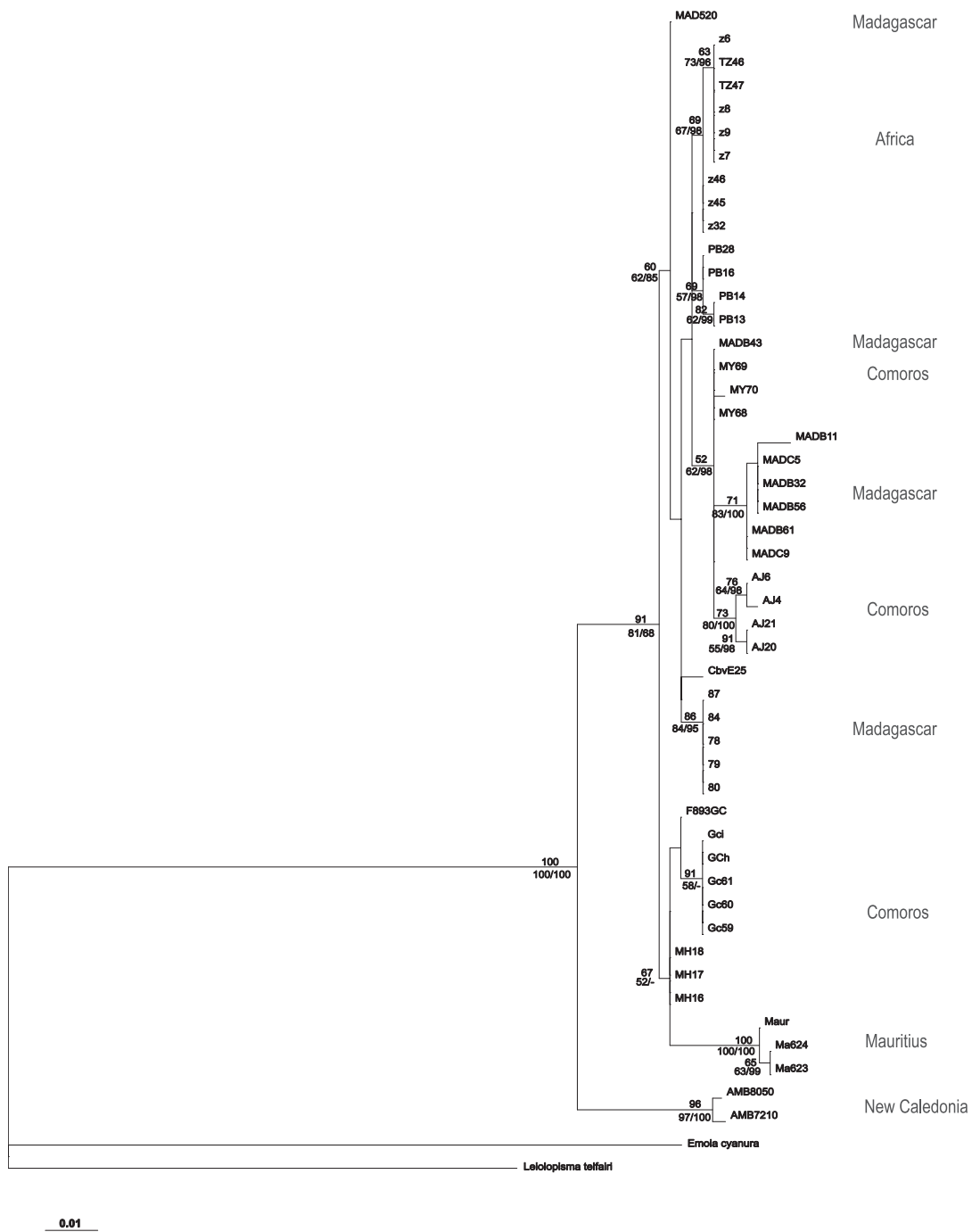


Figure 3 Maximum likelihood phylogram of all the *Cryptoblepharus* individuals sequenced for 12s and 16s rRNA. *Emoia cyanura* and *Leiopisma telfairi* are used as outgroups. Individual codes are as used in Table 1 and Fig. 2. Bootstrap values for MP and ML and Bayesian posterior probabilities (PP) above 50% are shown (in percentage: ML, MP/PP).

ongoing or past hybridization between the two forms is a matter for future investigation.

Despite the few individuals analysed, more haplotypes were detected within Anjouan and Mayotte (three in each island) than in Moheli and Grand Comore (one and two, respectively). This is congruent with the older age of the first two islands. In fact, these hotspot-originated volcanic islands span a wide range of ages: Mayotte, 10–15 Myr;

Anjouan, 11.5 Myr; Moheli, *c.* 5 Myr; and Grand Comore 0.5 Myr – age of the oldest exposed lavas in the case of Moheli and estimated age for the volcanic origin of the other three islands (Montaggioni & Nougier, 1981; Emerick & Duncan, 1982; Nougier *et al.*, 1986). The haplotypes from Mauritius are closest to those from Moheli and Grand Comore, but have a relevant genetic differentiation of 10 mutational steps.

Concerning the east African populations, some variation was observed: two closely related haplotypes were detected in individuals from Zanzibar and mainland Tanzania (with sharing of haplotypes between west of Zanzibar and mainland Tanzania), and two other slightly divergent ones were detected in Pemba populations (the other small offshore island). All these east African haplotypes form a monophyletic unit derived from the southern Malagasy haplotypes.

DISCUSSION

Origin of *Cryptoblepharus* in the Western Indian Ocean

As stated in the Introduction, three mechanisms can be invoked to explain the origin of *Cryptoblepharus* in the Western Indian Ocean region: (1) ancient vicariance, (2) transoceanic dispersal, and (3) translocation by humans. Our data provide novel information to assess these hypotheses. If vicariance was the origin of the disjoint *Cryptoblepharus* distribution, the separation of the main *Cryptoblepharus* populations would be related to the break-up of Gondwana in Mesozoic times. Much higher genetic divergences than the 3.1% pairwise divergences would be expected between *C. novocaledonicus* and the Western Indian Ocean forms under such a scenario. If human-mediated transportation were responsible for the presence of *Cryptoblepharus* in the Western Indian Ocean region, no geographically structured genetic variation at these mitochondrial markers would be expected, as in the case of some *Hemidactylus* species from this same region (Vences *et al.*, 2004b; Rocha *et al.*, 2005), or in introduced Polynesian lizards (Austin, 1999). Hence, our data strongly support an origin of *Cryptoblepharus* in the Western Indian Ocean region by natural transoceanic dispersal. Because no close relatives of *Cryptoblepharus* occur in the Western Indian Ocean, we assume that the direction of dispersal was from the Indo-Pacific region towards Madagascar.

Despite the lack of Australian and Indonesian taxa in this study, given the relatively low degree of variation among populations from the Western Indian Ocean and the divergence observed between these and the New Caledonian individuals, the most parsimonious hypothesis is to assume only one colonization event to the Western Indian Ocean, probably to Madagascar with subsequent dispersal to surrounding islands. Furthermore, the hypothesis of two colonization events, by individuals belonging to very closely related lineages, is much less likely.

The data therefore suggest a geologically recent long-distance overwater dispersal followed by several minor dispersal events. Variation between the two major Malagasy haplotype clades is at least 1.82%. Assuming a rRNA evolution rate of 0.625% per Myr since the last common ancestor (Lin *et al.*, 2002; Podnar *et al.*, 2005), and in the case that the differentiation among these clades took place in Madagascar, this would indicate a colonization of Madagascar at least 1.5 Ma (but possibly during the Pleistocene).

A similar pattern is suggested for several other taxa, such as the gekkonids *Nactus* and *Lepidodactylus* and the *Leiopisma* skinks (Austin *et al.*, 2004), which arose in Southeast Asia and reached the Mascarenes archipelago via the west-running Equatorial current. Several other long-distance transoceanic dispersals are known in reptiles: the ancestor of *Phelsuma andamanensis* colonized the Andaman and Nicobar islands from Madagascar, 6000 km away (Austin *et al.*, 2004); *Tarentola (Neotarentola) americana* reached Cuba from West Africa via the North Equatorial current, in a journey of at least 6000 km; and skinks from the genus *Mabuya* invaded tropical America from Africa twice, reaching the American mainland and the oceanic island of Fernando de Noronha independently, in two journeys of at least 3000 km (Carranza *et al.*, 2000; Mausfeld *et al.*, 2002; Carranza & Arnold, 2003). Thus, extremely long-distance colonization events in these reptile groups may be less rare than previously thought, and *Cryptoblepharus* provides another example. Some lizard groups appear to be much more prone to transmarine journeys than others, and geckos and skinks are much better island colonizers than agamids, lacertids and teids (Carranza & Arnold, 2003). As recently stressed (de Queiroz, 2005), the importance of oceanic dispersal in biogeography has been strongly underestimated, and the overall role of oceanic dispersal for continental landmasses, such as Madagascar, is considerable. Molecular dating of lineage divergences favours oceanic dispersal over tectonic vicariance as an explanation for disjunct distributions of a wide variety of taxa, including carnivores, lemurs, monkeys, squamate reptiles, frogs, flightless insects, and many others.

Phylogeography of Malagasy, Mascarene, Comoroan and East African *Cryptoblepharus*

The colonization of Madagascar seems to have been followed by rapid diversification, giving origin to at least two groups of *Cryptoblepharus* in Madagascar, one present in the extreme north and north-west offshore islets, namely *C. b. cognatus*, and the other in more central and southern territories, namely *C. b. voeltzkowi*. These two groups have been recognized by morphological characteristics (Mertens, 1934; Greer, 1974; Brygoo, 1986; Andreone & Greer, 2002; P. Horner, pers. comm.), and are also shown to be distinct in this study using molecular data, but their detailed geographical distributions and within-group genetic diversity remain undetermined. Thus, it is not yet possible to give any biogeographical scenario for these divergent forms.

Interestingly, this pattern of divergent lineages in northern Madagascar is also seen in other unrelated herpetofauna such as the geckos *Hemidactylus* and the frog *Ptychadena* (Vences *et al.*, 2004a,b) and may well represent a real biogeographical pattern inside Madagascar. Moreover, the northern region where these lineages are found is one of the 'over-prediction' areas identified by Raxworthy *et al.* (2003) in a study of ecological niche distribution modelling for reptile species in Madagascar, and is considered as one of the potential unrecognized areas of endemism in the island.

From Madagascar, *Cryptoblepharus* may have dispersed overseas, colonizing the Comoros at least twice, Africa and Mauritius, although the basal position of the Mauritian haplotypes in the tree (Fig. 3) indicates a need for further analysis. The Comoroan forms from Anjouan (*C. b. degrisii*) and Mayotte (*C. b. mayottensis*) are clearly derived from north-western Madagascar haplotypes, possibly in a stepwise fashion, with Malagasy individuals colonizing Mayotte and originating *mayottensis*, and this form later colonizing Anjouan and originating the *degrisii* form. The east African populations analysed, in which a considerable amount of variation is already present, are clearly derived from southern Madagascan populations, despite their being geographically closer to north Madagascar. Two distinct genetic lineages were found: one in Pemba Island, and the other in Zanzibar Island and mainland Tanzania. Despite the degree of genetic variation observed within this group, which is higher than that between some of the Comoron forms recognized as distinct, all the African populations studied form a monophyletic unit and are morphologically similar (in the dorsal colouration patterns). Nonetheless, *Cryptoblepharus* has a patchy distribution along the East African coast, from Somalia to Black Rock in KwaZulu-Natal, South Africa, possibly occurring in several isolated populations along the coast (Branch, 1988). Interestingly, a separate form, *C. b. ahli*, from the Island of Mozambique (a continental island close to the coast of mainland Mozambique) was described by Mertens (1928). This form was later synonymized with *C. b. africanus* by Brygoo (1986), but recently recognized again as a distinct form on a morphological basis (P. Horner, pers. comm.). Thus, the variation within *Cryptoblepharus* along the east African coast still warrants further investigation.

The origin of the forms from Grand Comore and Moheli, *C. b. ater* and *C. b. mohelicus*, is still uncertain. Considering the position of their respective haplotypes in the network, Grand Comore was probably colonized from Moheli, and the two forms seem to share a common ancestor with the Mauritian *C. b. boutoni* subspecies, but their exact relationship with the Malagasy forms is still unclear. The genetic differentiation between the Grand Comore and Moheli forms is minimal: only one mutation separates these two forms, and yet two mutations exist between the two haplotypes detected in individuals from Grand Comore. One of the most interesting aspects of *Cryptoblepharus* is the difference between the morphological (chromatic) and molecular data: considerable amounts of genetic variation are observed inside some morphologically uniform forms, while in other cases distinct chromatic forms exhibit the same (in the case of Mayotte and Nosy Be) or very closely related (in the case of Moheli and Grand Comore) haplotypes. If the short genetic distances reflect recent or ongoing 'speciation' events, what drives this quick morphological change between the forms/subspecies? Obviously, phenotypic differentiation could arise solely as the result of the action of random microevolutionary processes of genetic drift and mutation (Wright, 1931), but we may also be observing a system in which other selective forces are acting.

For the *ater* form, from Grand Comore, for example, some authors (Meirte, 2004) have argued that its melanic colouration could be somehow linked with the dark colour of the soil, which mostly comprises volcanic lavas. However, all the forms from the Comoros were found on similar-coloured coastal rags or artificial harbours (DJH and MAC, pers. obs.). In Madagascar, the southern populations have a lighter dorsal colouration than the northern forms, and they partly occur on lighter substrate (MV and FG, pers. obs.). Traits that have antipredator functions, such as blue tails, are more effective if all the individuals in a population display the same pattern (Margalef, 1974). The same may apply to traits used for social communication. Thus there may be strong selection on populations on recently colonized islands to converge quickly to the same pattern.

Despite the chromatic distinctiveness of all the forms, the low genetic distances observed within and between them lead us to propose that maintaining the subspecific status of all the forms seems to be, so far, the most appropriate taxonomic ranking for these Western Indian Ocean forms. For the 12s and 16s rRNA gene regions, values of intraspecific variation of 4.4% (*Chalcides sexlineatus*) and 2.3% (*Chalcides viridanus*) are known from other island skinks (Brown & Pestano, 1998), and for the 16s region only, in the skinks of the genus *Mabuya sensu lato*, sister species such as *M. spilogaster*/*M. striata* and *M. capensis*/*M. occidentalis* (all in the genus *Trachylepis* if the partitioning of *Mabuya* into separate genera is accepted; see Mausfeld *et al.*, 2002; Bauer, 2003; but see also Jesus *et al.*, 2005) present levels of divergence of 3.7–4.3% and 4.1%, respectively (Mausfeld *et al.*, 2000).

In addition to the subspecies *C. b. ahli*, the subspecies *C. b. aldabrae* (Aldabra island), *C. b. bitaeniatus* (Europa island), *C. b. caudatus* (Juan de Nova island) and *C. b. gloriosus* (Glorioso island) were not included in this analysis. Molecular data on these taxa are crucial for any further assessment of their taxonomic status.

Besides questions of classification, one of the important findings of this work is the low or complete lack of genetic variation within some groups. Although within-island sampling is reduced to a few samples, the samples are geographically dispersed and should reflect the existent variation. Island populations are more prone to extinction than mainland populations (Frankham, 1998), and one of the main causes of this 'extinction proneness' is inbreeding depression. Populations of *Cryptoblepharus* from Reunion in the Mascarenes are thought to have gone extinct recently (Probst, 2002). In the light of these new results, these insular '*Cryptoblepharus*' populations should be carefully considered from a conservation standpoint.

ACKNOWLEDGEMENTS

We especially thank Paul Horner and Allen Greer for valuable comments and providing information and to A. Bauer for the outgroup material. This study was supported by project POCTI/BSE/46647/2002 and grants SFRH/BPD/3596/2000

(to MAC) and SFRH/BPD/5702/2001 (to DJH), all from Fundação para a Ciência e Tecnologia (Portugal).

REFERENCES

- Andreone, F. & Greer, A. (2002) Malagasy scincid lizards: descriptions of nine new species, with notes on the morphology, reproduction and taxonomy of some previously described species (Reptilia, Squamata, Scincidae). *Journal of Zoology*, **258**, 139–181.
- Austin, C.C. (1999) Lizards took express train to Polynesia. *Nature*, **397**, 113–114.
- Austin, J.J., Arnold, E.N. & Jones, C.G. (2004) Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene islands. *Molecular Phylogenetics and Evolution*, **31**, 109–122.
- Bandelt, H.J., Forster, P. & Rohlf, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.
- Bauer, A.M. (2003) On the identity of *Lacerta punctata* Linnaeus 1758, the type species of the genus *Euprepis* Wagler 1830, and the generic assignment of Afro-Malagasy skinks. *African Journal of Herpetology*, **52**, 1–7.
- Branch, B. (1988) *Field guide to the snakes and other reptiles of southern Africa*. New Holland, London.
- Brown, R.P. & Pestano, J. (1998) Phylogeography of skinks (*Chalcides*) in the Canary Islands inferred from mitochondrial DNA sequences. *Molecular Ecology*, **7**, 1183–1191.
- Brygoo, E. (1986) Systématique des lézards Scincidés de la région malgache. XVIII. Les *Cryptoblepharus*. *Bulletin du Muséum National d'Histoire Naturelle, Paris*, **8**, 643–690.
- Carranza, S. & Arnold, E.N. (2003) Investigating the origin of transoceanic distributions: mtDNA shows *Mabuya* lizards (Reptilia, Scincidae) crossed the Atlantic twice. *Systematics and Biodiversity*, **1**, 275–282.
- Carranza, S., Arnold, E.N., Mateo, J.A. & López-Jurado, L.F. (2000) Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. *Proceedings of the Royal Society of London Series B*, **267**, 637–649.
- Emerick, C.M. & Duncan, R.A. (1982) Age progressive volcanism in the Comores Archipelago, western Indian Ocean and implications for Somali plate tectonics. *Earth and Planetary Science Letters*, **60**, 415–428.
- Felsenstein, J. (1985) Confidence-limits on phylogenies – an approach using the bootstrap. *Evolution*, **39**, 783–791.
- Frankham, R. (1998) Inbreeding and extinction: island populations. *Conservation Biology*, **12**, 665–675.
- Fricke, H.W. (1970) Die ökologische Spezialisierung der Eidechse *Cryptoblepharus boutonii cognatus* (Boettger) auf das Leben in der Gezeitenzone (Reptilia, Skinkidae). *Oecologia*, **5**, 380–391.
- Gascuel, O. (1997) BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution*, **14**, 685–695.
- Gillespie, R.G. (2002) Biogeography of spiders on recent oceanic islands of the Pacific: archipelagos as stepping stones? *Journal of Biogeography*, **29**, 655–662.
- Gillespie, R.G. (2004) Community assembly through adaptive radiation in Hawaiian spiders. *Science*, **303**, 356–359.
- Greer, A.E. (1974) The generic relationships of the Scincid lizards genus *Leiopisma* and its relations. *Australian Journal of Zoology*, **31**, 1–67.
- Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **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 Symposium Series*, **41**, 95–98.
- Harris, D.J., Arnold, E.N. & Thomas, R.H. (1998) Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proceedings of the Royal Society of London Series B*, **265**, 1939–1948.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.
- Jesus, J., Brehm, A. & Harris, D.J. (2005) Relationships of scincid lizards (*Mabuya* spp.) from the islands of the Gulf of Guinea based on mtDNA sequence data. *Amphibia-Reptilia*, in press.
- 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 mammals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences USA*, **86**, 6196–6200.
- Lin, S.M., Chen, C.A. & Lue, K.Y. (2002) Molecular phylogeny and biogeography of the grass lizards genus *Takydromus* (Reptilia: Lacertidae) of East Asia. *Molecular Phylogenetics and Evolution*, **22**, 276–288.
- Margalef, R. (1974) *Ecología*. Ediciones Omega, Barcelona.
- Mausfeld, P., Vences, M., Schmitz, A. & Veith, M. (2000) First data on the molecular phylogeography of scincid lizards of the genus *Mabuya*. *Molecular Phylogenetics and Evolution*, **17**, 11–14.
- Mausfeld, P., Schmitz, A., Böhme, W., Misof, B., Vrcibradic, D. & Rocha, C.F.D. (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). *Zoologischer Anzeiger*, **241**, 281–293.
- Meirte, D. (2004) Les Reptiles. *La faune terrestre de l'Archipel des Comores* (ed. by M. Louette, D. Meirte and R. Jocqué), pp. 209–210. Studies in Afrotropical Zoology, 293, MRAC, Tervuren.
- Mertens, R. (1928) Neue Inselrassen von *Cryptoblepharus boutonii* (Desjardin). *Zoologischer Anzeiger*, **78**, 82–89.
- Mertens, R. (1931) *Ablepharus boutonii* (Desjardin) und seine geographische Variation. *Zoologische Jahrbuecher Systematik*, **61**, 63–210.
- Mertens, R. (1934) Weitere Mitteilungen über die Rassen von *Ablepharus boutonii* (Desjardin), II. *Zoologischer Anzeiger*, **108**, 40–43.

- 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. *Bulletin de la Société de Géologie de 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. *Journal of African Earth Sciences*, **5**, 135–145.
- 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 document compiled by S. Palumbi). Department of Zoology, University of Hawaii, Honolulu, HI.
- Podnar, M., Mayer, W. & Tvrktović, N. (2005) Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Molecular Ecology*, **14**, 575–578.
- 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. *Systematic Biology*, **53**, 793–808.
- Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.
- Posada, D. & Crandall, K.A. (2001) Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution*, **16**, 37–45.
- Probst, J.M. (2002) *Animaux de la Réunion*. Azalées Editions, Saint Denis de la Réunion.
- de Queiroz, A. (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution*, **20**, 68–73.
- Raxworthy, C., Martinez-Meyer, E., Horning, N., Nussbaum, R.A., Schneider, G.E., Ortega-Huerta, M.A. & Peterson, A.T. (2003) Predicting distributions of known and unknown reptile species in Madagascar. *Nature*, **426**, 837–841.
- Rocha, S., Carretero, M.A. & Harris, D.J. (2005) Diversity and phylogenetic relationships of *Hemidactylus* geckos from the Comoro islands. *Molecular Phylogenetics and Evolution*, **35**, 292–299.
- Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular cloning, a laboratory manual*, 2nd edn, Vols 1 and 3. Laboratory Press, Cold Spring Harbor, NY.
- Schmitz, A., Ineich, I. & Chirio, L. (2005) Molecular review of the genus *Panaspis* sensu lato (Reptilia: Scincidae) in Cameroon, with special reference to the status of the proposed subgenera. *Zootaxa*, **863**, 1–28.
- Swofford, D.L. (2002) *PAUP*. Phylogenetic analysis using Parsimony (* and other methods)*, Version 4.0b10. Sinauer Associates, Sunderland, MA.
- Thorpe, R.S., McGregor, D.P., Cumming, A.M. & Jordan, W.C. (1994) DNA evolution and colonization sequence of island lizards in relation to geological history: mtDNA RFLP, cytochrome B, cytochrome oxidase, 12s rRNA sequence, and nuclear RAPD analysis. *Evolution*, **48**, 230–240.
- Vences, M., Kosuch, J., Rödel, M.-O., Lötters, S., Channing, A., Glaw, F. & Böhme, W. (2004a) Phylogeography of *Ptychadena mascareniensis* suggests transoceanic dispersal in a widespread African-Malagasy frog lineage. *Journal of Biogeography*, **31**, 593–601.
- Vences, M., Wanke, S., Vieites, D.R., Branch, W.R., Glaw, F. & Meyer, A. (2004b) Natural colonization or introduction? Phylogeographical relationships and morphological differentiation of house geckos (*Hemidactylus*) from Madagascar. *Biological Journal of the Linnean Society*, **83**, 115–130.
- Whiting, A.S., Bauer, A.M. & Sites, J.W. (2003) Phylogenetic relationships and limb loss in sub-Saharan African scincine lizards (Squamata: Scincidae). *Molecular Phylogenetics and Evolution*, **29**, 582–598.
- Wright, S. (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.

BIOSKETCHES

Sara Rocha is a PhD student at CIBIO/UP focusing on the molecular phylogeny and phylogeography of Indian Ocean island reptiles. This work formed part of her MSc.

Miguel A. Carretero is a post-doctoral researcher at CIBIO/UP. In his research, he integrates different aspects of the ecology, morphology, evolution and conservation of reptiles.

Miguel Vences, Assistant Professor at the University of Amsterdam, and **Frank Glaw**, curator for herpetology at the Zoological State Collection in Munich, have a long-standing interest in the herpetofauna of Madagascar and their biogeographical origins.

D. James Harris is an invited professor at the University of Porto. He is interested in island biogeography using reptiles as model organisms.

Editor: Jon Sadler