



Short communication

Diversity and phylogenetic relationships of *Hemidactylus* geckos from the Comoro islandsSara Rocha^{a,b}, Miguel A. Carretero^a, D. James Harris^{a,b,*}^a Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO/UP), ICETA, Campus Agrário de Vairão, 4485-661 Vila do Conde, Portugal^b Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, Praça Gomes Teixeira, 4099-002, Portugal

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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,

* Corresponding author. Fax: +351 252 661780.

E-mail address: james@mail.icav.up.pt (D.J. Harris).

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 (Emerick 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.

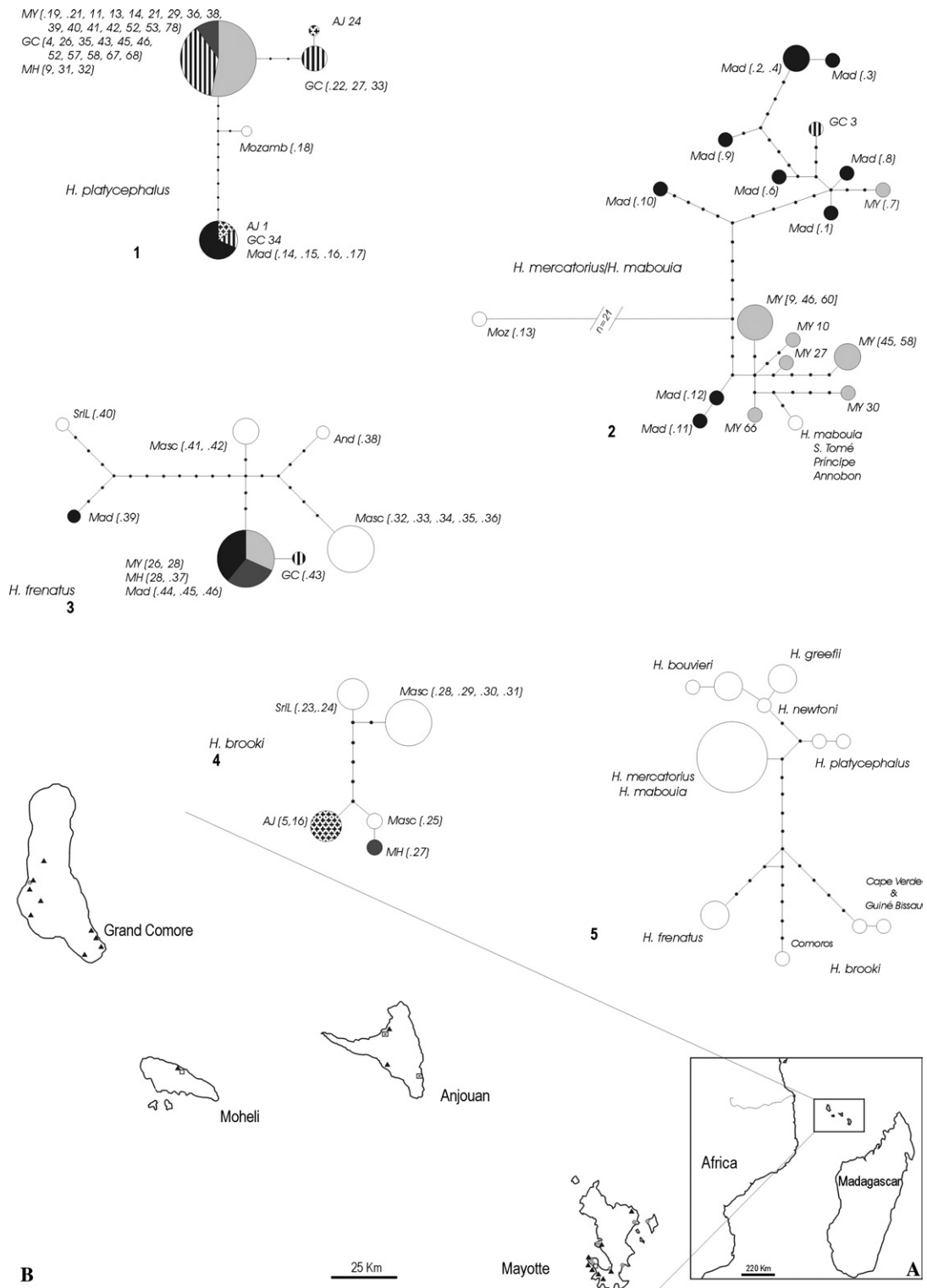


Fig. 1. (1–4) Haplotype (median-joining) networks for the four species of *Hemidactylus*, based on about 500 bp of the 16S rRNA gene. All individuals analyzed are identified by their code, next to the respective haplotype. Haplotypes found in Madagascar and Comoros are highlighted with different colors: black, Madagascar; light gray, Mayotte; dark gray, Moheli; crosses, Anjouan; and vertical stripes, Grand Comoro. In *H. mercatorius* some homoplasic positions inside the main groups make the original network too complicate 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	Chiroungoui, Mayotte	AY517573
<i>H. platycephalus</i>	MY 13	Chiroungoui, Mayotte	AY517573
<i>H. platycephalus</i>	MY 14	Chiroungoui, 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	Chiroungoui (Plage Mtihi), Mayotte	AY517573
<i>H. platycephalus</i>	MY 41	Chiroungoui (Plage Mtihi), Mayotte	AY517573
<i>H. platycephalus</i>	MY 42	Chiroungoui (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	Hantoindzi (Chezani), Grand Comore	AY517573
<i>H. platycephalus</i>	GC 27	Hantoindzi (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	Hantoindzi (Chezani), Grand Comore	AY517573
<i>H. platycephalus</i>	GC 68	Hantoindzi (Chezani), Grand Comore	AY517573
<i>H. platycephalus</i>	AJ 1	Moutsamoudu, 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. Comani, Mayotte	AY517573
<i>H. platycephalus</i>	.15	Nosy Be, Madagascar	AY517574

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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, Mascaren.	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	Moutsamoudu, urban, Anjouan	AY863040 / AY863047
<i>H. brookii</i>	AJ 16	Mboûeladougou, Anjouan	AY863040
<i>H. brookii</i>	.23	Kitulgala, Sri Lanka	AY517 570
<i>H. brookii</i>	.24	Kitulgala, Sri Lanka	AY517 570
<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, Reunion, 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. greefii</i>	703	Nova Estrela—P2	AY863044
<i>H. greefii</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. greeffii*, *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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