

**FACULDADE DE CIÊNCIAS DA UNIVERSIDADE DO PORTO**  
**MESTRADO EM BIODIVERSIDADE E RECURSOS GENÉTICOS**

**DIVERSITY OF REPTILES IN THE COMORO ISLANDS:  
A PHYLOGEOGRAPHIC ANALYSIS**

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SARA ROCHA  
2005



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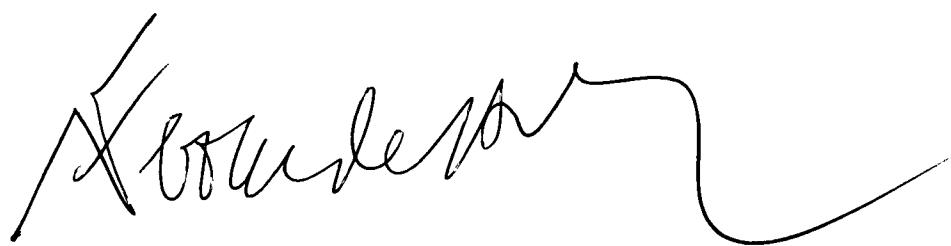
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## DIVERSITY OF REPTILES IN THE COMORO ISLANDS: A PHYLOGEOGRAPHIC ANALYSIS



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Orientador: Doutor David James Harris

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## RESUMO.

Os sistemas de ilhas vulcânicas constituem cenários privilegiados para o estudo de fenómenos evolutivos, sendo muitas vezes denominados de "laboratórios naturais". Neste trabalho, procurou-se investigar os padrões de variação genética e as relações filogenéticas existentes em três grupos de répteis das ilhas Comoros e áreas continentais adjacentes, a partir da análise de sequências de DNA mitocondrial e nuclear. Em particular, foi analisada a variação genética em camaleões do género *Furcifer*, escincídeos do género *Cryptoblepharus* e geckonídeos do género *Hemidactylus*. As Ilhas Comoros constituem um arquipélago formado por quatro ilhas de origem vulcânica, localizadas entre Madagáscar e a costa Africana, com idades estimadas entre 0.5 e 10-15 milhões de anos.

Os resultados obtidos sugerem que ambas as espécies do género *Furcifer* terão colonizado as Comoros, independentemente, a partir de Madagáscar. Também a presença de *Cryptoblepharus* nestas ilhas parece ser explicada pela ocorrência de pelo menos dois eventos de colonização, um deles claramente com origem em Madagáscar. Um terceiro evento de colonização, também a partir de Madagáscar, parece ser a origem das populações estudadas da costa africana. Tendo em consideração a baixa diferenciação genética encontrada dentro do género *Cryptoblepharus*, sugere-se a manutenção do estatuto de subespécies para as formas do Oceano Índico Ocidental.

Quanto ao género *Hemidactylus*, nas ilhas Comoros encontra-se *H. brooki* e mais três espécies que ocorrem também em Madagáscar, *H. mercatorius*, *H. frenatus* e *H. platycephalus*, sendo esta última a espécie mais abundante, apesar de praticamente não apresentar variação genética em todo o arquipélago. Pelo contrário, *H. mercatorius* é a espécie que apresenta maior diversidade genética, sugerindo que a sua presença em pelo menos uma das ilhas, Mayotte, poderá ter resultado de uma colonização natural a partir do Norte de Madagáscar, uma hipótese a ser testada pela análise de indivíduos da costa africana. No entanto, em duas das ilhas foram também encontrados indivíduos aparentemente introduzidos a partir de populações do Centro-Oeste de Madagáscar. *Hemidactylus frenatus* e *H. brooki* são as espécies menos abundantes nas Comoros: os indivíduos de *H. frenatus* são filogeneticamente muito próximos dos de Madagáscar e *H. brooki*, que ocorre pelo menos em Moheli e Anjouan, apresenta maior proximidade filogenética com indivíduos das Ilhas Mascarenhas. A diferenciação genética dentro da espécie *H. brooki* ao nível do gene nuclear C-mos indica ainda tratar-se de um complexo de espécies. Como esperado, a história evolutiva das espécies do género *Hemidactylus* revela-se complexa, moldada por múltiplos fenómenos de colonizações naturais e/ou antropogénicas.

Em comparação com outros arquipélagos de origem vulcânica, como as Canárias ou Cabo Verde, os grupos de répteis das ilhas Comoros mostram uma menor diferenciação genética relativamente às formas "continentais". A colonização destas ilhas parece ser assim um fenómeno relativamente recente, um resultado de certo modo já esperado tendo em conta a idade também mais recente deste arquipélago, e ocorreu maioritariamente a partir de grupos de Madagáscar.

## ABSTRACT.

Classically known as “natural laboratories”, volcanic island systems constitute exceptional environments to study evolutionary phenomena. An essential first step is to understand the biogeographic history of the insular taxa.

Using DNA sequence data, we approached questions about genetic diversity, relationships and levels of genetic differentiation between the populations (or species) from different islands and from the surrounding mainland areas, in three groups of lizards from the Comoro Islands, a setting of four major volcanic islands located between Madagascar and the East African coast. Their ages range from 10-15 to 0.5 Myr old, and they were supposedly colonised by fauna from both Africa and Madagascar. Collected data helped to unveil origins and colonisation pathways of island taxa, to clarify their taxonomy, to identify possible recent introductions and thus help in the establishment of effective conservation plans.

We analysed genetic variation within *Furcifer* chameleons, *Cryptoblepharus* skinks, and *Hemidactylus* geckos. Both species of *Furcifer* seem to have independently colonised the Comoros, from Madagascar. The presence of *Cryptoblepharus* in these islands is explained by a minimum of two colonisation events, at least one of them from Madagascar, with a third one, also from Madagascar, giving rise to the studied African populations. Genetic differentiation within *Cryptoblepharus* is however low, and we propose the maintenance of the subspecific status of the forms from the Western Indian Ocean region. Concerning the genus *Hemidactylus*, the Comoro islands are inhabited by the same three species that occur on Madagascar, *H. mercatorius*, *H. platycephalus* and *H. frenatus*, and by a fourth one, *H. brooki*. *Hemidactylus platycephalus* is by far the most abundant species on these islands, although it presents almost no genetic variation across the archipelago. *Hemidactylus mercatorius* is the species that exhibits more genetic variation, suggesting a natural and ancient colonisation of at least one of the islands, Mayotte, from North Madagascar; although further conclusions can not be made until genetic diversity in mainland Africa is properly assessed. Apparently introduced individuals of *H. mercatorius* from Western-Central Madagascar were also detected. *Hemidactylus frenatus* and *H. brooki* are the less abundant species in the Comoros: individuals from *H. frenatus* are closely related to the Malagasy ones and *H. brooki*, that occurs at least in Moheli and Anjouan, being most closely related to individuals from the Mascarenes islands. Differentiation within *Hemidactylus brooki* at the C-mos nuclear gene also indicates that this is, in fact, a species complex. As expected, it was within the genus *Hemidactylus* that we found more evidences of human-mediated transportations.

The majority of the Comoroan reptile fauna is related to the Malagasy one, and relative to other volcanic archipelagos, such as the Canary and the Cape Verde Islands, Comoroan reptile groups seem to have undergone less differentiation in relation to “continental” forms, which indicates their recent origin (a result already expected considering their relatively young age).

The continuous effort to obtain phylogenetic data across a diversity of taxa, together with geological and ecological data, seem the best way to evaluate diversification and ecosystem building in these (and other) island systems.

## **INTRODUCTION.**

Evolution on Oceanic Islands  
The Comoro Islands  
Thesis Aims  
Thematic Organisation  
References

## EVOLUTION ON OCEANIC ISLANDS

Islands have become synonymous with the study of evolution since the famous association between Darwin's theory of evolution by natural selection and the finches of the Galápagos Islands (DARWIN 1859). These later became one of the best-known cases of adaptive radiation.

In a recent review, EMERSON (2002) summarised some of the aspects that make island systems such attractive environments for studying evolution: they present discrete geographical entities with defined oceanic boundaries; gene flow between individual islands is reduced by oceanic barriers; their often small geographical size has made the cataloguing of flora and fauna easier than in continental systems; despite their small geographic size, they can contain a significant diversity of habitats and; they are often geologically dynamic, with historical and contemporary volcanic and erosional activity.

The combination of all these factors typically leads to high levels of endemism within oceanic island systems, presenting a microcosm of the evolutionary process. Moreover, different islands provide simplified, replicated natural experiments to study the processes of evolution.

### 1. Origins of Species Diversity on Oceanic Islands

The species diversity observed in oceanic islands can be the result of different factors, such as diversification of a founding population into an array of species differentially adapted to diverse environmental niches, *adaptive radiation*; multiple successful colonisations of an island from neighbouring islands or continental landmasses; the diversification of a founding population into a number of species caused by vicariant events such as lava flows and erosion, or increased rates of speciation through bottleneck and founder "flush" events (*sensu* CARSON AND TEMPLETON 1984). Moreover, recent results support that species diversity itself may be a driver of species diversification, and that the probability of speciation increases for each species on an island as total species number increases (EMERSON AND KOLM 2005).

Although islands harbour a great diversity of organisms, understanding their origins is not so obvious. The accurate reconstruction of the phylogenetic relationships within groups of species can be used to address this question and also to determine the processes that have led to species richness within island ecosystems.

The following part of this introduction intends to approach these processes, providing some examples of molecular studies that helped gaining an understanding of, and answering questions about the origins of species diversity on islands.

### 1.1 Adaptive radiations on islands

Oceanic islands show considerable habitat diversity produced by topology and humidity gradients, which, combined with their isolation, provides a powerful template for the evolution of species radiations (JUAN ET AL. 2000). Implicit in this concept is the formation of a suite of closely related species adapted to exploit different habitats or life styles (GILLESPIE 2001). This phenomenon presents an unusual opportunity for comparative studies on the processes of speciation and selection in natural populations.

The fourteen species of finches endemic from the Galápagos islands are thought to be the descendants of a single species that colonised these islands from the nearby South American continent only 2-3 Myr ago (SATO ET AL. 1999, 2001), and this is also the kind of evolutionary process that originated of the Hawaiian radiation of endemic species of spiders from the genus *Tetragnatha* (GILLESPIE 1997, 2004), the development of the same set of "ecomorphs" of *Anolis* lizards on each island of the Greater Antilles (LOSOS ET AL. 1998), or the Malagasy lemurs and carnivores (YODER ET AL. 1996, 2003).

### 1.2 Multiple colonisations

Two assumptions, or hypothesis, are often implicit when assessing the species diversity within a group of islands: the given species group is the product of a single colonisation event, in which case it will be monophyletic, or is the product of multiple colonisations from adjacent landmasses, that will be revealed by its paraphyly. These hypotheses can only be effectively tested by the inclusion of all closely related species from continental areas and neighbouring archipelagos.

Multiple colonisations from adjacent continental areas were identified by molecular phylogenetic analysis as giving rise to the warblers from Madagascar (CIBOIS ET AL. 1999) or *Tarentola* geckos from Macaronesia (NOGALES ET AL. 1998; CARRANZA ET AL. 2000, 2002).

### 1.3 Within island vicariant events

Once an island has been colonised, within island differentiation can also be promoted by vicariance events produced by the formation of new volcanoes or lava flows. The highly dissected island topography or glacially mediated fluctuation in sea levels, successively flooding and exposing connections between islands, or volcanoes, can also account for isolation and species formation in many groups.

These events provide multiple opportunities for the isolation of small populations. They have been implicated in generating the tremendous diversity among the Hawaiian *Drosophila* (KANESHIRO AND BOAKE 1987), and may as well be responsible for the rapid speciation in a number of other groups (RODERICK AND GILLESPIE 1998). Also some beetles and reptiles from the Canary islands exhibit phylogeographic patterns that are probably related to recent volcanic activities, both joining previously separated massifs (in Tenerife) or promoting the isolation of populations (in Grand Canaria) (JUAN ET AL. 1996; BROWN AND PESTANO 1998; NOGALES ET AL. 1998).

#### 1.4 Founder events and bottleneck

Colonisation events involving small number of individuals may trigger rapid species formation (CARSON AND TEMPLETON 1984, COATES 1992). Genetic drift, that is, the variation of allele frequencies from one generation to another due to chance, increased in small populations, may provide the key genetic variability for adaptive character change, mediated by selection (CARSON 1990). As a result, the founder events and associated bottlenecks, that are generally considered to accompany colonisation of new islands, may lead to rapid differentiation. Higher rates of molecular evolution within island lineages relative to mainland lineages are frequently observed (JOHNSON AND SEGER 2001).

## 2. Molecular Evolution and the Timing and Sequence of Colonisation

Estimating the sequence and timing of colonisation events are among the main goals in the research of island taxa evolution. Complex phylogenies, with paraphyletic island assemblages, can make the inference of colonisation pathways especially difficult.

The simplest of the models of island colonisation is that of *stepping-stone colonisation*, a step-like progression down the island chain from the oldest to the youngest islands, a pattern first described by CARSON (1970, 1974, 1987) for Hawaiian *Drosophila* and later considered appropriate to explain biogeographic patterns of other Hawaiian (see FUNK AND WAGNER 1995 for a review) and Canary islands taxa (reviewed in JUAN ET AL., 2000). This model is a good working null hypothesis but its acceptance from a molecular phylogeny may not be straightforward, and even a hypothetically clear example is open to different interpretations (Figure 1). Moreover, this simple pattern can be complicated by several factors: back colonisation, recent colonisation, within-island differentiation, adaptation, extinction and human mediated translocations are some of the events that confound an "island colonised as it emerges pattern" (JUAN ET AL. 2000).

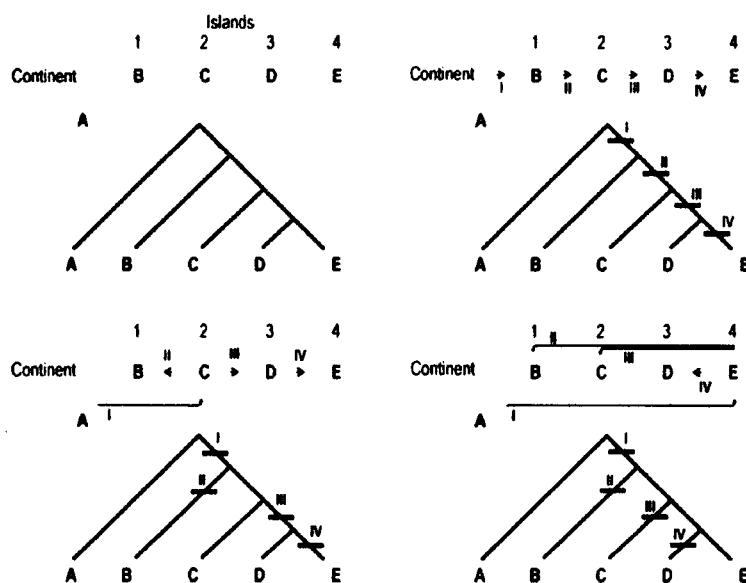


Figure 1 - The same phylogeny (cladogram) can be compatible with several island colonisation hypotheses. The distribution of several island endemic taxa (B, C, D and E), their continental relative (A) and colonisation events (I to IV) are represented in the figure. Colonisation events are also mapped into the tree. The phylogeny is compatible with a simple stepping-stone colonisation history (top right) but also more complex scenarios (bottom left and right) involving local extinctions and multiple colonisations (adapted from Emerson (2002)).

Inferring the ages of island colonisation, or divergence events, often requires the use of evolution rates estimated in other molecular studies. When information on the island's age is available, it may be possible to use it to calibrate molecular clocks for specific taxa and infer colonisation sequences and their ages (at least maximum ages of colonisation and minimum rates of evolution for a given gene fragment). This specific point has been target of much debate and controversy, with no clear definition about what should be the best procedure to improve accuracy of calibrations. BALDWIN AND SANDERSON (1998) pointed out that the use of an "external" calibration will be more reliable, by not magnifying eventual errors of assumptions on colonisation times in the deeper events within the tree. Lineage extinction, accelerated evolution within some lineages and sampling errors should also be taken into account when inferring divergence times from a molecular phylogeny (EMERSON ET AL. 2000).

### 3. Testing Ecological and Evolutionary Hypotheses

Molecular phylogenies are also powerful tools for both generating and testing ecological and evolutionary hypotheses (EMERSON 2002). LOSOS AND SCHLUTER (2000), for example, were able to quantitatively test hypotheses about species-area relationships (one of the main features of MACARTHUR AND WILSON's (1967) equilibrium theory of island biogeography), using a phylogeny of 53 species of *Anolis* lizards. In particular, they investigated the theory assumption that, on islands that generate most of their diversity *in situ*, species-area relationships should be driven by higher speciation rates in larger areas. Their results support a rate of species proliferation increasing with island area above a threshold island size. Molecular data was also useful in testing hypotheses about evolution of body size, size assortment or character displacement (Losos 1990, RADTKEY ET AL. 1997, CARRANZA ET AL. 2001); hypotheses about the causes of morphological variation (THORPE 1995, MALHOTRA AND THORPE 2000) or about the evolutionary consequences of ecological specialisation (ARMBRUSTER AND BALDWIN 1998, EMERSON ET AL. 1999). On this last topic, recent molecular phylogenetic analysis added support against the once commonly held viewpoint that extreme ecological specialisation is irreversible and limits the potential to adapt to new conditions (ARMBRUSTER AND BALDWIN 1998).

Island organisms, thus, are generally subject to diverse adaptational pressures, manifesting themselves through high levels of morphological diversity. There is still limited understanding of the interplay between time and isolation in the creation of such diversity, while even more limited is our knowledge of ecological determinants that can play critical roles in fostering colonisation and diversification. Accurate phylogenetic reconstructions using neutral molecular markers are needed to understand the origin of such groups of organisms (EMERSON 2002).

Perhaps the first step is gaining an understanding on the origins of a particular group on an archipelago, the colonisation routes between its islands, the number and their ages. This approach has the added advantage of allowing distinction between endemic island forms and human mediated introductions. This knowledge is crucial to implement conservation plans that take into account the evolutionary history of the organisms. Moreover, molecular phylogenetic analyses of island taxa are enabling biologists to get at the heart of some key ecological and evolutionary theories – there is much to be learned about the modes of speciation from the natural patterns of diversification on islands. At the same time, the current rates of anthropogenic perturbation in these insular ecosystems is threatening these particular biota, that are already more vulnerable than continental ones. Higher extinction rates in island species over the last 400 years than in any other habitat (REID AND MILLER 1989, WORLD CONSERVATION MONITORING CENTRE 1992) demonstrate the susceptibility of island populations to perturbations, and likely reflect their susceptibility to stochastic effects but also in some cases, lower levels of genetic diversity (FRANKHAM 1997, 1998). Research is needed to mitigate the impact of alien species and to determine the identity and distribution of introduced and native species.

## THE COMORO ISLANDS

### 1. Geographical and Geological Background

The Comoros are a set of four major oceanic islands - Mayotte, Anjouan, Moheli and Grand Comoro - and surrounding islets, situated in the middle of the Mozambique channel, approximately halfway from both Madagascar and the East African coast (about 300 km from each), in the southwestern Indian Ocean (Figure 2).

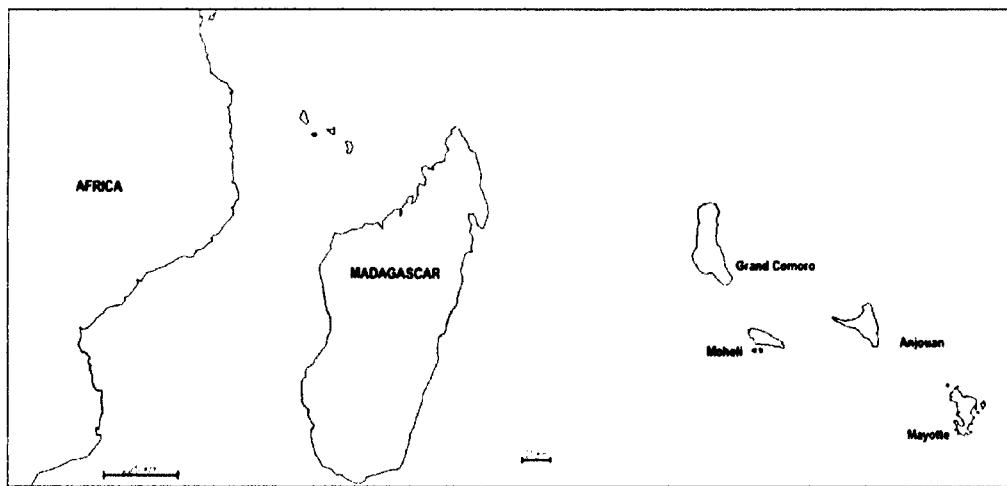


Figure 2 - Geographic location of Comoros archipelago.

Grand Comoro is, by far, the largest island, with 1148 km<sup>2</sup> and Moheli and Mayotte are the smaller ones, with less than half of its area. They are oriented in a roughly linear fashion along a southeast-northwest axis, starting from the northwest tip of Madagascar.

Their origins postdate the separation between Madagascar/India and Africa, known to have occurred 165 million years (Myr) ago, during the Cretaceous period, and the drift of Madadascar/India relative to Africa, that ended about 121 Myr ago (RABINOWITZ ET AL. 1983). They were formed by a progressive hotspot volcanic activity during the last 10 Myr, with its earliest manifestations to be found in the Seychelles plateau (EMERICK AND DUNCAN 1982). This volcanic activity originated Mayotte ( $7.7 \pm 1.0$  Myr), Moheli ( $5.0 \pm 0.4$  Myr), Anjouan ( $3.9 \pm 0.3$ ) and Grand Comoro ( $0.13 \pm 0.02$  Myr), this last one being the only island that is still volcanically active (ages of the oldest exposed lavas from EMERICK AND DUNCAN 1982, NOUGIER ET AL. 1986). The estimated age of the volcanic origin of the islands, calculated using the rate of plate motion (Somali plate) and the distance to the present volcanically active spot (Karthala volcano, Grand Comoro), considerably predates their oldest age estimates made from lavas and is of 10-15 Myr for Mayotte, 11.5 Myr for Anjouan and 0.5 Myr for Grand Comoro (MONTAGGIONI AND NOUGIER 1981, NOUGIER ET AL. 1986). These estimates are considered by some authors to be better estimators of their maximum

time of emergence above the sea, and thus, of their availability for colonisation for several taxa (WARREN PERS. COMM.). Presumably, the aerial emergence of the islands must fall in between these two dates, and probably close to the timing of first initiation (DUNCAN PERS. COMM.). The Comoros are surrounded by sea depths that vary from 400 to 3000m. The lowest historical sea level in the Indian Ocean was  $-145 \pm 5$ m at 18400 years ago (HAQ ET AL. 1987, COLONNA ET AL. 1996), and thus they are true oceanic islands, in the sense that they were never connected between themselves, or to Africa or Madagascar.

## 2. Origins of Comoroan Fauna

After their formation the Comoro islands were colonised mainly by fauna from Africa and Madagascar, most of which had already differentiated, and also eventually from Southeast Asia.

Madagascar is the fourth largest island in the world and is characterised by a remarkable degree of endemism of its fauna and flora. Species-level endemicity in reptiles is estimated around 93% and species diversity must yet be seen as preliminary, since ongoing taxonomic and molecular work is leading to the recognition of new species at an unprecedented pace (see VENCES 2004). The biogeographic origins of Madagascar's extant fauna have been much disputed. Many authors have speculated that the ancestral stocks were present on the island since the Late Cretaceous or even earlier, prior to its rifting from Africa (review in KRAUSE ET AL. 1997), but although it appears that certain taxa (e.g., some birds, bats, fishes and frogs) may be of Indian-Malagasy origin, and could have been originated by the vicariance imposed by the break-up of the Southern supercontinent Gondwana, the actual consensus is that most taxa arrived to Madagascar by random dispersal (rafting, swimming, and/or island hopping) from Africa, across the Mozambique channel, since the Late Cretaceous to recent times (KRAUSE ET AL. 1999, VENCES 2004). It is also now clear that many Malagasy taxa belong to species-rich endemic radiations, and in many cases, the sister taxa of the Malagasy lineages have been identified. In an analysis of 20 vertebrate clades represented in Madagascar, the closest relatives of the Malagasy taxa occur in Africa in 12 cases and in India and Asia only in three cases. The remaining (iguanas and boine snakes) occur on South America. Only three out of the 20 groups coincide with the plate tectonics events (VENCES ET AL. 2003). Thus, Madagascar almost exclusively harbours relatively modern lineages of non-marine vertebrates (VENCES ET AL 2001, 2003), and dispersal scenarios from Africa have been favoured to explain their biogeographic origins, to the detriment of Gondwanan vicariance. In fact, oceanic dispersal is a "resurrected" general pattern in historical biogeography, with recent evidences indicating that the importance of oceanic dispersal has been strongly underestimated and that such dispersal events may have been more common than thought by vicariance biogeographers (DE QUEIROZ 2005). Pleistocene dispersal, possibly using currently submerged islands as stepping-stones (KRAUSE ET AL. 1997, MCCALL 1997), seems to be an emerging pattern of colonisation of Madagascar for a variety of faunal elements (VENCES ET AL. 2004). Multiple additional dispersal events from Madagascar to Seychelles, Comoros and back to Africa, in some

cases, subsequently obscured this biogeographic signal (JANSA *ET AL.* 1999, RAXWORTHY *ET AL.* 2002, VENCES *ET AL.* 2003). In this context, the Comoro islands could, in the last 10 Myr, have possibly been used as stepping-stones, facilitating dispersal between Africa and Madagascar.

Eastern Africa is the other putative major source for Comoroan taxa. Estimating origins of island taxa, colonisation patterns and ages, requires an extensive assessment of the diversity within the taxa in its colonised and putative areas of origin, but unfortunately, as for other tropical areas, where most of the biodiversity resides, little is known about the extent and patterns of genetic diversity within the reptiles from mainland Africa. They are probably immense and complex (HEWITT 2001). The first phylogeographic studies in other tropical areas revealed congruent patterns of deep genetic divergence over short geographical distances, with distinct genomes confined to small areas (DA SILVA AND PATTON 1998, GARCIA-PARIS *ET AL.* 2000), and where recent and older lineages can co-occur in the same geographic place (ROY 1997, GARCIA-MORENO *ET AL.* 1999). As generators and retainers of much genetic divergence and speciation, it is most important that these tropical regions become studied phylogeographically, as they will provide a deeper understanding of the processes providing this biodiversity (HEWITT 2001).

Though impoverished and unbalanced relative to continental ones, the fauna of the Comoro islands comprises a diverse array of mammals, birds, reptiles, fishes and arthropods. Other classes are less abundant: amphibians, in particular, are represented only by two species, which, apparently, reached the archipelago by natural dispersion from Madagascar (VENCES *ET AL.* 2003). There is a high degree of endemism within the Comoroan taxa, across all classes, but also a considerable amount of widespread and introduced species (LOUETTE *ET AL.* 2004). Interestingly, despite being the youngest island, Grand Comoro shows the higher number of endemic bird species, probably because it is the larger and higher island, thus providing a considerable variety of habitats (LOUETTE *IN* LOUETTE *ET AL.* 2004). Among the endemisms, perhaps the most representative examples are the archipelago radiations of bats of the genus *Pteropus* and birds of the genera *Turdus*, *Terpsiphone*, *Zosterops*, *Foudia* and *Nectarina* (WARREN *ET AL.* 2003, LOUETTE *ET AL.* 2004): some are island endemisms; others are archipelago endemisms, being represented by different subspecies in different islands.

As mentioned above, the Comoroan taxa have diverse affinities: some have African ancestry, others Malagasy ancestry and even Asian related species are present. Still, for most of the taxa, their affinities remain unknown (LOUETTE *ET AL.* 2004). For several groups in the Comoros, it is still uncertain where they came from, when did they get to the Comoros, if there were single or multiple invasions and, if so, how frequent these were.

## STUDY ORGANISMS

### 1. Why Reptiles?

In this work we approached the kind of questions mentioned earlier in selected groups of reptiles found in the Comoro islands, investigating their origins, diversity and colonisation patterns across this region of the southwest Indian Ocean.

Reptiles are rapidly becoming model organisms for studying evolution on island systems mainly because they are non-volant (yet they have reached even remote islands), the alpha-taxonomy is not especially difficult, they do not require special equipment to catch and sampling methods are non-lethal (tail tips). In fact, reptiles are present on many islands and some are known to be excellent over-water dispersers, especially within lizards (CARRANZA ET AL. 2000, 2001, CARRANZA AND ARNOLD 2003, AUSTIN ET AL. 2004). Some lizard groups are more prone to transmarine journeys than others, and such voyages are more common in geckos and skinks than in lacertids, agamids or teids. Variation also occurs within geckos and skinks, with particular groups making repeated journeys while others, from the same source areas, fail to do so. This suggests colonisation across oceans is not a random process and the proclivities of the organisms concerned are important in determining whether it takes place. Such proclivities include frequent occurrence on marine beaches, tolerance to salt water and desiccation, and ability to maintain position on natural rafts (CARRANZA AND ARNOLD 2003). Molecular phylogenies of island taxa are powerful tools also to assess dispersal abilities of different groups.

### 2. Reptile Fauna from the Comoro Islands

As expected for an oceanic archipelago, the Comoroan reptile fauna is typically impoverished, unbalanced, but rich in endemisms. Recently introduced species are also present. Three groups of reptiles are known from the Comoro islands: turtles, snakes and lizards, of which lizards are clearly the most abundant. The lizard fauna is dominated by gekkonids and scincides, but two chameleons, one agamid and one oplurid are also present. *Oplurus comorensis* is probably endemic while *Agama agama* was clearly introduced from Africa (MEIRTE IN LOUETTE ET AL. 2004). Snakes are represented only by two typhlopidae and three colubrid species (MEIRTE 1992, GLAW AND VENCES 1994, HENKEL AND SCHMIDT 2000, MEIRTE 2004). The most represented group is undoubtedly the gekkonid genus *Phelsuma*, within which several endemisms are found together with more widespread species. Details on numbers of species present and distributions can be found in CARRETERO ET AL. (2005).

### 3. Groups to be Examined

In this work we focused on the origins and genetic diversity of three genera of lizards that occur in the Comoro islands: chameleons of the genus *Furcifer*, geckos of the genus *Hemidactylus* and skinks of the genus *Cryptoblepharus*.

The genus *Furcifer* is represented in this archipelago by two endemic species occurring in two distinct islands. The remaining 14 extant species of this genus all inhabit Madagascar, with one also present in the Mascarenes archipelago (located to the southeast of Madagascar). Previous studies considered both species from Comoros to be sister-taxa (RAXWORTHY ET AL. 2002), but phylogenetic analyses were only based on morphological data, known to be susceptible to adaptive ecological pressures.

*Hemidactylus* is a specious genus with more than 80 morphologically similar species widespread in tropical and subtropical areas; however no comprehensive phylogeny is available for this genus. Seven species are known from the East African coast (SPAOLS ET AL. 2001), although two of them, *H. mabouia* and *H. platycephalus*, are clearly the most abundant. VENCES ET AL. (2004) recently revised the Malagasy region *Hemidactylus*, confirming the occurrence of three species in Madagascar, *H. frenatus*, *H. platycephalus* and *H. mercatorius* (split again from its previous synonym *H. mabouia*; see appendix 1 from VENCES ET AL. 2004) plus a fourth one, *H. brooki*, in the Comoros. Intraspecific genetic variation implied an ancient natural colonisation of Madagascar by *H. mercatorius*, but both *H. platycephalus* and *H. frenatus* had little or no differentiation inside Madagascar, indicating more recent colonisations or introductions (VENCES ET AL. 2004). Concerning the Comoroan populations, *H. mercatorius* was assumed to have been recently introduced from Madagascar, *H. platycephalus* to have naturally colonised the Comoros from Africa while both *H. frenatus* and *H. brooki* had phylogeographic patterns across the Indian Ocean that suggested multiple human translocations. However, only a few individuals from the Comoros were included in this analysis (VENCES ET AL. 2004).

*Cryptoblepharus* is a genus of small-sized scincid lizards that occur mainly in coastal rags, and are distributed through the Indo-Pacific and Australian regions, with a disjunct unit in the Malagasy region (MERTENS 1934, GREER 1974). Besides Australia and Madagascar, it mostly inhabits small and isolated islands in the Pacific and Indian Ocean, reaching the African mainland, where it occurs from Somalia to South Africa in narrow areas along the East coast, possibly in isolated populations (BRANCH 1988). Taxonomy within this genus is controversial: different authors place the different forms (about 36) as distinct species or as subspecies of *C. boutonii*. Furthermore, the phylogenetic relationships between them are so far unknown.

**THESIS AIMS****1. General Objectives**

This work aims to contribute to a better understanding of the phylogeographic patterns of Indian Ocean reptiles, their biogeographic history and conservation priorities. Although Madagascar's biodiversity and the origin of their taxa are extensively studied, the diversity of reptiles in the Comoros and other Indian Ocean islands is still poorly known. This study will be focused exclusively in the three previously mentioned groups of reptiles from the Comoro islands, addressing both diversity and phylogenetic/phylogeographic relationships between them, Malagasy and East African taxa. It will help to clarify taxonomy, identify possible recent introductions and enable conservation plans to be established. The comparison of our data to others already collected for other volcanic island groups will contribute to a better understanding of general patterns of colonisation within reptiles and speciation phenomena within islands.

Within the study groups, our goals are:

1. To determine the number of species/Evolutionary Significant Units within the Comoros and assess genetic diversity within them
2. To assess (or use known data of) genetic diversity within Madagascar and East Africa, for taxa found in one of these areas and in the Comoros.
3. To infer colonisation events between islands and from continental landmasses.
4. To infer whether species have been introduced anthropogenically.
5. To compare levels of genetic variation within islands with the known geological ages of the islands.
6. To compare mitochondrial and nuclear DNA phylogenies and use both to reconstruct the history of each group in the region.
7. If possible, to calibrate molecular clocks using geological ages of the islands.
8. To compare predicted colonisation abilities of different reptile classes with our own inferences made from the phylogenies produced from the mitochondrial and nuclear DNA sequences.
9. To re-assess the taxonomic status of the taxa under study and determine their conservation priorities.
10. To compare the patterns of genetic diversity observed in the Comoro islands with that from other volcanic island archipelagos.

## 2. Specific Objectives

### 1. Genus *Furcifer*

To obtain molecular data from the species from the Comoros and analyse it together with the previously published sequences from this genus, in order to re-assess phylogenetic relationships within it, and also genetic diversity within both Comoroan species. To use the produced phylogenies to infer the number and if possible, ages of colonisation events to the Comoros.

### 2. Genus *Hemidactylus*

With a larger sampling within the Comoros, to use mitochondrial DNA data to re-assess origins, diversity and relationships within the four species of *Hemidactylus* from these islands. To use also slow-evolving nuclear gene sequences to make a wider comparison between Malagasy and West African species.

### 3. Genus *Cryptoblepharus*

To investigate the biogeography and genetic differentiation of the Malagasy region forms in order to understand their origin and history in the Western Indian Ocean. Using mitochondrial DNA sequences, to elucidate the phylogenetic relationships among these forms and address possible historical vicariant and dispersal patterns that may have shaped their current distribution.

## THEMATIC ORGANISATION

Following the objectives mentioned above and the particular questions posed by each group, three scientific articles compose the main body of this thesis, each one focusing on a distinct group.

In the first article, "*Mitochondrial DNA sequence data suggests two independent colonizations of the Comoros archipelago by Chameleons of the genus Furcifer*", recently published in the *Belgian Journal of Zoology*, we examined the relationships between *Furcifer* chameleons from the Comoro islands and some of the Malagasy species, using ND4 mitochondrial DNA sequences. The high genetic divergence between the two species from both islands, coupled with their young geological age, led us to suggest that these species are not sister-taxa, as previously hypothesised, but instead result from two independent colonisation events from Madagascar. Furthermore, the high degree of genetic divergence found within *F. cephalolepis*, given the age of the island it inhabits, suggests that this region of the ND4 gene and associated tRNA's may be evolving faster in *Furcifer*, than predicted by ectothermal vertebrate molecular clocks. This finding may have implications in estimated times of colonisation of other island groups within chameleons.

In the second article, "*Diversity and phylogenetic relationships of Hemidactylus geckos from the Comoro islands*", published in *Molecular Phylogenetics and Evolution*, we used mitochondrial (16s rRNA) and nuclear (C-mos) DNA sequences to examine the relationships within Comoroan *Hemidactylus*, and between them and Malagasy and West African insular populations of the same species/genus. Our results question previous ideas about colonisations of the islands by these species, and also add important data on their relative abundance and genetic diversity. Problems in differentiating *H. mabouia* from *H. mercatorius* are highlighted, and our results also indicate that *H. brooki* is in fact a species complex. In this work we take advantage of recently published molecular data on *Hemidactylus* (VENCES ET AL. 2004, JESUS ET AL. 2005) to make comparisons with our own data and to further the knowledge on the phylogeny of this complex genus.

In the third article, "*Deciphering patterns of trans-oceanic dispersal: the Evolutionary Origin and Biogeography of coastal lizards (Cryptoblepharus) in the Western Indian Ocean region*", in press in *Journal of Biogeography*, we use molecular data (12s and 16s rRNA mtDNA gene fragments) to explore the origin and phylogeographic relationships of a group of species of this genus that inhabits the Southwest Indian Ocean region and that has their closest relatives about 6000 km away, in the Indo-Pacific region. Natural over-water dispersals, both between the two disjunct areas and within the Southwest Indian Ocean group seem to have shaped this species distribution, as several independent colonisations were inferred.

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## **PHYLOGEOGRAPHY OF SELECTED GROUPS OF REPTILES IN THE COMORO ISLANDS.**

### *Article 1*

**Mitochondrial DNA sequence data suggests two independent colonizations of the Comoros archipelago by Chameleons of the genus *Furcifer*.**

Sara Rocha, Miguel A. Carretero, D. James Harris (2005)

*Belgian Journal of Zoology*, 135, 37-40.

### *Article 2*

**Diversity and phylogenetic relationships of *Hemidactylus* geckos from the Comoro islands**

Sara Rocha, Miguel A. Carretero, D. James Harris (2005)

*Molecular Phylogenetics and Evolution*, 35, 292-299.

### *Article 3*

**Deciphering patterns of trans-oceanic dispersal: the Evolutionary Origin and Biogeography of coastal lizards (*Cryptoblepharus*) in the Western Indian Ocean region**

Sara Rocha, Miguel A. Carretero, Miguel Vences, Frank Glaw and D. James Harris (in press)

*Journal of Biogeography*.

# Mitochondrial DNA sequence data suggests two independent colonizations of the Comoros archipelago by Chameleons of the genus *Furcifer*

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**ABSTRACT.** We used ND4 mtDNA sequences (815bp) to examine the relationships between *Furcifer* chameleons (Chamaeleonidae; Reptilia) from the Comoro Islands. High genetic divergence between *F. cephalolepis* from Grand Comoro and *F. polleni* from Mayotte is hardly compatible with the hypothesis of them being sister-taxa given the young geological age of both islands. Thus, each island was independently colonized, presumably from Madagascar. Genetic diversity within both islands is similar, despite their very different geological ages. The degree of divergence found within a recent island like Grand Comoro may indicate that the molecular clock calibration typically applied to reptiles is not appropriate for this species.

**KEY WORDS :** Comoros, *Furcifer polleni*, *Furcifer cephalolepis*, Chamaeleonidae, colonization, ND4, molecular clock.

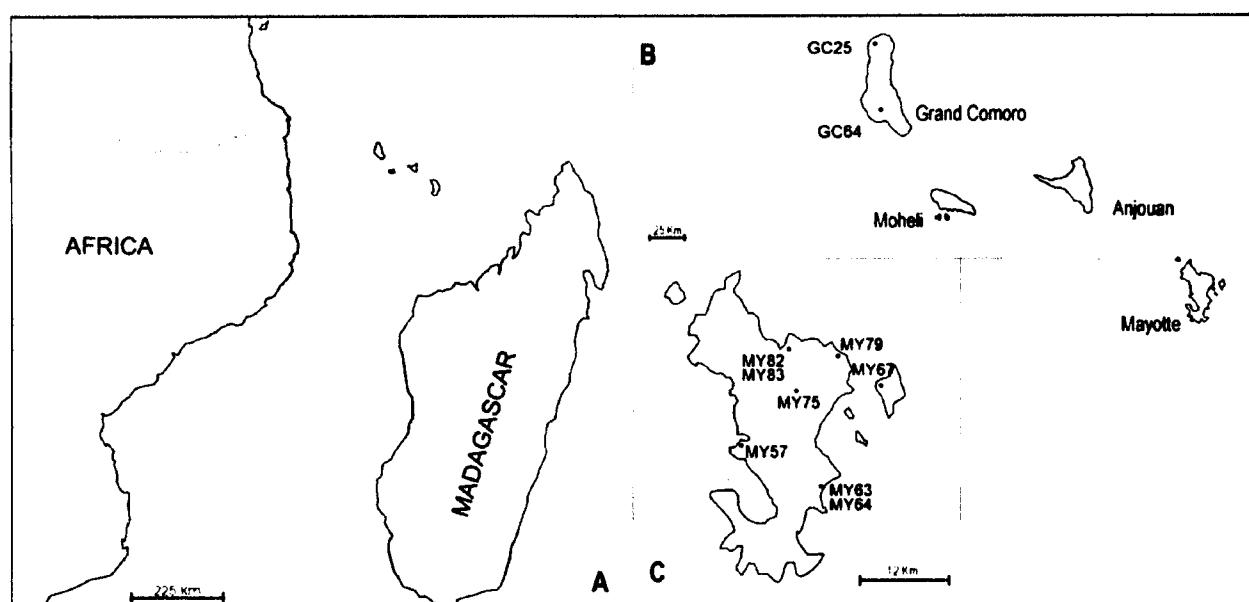


Fig. 1. – Maps showing : A) the position of Comoros archipelago relative to African mainland and Madagascar, B) the Comoros archipelago and the sampling sites in Grand Comoro and C) the sampling localities in Mayotte. Codes are given in Table 1.

## INTRODUCTION

The four major islands of the Comoros archipelago lie about 200 Km west of the northern tip of Madagascar, at the entrance of the Mozambique Channel (Fig. 1). After the separation of Madagascar from Mozambique, this volcanic chain of islands was formed, during the Miocene to the Late Pleistocene, and has been colonized by the flora and fauna of both Africa and Madagascar, which had

already differentiated. The youngest of the Comoros is Grand Comoro (0.5 My), dominated by the volcano Karthala, which is still active, giving this island a uniform topography. Mayotte is the oldest, with 10-15 My, and harbours several volcanoes, being the result of the union of previously independent massifs. These dates correspond to the estimated age of the volcanic origin of the islands (MONTAGGIONI & NOUGIER, 1981; NOUGIER et al., 1986). The age of the oldest exposed lavas is considerably

more recent:  $0.13 \pm 0.02$  My for Grand Comoro and  $7.7 \pm 1$  for Mayotte. These islands never had contact with other landmasses and are separated from each other and from Africa by sea depths of more than 3600 m (EMERICK & DUNCAN, 1982; NOUGIER et al., 1986).

The genus *Furcifer* (Chamaeleonidae : Reptilia) is represented in these islands only by two endemic species; *Furcifer cephalolepis* Günther, 1880, in Grand Comoro and *Furcifer polleni* Peters, 1874, in Mayotte. The extant 14 species of this genus all inhabit Madagascar, with one, *F. pardalis* Cuvier, 1829, also present in Mauritius and Reunion Islands, probably representing another natural oceanic dispersal (RAXWORTHY et al., 2002).

In a previous study involving many Chamaeleonidae species, RAXWORTHY et al. (2002) found support for a Madagascan origin for chameleons with multiple "out-of-Madagascar" dispersal events, one of them being the colonization of the Comoros archipelago by *Furcifer* species. Based only on morphological data, these authors placed *F. cephalolepis* and *F. polleni* as sister-taxa and related to the *F. oustaleti* and *F. lateralis* groups from Madagascar. However, attempts to interpret a morphological phylogenetic tree in terms of colonization sequence are compromised by

ecogenetic adaptation to current selective pressures influencing the tree (THORPE et al., 1994). This could be the case here, as they are placed together by two non-unique synapomorphies: both Comoros chameleons have reduced body size and lost lung diverticula, probably as function of body size. Dwarfism in island species is a fairly common evolutionary response, and is presumably adaptive, thus such adaptations may well have evolved in parallel. Therefore, to further assess the position of these species within the *Furcifer* clade on the basis of DNA sequence data, we obtained partial sequences of the ND4 gene, from the same region as RAXWORTHY et al. (2002). We also used ND4 sequences to assess intraspecific diversity within *F. cephalolepis* and *F. polleni*. Being a fast evolving gene, this is an adequate marker to use in recent evolutionary events. Considering the ages of the islands, and assuming just one colonization event, we expected minimal diversity within Grand Comoro, and also between *F. cephalolepis* and its presumed sister-taxa, *F. polleni*. Concerning Mayotte, its age and, in particular, its conglomerate nature, might have led to high genetic diversity within this island, as seen in reptiles from Tenerife, in the Canary Islands (THORPE et al., 1994, 1996; BROWN & PESTANO, 1998).

TABLE I

Sample code, locality and accession numbers of *Furcifer* specimens used in this study. All other samples were from RAXWORTHY et al. (2002).

Species	Locality	Code	Accession number
<i>Furcifer cephalolepis</i>	Forêt de la Guille, Grand Comoro	GC 25	DQ086038
<i>Furcifer cephalolepis</i>	Belvédère, Grand Comoro	GC 64	DQ086039
<i>Furcifer polleni</i>	Sada road, Mayotte	MY 57	DQ086040
<i>Furcifer polleni</i>	Bandrélé	MY 63	DQ086041
<i>Furcifer polleni</i>	Bandrélé	MY 64	DQ086042
<i>Furcifer polleni</i>	Airport, Dzaouzi islet, Mayotte	MY 67	DQ086043
<i>Furcifer polleni</i>	Vahibé	MY 75	DQ086044
<i>Furcifer polleni</i>	Mahicavo	MY 79	DQ086045
<i>Furcifer polleni</i>	Longoni	MY 82	DQ086046
<i>Furcifer polleni</i>	Longoni	MY 83	DQ086047

## MATERIALS AND METHODS

Tail tips from eight *F. polleni* and two *F. cephalolepis* were collected in Mayotte and Grand Comoro (geographic locations of the specimens are given in Table 1 and Fig. 1) and genomic DNA was extracted following standard high-salt protocols. A fragment including the terminal portion of the ND4 gene and the tRNA's for Serine, Histamine and Leucine was amplified by PCR using the primers published by ARÉVALO et al. (1994) and sequences from both strands were obtained on an automated sequencer (ABI 310). Alignment was performed using Clustal W 1.6 (THOMPSON et al., 1994; default parameters) and adjusted manually in BioEdit (HALL, 1999). Sequences from other *Furcifer* species from Madagascar and Reunion Island previously published by RAXWORTHY et al. (2002) were also included. *Chamaeleo jacksoni* and *Calumma cucullata* were used as outgroups. Ambiguous alignment regions (12 bp of the tRNA's) were excluded from all analyses. To select the model of nucleotide substitution that better fits our data set, the hierarchical likelihood-ratio test was carried out using Modeltest 3.06 (POSADA & CRANDALL, 1998). Sequences were then imported into PAUP\*4.0b10 (SWOFFORD, 2003) and

the chosen model used to perform Maximum Likelihood (ML) analysis with random sequence addition (10 replicate heuristic search). Maximum Parsimony (MP) analysis was also carried out with random sequence addition (100 replicate heuristic searches) and support for nodes was estimated through the bootstrap technique (FELSENSTEIN, 1985) with 1000 replicates. Bayesian analysis was implemented using MrBayes v.3.0 (HUELSENBECK & RONQUIST, 2001) with parameters estimated as part of the analysis and four incrementally heated Markov chains with the default heating values. All analysis started with randomly generated trees and ran for  $10^6$  generations, saving one tree in each 10 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, ensuring 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). Two independent replicates were conducted and inspected for consistency to check for local optima (HUELSENBECK & BOLLBACK, 2001). To assess variation within *Furcifer polleni* (from Mayotte), these sequences

(total length of 807 bp) were joined into a median network (BANDELT et al., 2000).

## RESULTS

Ten sequences were obtained and 22 sequences, representing 14 taxa, were included in the analyses, for an aligned length of 815 bp. The most appropriate model of evolution for this dataset was the GTR, with an estimate of invariable sites (0.4116) and a discrete approximation of the gamma distribution (1.1168). ML, MP and Bayesian analyses gave congruent estimates of relationships, with ML and Bayesian trees having identical topologies, and the MP tree having one difference in topology relative to these (Fig. 2). Concerning the intraspecific diversity, the two individuals from *F. cephalolepis* had distinct haplotypes, presenting 0.87% divergence (seven differences in 807 bp). Within *F. polleni*, five distinct haplotypes were found in a total of 8 individuals, with a maximum divergence of 0.74% (six differences in 807 bp) and without any clear geographic structure (Fig. 3).

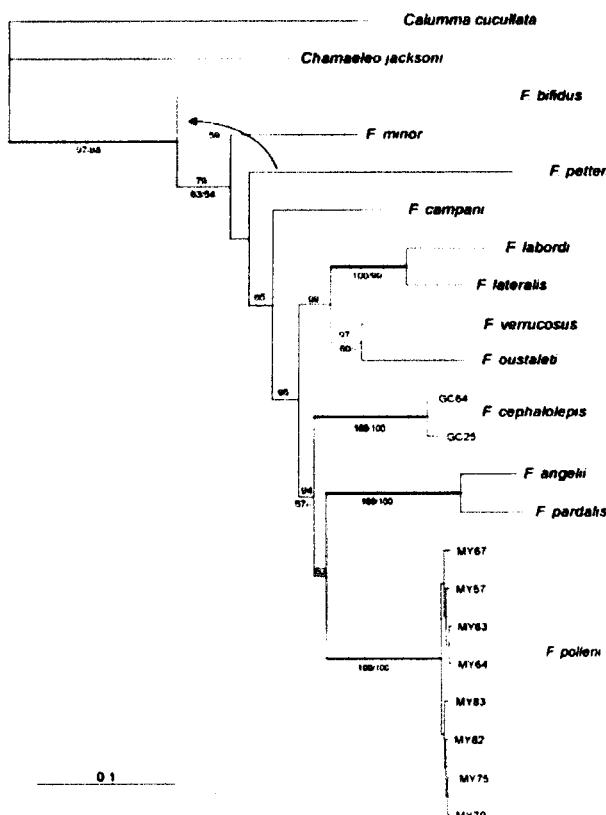


Fig. 2. – Tree derived from the Bayesian analysis of the 815 bp of the ND4 gene. Posterior probabilities are given above the branches with the bold branches having the value of 100%. Below the branches, bootstrap values for ML and MP are indicated (ML/MP). For both analyses, only bootstrap values above 50% are represented. The arrow indicates a variation in the position of one branch in the MP analysis and the respective bootstrap value is indicated below.

## DISCUSSION

Both *F. cephalolepis* and *F. polleni* represent distinct and very well supported branches. Their relative position

and long branch lengths show that they are probably not sister-taxa, as previously suggested. Indeed, independently of the method used in the analyses, *F. polleni* always appeared as sister-taxa of the *F. angeli* and *F. pardalis* group from Madagascar, with *F. cephalolepis* splitting first from their common ancestor. This, points to independent colonization of both Comoro Islands. While we cannot exclude alternative hypotheses, like the existence of a very divergent unsampled lineage in Mayotte that could be the "sister-group" of *F. cephalolepis* from Grand Comoro, these are less likely due to our geographically widespread sampling in Mayotte. Clearly, further sampling is needed, especially from the extant *Furcifer* species from Madagascar (eight species from this genus are not included in this analysis), to clarify the relationships between them, and to better understand the process of colonization of the Comoros Islands.

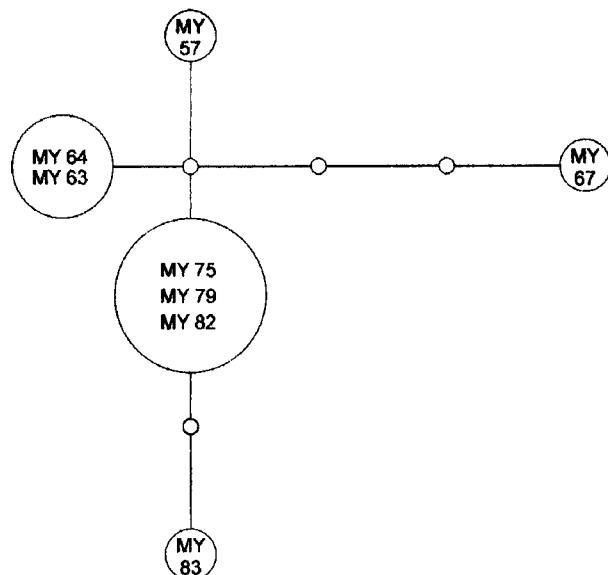


Fig. 3. – Network showing relationships between *Furcifer polleni* haplotypes found in Mayotte. Diameter of circles is proportional to the frequency of each haplotype and empty circles represent missing haplotypes.

The genetic divergence found inside *Furcifer cephalolepis* was surprisingly high, considering the age of the island. The two haplotypes from Grand Comoro have 0.87% genetic divergence (uncorrected p-distance), which, according to the vertebrate ectotherm mtDNA divergence rate – often used for this gene – of 0.4-0.6% per lineage per million year (RAND, 1994; CACCOME et al., 1999), would correspond to 0.73 to 1.1 million years divergence.

Even using a broader interval of sequence variation : 0.25-0.7% per lineage per million year (AVISE et al., 1992; CACCOME et al., 1997) we obtain estimates of divergence between 0.63 and 1.76 My, always higher than the oldest estimates for the age of the island – 0.5 My. So, the ND4 gene in *Furcifer* seems to be evolving faster than the rates generally used (at least at a value of 0.88% per lineage per million years if we use 0.5 My – age of Grand Comoro – as a calibration point. Moreover, these are minimum estimates of the divergence; based only in two individuals, as with more individuals even more divergent haplotypes could be found.

One possible explanation is that ND4 gene in *Furcifer* is evolving faster than predicted by these molecular clocks. However an alternative explanation for this result is that divergence within *F. cephalolepis* predates the colonization of Grand Comoro and this colonization was made by individuals including already differentiated mtDNA lineages.

Islands with known geological ages are often thought to be ideal for calibrating molecular clocks (CARRANZA et al., 2000) and the common procedure is that when sister-taxa are found on neighbouring islands to assume that the age of the younger island represents an approximate estimate for the maximum age of the split between the “offspring” population on the younger island and the “parental” population on the older island. However our results suggest that “universal” clocks are extremely inaccurate. Furthermore, precise phylogenies are needed – if divergence values between *F. polleni* and *F. cephalolepis* (assuming incorrectly they were sister taxa) were compared to the age of Grand Comoro we would obtain an erroneous estimated rate of evolution of at least 11% per lineage per million year. This type of calculation, focusing on the observed divergence between islands and using the age of the youngest one as a calibration point, is still commonly used (e.g. BROWN & PESTANO, 1998; WARREN et al., 2003). Our results highlight the importance of also assessing within-island diversity when estimating divergence rates.

In conclusion, our results suggest that the Comoros were independently colonized twice by *Furcifer* from Madagascar. They also suggest that this region of the ND4 gene and associated tRNA's may be evolving faster than that predicted by ectothermal vertebrate molecular clocks, which has implications for the estimated times of colonization of other island groups by chameleons. This further highlights the inaccuracies of generalized applications of molecular clocks, even when calibrated using known geological values such as the age of islands. The fast rate of evolution of this region of mtDNA makes it highly suitable for phylogeographic studies.

## ACKNOWLEDGEMENTS

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Short communication

## Diversity and phylogenetic relationships of *Hemidactylus* geckos from the Comoro islands

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### 1. Introduction

*Hemidactylus* (Gray, 1825) is a specious genus with more than 80 morphologically similar species, widespread in tropical and subtropical areas in the Pacific, Southern Europe, Asia, Central America, and Africa, where over 40 species have been described (Spawls et al., 2001). No comprehensive phylogeny is available for this genus, and the few phylogenetic studies made focused exclusively on specific areas like the Malagasy region (Vences et al., 2004a), or the Cape Verde and the Gulf of Guinea islands, off the West African coast (Jesus et al., 2001, 2005).

Like other geckos, *Hemidactylus* are particularly well suited to overseas dispersal (Kluge, 1969) and have naturally colonized both oceanic archipelagos like the Cape Verde islands (Jesus et al., 2001) and S. Tomé and Príncipe (Jesus et al., 2005) and continental but long-isolated islands like Madagascar (Vences et al., 2004a), inside which they can present considerable degrees of substructuring, in some cases producing endemic island species or subspecies. However, some species are also commensal and have been repeatedly translocated by humans (e.g., Jesus et al., 2002) resulting in unclear phylogeographic patterns shaped by both natural colonizations and anthropogenic introductions.

In a recent and extensive review of Malagasy *Hemidactylus* based on morphological and molecular data, Vences et al. (2004a) found that three *Hemidactylus* species were present in Madagascar (*H. mercatorius*, *H. platycephalus*, and *H. frenatus*) and a fourth one, *H. brooki*, in the Mascarenes and in the Comoros. The distribu-

tion and the phylogeographic structure of each species was, however, quite different.

*Hemidactylus mercatorius* is by far the most widespread species in Madagascar, occurring throughout the island, and is subdivided in two distinct genetic lineages, with the North-Eastern individuals presenting between 1.8 and 2.8% genetic divergence (data from the 16S rRNA gene) from all the others from Madagascar and with all Malagasy specimens differing strongly from the single African specimen included. This scenario points to an ancient and natural colonization of Madagascar by this species, or by its ancestor, questioning whether the Malagasy specimens are actually conspecific with the strongly differentiated African individual studied. The only Comoroan sample included in this study (one individual from Mayotte) clearly grouped inside the Central-Southern Madagascan clade and thus a recent anthropogenic introduction from Central Madagascar was considered as the probable origin for Mayotte populations (Vences et al., 2004a). *H. frenatus* appears to be a coastal species, so far only found in the West and North of Madagascar. The distribution of *H. platycephalus* seems to be even more restricted to only Northwestern and Northern lowland areas. The Malagasy individuals of *H. platycephalus* studied all shared the same haplotype and were clearly differentiated from the African and the Comoroan specimens studied. Vences et al. (2004a) suggested an African or Comoroan origin for the Malagasy populations of *H. platycephalus* which were likely a result of a very recent colonization or introduction.

*Hemidactylus frenatus* individuals analyzed included samples from the Andaman islands, Sri Lanka, Madagascar and the Comoros and Mascarenes archipelagos,

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within which no recognizable phylogeographic structure was observed. Nevertheless, and in parallel to what was observed in *H. mercatorius*, one individual from the extreme North of Madagascar presented a haplotype strongly divergent from others from the Northwestern coast and from the Comoros. Due to this congruence in phylogeographic patterns, of divergent haplotypes in Northern Madagascar, also observed in other taxa like *Ptychadena mascareniensis* (Vences et al., 2004b), the authors proposed that an unrecognized area of endemism exists in coastal Northern Madagascar and that, in this case, at least *H. frenatus* may have multiple origins—native and introduced—on Madagascar.

*Hemidactylus brooki* did not show any phylogeographic structure, with individuals from the Mascarenes having haplotypes close to ones from Sri Lanka but also from Comoros.

Our study focused in detail on the *Hemidactylus* of the Comoros archipelago, a volcanic chain of four major islands lying approximately halfway between Madagascar and the Eastern African coast (Fig. 1A), with ages of origin between 10–15 My (the oldest island, Mayotte) and 0.5 My (the youngest island, Grand Comore). These islands never had physical contact to other landmasses. They are separated from each other and from Africa and Madagascar by sea depths of more than 3600 m (Emeick and Duncan, 1982; Nougier et al., 1986). Studying the island forms and their affinities with the “continental” species may help to clarify the origins, evolutionary history, and patterns of dispersal of these species. Moreover, species-level phylogenies on island settings can potentially provide useful insights into the rate of species diversification or lineage accumulation with time (Barraclough and Nee, 2001; Lovette and Bermingham, 1999; Ricklefs and Bermingham, 2001).

We used nuclear and mitochondrial DNA sequence data to assess: (a) how many species of *Hemidactylus* occur on the Comoros, (b) what is the relationship between the populations from different islands, (c) what are the relationships between the populations of *Hemidactylus* from the Comoros and the ones from other islands of the Indian Ocean (especially Madagascar), African, and Asian, and (d) if the *Hemidactylus* populations from the Comoro islands are a result of natural colonizations or recent anthropogenic introductions.

## 2. Materials and methods

*Hemidactylus* specimens were collected from the four major islands of the Comoros archipelago, in total nine *H. mercatorius*, 33 *H. platycephalus*, two *H. brooki*, and three *H. frenatus* (see Table 1 and Fig. 1B) for geographical locations). Genetic samples were taken in the field by preserving muscle tissue (autotomized tails) in pure etha-

nol and total genomic DNA was extracted from tail tissue using standard methods (Sambrook et al., 1989).

We used primers 16SA-L and 16SB-H (Palumbi et al., 1991) to amplify a section of approximately 500 bp of the mitochondrial 16S ribosomal RNA gene, using conditions described in Harris et al. (1998). For the nuclear gene *C-mos* (about 300 bp) we used primers G73 and G74 and amplification conditions given in Saint et al. (1998). We chose these fragments so that we could combine our sequences with previously published data of Vences et al. (2004a) and Jesus et al. (2005). Amplified fragments were sequenced on a 310 Applied Biosystem DNA sequencing apparatus and sequences aligned using BioEdit (Hall, 1999).

For the 16S rRNA gene we analyzed separate sequence sets for each of the four *Hemidactylus* species. Only single indels were observed and alignment was unambiguous. These were aligned against published sequences of the same species from the Malagasy region (Vences et al., 2004a) and also from *Hemidactylus* from the Gulf of Guinea islands (Jesus et al., 2005). As the mitochondrial divergence inside each species was low, *C-mos* sequences were only collected from one individual from each species (and from distinct mtDNA lineages inside each species, in the case of *H. mercatorius*) and aligned against published sequences of *Hemidactylus* (Jesus et al., 2005; Whiting et al., 2003). For each dataset sequences were joined in median-joining networks (Bandelt et al., 2000).

## 3. Results and discussion

Understanding the biogeography of organisms is a challenge considerably increased in the cases of species that have extraordinary colonization capabilities and that are often anthropogenically transported, as is the case with *Hemidactylus*. Oceanic island systems such as the Comoros, that despite their small geographic size can contain a considerable diversity of habitats, are especially prone places to harbor endemic lineages of organisms but also introduced populations, that can increase the *within-population* genetic variation in a way that can have serious conservation implications (see Kolbe et al., 2004). A deep knowledge about the history of the populations is thus required and assessing genetic diversity at the species level is a powerful way to unveil patterns of colonization and distinguish between natural colonizations and introductions.

Our fieldwork and molecular analysis considerably further our understanding of the diversity and origins of the *Hemidactylus* from the Comoros archipelago. Four species were detected: *H. mercatorius*, *H. platycephalus*, *H. frenatus*, and *H. brooki*, although *H. platycephalus* was clearly the most abundant.

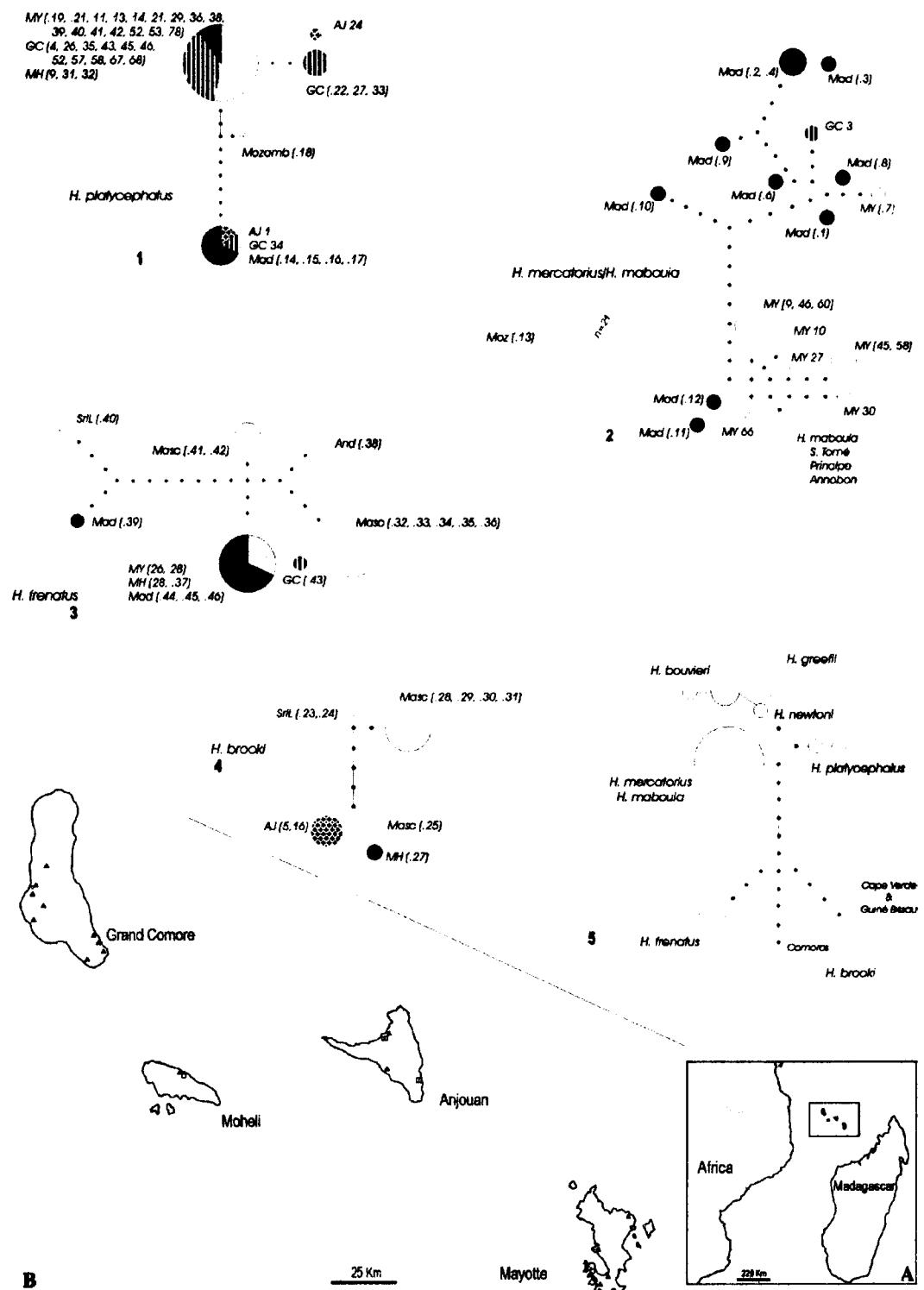


Fig. 1. (1–4) Haplotype (median-joining) networks for the four species of *Hemidactylus*, based on about 500 bp of the 16S rRNA gene. All individuals analyzed are identified by their code, next to the respective haplotype. Haplotypes found in Madagascar and Comoros are highlighted with different colors: black, Madagascar; light gray, Mayotte; dark gray, Moheli; crosses, Anjouan; and vertical stripes, Grand Comoro. In *H. mercatorius* some homoplasious positions inside the main groups make the original network too complicated to be represented. As the general topology does not change, only one of the possible trees is shown. (5) Median-joining network showing the relationships between *Hemidactylus* species from Malagasy and Gulf of Guinea regions, inferred from partial sequences of *C-mos* nuclear gene. In all networks small circles represent presumed missing haplotypes. (A) Location of the Comoros archipelago, approximately halfway between Madagascar and the East African coast. (B) Sampling places for *H. mercatorius* (●), *H. platycephalus* (▲), *H. frenatus* (□), and *H. brooki* (⊗) in the Comoros archipelago.

**Table 1**  
Individuals used in this study and respective locations

Species	Individual	Location, island	Accession number 16S/C-mos
<i>H. mercatorius</i>	MY 9	Mamoutzu, urban, Mayotte	AY863031
<i>H. mercatorius</i>	MY 10	Mamoutzu, urban, Mayotte	AY863032 / AY863046
<i>H. mercatorius</i>	MY 27	Bouéni (road to), Mayotte	AY863033
<i>H. mercatorius</i>	MY 30	Bouéni, Mayotte	AY863035
<i>H. mercatorius</i>	MY 45	Chiroungoui, Mayotte	AY863034
<i>H. mercatorius</i>	MY 46	Chiroungoui, Mayotte	AY863031
<i>H. mercatorius</i>	MY 58	Sada road, Mayotte	AY863034
<i>H. mercatorius</i>	MY 60	Bambo Est Plage, Mayotte	AY863031
<i>H. mercatorius</i>	MY 66	Dzaouzi road airport, Mayotte	AY863036
<i>H. mercatorius</i>	GC 3	Moroni, urban, Grand Comore	AY863037 / AY863046
<i>H. mercatorius</i>	.1	Ampirojoa (Ankarafantsika), Madagascar	AY517581
<i>H. mercatorius</i>	.2	Tolagnaro, Madagascar	AY517577
<i>H. mercatorius</i>	.3	Tolagnaro, Madagascar	AY517576
<i>H. mercatorius</i>	.4	Tolagnaro, Madagascar	AY517577
<i>H. mercatorius</i>	.6	Antananarivo, Madagascar	AY517579
<i>H. mercatorius</i>	.7	Mamoudzou, Mayotte	AY517580
<i>H. mercatorius</i>	.8	Ankarafantsika, Madagascar	AY517575
<i>H. mercatorius</i>	.9	Andranofotsy, Madagascar	AY517582
<i>H. mercatorius</i>	.10	Nosy Be, Madagascar	AY517583
<i>H. mercatorius</i>	.11	Montagne des Francais, Madagascar	AY517584
<i>H. mercatorius</i>	.12	Sambava, Madagascar	AY517585
<i>H. mercatorius</i>	.13	Zambezia, Mozambique	AY517586
<i>H. platycephalus</i>	MY 11	Chirongoui, Mayotte	AY517573
<i>H. platycephalus</i>	MY 13	Chirongoui, Mayotte	AY517573
<i>H. platycephalus</i>	MY 14	Chirongoui, Mayotte	AY517573
<i>H. platycephalus</i>	MY 21	Kani-Kéli, Mayotte	AY517573
<i>H. platycephalus</i>	MY 29	Bouéni, Mayotte	AY517573
<i>H. platycephalus</i>	MY 36	Bouéni (road to), Mayotte	AY517573
<i>H. platycephalus</i>	MY 38	Bambo Ouest, Mayotte	AY517573
<i>H. platycephalus</i>	MY 39	M'bouanatsa, Mayotte	AY517573
<i>H. platycephalus</i>	MY 40	Chirongoui (Plage Mtih), Mayotte	AY517573
<i>H. platycephalus</i>	MY 41	Chirongoui (Plage Mtih), Mayotte	AY517573
<i>H. platycephalus</i>	MY 42	Chirongoui (crossroads), Mayotte	AY517573
<i>H. platycephalus</i>	MY 52	Sada road, Mayotte	AY517573
<i>H. platycephalus</i>	MY 53	Sada road, Mayotte	AY517573
<i>H. platycephalus</i>	MY 78	Majicavo	AY517573
<i>H. platycephalus</i>	GC 4	Moroni, urban, Grand Comore	AY517573
<i>H. platycephalus</i>	GC 26	Hantoidzi (Chezani), Grand Comore	AY517573
<i>H. platycephalus</i>	GC 27	Hantoidzi (Chezani), Grand Comore	AY517571
<i>H. platycephalus</i>	GC 33	Itsoundzou, Grand Comore	AY517571
<i>H. platycephalus</i>	GC 34	Mouadzazi, Grand Comore	AY517574
<i>H. platycephalus</i>	GC 35	Mouadzazi, Grand Comore	AY517573
<i>H. platycephalus</i>	GC 43	Ifoundihé Chambouani, Grand Comore	AY517573
<i>H. platycephalus</i>	GC 45	Foumbouni, Grand Comore	AY517573
<i>H. platycephalus</i>	GC 46	Foumbouni, Grand Comore	AY517573
<i>H. platycephalus</i>	GC 52	Konbani, Grand Comore	AY517573
<i>H. platycephalus</i>	GC 57	Mvouni, Grand Comore	AY517573
<i>H. platycephalus</i>	GC 58	Mvouni, Grand Comore	AY517573
<i>H. platycephalus</i>	GC 67	Hantoidzi (Chezani), Grand Comore	AY517573
<i>H. platycephalus</i>	GC 68	Hantoidzi (Chezani), Grand Comore	AY517573
<i>H. platycephalus</i>	AJ 1	Moutsamoudou, urban, Anjouan	AY517574
<i>H. platycephalus</i>	AJ 24	Pomoni, Anjouan	AY863039 / AY863045
<i>H. platycephalus</i>	MH 9	Fomboni, urban, Moheli	AY517573
<i>H. platycephalus</i>	MH 31	Fomboni, urban, Moheli	AY517573
<i>H. platycephalus</i>	MH 32	Fomboni, urban, Moheli	AY517573
<i>H. platycephalus</i>	.22	Chomoni, Grand Comoro	AY517571
<i>H. platycephalus</i>	.18	Zambezia, Mozambique	AY517572
<i>H. platycephalus</i>	.21	Chissioua Mtsamboro, Mayotte	AY517573
<i>H. platycephalus</i>	.19	Mt. Combani, Mayotte	AY517573
<i>H. platycephalus</i>	.15	Nosy Be, Madagascar	AY517574

(continued on next page)

Table 1 (continued)

Species	Individual	Location, island	Accession number 16S/C-mos
<i>H. platycephalus</i>	.14	Nosy Sakatia, Madagascar	AY517574
<i>H. platycephalus</i>	.16	Antsiranana, Madagascar	AY517574
<i>H. platycephalus</i>	.17	Nosy Be, Madagascar	AY517574
<i>H. frenatus</i>	MY 26	Boueni (road to), Mayotte	AY517566
<i>H. frenatus</i>	MY 28	Boueni (road to), Mayotte	AY517566
<i>H. frenatus</i>	MH 28	Fomboni, urban, Moheli	AY517566
<i>H. frenatus</i>	.43	Moroni, Grand Comoro	AY517565
<i>H. frenatus</i>	.44	Ankarafantsika, Madagascar	AY517566
<i>H. frenatus</i>	.37	Fomboni, Moheli	AY517566
<i>H. frenatus</i>	.45	Antsiranana, Madagascar	AY517566
<i>H. frenatus</i>	.46	Nosy Be, Madagascar	AY517566
<i>H. frenatus</i>	.40	Negombo, Sri Lanka	AY517563
<i>H. frenatus</i>	.39	Montagne des Francais, Madagascar	AY517562
<i>H. frenatus</i>	.32	Porth Mathurin, Rodrigues, Mascarenes	AY517561
<i>H. frenatus</i>	.33	Cotton Bay, Rodrigues, Mascarenes	AY517561
<i>H. frenatus</i>	.34	near Grand Bay, Rodrigues, Mascarenes	AY517561
<i>H. frenatus</i>	.35	Morne Brabant, Mauritius, Mascarenes	AY517561
<i>H. frenatus</i>	.36	Pointe aux Roches, Mauritius, Mascarenes	AY517561
<i>H. frenatus</i>	.38	Port Blair, Andamane Islands	AY517564
<i>H. frenatus</i>	.41	La Saline des Baines, Réunion, Mascaren.	AY517560
<i>H. frenatus</i>	.42	La Saline des Baines, Réunion, Mascaren.	AY517560
<i>H. frenatus</i>		Port Moresby, Papua New Guinea	AY217852
<i>H. brookii</i>	AJ 5	Moutsamoudou, urban, Anjouan	AY863040 / AY863047
<i>H. brookii</i>	AJ 16	Mboüeladoungou, Anjouan	AY863040
<i>H. brookii</i>	.23	Kitulgala, Sri Lanka	AY517570
<i>H. brookii</i>	.24	Kitulgala, Sri Lanka	AY517570
<i>H. brookii</i>	.28	Porth Mathurin, Rodrigues, Mascarenes	AY517569
<i>H. brookii</i>	.29	Pointe aux Roches, Rodrigues, Mascaren.	AY517569
<i>H. brookii</i>	.30	La Saline les Bains, Réunion, Mascarenes	AY517569
<i>H. brookii</i>	.31	Porth Mathurin, Rodrigues, Mascarenes	AY517569
<i>H. brookii</i>	.25	La Saline des Baine, Réunion, Mascaren.	AY517567
<i>H. brookii</i>	.27	Nioumachoua, Moheli	AY517568
<i>H. brookii</i>	121	Bissau—Guinea	AY863048
<i>H. brookii</i>	HB38	Santo Antão—Caeo Verde	AY863049
<i>H. mabouia</i>	705	São Tomé e Príncipe, Príncipe	AY863038 / AY863046
<i>H. mabouia</i>	557	Ilhéu das Rolas—ST6	AY863038 / AY863046
<i>H. mabouia</i>	558	Ilhéu das Rolas—ST6	AY863038 / AY863046
<i>H. mabouia</i>	668	Annobon	AY863038 / AY863046
<i>H. mabouia</i>	669	Annobon	AY863038 / AY863046
<i>H. bowieri</i>	168	Boavista—Sal Rei—Cape Verde Islands	AY863041
<i>H. bowieri</i>	CV38	Boavista—Sal Rei—Cape Verde Islands	AY863042
<i>H. bowieri</i>	CV127	Sal—Cape Verde Islands	AY863042
<i>H. newtoni</i>	667	Annobon	AY863043
<i>H. greeffi</i>	703	Nova Estrela—P2	AY863044
<i>H. greeffi</i>	569	Vale do Contador—ST7	AY863044

Codes are the same used in Fig. 1. When referring to data from other authors, codes refer to individual's identification in the original article (and Fig. 1) and accession numbers are also provided. When referring to the same haplotype just one accession number is provided.

The sizes of the fragments of the 16S rRNA gene used to construct the median-joining networks (Fig. 1(1–4)) were slightly different between the different species and varied between 481 bp in *H. mercatorius* and 496 bp in *H. frenatus* and *H. brooki*.

*Hemidactylus mercatorius* is more abundant in the island of Mayotte than in the remaining archipelago. Seven haplotypes were found in the 10 individuals sampled, showing a considerable degree of genetic diversity. Most of them were closely related (five to nine substitutions) and clearly grouped with the haplotypes from

Northern Madagascar described by Vences et al. (2004a). However, one of the individuals from Grand Comore (GC3) differed from these by a high number of mutational steps (20–25 substitutions) clustering inside the Western-Central group from Madagascar, as was the case of one individual from the island of Mayotte analyzed by Vences et al. (2004a). The individual representing the *H. mabouia* from the Gulf of Guinea islands of S. Tomé, Príncipe, and Annobon (*H. mabouia* from these islands all share the same haplotype for the 16S rRNA gene; Jesus et al., 2005) clearly grouped with the *H. mercatorius*

group from Northern Madagascar and Comoros (differing from individuals from Mayotte by four mutational steps), and not with the African individual analyzed by Vences et al. (2004a) assigned to the species *H. mercatorius*, that differs from the other individuals from Madagascar and Comoros by at least 25–31 mutational steps.

These results clearly change the scenario proposed by Vences et al. (2004a) for the origins of this species in the Comoros archipelago. Thus, instead of only introduced individuals from Western-Central Madagascar, an endemic lineage, arising from a natural and ancient colonization of Mayotte, presumably from Northern Madagascar is also present. Nonetheless, sampling in North Madagascar is limited, and more samples from this region are needed to assess more precisely variation in this lineage. Furthermore, the presence of one individual on Grand Comore that, as the one previously identified by Vences et al. (2004a), groups with the Western-Central haplotypes from Madagascar, further supports hypothesis that occasional anthropogenic translocations and introductions also occur (Raselimanana and Vences, 2003).

The results were quite different in the case of *H. platycephalus*, from which 33 individuals from widespread locations across the four islands were analyzed and only four haplotypes were found (Fig. 1(1)). Furthermore, these haplotypes presented no phylogeographic structure at all: most of the individuals (29, from three distinct islands—Mayotte, Moheli, and Grand Comore) shared the same haplotype, a sign of a rapid and recent expansion of this species in the Comoros, and four individuals (one from Anjouan and three from Grand Comore) had haplotypes that differed from this only by three and four mutations, respectively. Another two individuals (one from Anjouan and one from Grand Comore) had an haplotype identical to all the Malagasy individuals analyzed by Vences et al. (2004a) and that differed by 10 mutational steps from the first one. The only African individual analyzed (Vences et al., 2004a) is located approximately halfway between these two lineages (Fig. 1(1)). This species is distributed across Central and East Africa, the Comoros, and Madagascar. We could hypothesize a scenario of two colonizations from Africa, one to Comoros and another to Madagascar, with later occasional introductions to the Comoros of Malagasy individuals or two independent colonizations of Comoros, with Madagascar being colonized from there. Nevertheless, further sampling in Madagascar and Africa is needed to understand the genetic complexity of this species outside of the Comoros and answer this question.

In *H. frenatus*, the three individuals sampled (two from Mayotte and one from Moheli) exhibited the same haplotype that another individual from Moheli and specimens from North, Northwest and West Madagascar (Vences et al., 2004a) and the fourth species, *H. brooki*, was found only in one of the islands, Anjouan (two individuals), where both individuals shared the same

haplotype, which is closely related to an haplotype found in the Mascarenes and to the one exhibited by another individual from the Moheli island in the Comoros archipelago (Fig. 1(4)). These are apparently the least abundant species in the Comoros, and at least in *H. frenatus*, the share of the same haplotype between Malagasy and Comoroan populations points to a very recent presence of this species both in Comoros and Madagascar, again possibly due to human introductions.

The analyses of variation in C-mos DNA sequences (a slow-evolving nuclear gene) allowed us a wider comparison and a picture of the relationships between several *Hemidactylus* species from West Africa and the Malagasy region (Fig. 1(5)). We chose to use only this fragment for this purpose because large portions of the 16S rRNA gene fragment were not unambiguously alignable across all species. It is clear from these analyses that *H. brooki* from the Malagasy region (Comoros) and from West Africa (Cape Verde and Guiné Bissau) constitute two genetically differentiated lineages sharing a common ancestor but with a minimum of 11 mutational steps between them (Fig. 1(5)). This level of divergence is similar to the divergence found between each of the *H. brooki* lineages and *H. frenatus* and much greater than the divergence found between the other *Hemidactylus* species or between other gecko species such as *Tarentola* sp. (Carranza et al., 2002; Harris et al., 2004a), suggesting a species complex, which deserves further investigation, including analysis of morphological traits. There are very different levels of differentiation between species; while the two *H. brooki* lineages and *H. frenatus* are very differentiated between themselves and in relation to the other species, *H. bouvieri*, *H. greefi*, *H. newtoni*, *H. platycephalus*, and *H. mercatorius/mabouia* form a group of closely related species, which differ from each others by no more than three substitutions. Sampling of other species for this gene region may help to resolve the phylogeny of the group.

Concerning the *H. mercatorius/H. mabouia* “species complex” our data once again indicates that the exact species delimitations and phylogeographic structure are far from being understood. Vences et al. (2004a), following the taxonomic arrangement of Kluge (1991, 2001), considered *H. mercatorius* as a valid species assigning this name to Malagasy and African populations previously considered *H. mabouia*. However, they found a great differentiation between the African and the Malagasy individuals (24–29 substitutions in the 16S rRNA gene fragment analyzed). Interestingly, when we compared these with individuals of *H. mabouia* from the Gulf of Guinea islands, we found that they clearly nest inside the Northern Madagascar-Mayotte clade and not with the African individual (see *H. mercatorius* network, Fig. 1(2)). For C-mos there was no difference between both Malagasy lineages and the individuals from the Gulf of Guinea islands analyzed by Jesus et al. (2005).

Two hypothesis can explain these observations: (1) the individuals from Gulf of Guinea islands were introduced there from Mayotte, and maybe also on the West African mainland, where its distribution is spotty, perhaps indicating multiple introductions through seaports (Spawls et al., 2001), or (2) the picture is far more complicated with a probable species complex existing in the African mainland. Extensive sampling in East Africa and North Madagascar is crucial to address this question. The degree of genetic diversity observed in the Malagasy–Comoroan populations strongly contrasts with the unique haplotype shared by all the populations from the Gulf of Guinea islands, and the divergence between these and the African individual analyzed is between 4.8 and 5.8%. Although geckos often present very high degrees of intraspecific mitochondrial sequence divergence (Harris et al., 2004b), this is a high value and deserves further investigation.

#### 4. Conclusions

The Comoro islands are inhabited by the same three species of *Hemidactylus* that occur on Madagascar; *H. mercatorius*, *H. platycephalus*, and *H. frenatus* and by a fourth one, *H. brooki*. Although *H. platycephalus* is by far the most abundant species on these islands, and its presence may be due to a natural colonization from Africa, *H. mercatorius* is the species that exhibits more genetic variation, pointing to a natural and ancient colonization of at least one of the islands, Mayotte, from Madagascar. Apparently introduced individuals of *H. mercatorius* from Western-Central Madagascar were also detected, that can have serious conservation implications. *H. frenatus* and *H. brooki* are the least abundant species in the Comoros: individuals from *H. frenatus* are closely related to the Malagasy ones and *H. brooki* occurs at least in Moheli and Anjouan, with individuals from the Mascarenes islands being the most closely related to them.

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

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### 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.

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## INTRODUCTION

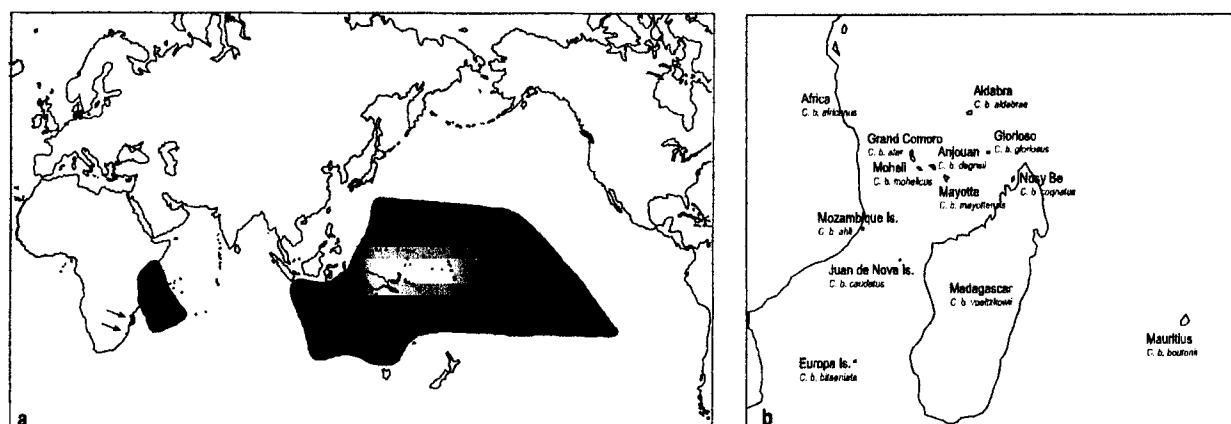
*Cryptoblepharus* is a pan-Pacific genus of c. 36 morphologically similar skinks, formerly considered as subspecies of a single variable species, *Cryptoblepharus boutonii* (Mertens, 1934; Greer, 1974). These lizards occur in two disjunct areas: (1) the eastern end of the Indo-Australian archipelago, 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. glorus* (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).

Biogeography of *Cryptoblepharus* in the Western Indian Ocean

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 *Leiolopisma telfairi* (Caranza & 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 (Helsenbeck & 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  $2 \times 10^6$  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 (Helsenbeck & 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

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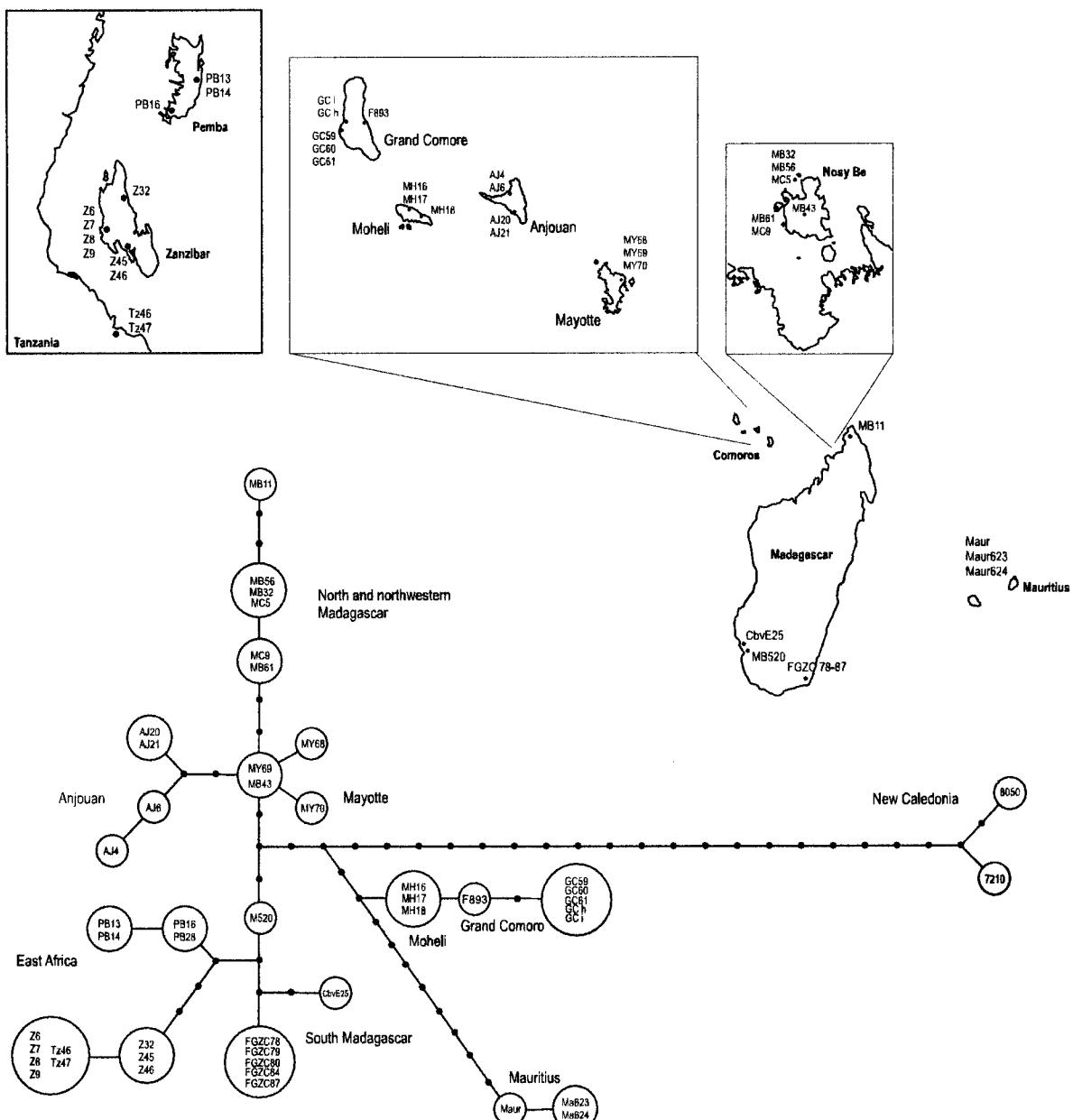
**Table 1** List of samples used for analysis, geographic locations, and GenBank accession numbers for 12s and 16s

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. degrisi</i>	AJ4	Moutsamoudu	Anjouan; Comoros	DQ118047/DQ118068
<i>C. b. degrisi</i>	AJ6	Moutsamoudu	Anjouan; Comoros	DQ118046/DQ118067
<i>C. b. degrisi</i>	AJ20	Moya	Anjouan; Comoros	DQ118045/DQ118066
<i>C. b. degrisi</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	DarEsSalam, Msanani penins.	continental Tanzania	DQ118050/DQ118071
<i>C. b. africanus</i>	TZ47	DarEsSalam, 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>Leiolopisma 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

Biogeography of *Cryptoblepharus* in the Western Indian Ocean

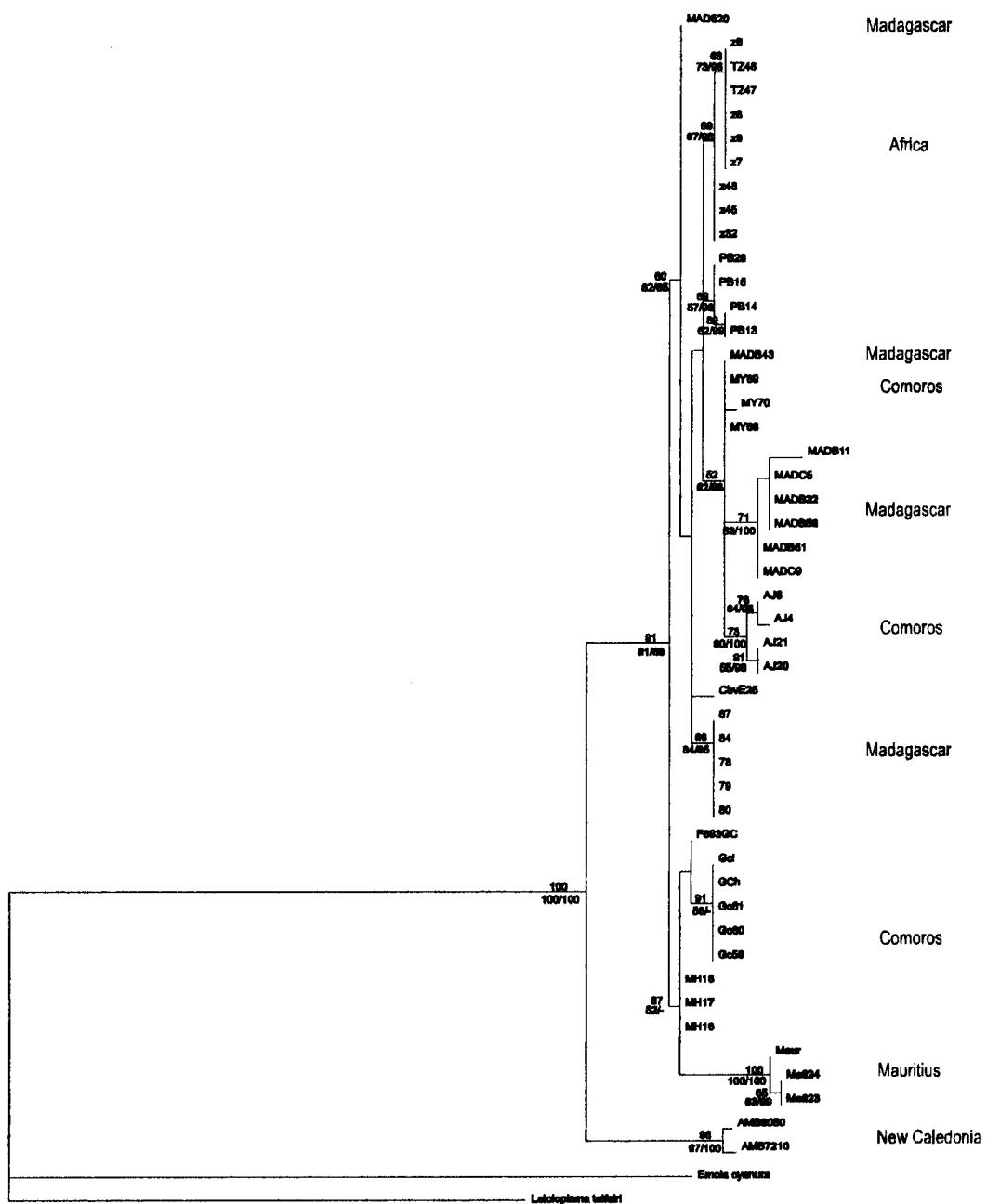
**Figure 2** Sampling localities in Tanzania (east Africa), the Comoros archipelago, Madagascar and Mauritius (grey dots), and haplotype median-joining network of the analysed subspecies of *Cryptoblepharus boutonii*, using combined 12s and 16s rRNA gene fragments. Each circle corresponds to one observed haplotype, with the size of the circle proportional to the number of individuals in which the haplotype was found (inside). Small black dots represent missing haplotypes.

*Cryptoblepharus* (Fig. 2) revealed clear geographical structuring. Madagascar harbours the highest haplotype diversity, as seven haplotypes were found from seven localities, with as many as 14 mutations between them. These can be placed into two putative groups distributed in north-western Madagascar, (1) Nosy Be and surrounding islets and (2) southern Madagascar. The haplotypes from the individuals from the Comoros archipelago can also be divided into two clades: one representing the populations from Mayotte and Anjouan (the two islands closest to Madagascar), and the

other formed by the populations from Moheli and Grand Comore. The haplotypes from Mayotte and Anjouan are clearly derived from the north-western Madagascar ones, while the origin of the Moheli and Grand Comore ones, with just one mutational step between them, cannot be ascertained with certainty.

The only case of haplotype sharing between distinct morphological forms occurred between one individual from Nosy Be (2000B43) and one from Mayotte (MY69). Whether this represents incomplete lineage sorting or is the result of

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**Figure 3** Maximum likelihood phylogram of all the *Cryptoblepharus* individuals sequenced for 12s and 16s rRNA. *Emoia cyanura* and *Leiolopisma telfari* 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.

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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 *Leiolopisma* 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.

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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. degrisi*) 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 *degrisi* 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 Comoroan 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 Réunion 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.

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## **DISCUSSION.**

Specific Diversity in the Study Groups

Origins, Diversity and Colonisation Pathways of Comoroan *Furcifer*, *Hemidactylus* and *Cryptoblepharus*

Within Island Differentiation

Dispersal Abilities of Different Groups

Comparison with Colonisation Patterns of Other Volcanic Islands

Rates of Molecular Evolution

Probable Species Complexes

Probable Instances of Anthropogenic Introductions

Taxonomic Remarks

Conservation Issues

Concluding Remarks

References

The present study investigates mitochondrial DNA differentiation within some reptile groups from the Comoro islands (in one of the groups also nuclear DNA data was acquired), and represents a first step towards understanding evolution and biogeography within this archipelago. Relationships and levels of differentiation between lineages and island geology are of considerable use when evaluating historical colonisation patterns. We used all these kinds of information in an attempt to understand the origins and evolution of these groups in the Malagasy region and to infer putative biogeographic scenarios.

Two plausible sources of colonisers flank the Comoros archipelago: Madagascar and East Africa. Within the analysed groups we found species with their origin in both these areas. In some cases, the ancestors of the Comoroan species are well and long-time established groups in their areas of origin but in others they are also recent-arrived to these, in some cases probably from South East Asia. Some species colonised the Comoros a long time ago and have since then been evolving independently, now representing well-differentiated forms from their ancestors. Others may represent recent colonisations or introductions. Examples of diversification within the archipelago and incipient speciation processes (subspecific divergence) can also be found, with the *Cryptoblepharus* genus being, among the studied ones, the most striking example. Data collected in the Comoros and their comparison with other available data helped to unveil some probable species complexes within the genus *Hemidactylus* and also provided some insights on mutation rates of specific gene fragments and groups. Significative intra-island genetic differentiation was only observed in one species (*Furcifer cephalolepis*), thus relationships between within-island volcanism and genetic variation could not be further explored.

By comparing our own data with data from other volcanic archipelagos such as the Canary and Cape Verde Islands we discuss general trends on islands colonisation and/or stochasticity as primer factor determining island biota composition. These kinds of assessments are of major importance for conservation of island biotas, and this topic is also highlighted in this discussion. Some taxonomic implications of the results are discussed.

## 1. Specific Diversity in the Study Groups

Within the studied genera, distinct numbers of species are found in the archipelago. *Furcifer* chameleons are represented only by two species, both single island endemics; *Hemidactylus* geckos by four species, none of them endemic from the islands; and the skinks of the genus *Cryptoblepharus* by four subspecies, also each one being a single island endemism.

## 2. Origins, Diversity and Colonisation Pathways of Comoroan *Furcifer*, *Hemidactylus* and *Cryptoblepharus*

### 1. Genus *Furcifer*

Besides the Comoroan endemics *Furcifer cephalolepis*, from Grand Comoro, and *Furcifer polleni*, from Mayotte, all the fourteen species of this genus inhabit Madagascar, with one also present in Mauritius and Reunion islands. The main question concerning this group was whether the Comoroan species resulted from two independent colonisation events from Madagascar, one to each island, or of only one colonisation from Madagascar, to one of the islands, with posterior colonisation of the other. If this was the case, direction of colonisations was also to be determined. Previous studies based on morphological characters placed the species from the Comoros as sister species (RAXWORTHY ET AL. 2002), making the second hypothesis more likely. However, our DNA sequence based phylogeny provided some support for the first hypothesis: *Furcifer polleni* and *Furcifer cephalolepis* are too differentiated (11% of an 807bp fragment of the mitochondrial ND4 gene) for it to be reasonable to hypothesise a divergence posterior to any island colonisation, even considering broad intervals of evolution rates for the ND4 gene fragment (0.25-0.7% per lineage per Myr; AVISE 1992, CACCONE ET AL. 1997), if we take into account islands ages. Plus, *F. polleni* always results as sister-species of the *F. angeli* and *F. pardalis* group from Madagascar, with *F. cephalolepis* splitting first from them. Eight species from this genus from Madagascar could not be included in the analysis. We hypothesise that their inclusion will clarify deeper relationships within the genus and also provide support for our double origin hypothesis.

### 2. Genus *Hemidactylus*

The four species of *Hemidactylus* geckos (*H. mabouia/mercatorius*, *H. platycephalus*, *H. frenatus* and *H. brooki*) present in the Comoros have distinct evolutionary histories that still remain largely undeciphered.

*Hemidactylus mercatorius* (*sensu* KLUGE 1991, 2001) is distributed across the Eastern African coast, Madagascar and the Comoros (Figure 3). The relationships of this taxon with *H. mabouia*, distributed in all sub-saharan Africa, South America and the Caribbean are complex and, clearly, further genetic research will be needed to clarify the status of both forms. Genetic diversity across the African mainland is unknown but deep divergence was observed between a single haplotype from Mozambique (East Africa) and all the Malagasy ones, and two distinct and geographically structured groups were observed within Madagascar (VENCES ET AL. 2004). In the Comoros, this species was mostly found in the easternmost island, Mayotte, exhibiting several mtDNA haplotypes that are closer to the northern Madagascar group (article 2: Fig 1.2). However, individuals assigned to *H. mabouia* from the Gulf of Guinea islands (West Africa) clearly cluster within this North Madagascar-Comoros group. An ancient colonisation from East Africa to Madagascar and a North-South vicariance inside Madagascar was proposed by VENCES ET AL. (2004) to explain their observed patterns. Although our Comoroan haplotypes are clearly related to the North Madagascar ones, leading us to postulate an old colonisation of Mayotte from North Madagascar, given the position of the

Gulf of Guinea haplotypes we cannot unambiguously propose a Northern Madagascar origin for the Comoroan individuals until genetic diversity in mainland Africa is properly assessed.

*Hemidactylus platycephalus* is distributed through East Africa, Comoros and Madagascar, where it is restricted to Northwestern and Northern lowland areas (Figure 3). Homogeneity was found within individuals from Madagascar, all sharing the same haplotype, almost equally differentiated from the one found in Africa and the ones from the Comoros. Thus, a recent African or Comoroan origin was proposed by VENCES ET AL. (2004) for Malagasy populations. In the Comoros we found two distinct groups of haplotypes: most individuals, widespread across the four islands share the same mtDNA haplotype or very closely related ones. Two individuals, from Anjouan and Grand Comoro, share the haplotype from Madagascar (article 2: Fig 1.1). Once again, the history of these populations remains unclear. The low levels of mtDNA variation across all the islands suggest a recent colonisation from Africa and expansion of this species, with one almost non-variable lineage widespread across the four islands. The presence of the Malagasy haplotype may be explained by posterior colonisations or introductions from Madagascar to Comoros. An alternative hypothesis, although, in our opinion, less likely, is that Madagascar was in fact colonised recently, from the Comoros.

*Hemidactylus frenatus* and *Hemidactylus brooki* are the least abundant species within this genus in the Comoros. The sharing of mtDNA haplotypes between geographically-spread Malagasy and Comoroan individuals lead us to propose a recent origin for both Malagasy and Comoroan populations of *H. frenatus*, which is primarily an oriental species (SPAUALS ET AL. 2001). A strongly divergent haplotype found in the extreme north of Madagascar (VENCES ET AL. 2004) may indicate more than one colonisation or introduction of this species to Madagascar. Variation and distribution of both lineages within Madagascar has still to be determined. *Hemidactylus brooki* is widely spread across Central Africa, including Cape Verde and Gulf of Guinea islands, the Indian subcontinent and Southeast Asia. Differentiation within the slow-evolving nuclear gene C-mos indicates that *H. brooki* is in fact a species complex (see page 69 and article 2 – Fig. 1.5), and distribution and variation within lineages is completely unknown. Due to the lack of mainland or insular East African samples, we can only state that the individuals from the Comoros exhibit mtDNA haplotypes closely related to another found in the Mascarene islands (article 2: Fig 1.4).

### 3. *Cryptoblepharus*

The distribution of *Cryptoblepharus* in the Western Indian Ocean region ranges from the coasts of East Africa through Comoros, Madagascar and the Mascarenes. Then, it is only found far to the East, in the Indo-Pacific and Australian regions (article 3: Fig. 1A). Thirteen subspecies (or forms) are known from the Western Indian Ocean region (article 3: Fig. 1B), while 23 are known from the Indo-Pacific and Australian regions. Previous authors (MERTENS 1931, GREER 1974, ISKANDAR AND NIO 1996) agree that the ancestral form of this genus evolved in South East Asia and migrated to Australia/ Australo-Papuan region, where the genus evolved and diversified, and by passive means of dispersal radiated to its present distribution in the Australian and Indo-Pacific regions. Given the morphological variation and distinctiveness of the forms

from the Western Indian Ocean region, previous authors also considered likely that their distribution reflects natural processes of dispersal rather than human introductions. Using two mitochondrial DNA markers we unveiled degrees of differentiation and dispersal patterns within *Cryptoblepharus*. The absence of close relatives of *Cryptoblepharus* in the Western Indian Ocean, the level of divergence between the populations from Western Indian Ocean and Australian regions and the geographical coherence of the variation within the Western Indian Ocean group are concordant with the hypothesis of a colonisation of this region by a natural transoceanic dispersal (from Australia or Indonesia), most probably to Madagascar. The group may then have diversified in Madagascar, from where it separately colonised the East African coast, the Comoro islands (at least twice) and Mauritius. After the colonisation of Madagascar, apparently two groups were formed, one in the North and Northwestern islets, and the other in the Southern territories. Colonisation of surrounding areas happened from there, more than once and independently. The ancestors of the forms from Anjouan and Mayotte clearly belong to Northwestern Malagasy populations, and colonised these islands possibly in a stepwise fashion first arriving to Mayotte and originating *C. b. mayottensis*, that later colonised Anjouan originating *C. b. degrisi*. A similar case may have happened with the colonisation of Grand Comoro and Moheli (Grand Comoro seems to have been colonised from Moheli), although in this case the source population of Moheli is so far unclear. Both share a common ancestor with individuals from Mauritius. East African populations result from another colonisation event, this one apparently from South Madagascar.

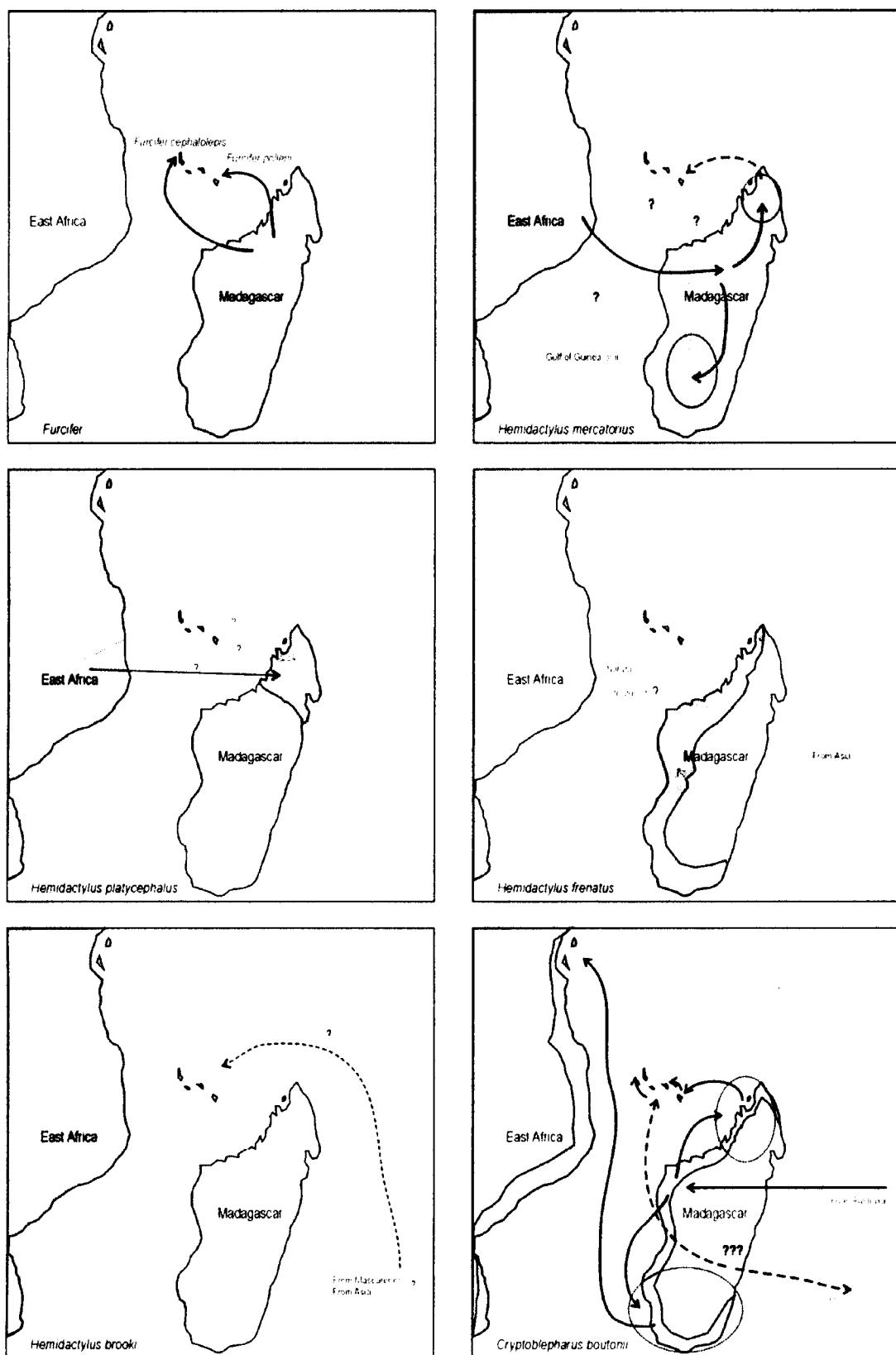


Figure 3: Putative biogeographic scenarios for the species studied in this work in the Western Indian Ocean. Known distributions of the taxa are in grey (distributions of *Hemidactylus* adapted from VENCES ET AL. 2004, distribution of *Furcifer* and *Cryptoblepharus* adapted from GLAW AND VENCES 1994 and GREER 1974). Arrows represent dispersal/colonisation events: bold arrows, natural dispersals; light arrows, recent/eventual anthropogenic dispersals; and dashed arrows, when uncertain sources of colonisation or arriving places are pointed. Circles represent putative distribution of lineages.

### 3. Within Island Differentiation

As the Comoro islands exhibit a gradient in ages, from 10-15 Myr to 0.5 Myr, we expected that genetic variation within the colonising species could reflect this difference in ages of island availability. Another aspect of the geological history of the islands that created some of the initial expectations was the fact that different volcanic massifs constitute at least two of the islands, Mayotte and Grand Comoro. Grand Comoro is formed by two, respectively the northern and southern part of the island, whereas Mayotte harbours several volcanoes, and is the result of the union of previously independent massifs. Considering this conglomerate nature, one could expect coherent genetic variation, as seen in reptiles from Tenerife, in the Canary Islands, where the junction of previously separated massifs led to secondary contact between previously allopatric populations (THORPE ET AL. 1994, 1996; BROWN AND PESTANO, 1998, GÜBITZ ET AL. 2000).

Actually, available data allow us little inference about within island genetic variation in the studied taxa. Among widespread species like *Hemidactylus platycephalus*, lack of variation was found across the whole archipelago, providing no signal of any kind of differentiation, and within *Hemidactylus mercatorius*, the other abundant *Hemidactylus* species, for which this kind of data could be available, and that furthermore is widespread within Mayotte, also no geographically correlated genetic variation was found.

The skinks of the genus *Cryptoblepharus* could provide a good system to study within island differentiation, even because each form/subspecies is restricted to a single island and seems to be the result of a unique colonisation event, but again, limited variation within the studied markers, and in some cases, limited geographical sampling prevent us from inferring about any possible genetic differentiation within each form.

Considerable differentiation was observed within the chameleons *Furcifer polleni* and *Furcifer cephalolepis*. For the first species, endemic of Mayotte, eight widespread individuals were available, exhibiting five different haplotypes, but genetic variation presented no geographical structure. The only case of within island variation congruent with geography or geological history may perhaps be the case of *Furcifer cephalolepis*, endemic from Grand Comoro: considerable divergence (0.87% of an 807bp fragment of the ND4 gene fragment and associated tRNA's) was found between the two individuals analysed, one from the North and the other from the South of the island. However, more data would be needed before further conclusions can be made.

### 4. Dispersal Abilities of Different Groups

Some lizard groups are much more prone to transmarine journeys than others. Such kinds of voyages are generally much commoner in geckos and skinks than in agamids, lacertids and teiids (CARRANZA AND

ARNOLD 2003). Thus, we had different *a priori* expectations on the population structure and levels of inter-island gene flow of our study groups, as well as on their timings of colonisation.

Nevertheless, chameleons were recently shown to be better water-crossers than previously thought (RAXWORTHY ET AL. 2002, but see also TOWSEND AND LARSON 2002), with their current distribution involving several over-water dispersal events between Madagascar, Africa, India, Seychelles, Mascarenes and, as shown in this work, most probably two to the Comoros.

As stated before, variation also occurs within geckos and skinks, with particular groups making repeated journeys while others from the same source areas fail to do so, suggesting that colonisation across oceans is not a random process and can be related to the particular characteristics of each group.

Geckos are known to have excellent dispersal abilities, especially to be persistent transmarine colonisers. Species of the genus *Tarentola*, for example, are known to have colonised many oceanic archipelagos, including Cuba and the Bahamas, from Africa, in a journey of more than 6000 km. The Canary and Cape Verde islands colonisation involved several dispersals from Africa and repeated inter-islands movements (NOGALES ET AL. 1998; CARRANZA ET AL. 2002, 2002). Also *Hemidactylus* have naturally colonised both oceanic archipelagos, like the Cape Verde islands (JESUS ET AL. 2001) and S. Tomé and Príncipe (JESUS ET AL. 2005), and continental but long-isolated landmasses like Madagascar (VENCES ET AL. 2004). Plus, they are also known for their commensal relationship with man, being easily translocated with human systems (VENCES ET AL. 2004). Thus, as expected, in this group we observed more evidences of human-mediated transportation.

Skinks are also known to be good colonisers; *Cryptoblepharus*, in particular, has many attributes that aid natural dispersal, including low metabolic requirements associated with ectothermy and an adaptation to habitats devoid of fresh water. Thus, we also expected that it had promptly colonised the Malagasy region, which appears to be the case. Apparently, they were able to colonise Madagascar from Australia, in a journey of more than 6000 km. As recently stressed by DE QUEIROZ (2005), even entire oceans do not seem to be effective barriers to reptile dispersals, and some equally long-distance over-water dispersals are known in reptiles (CARRANZA ET AL., 2000; MAUSFELD ET AL., 2002; RAXWORTHY ET AL. 2002; CARRANZA & ARNOLD 2003; VICARIO ET AL. 2003; AUSTIN ET AL., 2004).

## 5. Comparison with Colonisation Patterns of Other Volcanic Islands

In other volcanic islands groups, colonisation patterns also vary. In the Canary Islands, the extant groups of lizards (*Tarentola*, *Gallotia* and *Chalcides*) exhibit distinct patterns of genetic diversity and distinct biogeographic histories. Two of the groups, *Gallotia* and *Chalcides*, exhibit patterns partially compatible with time and sequence of island formation (THORPE ET AL. 1994, BROWN AND PESTANO 1998). More complicated ecological and historical factors have influenced the evolutionary history of the genus *Tarentola*, leading to several colonisation events and probably to replacements of some species for others from different origins (NOGALES ET AL. 1998). This group presents higher genetic divergences compared

with *Gallotia* and *Chalcides*, reflecting the earlier age of Canary Islands colonisation by these geckos, or, as it has been suggested, a faster rate of mtDNA evolution within gekkonids (JESUS ET AL. 2002).

All the groups in Canary Islands exhibit, in general, more genetic variation than the groups in the Comoros, indicating a more ancient origin for these, than the one we can so far infer for the Comoroan groups: *Chalcides* first colonised one of the central Canary Islands around four Myr ago, and *Tarentola* colonisation involved both older and more recent colonisation events, with the oldest one (by the ancestor of *Makariogecko*) estimated around 17 Myr ago (CARRANZA ET AL. 2000). Nevertheless, the Canary Islands are much older than the Comoros, ranging from 20 to one million years old.

Also in the Cape Verde Islands, skinks are represented by a monophyletic group of six species of the genus *Mabuya*, whose ancestor colonised the islands more than six Myr ago. The low levels of differentiation between the remaining species of this genus suggest that all other colonisations and dispersals occurred much more recently, and were in fewer numbers (BREHM ET AL. 2001, BROWN ET AL. 2001, CARRANZA ET AL. 2001). Also geckos of the genus *Hemidactylus* occur on Cape Verde, with possibly two introductions and at least two independent natural colonisations, but within species variation is basically low or nonexistent (JESUS ET AL. 2001). Only the endemic *H. bouvieri* shows some genetic differentiation between the two islands it inhabits. In parallel with what happens in Comoros, dispersal within this genus appears to be more related with human-mediated movements than in the ecologically similar *Tarentola*, what seems to be a general trend. Representatives of *Tarentola* (subgenus *Makariogecko*) also occur on Cape Verde islands, having colonised them from La Gomera or Tenerife (Canary islands) around seven Myr ago, in one dispersal event of more than 1400 Km, and have then undergone considerable radiation, with now nine major genetic groups identified (CARRANZA 2000, JESUS ET AL. 2002) and considerable within-species variation and geographic structure. But also the Cape Verde islands are much older than Comoros, dating back from the Mesozoic period until at least 5.5 Myr (Brava) (MITCHELL-THOMÉ 1985), so the existence of older and more diverse groups was expected.

Common aspects to all volcanic island systems studied so far are the long general interval between islands emergence and first (putative) colonisation, except for some younger islands, that can be rapidly colonised from the older and closer ones. Concerning the colonising species we can also infer some general trends, like the obvious proneness of geckos for overseas dispersal, and the tendency of *Hemidactylus* sp. to undergo human-mediated colonisations. Nevertheless, the overall aspects of colonisation can be mainly attributable to chance in their essence, and stochasticity must be seen as a primary factor determining species compositions in islands.

## 6. Rates of Molecular Evolution

Islands with known geological ages are often used to calibrate molecular clocks, i.e., to estimate rates of mutation for specific gene fragments and specific taxa, assuming, for example, that divergence seen between different island taxa (sister-taxa) is due to their separation that can be dated with the younger

island age. Another more conservative way of estimating mutation rates is dating the observed within island divergence with respective island age. Several assumptions have to be made when using these methodologies, which can considerably affect our estimates. Specifically, we can only estimate maximum colonisation ages and minimum evolution rates that, even so, can be considerably misleading. Estimating molecular evolution rates and divergence times are central to evolutionary biology, however, still little attention is given to specific rates, whether lineage specific and due to life-history traits of organisms (BROMHAM 2002; GILLOOLY ET AL. 2005), or gene specific rates. Usually, general molecular clocks are applied.

We used genetic variation within *Furcifer cephalolepis* to try to estimate the rate of molecular evolution of the ND4 gene fragment and associated tRNA's in this species. Genetic divergence values found were surprisingly high, considering the age of the island they are endemic from: the two haplotypes from Grand Comoro have 0.87% genetic divergence (uncorrected p-distance). According to the vertebrate ectotherm mtDNA evolution rate (often used for this gene) of 0.4-0.6% per lineage per Myr (RAND 1994, CACCONE ET AL. 1999), the divergence between the two individuals would correspond to 0.73 to 1.1 million years, and even using a broader interval of evolution rates (0.25-0.7% per lineage per Myr: AVISE 1992, CACCONE ET AL. 1997) we obtain estimates of diverge times between 0.63 and 1.76 million years. All estimates are higher than the oldest estimate for the age of the island that is 0.5 million years. This way the ND4 gene fragment in *Furcifer* seems to be evolving faster than the rates generally used, at least at a value of 0.88% per lineage per million year, if we use the age of Grand Comoro as a calibration point. Furthermore, these are minimum estimates, based only in two individuals. Values would be higher if more divergent haplotypes were found with an increased sampling. An alternative explanation for this result is that divergence within *Furcifer cephalolepis* predates the colonisation of Grand Comoro and this colonisation was made by individuals with already differentiated mtDNA lineages.

Still, this result should be taken into consideration in posterior studies, since it has implications for the estimated times of colonisation of other island groups by chameleons, and further highlights the inaccuracies of generalised applications of molecular clocks.

## 7. Probable Species Complexes

Perhaps due to their morphological conservativeness, variation within geckos has often been overlooked. Many species are morphologically similar, and molecular data has been widely used to detect and describe cryptic differentiation (eg. NOGALES ET AL. 1998, KRONAUER ET AL. 2005). Two important aspects arose from our nuclear DNA phylogeny of *Hemidactylus* (article 2). First, we showed that the African "species" *H. mercatorius/mabouia* and *H. platycephalus* and the Cape Verde islands endemics *H. greeffi*, *H. newtonii* and *H. bouvieri*, constitute a group of closely related species within this genus, very divergent from either *H. frenatus* or *H. brooki*. Second, we showed that *H. brooki* from the Cape Verde islands and

Guinea Bissau (West Africa) constitutes a very divergent lineage of *H. brooki* from the Comoros (these ones were closer to Mascarene and Asian specimens). The degree of divergence between these two lineages (article 2: Fig. 1.5) is several times higher than the generally observed between distinct species of *Hemidactylus* sp. or other gecko species such as *Tarentola* sp. (CARRANZA ET AL. 2002, HARRIS ET AL. 2004), thus suggesting that *H. brooki* is in fact a species complex. This highlights the need of obtaining molecular data from the entire distribution of this "species", where cryptic differentiation must be occurring.

Another case of unclear taxonomic boundaries is the case of *Hemidactylus mercatorius* and *Hemidactylus mabouia* "species", for which our data provide additional evidence that exact species delimitations and phylogeographic structure are far from being understood. VENICES ET AL. (2004) resurrected *H. mercatorius* from its congener *H. mabouia*, assigning to it Malagasy and East African populations that seemed to represent a distinct evolutionary unit within the known *H. mabouia* "species-complex". Surprisingly, individuals from the Gulf of Guinea islands (West Africa) assigned to *H. mabouia* clustered within the North Madagascar-Comoros mtDNA lineage of *H. mercatorius* and not with the African individual analysed by VENICES ET AL. (2004). For the nuclear gene C-mos, both Malagasy lineages (including the individuals from Gulf of Guinea islands) share the same haplotype. Information about this gene fragment was unavailable for the East African individual, however. Although it is clear, from the available data, that distinct evolutionary lineages exist (see article 2: Fig. 1.1), the exact species delimitations remain a future challenge, besides the lack of nomenclatural stability of *mabouia*.

#### **8. Probable Instances of Anthropogenic Introductions**

Several house geckos of the genus *Hemidactylus* are almost cosmopolitan, with distributions probably shaped by both natural dispersals and more recent human mediated translocations. In fact, many geckos are commensal and therefore more likely to be anthropogenically introduced. This is probably the case of some of the *Hemidactylus* species found in the Comoros. For every species recorded there we found signs of what could be human mediated transportation.

The predominance of a single and widespread haplotype within *Hemidactylus platycephalus* populations may indicate recent colonisation and rapid expansion throughout the archipelago but may as well be the result of constant human aided dispersals. For this species, also widespread individuals in Madagascar shared the same haplotype, also found in two Comoroan individuals. Once again, human mediated transportation may be invoked as a plausible explanation.

VENICES ET AL. (2004) proposed that Comoroan populations of *Hemidactylus mercatorius* (at least populations from Mayotte) should have been recently introduced, on the basis of the observation that the individual from Mayotte analysed by them was found in the main harbour of Mayotte and exhibited a mtDNA haplotype very similar to the one of a specimen from Central Madagascar, from a place where many Comoroans live and regularly travel to Mayotte. Although we found a distinct and widespread lineage in Mayotte, one more individual clustering within this Western-Central Malagasy clade was also

found, in Grand Comoro, suggesting that occasional anthropogenic translocations and introductions do occur.

*Hemidactylus frenatus* and *Hemidactylus brooki* showed relatively low degrees of genetic differentiation over the whole Indian Ocean region (Madagascar, Comoros, Mascarene and Andaman islands and Sri Lanka), with no recognisable phylogeographic structure, also suggesting distributions driven by recent colonisations or introductions (article 2: Figs. 1.3 and 1.4).

### 9. Taxonomic remarks

Although in a recent work VENCES ET AL. (2004) provided, for the first time, some clarification on the taxonomy of *Hemidactylus* in the Indian Ocean region, reporting three species for Madagascar (*H. mercatorius*, *H. platycephalus* and *H. frenatus*) and a fourth one for the Comoro islands (*H. brooki*), their work was especially focused on Madagascar, and data for the Comoro islands was scarce, and deserving further investigations. We confirmed the existence of these four species in the Comoros archipelago (CARRETERO ET AL. 2005, ROCHA ET AL. 2005), and further highlighted some of the problems of unclear taxonomic boundaries within this genus, namely the cases of the *H. mabouia/H. mercatorius* and *H. brooki* "species complexes". As stated above, given the close relatedness of Gulf of Guinea islands populations of *H. mabouia* with the *H. mercatorius* related lineage from Mayotte, diversity within this group becomes more and more puzzling. It's now evident that more genetic and morphological data need to be collected before any, more than speculative, conclusion can be drawn, and taxonomic boundaries established. Very divergent nuclear lineages were also identified within *H. brooki* (article 2: Fig. 1.5), where genetic diversity must also be assessed and taxonomic revision undertaken.

Within *Cryptoblepharus* much controversy exists about the true taxonomic status of its "morphological forms", which have been alternatively treated as different species or as subspecies of *Cryptoblepharus boutonii*. The long appreciated distinct colour patterns and ecologies of at least some of the forms and the more recent recognition of sympatric occurrence of others, such as those in Australia, calls into question the interpretation of one polytypic species. It is now quite certain that many forms are distinct species while the status of other forms is uncertain (ANDREONE AND GREER 2002). Especially difficult is the taxonomy of the separate island forms such as the ones that occur on Madagascar and neighbouring islands and it was recently suggested that, in the absence of a review of this problem, treating this forms as separate species would be probably closer to the real situation (ANDREONE AND GREER 2002). Our data for the Western Indian Ocean region, however, do not fit this interpretation. The level of differentiation between the different forms is very low (article 3: Fig. 3), and we propose that a subspecific treatment of these forms is the most appropriate taxonomic ranking for them.

The usage of only mitochondrial or nuclear divergences as indicators for taxonomic distinctiveness, or lack of it, is controversial, nevertheless, we consider this is a reasonable first approach to these problems that need to be clarified with more genetic and ecological data.

## 10. Conservation Issues

In global terms, for a variety of taxa, islands make a contribution to biodiversity out of proportion to their land area, and in this sense collectively they can be thought of as "hot spots". With increasing isolation, island size and topographic variety, the number and proportion of endemics tends to increase (WHITTAKER, 1998). Some lineages have done particularly well out of islands and particular genera have radiated spectacularly.

Nevertheless, islands in general are known for elevated levels of extinction (REID AND MILLER 1989, MAWDSLAY AND STORK 1995, FRANKHAM 1997). Small population size is generally identified as the main problem affecting island populations, since because of it they are more susceptible to extinction by demographic accidents and habitat modification. Also non-native species invasions are an additional concern when dealing with island populations.

Given the clear link between genetic diversity and the potential for adaptive response, such assessments are critical for the long-term maintenance of individual species and species assemblages. Understanding of biogeographic patterns is thus crucial to determine conservation priorities, but the knowledge about what are and are not native and non-native forms is sometimes scarce, as was (and for several species still is) the case with the Comoro islands species. Phylogenetic/phylogeographic studies like this one are thus useful in determining origins of island taxa and establishing these conservation priorities.

Possibly, several introduced species are found in the Comoros, and clarification of the spatial and temporal origin of each species in these islands, and their interspecific ecological relationships are of great importance. *Agama agama*, for example, was introduced supposedly a few years ago (MEIRTE 2004), but so far competitive relationships with the native ecological similar *Oplurus comorensis* seem negligible, since the later is restricted to small areas in the north and northeast coasts of Grand Comoro (CARRETERO ET AL. 2005). A different case may be that of *Hemidactylus* species, where we still lack information to be able to state which, if any, forms are native, and competition and ecological exclusion of native forms may actually be happening. In other oceanic archipelagos, like the Mascarenes for example, the spread of introduced *Hemidactylus*, has had devastating consequences for endemic species (ARNOLD 2000).

The special interest in conservation of island biotas is maintaining the result of the special process of evolution that generated it: islands sampled continental fauna and flora from different geological times; preserved some of it and "reinvented" some of it. Each island has also its peculiarities due to age, size, geography and other factors. Understanding and maintaining the peculiarities and processes on the basis of island biota is the point of island taxa conservation (CRONK 1997).

KOLBE ET AL. (2004) recently showed how within-population genetic variation can be higher in populations resulting from multiple introduction events, than in the native-range populations, and how that may enhance the ability of invasive populations to spread and adapt to new environments. This is an important aspect to take into account in easily human-translocated species, such as *Hemidactylus* sp. for example. The biogeographic relationships between the different populations of these species are

obscured by their proven abilities for natural overseas dispersal as well as the association with humans involving repeated introductions. A greater effort should be made, within this genus, to sample throughout the islands and mainland and clarify Comoroan species origins, in order to be able to preserve autochthonous lineages that may exist.

Concerning the other species, it is possible that two distinct evolutionary lineages exist within *F. cephalolepis*, in Grand Comoro, an interesting aspect that could also be further investigated. Each form of *Cryptoblepharus* seems to be island-endemic, and despite the low differentiation between some of the forms, they should be regarded as independent "lineages".

Instead of only a subset of either African or Malagasy mainland fauna, and despite its general recent origin, Comoroan herpetofauna can be regarded as a distinct "evolutionary unit" and this aspect taken into account when designing conservation plans.

#### 11. Concluding Remarks

Existing molecular phylogenies of other recently diverged island organisms frequently conform to one of two generalized patterns. Radiations which are contemporary with island formation very frequently show a stepwise "island colonised as it emerges" pattern, from older to younger islands in the group. This has been observed in Hawaiian island birds and crickets (SHAW 1996, FLEISHER ET AL. 1998) as well as in Canary island skinks, geckos and beetles (BROWN AND PESTANO 1998, NOGALES ET AL. 1998, EMERSON ET AL. 2000). By contrast, several studies showed radiations which post-date island formation and have shown relatively rapid expansion and speciation, with relatively short coalescence time within an archipelago.

A mixture of patterns is found in the Comoro islands: ancient colonisations (although they seem to be much younger than the older islands), more recent dispersal movements with low differentiation, and even more recent and eventually human attributable translocations. The majority of Comoroan reptile groups are related with Malagasy ones, nevertheless, there is little general pattern for these islands colonisation and each species "history" is probably more driven by chance than by any other factor. Stochasticity must be seen as a primary factor in determining species composition on islands, and even when colonisation occurs, subsequent evolution will be dependant on several factors.

Existing Indian Ocean phylogenies have largely focused on the origins of insular Malagasy forms, all probably post-dating the separation of Madagascar from Africa and Greater India and arrived to Madagascar by dispersal. Nonetheless, in general they show long histories of isolation on the island. By contrast, Comoros colonisation seems to be extremely recent: the history of the studied groups in the islands seems to be far more recent than islands history itself.

Several authors suggested that these islands have played a key role as "stepping-stones" for dispersal between Africa, Madagascar and other Indian Ocean islands, which appears to be the case in some taxa, like the Indian Ocean sunbirds (WARREN ET AL. 2003). Although we do not have clear

evidences of such a pattern in the analysed groups, we cannot exclude such hypothesis, especially in the case of the genus *Hemidactylus*, where, for certain, much still has to be done before we can uncover their biogeographic history.

Each of the studied species presents distinct biogeographic scenarios. Their knowledge is of great importance to understand their evolutionary history, the evolution of island systems as a whole, and to be able to draw effective conservation measures that maintain the evolutionary processes that are in the origin of these taxa. The continuous effort to obtain phylogenetic data across a diversity of taxa, together with geological and ecological data, seem the best way to evaluate diversification and ecosystem building in these (and other) island systems.

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