

Natural colonization or introduction? Phylogeographical relationships and morphological differentiation of house geckos (*Hemidactylus*) from Madagascar

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Several house gecko species of the genus *Hemidactylus* are almost cosmopolitan lizards, with distributions that have probably been shaped by natural transoceanic dispersal as well as by more recent human introductions. Here we revise the *Hemidactylus* populations of Madagascar and compare them genetically with populations from other sites in the Indian Ocean region. Morphological data strongly confirm the occurrence of three *Hemidactylus* species on Madagascar: *Hemidactylus frenatus*, distributed along the western coast of Madagascar; *H. platycephalus*, restricted to the north-west and the widespread *H. mercatorius* that occurs throughout the island, including coastal areas at sea level as well as big cities (Antananarivo, Fianarantsoa) at altitudes of 1200–1300 m above sea level. Analyses of partial sequences of the 16S rRNA gene in 46 *Hemidactylus* specimens from Madagascar, East Africa, South Asia, and the Comoro and Mascarene archipelagos demonstrated the presence of a fourth species, *H. brooki*, on the Mascarenes (Réunion, Rodrigues, and Mauritius) and Comoros (Moheli). The Malagasy populations of *H. platycephalus* were genetically uniform and differentiated from the African and Comoroan specimens studied. *H. frenatus* had a relatively low genetic differentiation over the whole region with no recognizable phylogeographical structure, indicating more recent colonizations or introductions. In contrast, *H. mercatorius* showed a strong phylogeographical structure of haplotypes, with two distinctly different lineages in Madagascar. Moreover, all Malagasy specimens differed strongly from the single African specimen included. This indicates that populations of *H. mercatorius* in Madagascar have a long history that predates human settlement. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, 83, 115–130.

ADDITIONAL KEYWORDS: dispersal – distribution – Gekkonidae – *Hemidactylus brooki* – *Hemidactylus frenatus* – *Hemidactylus mercatorius* – *Hemidactylus platycephalus* – Indian Ocean – Squamata.

INTRODUCTION

Madagascar is populated by a fascinating fauna with an extraordinary degree of endemism (Glaw & Vences, 2000). Species-level endemism is almost complete in

amphibians (>99%), with one species, *Hoplobatrachus tigerinus* Daudin, 1803 introduced from India (Kosuch *et al.*, 2001) and a second species, *Ptychadena mascareniensis* Duméril & Bibron, 1841, closely related to (although probably not conspecific with) African populations. Among reptiles, more nonendemic species or populations of questionable status exist (Raseli-

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manana & Vences, 2003), but endemism at the species level is still very high (93%; Glaw & Vences, 2000).

Madagascar has been separated from other fragments of the ancient supercontinent Gondwana since the Mesozoic era. Separation from Africa dates to about 130 million years ago (Mya), and separation from the northwards drifting Indian plate occurred 88–63 Mya (Rabinowitz, Coffin & Falvey, 1983; Storey *et al.*, 1995). This long isolation and the biogeographical origins of Madagascar's biotas has attracted the interest of many authors. Debates centre around the question of whether the extant fauna is derived from Mesozoic ancestors by vicariance (e.g. Farias *et al.*, 1999, Farias, Orti & Meyer, 2000; Bossuyt & Milinkovitch, 2001) or by Cenozoic dispersal (e.g. Krause, Hartman & Wells, 1997; Vences *et al.*, 2001), with colonists arriving by overseas dispersal (e.g. Palkovacs, Gerlach & Caccone, 2002; Raxworthy, Forstner & Nussbaum, 2002) or via land bridges (McCall, 1997). Before any comprehensive scenario can be considered as sufficiently corroborated, it will be necessary to gather data on the phylogeny and genetic differentiation of as many lineages as possible. To understand these processes in deep time (Krause *et al.*, 1997) it may also be relevant to trace the more recent colonization patterns in the Indian Ocean region.

Geckos are a family of lizards that are particularly well suited to overseas dispersal (Kluge, 1969). They have naturally colonized most major tropical and subtropical oceanic islands and archipelagos, often producing endemic island species or subspecies (e.g. the genera *Phelsuma* and *Tarentola*; Joger, 1984; Nogales *et al.*, 1998; Arnold, 2000; Carranza *et al.*, 2000). Many geckos are commensal and therefore they are also likely to be translocated by humans. In the Indian Ocean this is especially true for several species of house geckos of the genus *Hemidactylus* Gray, 1825. The distribution of these geckos is almost cosmopolitan (whether by natural or human-mediated dispersal), and many species are morphologically similar.

The *Hemidactylus* from Madagascar have never been thoroughly revised. *Hemidactylus frenatus* Duméril & Bibron, 1836 (restricted type locality: Java), is relatively easy to recognize by its almost smooth dorsal skin and low number of dorsal tubercle rows, and has been unanimously identified from Madagascar (Angel, 1942; Loveridge, 1947; Glaw & Vences, 1994). On the contrary, no consensus exists on the status and presence of one or two additional species on Madagascar. Kluge (1969) analysed Malagasy specimens and considered them conspecific with African, Antillean and South American specimens of *Hemidactylus mabouia* (Moreau de Jonnés, 1818). Later he resurrected *Hemidactylus mercatorius* Gray, 1842 (type locality: Madagascar) from the

synonymy of *H. mabouia* (Kluge, 1991, 2001), whilst Broadley (1977) considered *H. platycephalus* Peters, 1854, from Anjouan (Comoros) and Moçambique as a valid species. Glaw & Vences (1994) distinguished *Hemidactylus frenatus*, *H. mercatorius* and *H. mabouia* from Madagascar, but gave sizes and meristic characters for these species that did not agree with the revisions of Kluge (1969) for New World populations and of Broadley (1977) for south-east Africa. Consequently, the taxonomic and nomenclatural status of *Hemidactylus* from Africa, Madagascar and the islands in the western Indian Ocean remains confused (see Appendix 1). Moreover, the phylogeographical relationships of these populations have, as of yet, not been studied.

The application of molecular methods in combination with morphological examination provides a highly effective approach to the understanding of the taxonomy of morphologically conservative and widespread groups. The analysis of phylogeographical structuring of populations (Avice, 2000) can be useful to unweave old natural island colonizations from more recent human-mediated dispersal (Austin, 1999; Jesús *et al.*, 2001, 2002). In this paper, we use morphological and mitochondrial DNA data to assess: (a) how many species of *Hemidactylus* occur on Madagascar and how they are distributed over the island; and (b) which are the phylogeographical relationships of the Malagasy populations to geckos from other Indian Ocean islands and from the Asian and African mainland. Our main focus was to determine if the Malagasy *Hemidactylus* represent native populations or even endemic species, or have been introduced by humans more recently.

MATERIAL AND METHODS

TAXON SAMPLING AND CLASSIFICATION

Morphological data were recorded from available material of *Hemidactylus* from Madagascar, housed in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN), Zoologisches Forschungsinstitut und Museum Koenig, Bonn (ZFMK) and Zoologische Staatssammlung München (ZSM). Additionally, we examined some type specimens from the Natural History Museum, London (BMNH). Because our aim was not a comprehensive revision of *Hemidactylus* in the Indian Ocean but was restricted to studying the identity of the Malagasy populations, we did not undertake detailed morphological studies of non-Malagasy populations. Specimens examined morphologically are listed in Appendix 2.

Genetic samples were taken in the field, by preserving muscle tissues (usually autotomized tails) in pure ethanol. In most cases, the corresponding voucher

specimens were collected and preserved in the ZSM (see Appendix 3).

We herein follow the taxonomic arrangement of Kluge (1991, 2001) and consider *Hemidactylus mercatorius* as a valid species, assigning this name to Malagasy and African populations previously (Kluge, 1969; Broadley, 1977) considered as *H. mabouia*. In anticipation of our results we follow Broadley's (1977) analysis for south-east Africa and use the name *Hemidactylus platycephalus* for a species characterized by a large body size (see Appendix 1) found in Africa, on the Comoros and (as documented below) in Madagascar.

MORPHOMETRIC AND MERISTIC EXAMINATION

The following morphometric measurements were taken with a calliper to the nearest 0.1 mm: snout-vent length, SVL; tail length, TAL; hindlimb length from groin of straightened limb to tip of longest toe, HIL; interlimb distance, ILD, measured between axilla and anterior insertion of hindlimb; head width at maxillary commissure, HW; distance between anterior margin of tympanum and snout tip, TSD; distance between anterior margin of eye and posterior margin of nostril, END; dorsal distance between centres of eyes, EED; distance between anterior margin of tympanum and posterior margin of eye, TED. Meristic characters recorded were as follows: number of supralabials and infralabials, SUPL and INFL; number of longitudinal rows of tubercles on the dorsum, DTR; longitudinal count of tubercles on central dorsal row, LTUB; number of longitudinal rows of tubercles on original tail, TTR; number of preanofemoral pores in males, PAFP. Additionally, we recorded the number of adhesive lamellae on the fifth finger and the fourth toe. In all specimens examined by us, these lamellae were arranged (proximally to distally) in the following way: one undivided lamella (FL1, TL1), followed by a variable number of paired lamellae (FL2, TL2), and a variable number of undivided lamellae (FL3, TL3).

Morphometric and meristic data were included in principal component analyses (PCA) using SPSS for Windows, version 10. Factors with an eigenvalue over 1 were extracted and kept unrotated. (1) A first PCA was performed with morphometric variables only; in this analysis, the first principal component that largely corresponds to the size factor was excluded. (2) In a second analysis we included meristic characters together with snout-vent length to account for the diagnostic larger size of *Hemidactylus platycephalus*. (3) In a third PCA, only meristic data were analysed. To assess significance of differences among taxa we performed univariate analyses of variance (ANOVA) or, for the morpho-

metric characters, analyses of covariance (ANCOVA) with SVL as covariate.

DNA SEQUENCING

DNA was extracted using QIAmp tissue extraction kits (Qiagen) from muscle or tail tissue samples preserved in pure ethanol. We used the primers 16SA-L (light chain; 5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SB-H (heavy chain; 5'-CCG GTC TGA ACT CAG ATC ACG T-3') of Palumbi *et al.* (1991) to amplify a section (approximately 500 bp) of the mitochondrial 16S ribosomal RNA gene. PCR conditions were as described previously Vences *et al.* (2000). PCR products were purified using QIAquick purification kits (Qiagen) and sequenced (single-stranded) using automated DNA sequencers (ABI 377 and 3100). Sequences were deposited in GenBank (accession numbers AY517559–AY517586).

SEQUENCE ANALYSIS

Initial alignments were obtained using Clustal X (Thompson *et al.*, 1997), followed by manual editing using QuickAlign 1.5.5 (Müller & Müller, 2003). Because a large number of gaps had to be added to the sequences to account for indel mutations, several highly variable regions could not be reliably aligned across all taxa. These hypervariable regions were therefore excluded from the phylogenetic analyses that included all species. In addition, we analysed separate sequence sets for each of the four *Hemidactylus* species. In these, only single indels were observed and the alignment was unambiguous.

Phylogenetic analyses were carried out using PAUP*, version 4b10 (Swofford, 2002). Prior to phylogenetic reconstruction, we explored which substitution model fits our sequence data best. We applied a hierarchical likelihood method to test the goodness-of-fit of nested substitution models, using the program Modeltest (Posada & Crandall, 1998). The substitution model estimated as best fitting our data (see Results) was used to obtain maximum likelihood (ML) trees using the heuristic search option, and a random sequence-addition sequence with ten replicates. To test the robustness of nodes we ran 100 heuristic bootstrap replicates under ML.

To visualize the number of specimens sharing certain haplotypes, minimum spanning trees were constructed using the TCS software package (Clement, Posada & Crandall, 2000), which employs the method of Templeton, Crandall & Sing, (1992) to calculate the number of mutational steps by which pairwise haplotypes differ using the parsimony criterion. Gaps were treated as fifth character state in the TCS analysis.

RESULTS

MORPHOMETRIC ANALYSIS

Mean values of morphometric and meristic characters obtained for Malagasy *Hemidactylus*, assigned to different species by a priori diagnosis based on body size and amount of tubercles on the body, are shown in Tables 1 and 2.

A PCA using the meristic data and SVL extracted three principal components with an eigenvalue >1 in both the analysis of males and females; together these factors explained 71% (males) and 62% (females) of the total variance, respectively. Scatterplots using the first and second factor (Fig. 1) separated three main groups that fully corresponded to the a priori species assignments. For females the separation was less

Table 1. Meristic characters of Malagasy *Hemidactylus* species (mean \pm SD, followed by minimum and maximum values). SUPL, INFL, number of supralabials and infralabials; DTR, number of longitudinal rows of tubercles on the dorsum; LTUB, longitudinal count of tubercles on central dorsal row; TTR, number of longitudinal rows of tubercles on original tail; PAFP, number of preanofemoral pores in males. FL2, TL2, FL3, TL3, number of divided and terminal undivided adhesive lamella on fifth finger and fourth toe, respectively

| Sex | FL2 | FL3 | TL2 | TL3 | SUPL | INFL | DTR | LTUB | TTR | PAFP |
|-------------------------|---------------|---------------|---------------|---------------|----------------|----------------|----------------|----------------|---------------|----------------|
| <i>H. frenatus</i> | | | | | | | | | | |
| Males | 1.7 \pm 0.5 | 2.3 \pm 0.6 | 5.3 \pm 0.8 | 3.7 \pm 0.8 | 11.0 \pm 1.0 | 9.0 \pm 0.9 | 6.3 \pm 1.4 | 18.1 \pm 3.8 | 6.0 \pm 0.0 | 30.1 \pm 2.1 |
| <i>N</i> = 24 | 1–2 | 1–3 | 4–7 | 2–5 | 9–12 | 7–11 | 4–8 | 7–24 | 6–6 | 26–36 |
| Females | 1.6 \pm 0.5 | 2.3 \pm 0.5 | 5.2 \pm 1.1 | 3.5 \pm 1.0 | 11.6 \pm 0.9 | 8.9 \pm 0.7 | 5.0 \pm 1.5 | 16.8 \pm 3.4 | 6.2 \pm 0.5 | |
| <i>N</i> = 26 | 1–2 | 2–3 | 1–7 | 1–6 | 10–14 | 8–10 | 2–8 | 11–24 | 6–8 | |
| <i>H. mercatorius</i> | | | | | | | | | | |
| Males | 2.0 \pm 0.3 | 2.0 \pm 0.4 | 5.0 \pm 0.4 | 1.0 \pm 0.2 | 10.8 \pm 1.0 | 8.6 \pm 0.7 | 13.3 \pm 1.3 | 30.9 \pm 2.3 | 6.2 \pm 0.5 | 28.5 \pm 2.0 |
| <i>N</i> = 42 | 1–3 | 1–3 | 4–6 | 0–2 | 9–13 | 7–10 | 11–16 | 25–35 | 6–8 | 25–32 |
| Females | 2.1 \pm 0.3 | 2.2 \pm 0.5 | 5.0 \pm 0.4 | 1.1 \pm 0.4 | 10.8 \pm 0.9 | 8.7 \pm 0.8 | 13.3 \pm 1.4 | 31.9 \pm 1.9 | 6.4 \pm 0.7 | |
| <i>N</i> = 38 | 1–3 | 1–3 | 4–6 | 0–2 | 9–13 | 7–10 | 8–16 | 29–37 | 6–8 | |
| <i>H. platycephalus</i> | | | | | | | | | | |
| Males | 2.8 \pm 0.6 | 2.0 \pm 0.6 | 5.9 \pm 0.3 | 1.5 \pm 0.5 | 13.2 \pm 1.3 | 10.4 \pm 1.0 | 10.5 \pm 0.8 | 31.6 \pm 4.2 | 6.0 \pm 0.0 | 49.9 \pm 4.3 |
| <i>N</i> = 12 | 2–4 | 1–3 | 5–6 | 1–2 | 11–16 | 9–12 | 10–12 | 23–37 | 6–6 | 42–59 |
| Females | 2.6 \pm 0.5 | 2.2 \pm 0.6 | 6.0 \pm 0.0 | 1.0 \pm 0.0 | 12.8 \pm 1.3 | 10.0 \pm 1.0 | 10.4 \pm 1.0 | 31.4 \pm 4.6 | 5.8 \pm 0.7 | |
| <i>N</i> = 12 | 2–3 | 1–3 | 6–6 | 1–1 | 11–15 | 9–12 | 8–12 | 22–35 | 4–6 | |

Table 2. Morphometric measurements (in mm) of Malagasy *Hemidactylus* species (mean \pm SD, followed by minimum and maximum values). SVL, snout–vent length; TAL, tail length; HIL, hindlimb length; ILD, interlimb distance; HW, head width; TSD, distance between tympanum and snout tip; END, eye–nostril distance; EED, distance between eyes; TED, distance between tympanum and posterior eye corner

| Sex | SVL | TAL | HIL | ILD | HW | TSD | END | EED | TED |
|-------------------------|----------------|-----------------|----------------|----------------|----------------|----------------|----------------|---------------|---------------|
| <i>H. frenatus</i> | | | | | | | | | |
| Males | 48.3 \pm 4.8 | 45.7 \pm 8.2 | 20.4 \pm 2.1 | 24.8 \pm 3.0 | 8.1 \pm 0.8 | 13.0 \pm 1.2 | 6.2 \pm 0.6 | 5.3 \pm 0.4 | 6.4 \pm 0.7 |
| <i>N</i> = 24 | 39.1–55.4 | 32.8–62.9 | 16.1–24.3 | 19.5–29.6 | 6.6–9.6 | 10.7–15.1 | 5.1–7.4 | 4.0–6.0 | 4.7–7.6 |
| Females | 45.9 \pm 4.4 | 38.8 \pm 6.3 | 19.4 \pm 1.8 | 23.4 \pm 3.0 | 7.7 \pm 0.7 | 11.8 \pm 2.0 | 5.9 \pm 0.5 | 4.9 \pm 0.7 | 6.0 \pm 0.6 |
| <i>N</i> = 26 | 39.0–53.6 | 27.8–49.3 | 16.4–23.4 | 18.5–29.2 | 6.5–8.7 | 5.7–13.7 | 5.0–6.8 | 3.6–6.0 | 5.0–7.1 |
| <i>H. mercatorius</i> | | | | | | | | | |
| Males | 48.4 \pm 4.8 | 47.9 \pm 6.0 | 21.2 \pm 2.0 | 23.5 \pm 3.2 | 8.8 \pm 0.8 | 13.7 \pm 1.2 | 6.6 \pm 0.6 | 5.1 \pm 0.6 | 6.5 \pm 0.6 |
| <i>N</i> = 42 | 39.0–56.1 | 35.1–58.7 | 16.3–25.1 | 17.7–30.2 | 6.9–10.7 | 11.0–16.0 | 5.3–7.8 | 3.6–6.2 | 5.4–7.4 |
| Females | 48.3 \pm 5.6 | 43.8 \pm 8.4 | 21.5 \pm 2.3 | 24.3 \pm 3.6 | 8.7 \pm 0.9 | 13.5 \pm 1.3 | 6.5 \pm 0.8 | 5.0 \pm 0.6 | 6.4 \pm 0.7 |
| <i>N</i> = 38 | 38.6–56.1 | 26.9–60.7 | 16.5–24.4 | 16.9–31.8 | 6.7–10.2 | 11.0–15.4 | 4.9–8.4 | 4.0–6.3 | 5.1–7.3 |
| <i>H. platycephalus</i> | | | | | | | | | |
| Males | 75.2 \pm 5.3 | 84.1 \pm 10.6 | 33.5 \pm 2.5 | 38.0 \pm 4.2 | 13.1 \pm 0.9 | 20.7 \pm 1.2 | 10.9 \pm 1.2 | 7.6 \pm 0.7 | 9.3 \pm 0.8 |
| <i>N</i> = 12 | 65.4–85.2 | 64.2–99.2 | 28.3–38.7 | 29.6–43.8 | 11.8–14.8 | 18.9–22.8 | 9.9–13.4 | 6.2–8.5 | 8.1–10.8 |
| Females | 69.8 \pm 3.6 | 71.9 \pm 9.8 | 31.0 \pm 1.4 | 35.4 \pm 2.6 | 12.4 \pm 0.6 | 19.3 \pm 0.9 | 9.9 \pm 0.6 | 7.2 \pm 0.6 | 8.8 \pm 0.6 |
| <i>N</i> = 12 | 64.6–76.9 | 54.5–87.5 | 28.2–33.2 | 30.1–39.6 | 11.4–13.4 | 17.6–20.3 | 8.9–10.9 | 6.1–8.2 | 7.8–9.5 |

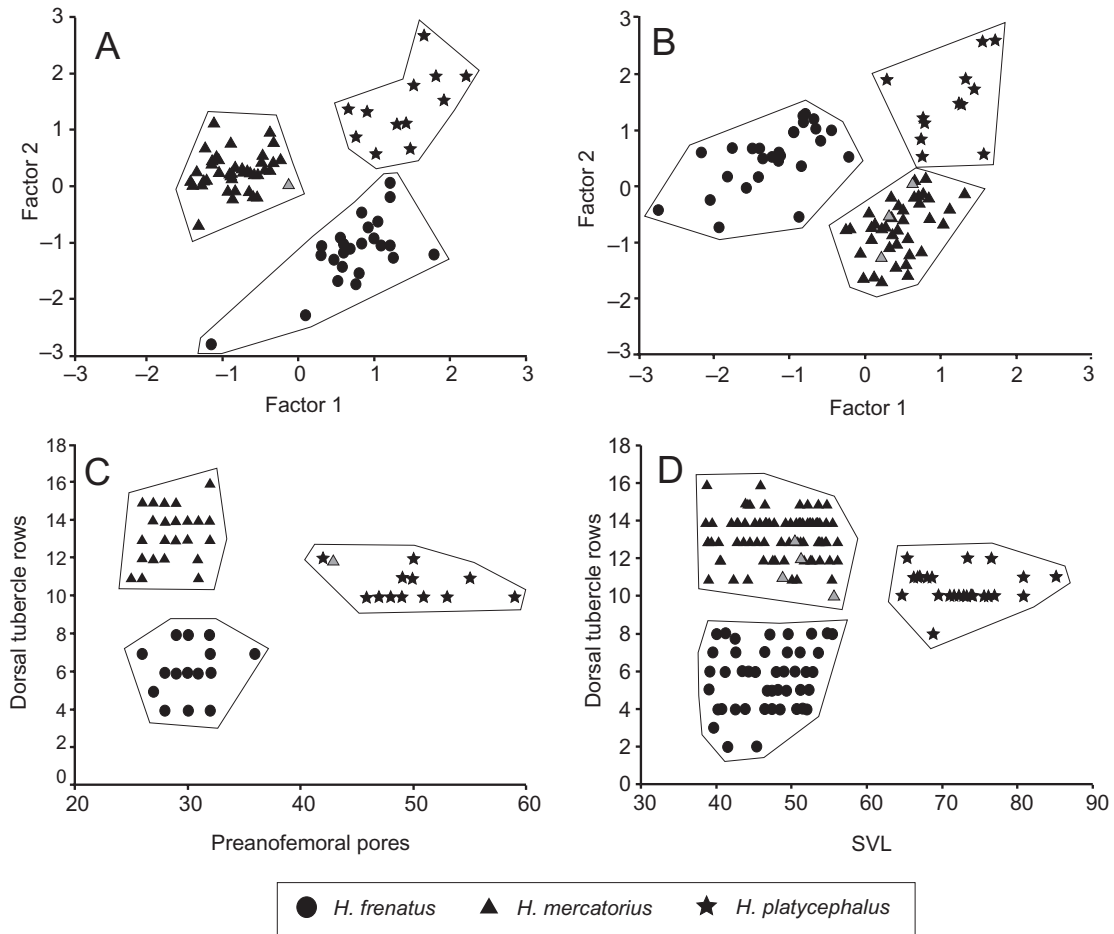


Figure 1. Morphological differentiation among *Hemidactylus* specimens from Madagascar. The scattergrams show males (A) and females (B) ordered along first and second principal components of a PCA based on meristic data from Table 1, including snout-vent length (SVL) as additional character; (C) males along the variables PAFP (number of preanofemoral pores) and DTR (number of dorsal tubercle rows); and (D) males and females arranged along the variables DTR and SVL. Grey triangles are type specimens of *Hemidactylus mercatorius* (one female, BMNH, 1946.8.19.92) and *H. gardineri* (junior synonym of *H. mercatorius*; one male, two females, BMNH, 1946.9.7.2, 1946.8.19.91, 1946.8.19.93).

clear although no overlap between the three groups was observed. This difference between sexes was probably due to the lack of one highly informative character (number of preanofemoral pores) in the female sample. Exclusion of the only non-meristic character (SVL) resulted in less resolved plots. Again, three factors with eigenvalues >1 were extracted that together explained 72% (males) and 69% (females) of the variance, respectively. In the analysis of the male sample, the three species were still separated without overlap, although the three groups appeared to be somewhat less distinct. In the female samples, a limited overlap between *H. mercatorius* and *H. platycephalus* was observed (data not shown).

A PCA using the morphometric data from Table 2 extracted only a single factor with an eigenvalue >1 . This first factor explained 91% (females) and 94%

(males) of the total variance while the second and third factors together explained less than 5% in both cases. The first factor that corresponds to the size component was not analysed further. Instead we extracted the second and third factors, but scatterplots based on these variables (not shown) did not result in any substantial separation of the species for either sex.

Four morphometric characters showed significant differences among species as revealed by an ANCOVA analysis with SVL as covariate (HIL, HW, TDIST, END), in separate comparisons among males and females ($P < 0.001$; $P < 0.05$ after Bonferroni correction over all 14 analyses). A slight significance of interspecies differences among male TED ($P < 0.05$) cannot be considered as significant after Bonferroni correction, and no significant differences were detected in EED and ILD. All meristic characters except for FL3

and TTR were significantly different among species both in comparisons among males and females (ANOVA; $P < 0.001$; $P < 0.05$ after Bonferroni correction); see Table 3 for significance of pairwise differences as revealed by ANOVA.

The type specimen of *Hemidactylus mercatorius* examined (a female) clustered with other Malagasy specimens assigned to this species, as did the types of *H. gardineri* (described from the Seychelles) in the multivariate analysis (Fig. 1). However, the male specimen out of the *gardineri* type series (BMNH, 1946.9.7.2) had a high number of preanofemoral pores (42) and therefore showed similarities to *H. platycephalus* in this character (Fig. 1C), indicating a possible taxonomic distinctness of the Seychelles populations which was not studied further here.

MOLECULAR PHYLOGENY

The hierarchical likelihood ratio test implemented in Modeltest selected a TVMef+G substitution model ($-\ln L = 1456.3046$) with a proportion of invariable sites of 0, a gamma distribution shape parameter of 0.1989, equal base frequencies, and empirical nucleotide substitution rates ($R_{A-C} = 4.8671$, $R_{A-G} = 10.2387$, $R_{A-T} = 1.3885$, $R_{C-G} = 0.1552$, $R_{C-T} = 10.2387$, $R_{G-T} = 1.0000$).

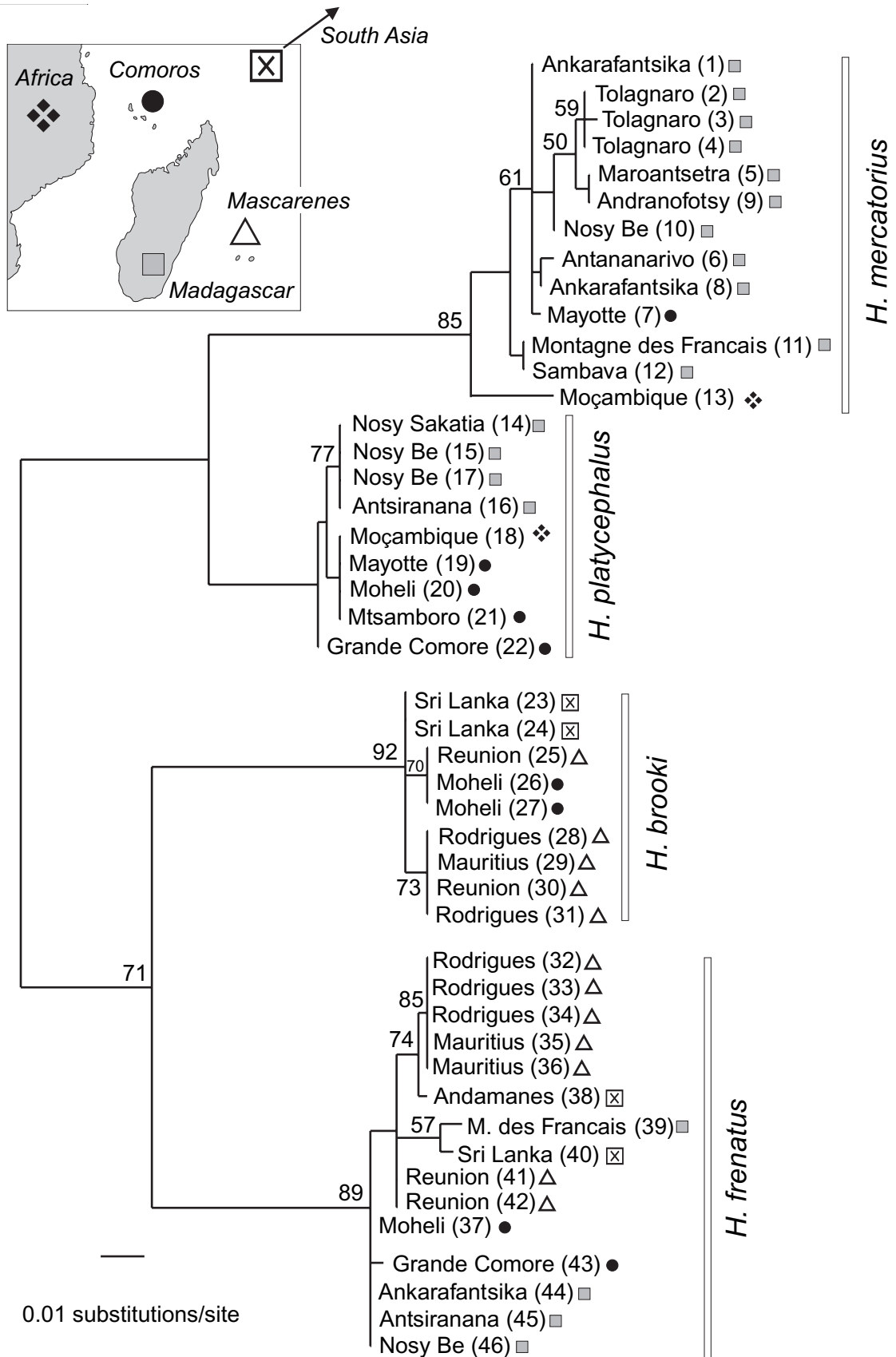
The maximum likelihood tree (Fig. 2) clearly distinguishes four main clades that represent four *Hemidactylus* species. These are supported by relevant bootstrap values except for the *Hemidactylus platycephalus* clade, which receives no significant support, almost certainly because of the low number of informative positions under the substitution model selected. Apart from the species known from Madagascar, the tree also reveals the occurrence of a fourth species in this region, namely *H. brooki* from the Comoro island Moheli and the three Mascarene islands La Réunion, Mauritius and Rodrigues. African specimens of *H. mercatorius* and *H. platycephalus* are clearly grouped with the Malagasy and Comoroan representatives assigned to these species. Also, Malagasy, Comoroan, Mascarene and South Asian specimens of *H. frenatus* and Mascarene, Comoroan and South Asian specimens of *H. brooki* clearly cluster together. In *H. mercatorius*, the African specimen is placed basal to the Malagasy and Comoroan populations, but in the other species there is no trend of a basal position of the continental representatives.

The minimum spanning trees constructed for the four species (Fig. 3) show clear differences of phylogeographical pattern. In *H. mercatorius*, a large number of haplotypes were found, many of which differ by a relevant number of steps. The pairwise total sequence

Table 3. Results of pairwise ANOVA comparisons between *Hemidactylus* species from Madagascar (P -values before Bonferroni-corrections). The left values refer to comparison among males, the right values to comparisons among females. Three asterisks mark significance values below 0.00077, the threshold value for the significance ($P < 0.05$) after a Bonferroni correction over all 67 comparisons

| Comparison | <i>H. frenatus</i> – <i>H. mercatorius</i> | <i>H. frenatus</i> – <i>H. platycephalus</i> | <i>H. mercatorius</i> – <i>H. platycephalus</i> |
|------------|---|---|--|
| SVL | –/– | ***/** | ***/** |
| LAM1B | 0.007/** | ***/** | ***/0.005 |
| LAM1C | –/– | –/– | –/– |
| LAM4B | –/– | 0.011/0.006 | ***/** |
| LAM4C | ***/** | ***/** | 0.020/– |
| SUPL | –/0.005 | ***/0.005 | ***/** |
| INFL | –/– | ***/0.001 | ***/** |
| TUBROW | ***/** | ***/** | ***/** |
| TUBCENT | ***/** | ***/** | –/– |
| TATUBR | –/– | –/– | –/– |
| PORALL | – | *** | *** |

Figure 2. Maximum likelihood tree based on 391 bp of the mitochondrial 16S rRNA gene in *Hemidactylus* specimens from the Indian Ocean region (hypervariable regions and gapped characters excluded). The numbers are bootstrap values in per cent (maximum likelihood; 100 replicates; values below 50% not shown). The symbols represent the general geographical origin of the specimens (Africa, Comoros, Madagascar, Mascarenes, South Asia) as shown in the inset map. Numbers after locality names are specimen labels as also used in Fig. 4 and Appendix 3.



divergence is 24–29 substitutions between the individual from Africa (specimen number 13) and any of the other individuals. Among the Malagasy populations, two individuals from northern Madagascar (Sambava and Montagne des Francais; 11 and 12) have divergences of 9–14 substitutions compared with those from other regions. Also in addition to these two divergent specimens, the *H. mercatorius* network shows a rather clear phylogeographical structure, with one group of specimens from the east of Madagascar (2–5 and 9), one group from the west and centre (1, 6 and 8), and one specimen from the north-west (10). Only two specimens (2 and 4, both from Tolagnaro in south-eastern Madagascar) have identical haplotypes. The single Comoro specimen is placed close to the specimens from western-central Madagascar.

In contrast, the trees of the other species contain more specimens with identical haplotypes and less distinct phylogeographical clustering. In *H. platycephalus*, all four Malagasy specimens share an identical haplotype. In *H. frenatus*, two haplotypes are found in Madagascar. One is shared by three specimens from the north, north-west and west (44–46) and is the closest to a haplotype found on the Comoros; a second haplotype is found in one specimen (39) from the north and is closest to a haplotype identified from Sri Lanka.

DISTRIBUTION OF *HEMIDACTYLUS* IN MADAGASCAR

Combining the data from specimens examined morphologically (Appendix 2) with those sampled for genetic analysis (Fig. 2), we plotted the distribution of the three Malagasy *Hemidactylus* species (Fig. 4). Altogether, 22 localities of *H. frenatus*, 30 localities of *H. mercatorius* and nine localities of *H. platycephalus* were included in the maps. Many additional localities are known to us, but only from personal observations or photographic records and are therefore not considered. The maps indicate that *Hemidactylus frenatus* is a coastal species that so far has only been collected in the west and north of Madagascar. *Hemidactylus platycephalus* seems to be restricted to the north-western and northern lowland areas, with a single observation from Mananara on the east coast (two female specimens, MNHN, 1989.3703–3704). *Hemidactylus mercatorius* is by far the most widespread species. It occurs in lowland coastal areas all around the island as well as in highland areas, although at higher altitudes (>1000 m elevation) it seems to be restricted to large towns (Antananarivo, Fianarantsoa; 1290 and 1160 m above sea level).

Where their distributions overlap, all three *Hemidactylus* can occur sympatrically according to our field observations. In areas in which *H. frenatus* and *H. mercatorius*, and *H. frenatus* and *H. platycephalus*

co-occur, they are regularly seen in close syntopy, i.e. even on walls of the same buildings. In coastal areas a third nocturnal species (*Gehyra mutilata*) is often also a component of the commensal gecko community. In contrast, instances of syntopy of *H. mercatorius* and *H. platycephalus* are less common. On the Malagasy offshore island Nosy Be, we only observed *H. platycephalus* on buildings, while *H. mercatorius* seemed to be restricted to secondary forest and banana plantations.

DISCUSSION

TAXONOMY OF *HEMIDACTYLUS* IN THE INDIAN OCEAN REGION

The almost cosmopolitan distribution of geckos of the genus *Hemidactylus*, and their colonization capabilities, constitute important challenges for systematists and evolutionary biologists who aim to understand their biogeography. Revisions and other accounts dealing with these lizards usually focus on single areas (Kluge, 1969; Broadley, 1977; Vanzolini, 1978; Branch, 1998; Jesús *et al.*, 2001) and therefore it is still uncertain which populations assigned to *Hemidactylus mercatorius*, *H. mabouia*, *H. frenatus* and *H. brooki* from all over the globe are actually conspecific. Our data provide for the first time some clarification of this question for the Malagasy region, an area in which the lack of revisionary work was especially notorious (see Appendix 1).

The meristic data for Malagasy *H. mercatorius* specimens provided herein agree with those presented by Kluge (1969), Broadley (1977) and Vanzolini (1978) for Africa and the Neotropics (under the name *mabouia*). This indicates that these populations are actually closely related, but the exact species delimitations of *H. mercatorius* and *H. mabouia* remain a future challenge besides the lack of nomenclatural stability of *mabouia* (see Appendix 1). Our morphological data also provide strong evidence that the large *Hemidactylus* specimens from north-western Madagascar, considered to be *H. mabouia* by Glaw & Vences (1994), actually belong to the species *H. platycephalus* as defined by Broadley (1977). This is supported by their large size, high number of preanofemoral pores, and relatively low number of dorsal tubercle rows: Madagascar (Tables 1 and 2), SVL 65–85 mm, DTR 8–12, PAFP 42–59; Africa (Broadley, 1977), SVL up to 66 mm, DTR 8–12, PAFP 45–57.

Our morphological and genetic results corroborate the existence of three distinct *Hemidactylus* taxa on Madagascar. According to our data, these species are not restricted to Madagascar, although in the case of *H. mercatorius* it is questionable whether the Malagasy specimens are conspecific with the strongly

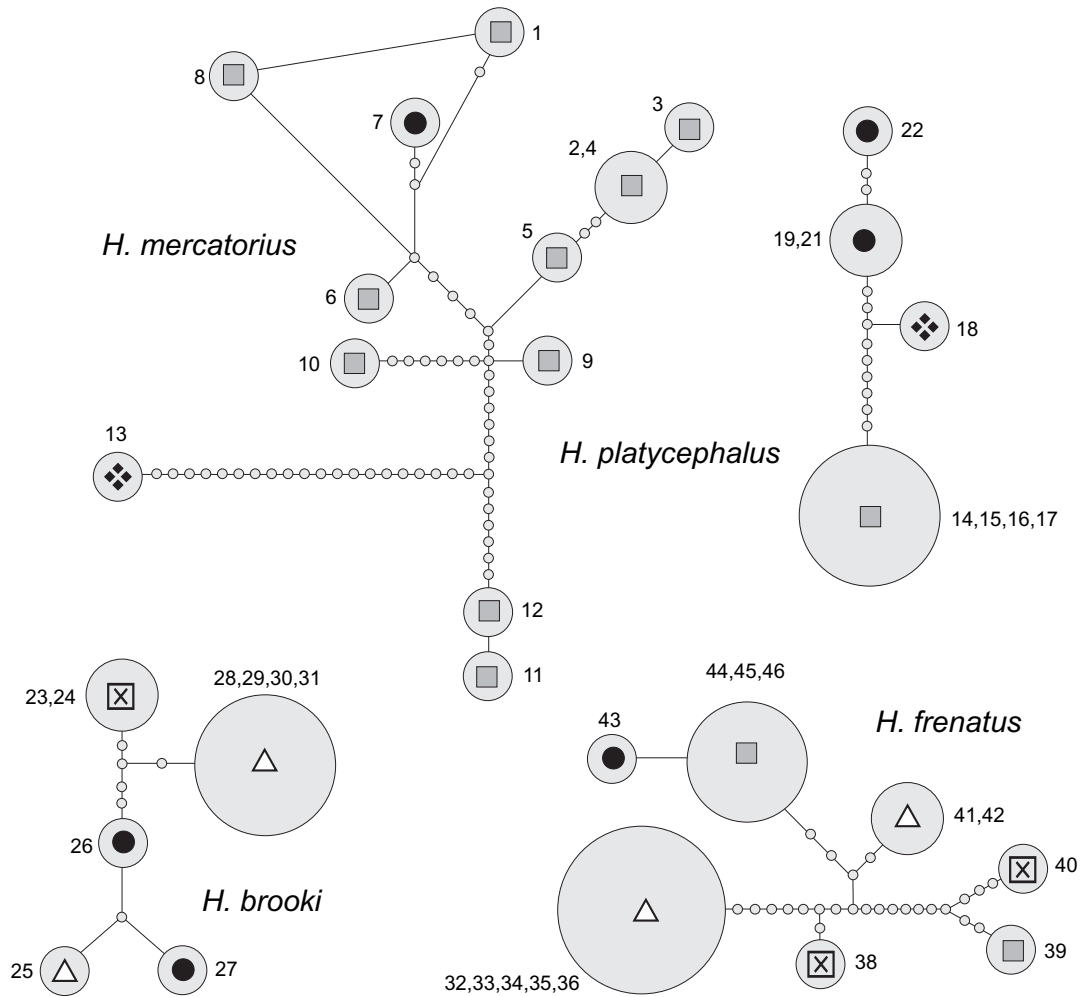


Figure 3. Haplotype networks (minimum spanning trees) for the four species of *Hemidactylus* studied, based on analysis of about 500 bp of the mitochondrial 16S rRNA gene (different sequence lengths for each analysis because of slightly different alignments for each species). Numbers next to and symbols within haplotype symbols refer to specimen numbers and geographical origins as specified in Fig. 2 (grey squares, Madagascar; black circles, Comoros; white triangles, Mascarenes; crossed square, Sri Lanka and Andamanes; composed rhomboid, Africa). Small dots refer to putative haplotypes needed to connect the network but not observed in the individuals studied.

differentiated African individuals studied. The phylogeography and taxonomic status of African geckos currently assigned to *Hemidactylus mabouia* requires further investigation and will be dealt with in a forthcoming paper. Our data further confirm the presence of a fourth species in the Malagasy region: *H. brooki* occurs on the Mascarenes and on the Comoro island of Moheli.

INSTANCES OF NATURAL COLONIZATION

Malagasy populations of *Hemidactylus mercatorius* are highly differentiated, and their haplotype distri-

bution seems to correlate with geography. This pattern is comparable with that found in endemic insular gekkonids, such as Makkaronesian *Tarentola* (Carranza *et al.*, 2000) and Cape Verdian *Hemidactylus* (Jesús *et al.*, 2001). Considering the wide distribution of *H. mercatorius* over Madagascar, in anthropogenous as well as natural habitats (Glaw & Vences, 1994), it is likely that it has a long history of occurrence that predates human settlement.

Our data also provide some indications for a natural colonization of the Comoro islands by *H. platycephalus*. In this species, the Comoroan populations show some phylogeographical structuring.

In both these cases, it is difficult to assess the direction of the dispersal events. For *H. mercatorius*, it would be necessary to obtain genetic data from other populations of the *H. mabouia-mercatorius* complex from Africa and the Neotropics to reconstruct the pathways of colonization. For Comoroan *H. platycephalus* an origin from Africa is probable, because the Malagasy specimens were genetically uniform and therefore unlikely to be the source of the more diverse Comoroan individuals.

PROBABLE INTRODUCTIONS

Although our data do not in all cases suffice to draw definitive conclusions, they provide indications of some more recent origins of *Hemidactylus* populations in the Malagasy region, which may represent introductions by humans.

The *H. mercatorius* specimen sequenced from the Comoro islands was found in the main (harbour) town of Mayotte (Mamoudzou), and the species has not been recorded elsewhere on the island. Its haplotype is very similar to that of the Malagasy specimen from Ankarafantsika, a nature reserve close to

Mahajanga where many Comoroans live and regularly travel to Mayotte. We therefore consider it probable that the Mayotte population has been recently introduced.

Similarly, Malagasy specimens of *H. platycephalus* from three localities in north-western Madagascar had identical haplotypes, which could be interpreted as representing a recent colonization or introduction. Although the minimum spanning tree (Fig. 4) would suggest an African origin of the Malagasy populations, a Comoroan origin cannot be excluded until a wider haplotype survey becomes available from this archipelago and from continental Africa. In this context it is relevant that the main distribution area of this species on Madagascar, the north-western coast, is a region harbouring a significant number of species with biogeographical links to the Comoros and Africa. In several cases the allochthonous origin of these species in Madagascar cannot be excluded or is probable (e.g. *Mabuya comorensis* and *Kinixys belliana*; Raselimanana & Vences, 2003), whilst in other cases the Comoroan and Malagasy species are differentiated at the species or subspecies level (e.g. *Oplurus cuvieri*, species of *Amphiglossus* and *Furcifer*; Glaw & Vences,

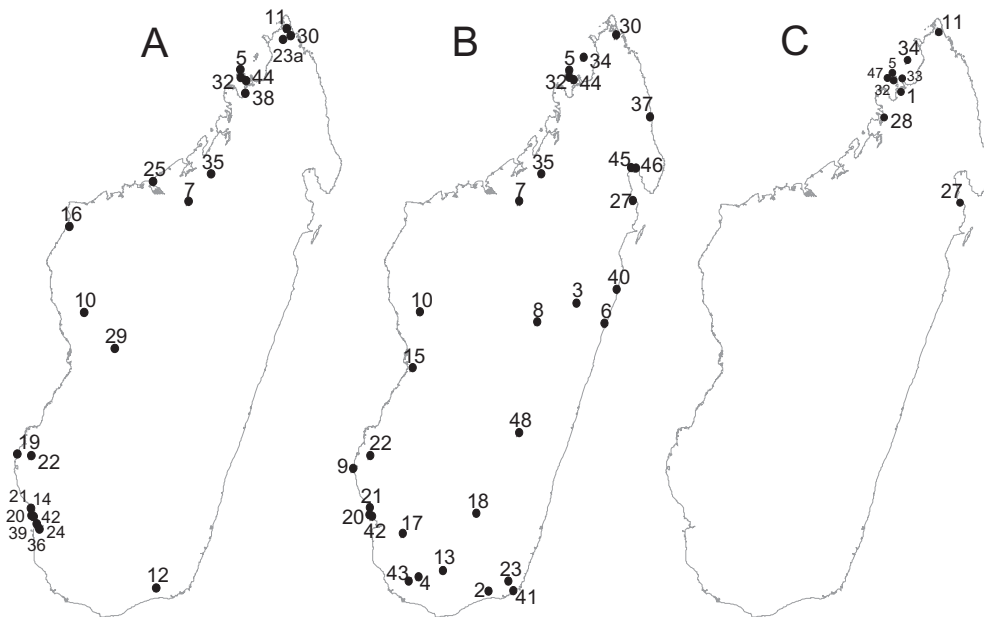


Figure 4. Distribution of *Hemidactylus* in Madagascar, based upon locality data from specimens examined for morphology (Appendix 2) and DNA sequences (Fig. 2). A, *Hemidactylus frenatus*; B, *H. mercatorius*; C, *H. platycephalus*. Localities were numbered alphabetically and are as follows: 1, Ambanja; 2, Amboasary; 3, Ampahana forest; 4, Ampanihy; 5, Amporaha; 6, Andevoranto; 7, Ankarafantsika; 8, Antananarivo; 9, Antsepoka; 10, Antsingy; 11, Antsiranana; 12, Behara; 13, Bekitro; 14, Belalanda; 15, Beroboka Ava.; 16, Besalampy; 17, Betioky; 18, Betroka; 19, Bevato; 20, Fiherenana; 21, Ifaty; 22, Ihotry; 23, Isaka-Ivondro; 23a, Joffreville; 24, Lavenombato; 25, Mahajanga; 26, Maintirano; 27, Mananara; 28, Maromandia; 29, Miandrivazo; 30, Montagne des Français; 31, Morafenobe; 32, Nosy Be; 33, Nosy Faly; 34, Nosy Mitsio; 35, Port-Bergé Vaovao; 36, Saint Augustin; 37, Sambava; 38, Sambirano; 39, Sarodrano; 40, Toamasina; 41, Tolagnaro; 42, Toliara; 43, Itrobeka; 44, Nosy Vorona; 45, Maroantsetra; 46, Andranofotsy; 47, Nosy Sakatia; 48, Fianarantsoa.

1994), or they are native to Madagascar but were introduced to the Comoros (e.g. *Leioheterodon*; Meirte, 1992).

The available data also suggest that the current distribution patterns of *Hemidactylus brooki* and *H. frenatus* in the Indian Ocean region have been, at least partly, shaped by multiple human translocation. *H. brooki* shows little phylogeographical structuring, with individuals from the Mascarenes having haplotypes very similar to those from the Comoros on one hand (specimen 25 in Fig. 3), and to Sri Lanka on the other hand (specimens 28–31). A similar, though more complex picture is also shown by *H. frenatus* in which Malagasy specimens from all over the north-western coast (specimens 44–46) had identical haplotypes, similar to that from Grande Comoro.

DIVERGENT HAPLOTYPES IN NORTHERN MADAGASCAR

The haplotype networks of *Hemidactylus frenatus* and *H. mercatorius* (Fig. 4) reveal one additional remarkable aspect. One *H. frenatus* from Montagne des Français in the extreme north of Madagascar (specimen 39) was the only Malagasy specimen with a haplotype different from that of the other Malagasy specimens. Also a specimen of *H. mercatorius* from Montagne des Français, and another one from Sambava in the north-east (11 and 12), had strongly divergent haplotypes. One of the two possibly non-endemic frogs from Madagascar (*Ptychadena mascareniensis*) shows an identical pattern, with specimens from the extreme north and north-east having divergent and unique haplotypes (Vences *et al.*, 2004). All involved localities are located between Antsiranana and Sambava, at very low altitudes in northern Madagascar. This congruence in phylogeographical pattern among unrelated widespread species may point to a previously unrecognized area of endemism in coastal northern Madagascar that does not include the Sambirano region in the north-west (Glaw & Vences, 1994).

This pattern also has some impact on the reconstruction of the origins (natural vs. introduced) of these populations. Thus far, similar observations were made in two gecko and one frog species. It appears to be unlikely that these unrelated taxa were simultaneously introduced into this area by humans. Therefore, these populations may be native to northern Madagascar. In this case at least one of them, *Hemidactylus frenatus*, may have multiple origins – native and introduced – on Madagascar. If this hypothesis was further corroborated, it would not only be interesting from a biogeographical perspective. The possible replacement of endemic Malagasy gene lineages by introductions, even of conspecific specimens, should also be monitored from a conservation standpoint.

CONCLUSIONS

The phylogeographical patterns documented by our genetic analyses provide some information on the probable colonization history of the Malagasy region by *Hemidactylus*, despite the somewhat limited number of individuals sequenced. We found strong evidence for the occurrence of three species on Madagascar (*H. frenatus*, *H. mercatorius* and *H. platycephalus*), four species on the Comoros (*H. frenatus*, *H. platycephalus*, *H. mercatorius* and *H. brooki*), and two species on the Mascarenes (*H. frenatus* and *H. brooki*). These distributions might be explained by different processes. There is strong evidence for a natural colonization of Madagascar by *H. mercatorius*, based on the diversity and phylogeographical structuring of its haplotypes. Also the populations of *H. platycephalus* on the Comoros may be due to natural colonization, whereas some other populations of *Hemidactylus* in the Malagasy area (especially *H. mercatorius* and *H. brooki* on the Comoros) were most probably introduced by humans.

Apart from *Hemidactylus mercatorius* and *H. platycephalus*, several other vertebrates on Madagascar are thought to be conspecific or very closely related to African taxa, including the crocodile (*Crocodylus niloticus*), several freshwater turtles (*Pelusios castanoides*, *P. subniger*, *Pelomedusa subrufa*), one frog (*Ptychadena mascareniensis*), and a bush pig (*Potamochoerus larvatus*). Species of *Hemidactylus* – at least *H. mercatorius* – may be representatives of a more extensive Pleistocene colonization of Madagascar from Africa by a variety of faunal elements. These putative recent arrivals have often been overlooked in attempts to reconstruct Malagasy biogeography. The phylogeography of these species may prove to be informative for understanding the pathways and means of overseas dispersal that probably gave rise to most of Madagascar's extraordinary fauna (Krause *et al.*, 1997).

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APPENDIX 1

TAXONOMIC HISTORY OF THE
H. MABOUIA COMPLEX

The status and identification of commensal *Hemidactylus* from the western Indian Ocean islands and the adjacent African mainland have an involved taxonomic history and remain sources of confusion. The following is a chronological summary incorporating the observations of Kluge (1969) and Broadley (1977)

Hemidactylus platycephalus (Peters, 1854) was described from the coast of Moçambique and Anjoana Island (Anjouan) in the Comoro Archipelago, but when this taxon was more fully described and illustrated (Peters, 1882) it was treated as a synonym of *H. mabouia* and this was accepted by subsequent authors.

Barbour and Loveridge (1928) realized that there were two species included under the name *H. mabouia* in East Africa, but they regarded the large form (*H. platycephalus*) as true *H. mabouia* and described the smaller form as new under the name *H. persimilis*. Loveridge (1929, 1933, 1936b, 1942, 1947, 1951; 1953a, 1953b, 1955) thereafter continued to identify the large species of East Africa as *H. mabouia*, but the small species was listed under several names.

Loveridge (1936a) described *H. mandanus* from Manda Island, Kenya, but in 1942 he placed this taxon and *H. persimilis* in the synonymy of *H. gardineri* Boulenger, 1909, originally described from Farquhar island (Seychelles). Loveridge (1953a) finally placed *H. gardineri* in the synonymy of *H. mercatorius* Gray, described from Madagascar in 1842.

Hewitt (1932) described *H. tasmani* from a pair of geckos collected at Driefontein Mission, near Umvuma, Rhodesia (Zimbabwe). FitzSimons (1943) recognized *tasmani* as a full species, but Loveridge (1942) placed it in the synonymy of *H. mabouia* because he could not distinguish Birchenough Bridge specimens from Antillean material. However, these geckos were not *tasmani* but *H. mabouia*, which extends up the Sabi River to Birchenough Bridge, as previously indicated by FitzSimons (1939, 1943).

Vinson and Vinson (1969) identified the two *Hemidactylus* species on the Mascarene islands as *frenatus* and *mercatorius*.

Kluge (1969) revised the New World forms of the *Hemidactylus mabouia* and *H. brooki* complexes. He drew attention to the fact that the holotype of *Gecko Mabouia* Moreau de Jonnès is apparently an example of the Colombian population now called *Hemidactylus brooki leightoni* Boulenger. He stated that: 'I believe it is in the best interest of nomenclatural stability to ignore the first use of the name *mabouia* by Cuvier (not in a binomial form), and the true identity and probable geographical origin of Moreau de Jonnès' holotype (probably Cartagena, Colombia). I recommend that the name *mabouia* (Moreau de Jonnès) be retained for the Lesser Antilles and South American *Hemidactylus* species populations which are diagnosed on page 29.' The alternative would be to transfer the specific name *mabouia* to the equally cosmopolitan species now known as *H. brooki* Gray, while the species currently known as *H. mabouia* would take the name *H. incanescens* Wied-Neuwied, 1824 or *H. armatus* Wied-Neuwied, 1824, both described from

Brazil. Kluge (1969) also removed the names *platycephalus* Peters and *tasmani* Hewitt from the synonymy of *H. mabouia*, but did not define them or discuss their status. He returned *H. mercatorius* Gray to the synonymy of *H. mabouia*.

In his biogeographical review Blanc (1971) listed the three taxa *mabouia*, *frenatus* and *gardineri* for the Malagasy region. Sassi, Russell and Lanza (1972), possibly overlooking Kluge's work, recorded *H. mercatorius* from Southern Somalia, after comparing their specimens with the types of *H. mercatorius*, *H. gardineri* and *H. mandanus*. They also indicated the occurrence of *H. mercatorius* in eastern Africa, Madagascar, Mauritius and several Seychelles islands.

After an analysis of populations in south-east Africa, Broadley (1977) validated *platycephalus*, treated *tasmani* as an endemic 'race' of *H. mabouia* from central Zimbabwe, and continued to follow Kluge (1969) in treating *mercatorius* (as well as *gardineri* and *persimilis*) as synonyms of *mabouia*. He specifically excluded *H. benguellensis* Bocage from Angola as it lacked transversely enlarged median subcaudals. After including the southern Somalia specimens of Sassi *et al.* (1972), he noted that the distribution of typical *H. mabouia* in Africa extended from southern Somalia to KwaZulu-Natal in South Africa and west to Liberia and Sierra Leone, and also to Madagascar, the Comoro Islands, Seychelles and Mauritius (Broadley, 1977). He also quoted *H. platycephalus* from the Comoro island Anjouan, but not from Madagascar, and restricted the original type localities of *platycephalus* (coastal localities in Moçambique and Anjouan) to Moçambique by designation of a lectotype.

Pakenham (1983) followed the taxonomic conclusions of Kluge (1969) and Broadley (1977) and noted *Hemidactylus brooki angulatus*, *H. mabouia* and *H. platycephalus* from Zanzibar and Pemba Island. This arrangement was not followed by Lanza (1983, 1990), who continued to recognize *H. mercatorius* and *H. platycephalus* from coastal Somali. Cheke (1984) listed the species *H. frenatus* and *H. mercatorius* for several Seychelles islands, *H. brooki* for the island Desroches, and *H. frenatus* and *H. mabouia* on the Comoros.

In a checklist of world geckos, and without presenting further analysis or distributions of the Old World forms, Kluge (1991) recognized as full species *H. mercatorius* (including *gardineri*, *persimilis* and *mandanus* as synonyms), *H. platycephalus*, *H. tasmani* and *H. mabouia* (the latter including African *calabaricus* and *benguellensis* as synonyms).

Following this scheme, and because fieldwork had revealed the presence of two species with tubercular backs of which one was much larger and apparently occurring in north-western Madagascar, Glaw and

Vences (1994) used the names *Hemidactylus frenatus* (relatively small size, smooth dorsal skin), *H. mabouia* (large, tubercular) and *H. mercatorius* (small, tubercular) to refer to Malagasy *Hemidactylus*. Meirte (1999), besides *H. frenatus*, lists only *H. mabouia* from Mayotte (Comoros). Spawls *et al.* (2001) recorded only *H. mabouia* (presumably including *mercatorius*) and *H. platycephalus* from coastal regions of East Africa. Kluge (2001), in the latest updated checklist of gekkotan lizards, continued to consider *H. mercatorius* as valid species besides *H. mabouia*.

APPENDIX 2

SPECIMENS EXAMINED MORPHOLOGICALLY

Hemidactylus frenatus: Males

Amporaha, Nosy Be (MNHN 1986.220, Brygoo); Ankarafantsika (MNHN 1950.270, Millot); Antsingy nord, between Maintirano and Morafenobe (MNHN 1950.271, Paulian); Antsiranana [Diego] (MNHN 1990.1655, 1658, Pasteur & Blanc; MNHN 1893.191); Bas Sambirano (MNHN 1950.269, Millot); Besalampy (MNHN 1990.838, Domergue); Fiherenana plain (MNHN 2000.700, Geay); Ihotry Lake (MNHN 1970.327–328, Arnoult); Mahajanga [Majunga] (MNHN 1974.1014, Waterlot); Montagne des Francais (ZSM 539/2000, Glaw & Vences); Nosy Be (ZSM 454/2000, Glaw & Vences); Port Berge (MNHN 1990.1644, Pasteur & Blanc); St. Augustin valley (MNHN 1899.327, 329–330, Grandidier); Toliara [Tulear] (MNHN 1950.268, Millot; ZFMK 59802); West Coast [Cote Ouest] (MNHN 1895.157); ZSM 535/2000, Glaw & Vences).

Females

Antsingy nord, between Maintirano and Morafenobe (MNHN 2000.2902, 2905, Paulian); Antsiranana [Diego] (MNHN 1990.1654, 1656–1657, Pasteur & Blanc); Behara (MNHN 1950.275, Millot); Belalanada-Toliara (MNHN 1990.1662, Domergue); Besalampy (MNHN 1990.836–837, Domergue); Bevato (MNHN 1970.273, Koechlin); Fiherenanan plain (MNHN 1905.120, Geay); Ifaty (ZSM 599/2000, Glaw); Lavenombato (MNHN 1929.86); Miandrivazo (MNHN 1990.1620, Domergue); Montagne des Francais (ZSM 541/2000, Glaw & Vences); Nosy Vorona (MNHN 1956.78, 2000.686, Guibe); Port Berge (MNHN 1990.1646, 1659, Pasteur & Blanc); Sarodrano (ZSM 597/2000, Glaw); St. Augustin valley (MNHN 1899.325, 328, Grandidier); Toliara, Restaurant La Mangrove (ZSM 598/2000, Glaw); Joffreville (ZFMK 19334).

Hemidactylus mercatorius: Males

Amboasary, Mandrare (MNHN 1935.125, Catala); Amove (gargotte du bac d'Amove?) (MNHN 1990.893,

Domergue); Ampahana (ZFMK 61498); Ampanihy (MNHN 1933.39, Petit); Ampanihy (MNHN 1933.41, Petit); Amporaha, Nosy Be (MNHN 1986.223–224, Brygoo); Analamary (MNHN 1989.3322, Brygoo); Andevoranto (MNHN 1990.887, Domergue); Antandroy-Nord (MNHN 1901.142); Antsepoka (MNHN 1929.77); Antsingy (MNHN 1933.140, Leandri); Baraiketa (MNHN 1899.324); Bekitro (MNHN, 1990.880, Domergue); Betioky (ZFMK 19348–19349); Betroka (MNHN 1950.276, Millot); Fiherenana plain (MNHN 1905.119); Ifaty (ZSM 595–596/2000, Glaw); Isaka (MNHN 1901.141); Mananara (MNHN 1990.889, 892, Domergue); Sambava (ZSM 626/2000, Vences); Toamasina [Tamatave] (MNHN 1974.294, Martin); Tolagnaro [Fort Dauphin] (MNHN 1901.144); Toliara [Tulear] (MNHN 1990.881–882).

Females

Amboasary, Mandrare (MNHN 1935.126–127, Catala); Ampahana (ZFMK 61497); Ampanihy (MNHN 1933.40, 2000.697–698, Petit); Amporaha, Nosy Be (MNHN 1986.221–222, Brygoo); Andevoranto (MNHN 1990.888, Domergue); Antananarivo (ZSM 538/2000, Glaw and Vences); Antandroy-Nord (MNHN 1901.143); Beroboka [Berobaka] (MNHN 1990.884); Betioky (ZFMK 19350–19351); Fianarantsoa (ZFMK 62326); Forest S Port Berge (MNHN, 1990.1606, Pasteur and Blanc); Ihotry (MNHN 1990.1198, Domergue); Itrobeka (MNHN 1990.896, Domergue); Mahajanga [Majunga] (MNHN 1974.1013, Waterlot); Mananara (MNHN, 1990.890, Domergue); Nosy Mitsio (ZFMK 61559); Nosy Vorona (MNHN, 2000.666–668, 669, 672, 675, 677, 683, 689, Guibe); Toamasina [Tamatave] (MNHN 1974.293, Martin; MNHN 1990.885, Domergue; MNHN 1898.283); Toliara [Tulear] (MNHN 1899.323); Tolagnaro [Fort Dauphin] (MNHN 1901.145–146); Port Berge – Le Vieux (MNHN 1990.1608–1609, Pasteur & Blanc).

Hemidactylus platycephalus: Males

Ambanja (ZSM 540/2000, Glaw & Vences); Amporaha, Nosy Be (MNHN 1986.219, Brygoo); Antsiranana [Diego Suarez] (MNHN 1990.1665; ZSM 537/2000); Maromandia (MNHN 1930.262, 2000.692; 1990.1615, 1617, Pasteur & Blanc); Nosy Be (ZSM 450–451/2000, Glaw & Vences); Nosy Faly (MNHN, 1990.1611, 1613, Pasteur & Blanc); Nosy Mitsio (ZFMK 61562).

Females

Ambanja (MNHN 1990.1619, Pasteur & Blanc); Amporaha, Nosy Be (MNHN 1986.218, Brygoo); Mananara (MNHN 1989.3703–3704, Domergue); Maromandia (MNHN 2000.694–695; MNHN, 1990.1616, Pasteur & Blanc); Nosy Be/Nosy Komba? (MNHN 1990.883, Pasteur); Nosy Faly (MNHN

1990.1612, Pasteur & Blanc); Nosy Mitsio (ZFMK 61557–61558, 61563).

APPENDIX 3

LIST OF SPECIMENS AND LOCALITIES USED FOR GENETIC ANALYSIS

Additional collection acronyms used: MVTIS (tissue collection of M. Vences, voucher specimen not collected); PEM, Port Elizabeth Museum, Eastern Cape Museum, South Africa; UADBA-FG/MV and UADBA-MV, Université d'Antananarivo, Département de Biologie Animale (field numbers of F. Glaw & M. Vences); WRBTIS (tissue collection of W. R. Branch); ZSM, Zoologische Staatssammlung München.

Hemidactylus mercatorius

(1) MV 2001.309, Ampijoroa (Ankarafantsika), Madagascar, 2.2001; (2) MVTIS 2002-B50, Tolagnaro, Madagascar, 12.2001; (3) UADBA-MV 1490, Tolagnaro, Madagascar, 12.2001; (4) MVTIS 2002-B45, Tolagnaro, Madagascar, 12.2001; (5) ZSM 205/2002, Maroantsetra, Madagascar, 12.2001; (6) ZSM 209/2002, Antananarivo, Madagascar, 12.2001; (7) ZSM 674/2000, Mamoudzou, Mayotte, 2.2000; (8) ZSM 488/2001, Ankarafantsika, Madagascar, 2.2001; (9) MVTIS 2002-A24, Andranofotsy, Madagascar, 12.2001; (10) ZSM 207/2002, Nosy Be, Madagascar, 12.2001; (11) MVTIS 2000-C33, Montagne des Français, Madagascar, 3.2000; (12) MVTIS 2000-D26, Sambava, Madagascar, 3.2000; (13) PEM R13251, Moebase camp, Zambezia district, Mozambique, 5.1997; (14) ZSM 452/2000, Nosy Sakatia, Madagascar, 3.2000.

Hemidactylus platycephalus

(15) MVTIS 2000-C22, Nosy Be, Madagascar, 3.2000; (16) MVTIS 2000-C41, Antsiranana, Madagascar, 3.2000; (17) MVTIS 2002-D20, Nosy Be, Madagascar, 12.2001; (18) PEM R5529, Moebase camp, Zambezia

district, Mozambique, 5.1997; (19) ZSM 673/2000 Mt. Combani, Mayotte, 2.2000; (20) ZSM 374/2002, islet Chissioua Kanzoni (off Moheli), 4.2002 (21) ZSM 675/2000 islet Chissioua Mtsamboro (off Mayotte), 2.2000; (22) ZSM 682/2000, Chomoni, Grande Comoro, 2.2000.

Hemidactylus brooki

(23) MVTIS unnumbered, Kitulgala, Sri Lanka, 1999; (24) MVTIS unnumbered, Kitulgala, Sri Lanka, 1999; (25) ZSM 1015/2000, La Saline les Bains, La Réunion, 7.12.2000; (26) ZSM 370/2002, Itsamia, Moheli, 4.2002; (27) ZSM 369/2002, Nioumachoua, Moheli, 4.2002; (28) ZSM 990/2000, Port Mathurin, Rodrigues, 11.2000; (29) ZSM 961/2000, Pointe aux Roches, Mauritius, 11.2000; (30) ZSM 1014/2000, La Saline les Bains, 12.2000, La Réunion; (31) ZSM 989/2000, Port Mathurin, Rodrigues, 11.2000.

Hemidactylus frenatus

(32) ZSM 991/2000, Port Mathurin, Rodrigues, 11.2000; (33) ZSM 993/2000, Cotton Bay, Rodrigues, 11.2000; (34) ZSM 994/2000, near Grand Bay, Rodrigues, 11.2000; (35) ZSM 959/2000 near Morne Brabant, Mauritius, 11.2000; (36) ZSM 957/2000 near Pointe aux Roches, Mauritius, 11.2000; (37) ZSM 373/2002, Fomboni, Moheli, 4.2002; (38) MVTIS unnumbered, Port Blair, Andamane Islands, 1999; (39) MVTIS 2000-C29, Montagne des Français, Madagascar, 3.2000; (40) MVTIS unnumbered, Negombo, Sri Lanka, 1999; (41) ZSM 1018/2000, La Saline les Baines, La Réunion, 12.2000; (42) ZSM 1019/2000, La Saline les Baines, La Réunion, 12.2000; (43) ZSM 683/2000, Moroni, Grande Comoro, 2.2000; (44) ZSM 458/2001, Ankarafantsika, Madagascar, 2.2001; (45) MVTIS 2000-C37, Antsiranana, Madagascar, 3.2000; (46) MVTIS 2001-G12, Hell-Ville, Nosy Be, Madagascar, 1.2001.