Phylogeography of *Ptychadena mascareniensis* suggests transoceanic dispersal in a widespread African-Malagasy frog lineage

Miguel Vences[1], Joachim Kosuch[2], Mark-Oliver Rödel[3], Stefan Lötters[2], Alan Channing[4], Frank Glaw[5] and Wolfgang Böhme[6]

[1]*Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, University of Amsterdam, Amsterdam, The Netherlands,* [2]*Institute of Zoology, Department of Ecology, University of Mainz, Saarstrasse, Mainz, Germany,* [3]*Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Am Hubland, Würzburg, Germany,* [4]*Zoology Department, University of the Western Cape, Bellville, South Africa,* [5]*Zoologische Staatssammlung, Münchhausenstrasse, München, Germany, and* [6]*Zoologisches Forschungsinstitut und Museum A. Koenig, Adenauerallee, Bonn, Germany*

*Correspondence: Miguel Vences, Institute for Biodiversity and Ecosystem Dynamics, Zoological Museum, University of Amsterdam, PO Box 94766, 1090 GT Amsterdam, The Netherlands. E-mail: vences@science.uva.nl

ABSTRACT

Aim The Mascarene ridged frog, *Ptychadena mascareniensis*, is the only African amphibian species thought to occur on Madagascar and on the Seychelles and also Mascarene islands. We explored its phylogenetic relationships and intraspecific genetic differentiation to contribute to the understanding of transoceanic dispersal in amphibians.

Methods Fragments of the mitochondrial 16S rRNA gene were sequenced from specimens collected over most of the distribution area of *P. mascareniensis*, including populations from Madagascar, Mascarenes and Seychelles.

Results We identified five deeply divergent clades having pairwise divergences >5%, which probably all represent cryptic species in a *P. mascareniensis* complex. One of these seems to be restricted to Madagascar, the Mascarenes and the Seychelles. Sequences obtained from topotypic material (Réunion) were identical to the most widespread haplotype from Madagascar. The single Mauritian/Seychellean haplotype differed by only one mutation from a Malagasy haplotype.

Main conclusions It is likely that the Mascarene and Seychellean populations were introduced from Madagascar by humans. In contrast, the absence of the Malagasy haplotypes from Africa and the distinct divergences among Malagasy populations (16 mutations in one divergent hapolotype from northern Madagascar) suggest that Madagascar was populated by *Ptychadena* before the arrival of humans *c.* 2000 years ago. Because Madagascar has been separated from Africa since the Jurassic, this colonization must have taken place by overseas rafting, which may be a more widespread dispersal mode in amphibians than commonly thought.

Keywords

Madagascar, Mauritius, Reunion, Seychelles, Africa, Anura, Ranidae, *Ptychadena*, transoceanic dispersal.

INTRODUCTION

Although some anurans are known to tolerate limited salinity (Balinsky, 1981), amphibians generally are extremely sensitive to salt water. For this reason, their capacity of transoceanic dispersal is considered to be very low or absent, and amphibian biogeography is usually interpreted as the result of vicariance (Duellman & Trueb, 1986). However, evidence from molecular clock calculations in Caribbean amphibians (e.g. Hedges *et al.*, 1992) indicated that overseas dispersal

may have played a major role in shaping the distribution of this fauna.

The Indian Ocean has in the recent years been intensively used as model region to explore transoceanic dispersal phenomena (e.g. Arnold, 2000; Vences *et al.*, 2001; Raxworthy *et al.*, 2002; Yoder *et al.*, 2003). The exceptional interest in this region stems from the fact that it contains, on one hand, old continental landmasses such as Madagascar and the Seychelles which are characterized by highly endemic faunas and have been completely isolated from continents since the Mesozoic,

and on the other hand, geologically dated volcanic archipelagos such as the Comoros and Mascarenes that are fully oceanic yet also contain endemic faunal radiations.

The anuran fauna of Madagascar is characterized by a remarkable degree of endemism. Only two of over 200 nominal species of Malagasy anurans are considered to be not endemic and are classified in genera occurring elsewhere (Blommers-Schlösser & Blanc, 1991; Glaw & Vences, 1994): the tiger frog, *Hoplobatrachus tigerinus* (Daudin, 1803) and the Mascarene ridged frog, *Ptychadena mascareniensis* (Duméril & Bibron, 1841). Molecular data recently corroborated the allochthonous origin of the Malagasy *Hoplobatrachus* (Kosuch *et al.*, 2001). In contrast, the origin of Malagasy *Ptychadena* populations so far remains unclear. This genus contains 47 species of frogs that are restricted to subsaharan Africa and represent a well differentiated clade within the family Ranidae (Clarke, 1981; Dubois, 1992; M. Vences, unpubl. data). *Ptychadena mascareniensis*, the only not exclusively African species, is thought to be distributed in a vast area of Africa, Madagascar, the Mascarene islands (Mauritius and Réunion) and Seychelles islands (Channing, 2001; Rödel, 2000). West African populations of this species were in the past often considered as subspecies (see Rödel, 2000). The Mascarene populations are thought to have originated by introductions from Madagascar (Staub, 1993; Probst, 1997) while the origin of the Seychellean populations remains uncertain (Nussbaum, 1984). Parasites of Malagasy *P. mascareniensis* are phylogenetically basal to those of other *Ptychadena* species, but those of African populations attributed to *mascareniensis* have not been studied so far (Bentz *et al.*, 2001).

In this paper, we analyse mitochondrial DNA sequences attributed to *P. mascareniensis* from large parts of its distribution area. Our main goal is to understand whether the insular populations (Madagascar, Seychelles, Mascarenes) are of allochthonous origin or reached these archipelagos by overseas dispersal.

MATERIALS AND METHODS

Specimens from different localities (see Table 1) were collected by opportunistic searches. In the present study, we included all *Ptychadena* samples available to us, and a representative of the sister group of *Ptychadena* as outgroup (see Clarke, 1981): *Hildebrandtia ornata* (Peters, 1878). Specimens with the following set of characters were considered as *P. mascareniensis*: three to four pairs of continuous dorsal folds; no sacral fold; vocal sacs located above forearm insertion; no external metatarsal tubercle; no extranumeral tubercles on fourth toes (Lamotte, 1967; Lamotte & Ohler, 1997; Rödel, 2000; Channing, 2001). Additionally, these specimens were almost always characterized by a thin light longitudinal dorsal line on shanks and thighs and, and often had a greenish vertebral band which is rare in other *Ptychadena*.

Samples of muscle tissue or toe clips were sampled and preserved in 98% ethanol, and DNA extracted from these using different standard protocols. Voucher specimens were deposited in the herpetological collections of the Département de Biologie Animale, Université d'Antananarivo, Madagascar (UADBA), the National Museums of Kenya, Nairobi (NMK), the Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn, Germany (ZFMK), and the Zoologische Staatssammlung, München, Germany (ZSM), or are kept in the working collections of the authors (see Table 1). Sequences were deposited in Genbank (see Table 1 for accession numbers).

We chose a fragment of the mitochondrial 16S rRNA gene for amplification and sequencing, using standard primers (16SAR-L and 16SBR-H of Palumbi *et al.*, 1991) and procedures (Vences *et al.*, 2002). Due to the presence of hypervariable regions, this fragment shows sufficient variation to assess the phylogeny both among and within species (e.g. Vences *et al.*, 2002; Veith *et al.*, 2003a, b).

The PCR products were purified using QIAquick purification kits (Qiagen, Hilden, Germany) and sequenced on ABI 377 and 3100 automated sequencers. Sequences were aligned using Sequence Navigator software (Applied Biosystems, Lincoln, CA, USA). Hypervariable regions that could not be reliably aligned without assumption of indels were excluded from analysis (Swofford *et al.*, 1996). To be able to include a maximum of information, we analysed two separate alignments: (1) an alignment of all available *Ptychadena* sequences to understand phylogenetic relationships within the genus; in this alignment, hypervariable regions were excluded from analysis; (2) an alignment of the sequences of *P. mascareniensis* sensu stricto (specimens from the Malagasy clade) only, which was used to calculate a haplotype network (see below); this alignment needed no indels, and no regions were excluded. Uncorrected pairwise divergences between sequences were calculated by hand by separate pairwise alignments, and not excluding any of the hypervariable sites; hence, the pairwise divergences given refer to the complete fragment in all cases.

Substitution model parameters were estimated using Modeltest version 3.06 (Posada & Crandall, 1998). The hierarchical likelihood ratio tests in this program selected a general time reversible (GTR + I + G) substitution model as best fitting the data, with base frequencies (freqA = 0.3277; freqC = 0.1995; freqG = 0.1952; freqT = 0.2775) and substitution rates (R[A–C] = 6.4755; R[A–G] = 17.1508; R[A–T] = 7.4767; R[C–G] = 1.7346; R[C–T] = 44.1646; R[G–T] = 1) estimated from the data, a proportion of invariable sites of 0.4954 and a gamma distribution shape parameter of 0.5523. Maximum parsimony (MP) and maximum likelihood (ML; using Modeltest settings) analyses were carried out using PAUP*, version 4.0b8 (Swofford, 2002), using the heuristic search option with tree-bisection-reconnection (TBR) branch swapping and 100 random addition sequence replicates.

Two thousand bootstrap replicates were calculated under the MP optimality criterion, while only 500 ML bootstrap replicates were performed due to computational constraints. All bootstrapping was carried out using heuristic searches with 10 random addition sequence replicates and TBR branch swapping. Bayesian posterior probabilities were calculated using MrBayes, version 2.01 (Huelsenbeck & Ronquist, 2001)

Table 1 List of samples used for analysis, their geographic origin, voucher specimens and Genbank accession numbers

Species	Origin	Locality	Voucher	Accession
P. mascareniensis	Réunion	St. Etienne River	ZSM 1007/2000	AY517587 (m1)
P. mascareniensis	Réunion	St. Etienne River	ZSM 1006/2000	AY517587 (m1)
P. mascareniensis	Mauritius	Pereybere	ZSM 984/2000	AY517589 (m3)
P. mascareniensis	Mauritius	Cascade Chamarel	ZSM 973/2000	AY517589 (m3)
P. mascareniensis	Seychelles	Praslin	ZFMK 62876	AY517589 (m3)
P. mascareniensis	Madagascar	Antsiranana	ZSM 504-506/2000	AY517594 (m8)
P. mascareniensis	Madagascar	Sambava	ZSM 562/2000	AY517594 (m8)
P. mascareniensis	Madagascar	Nosy Be	UADBA-FG/MV 2001.02	AY517592 (m6)
P. mascareniensis	Madagascar	Ambanja	ZSM 421/2000	AY517591 (m5)
P. mascareniensis	Madagascar	Torotorofotsy	Voucher not collected	AY517590 (m4)
P. mascareniensis	Madagascar	Fierenana	ZSM 252/2002	AY517590 (m4)
P. mascareniensis	Madagascar	Ambohimanarivo	UADBA-MV 2001.1111	AY517590 (m4)
P. mascareniensis	Madagascar	Ambohimanarivo	UADBA-MV 2001.1109	AY517590 (m4)
P. mascareniensis	Madagascar	Moramanga	ZFMK 66683	AY517590 (m4)
P. mascareniensis	Madagascar	Andasibe	ZFMK 52754	AY517590 (m4)
P. mascareniensis	Madagascar	Antananarivo	ZSM 423/2000	AY517593 (m7)
P. mascareniensis	Madagascar	Mantasoa	UADBA-FG/MV 2000.04	AY517593 (m7)
P. mascareniensis	Madagascar	Ambatolampy	Voucher not collected	AY517593 (m7)
P. mascareniensis	Madagascar	Ambatolampy	Voucher not collected	AY517593 (m7)
P. mascareniensis	Madagascar	Ambatolampy	Voucher not collected	AY517593 (m7)
P. mascareniensis	Madagascar	Tolagnaro	Voucher not collected	AY517587 (m1)
P. mascareniensis	Madagascar	Nahampoana	ZSM 190/2002	AY517587 (m1)
P. mascareniensis	Madagascar	Ankarafantsika	ZSM 702/2001	AY517587 (m1)
P. mascareniensis	Madagascar	Andohariana (Andringitra)	UADBA-MV 2001.531	AY517587 (m1)
P. mascareniensis	Madagascar	Andohariana (Andringitra)	ZSM 717/2001	AY517588 (m2)
P. aff. mascareniensis A	Tanzania	Kibebe Farm, Iringa	AC 1728	AY517595
P. aff. mascareniensis A	Tanzania	Ihafu, Usangu Swamp, N Mbeya	AC 1824	AY517595
P. aff. mascareniensis A	Egypt	probably Rashid	Voucher not collected	AY517596
P. aff. mascareniensis A	Egypt	Rashid	Voucher not collected	AY517596
P. aff. mascareniensis A	Egypt	Esna	Voucher not collected	AY517596
P. aff. mascareniensis A	Egypt	Gabala, Fayoum	ZFMK 77757-758	AY517596
P. aff. mascareniensis A	Kenya	Runda-Gigiri	NMK/A/3842	AY517596
P. aff. mascareniensis A	Kenya	Mount Elgon	NMK/A3843/1	AY517596
P. aff. mascareniensis A	Kenya	Aberdares Salient	NMK A/3844/7	AY517596
P. aff. mascareniensis A	Kenya	Aberdares Salient	NMK A/3840/2	AY517596
P. aff. mascareniensis A	Kenya	Aberdares Salient	NMK A/3844/3	AY517596
P. aff. mascareniensis A	Kenya	Aberdares Salient	NMK/A3841/2	AY517596
P. aff. mascareniensis A	Kenya	Aberdares Salient	NMK/A3844/6	AY517596
P. aff. mascareniensis A	Kenya	Aberdares Salient	NMK/A3841/1	AY517596
P. aff. mascareniensis B	Cameroon	unknown	ZFMK 68826	AF215408
P. aff. mascareniensis B	Benin	Lama forest	ZFMK 77100	AY517597
P. aff. mascareniensis C	Ivory Coast	Mont Sangbé National Park	MOR S01.40	AY517598
P. aff. mascareniensis D	Kenya	Kakamega Forest	NMK A/3840/5	AY517599
P. aff. mascareniensis D	Kenya	Kakamega Forest	NMK A/3840/1	AY517599
P. aequiplicata	Ivory Coast	Taï National Park	MOR T01.44	AY517618
P. aequiplicata	Ivory Coast	Taï National Park	MOR T01.3	AY517617
P. aequiplicata	Ivory Coast	Taï National Park	MOR T01.19	AY517616
P. aff. aequiplicata	Ivory Coast	Marahoué National Park, forest	Voucher not collected	AY517614
P. aff. aequiplicata	Ivory Coast	Mont Sangbé National Park	Voucher not collected	AY517615
P. aff. aequiplicata	Ghana	Wli Waterfalls	MOR G56	AY517613
P. aff. aequiplicata	Benin	Lama forest	ZFMK 77098	AY517613
P. aff. aequiplicata	Benin	Lama forest	ZFMK 77104	AY517613
P. anchietae	South Africa	Mtunzini	Voucher not collected	AF215404
P. anchietae	South Africa	St. Lucia	Voucher not collected	AF215405
P. anchietae	Kenya	Marich Field Study Center	ZFMK 70824	—
P. anchietae	Kenya	Runda-Gigiri	SL coll. (unnumbered)	AY517612

Table 1 *continued*

Species	Origin	Locality	Voucher	Accession
P. anchietae	Kenya	Runda-Gigiri	NMK A/3849/1	AY517611
P. anchietae	Tanzania	Makuyuni	Voucher not collected	AY517610
P. anchietae	Kenya	Kakamega Forest	NMK A/3845	AY517609
P. bibroni	Ivory Coast	Taï National Park	MOR T38	AY517603
P. bibroni	Ivory Coast	Mont Sangbé National Park	Voucher not collected	AY517602
P. aff. bibroni	Gabon	Monts Cristal, Tchimbélé	SL 1036	AY517604
P. longirostris	Ivory Coast	Mont Sangbé National Park	Voucher not collected	AY517605
P. longirostris	Ivory Coast	Mont Sangbé National Park	Voucher not collected	AY517605
P. longirostris	Ivory Coast	Mont Sangbé National Park	Voucher not collected	AY517606
P. porosissima	South Africa	Kwambonambi	Voucher not collected	AF215411
P. aff. porosissima	Tanzania	Mumba	AC 2122	AY517601
P. pumilio	Ghana	Nkwanta	MOR G79	AY517600
P. pumilio	Ghana	Nkwanta	MOR G80	AY517600
P. oxyrhynchus	Namibia	Rundu	Voucher not collected	AF215409
P. oxyrhynchus	South Africa	Kwambonambi	Voucher not collected	AF215403
P. schubotzi	Ivory Coast	Mont Sangbé National Park	Voucher not collected	AY517607
P. schubotzi	Ivory Coast	Mont Sangbé National Park	Voucher not collected	AY517607
P. aff. schubotzi	Kenya	Kakamega Forest	NMK/A3846	AY517608
P. aff. schubotzi	Kenya	Kakamega Forest	SL coll	AY517608
P. aff. schubotzi	Kenya	Kakamega Forest	SL coll	AY517608
P. aff. schubotzi	Kenya	Kakamega Forest	SL coll	AY517608
P. subpunctata	Namibia	Rundu	Voucher not collected	AF215410
Hildebrandtia ornata	Ivory Coast	Comoé National Park	Voucher not collected	AF215402

Collection acronyms as in Materials and Methods, except for: AC, working collection of Alan Channing; MOR, working collection of Mark-Oliver Rödel; SL, working collection of Stefan Lötters. For specimens deposited in UADBA, we give the original fieldnumbers of Frank Glaw and Miguel Vences (FG/MV) or Miguel Vences (MV). Some accession numbers are assigned to several samples because of shared haplotypes.

under a GTR substitution model with parameters estimated from the data. A total of 300,000 generations were run, every 10th tree collected, and the number of initial generations needed before convergence on stable likelihood values was empirically estimated at 70,000; the 'burn in' parameter was consequently set at 23%.

A minimum-spanning network was constructed using the TCS software package (Clement *et al.*, 2000), which employs the method of Templeton *et al.* (1992). It calculates the number of mutational steps by which pairwise haplotypes differ and computes the probability of parsimony (Templeton *et al.*, 1992) for pairwise differences until the probability exceeds 0.95.

RESULTS

We obtained a total of 78 *Ptychadena* and one outgroup sequences. Several specimens had haplotypes identical to other individuals; these were excluded from the phylogenetic analysis. The final matrix contained 40 sequences. After exclusion of some hypervariable sites (altogether 26 nucleotides), 521 characters were included in the analysis. Of these, 159 were parsimony-informative.

Maximum parsimony analysis recovered 48 equally most parsimonious trees (579 steps; consistency index 0.461; retention index 0.802). A strict consensus of these is shown in Fig. 1. The results of ML and Bayesian analyses were largely in agreement with the MP topology, differing mainly in the arrangement of the basal clades (not shown). Specimens from different localities in Africa and the Indian Ocean islands assigned to *P. mascareniensis* were placed into one monophyletic group in all analyses.

Pairwise comparisons of sequences revealed a remarkable genetic diversity among the samples assigned to *P. mascareniensis*. Five main clades were identified that had pairwise divergences of over 5% (more than 25 nucleotide substitutions). In contrast, only small differences were found among other populations, such as the haplotypes m1–m7 from Madagascar (<1%). Several populations from Kenya had haplotypes identical to those found in Egypt.

A similar situation was observed in specimens assigned to other species of *Ptychadena*; in *P. bibroni* (Hallowell, 1845), *P. porosissima* (Steindachner, 1867), *P. schubotzi* Sternfeld, 1917 and *P. aequiplicata* (Werner, 1898) we identified two or more distinct clades of more than 5% pairwise sequence divergence.

A detailed analysis of the haplotypes assigned to *P. mascareniensis* in a strict sense (the clade containing specimens from the type locality Réunion) using the TCS program revealed a distinct geographical structuring of the Malagasy haplotypes (Fig. 2). A very divergent haplotype (m8) was found in northern and north-eastern Madagascar, while common haplotypes or haplotype lineages were found in localities from the central-east (m4) and central highlands (m7) and northwest (m5 and m6). The haplotypes identified from Réunion were identical to the most widespread Malagasy
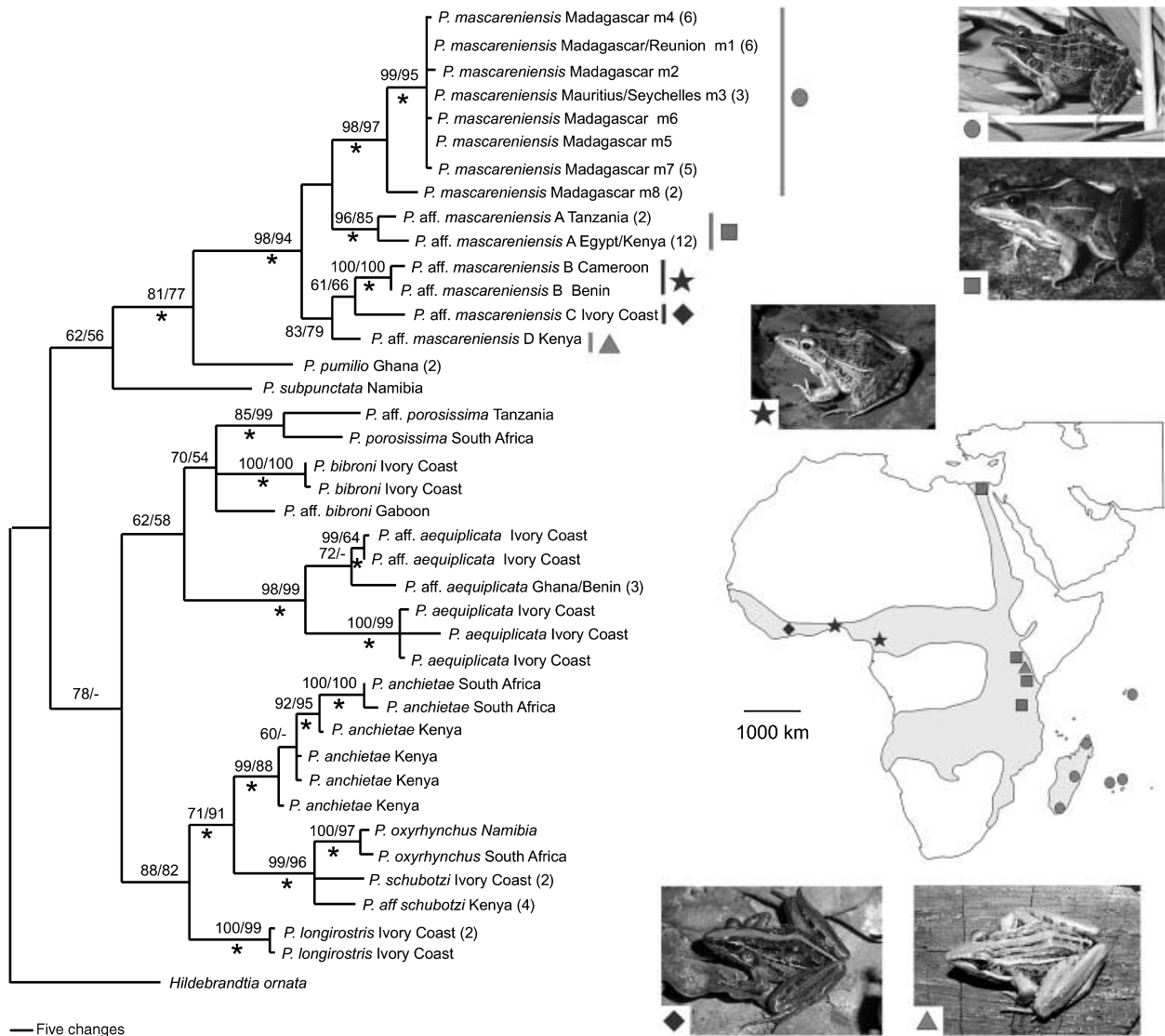
Figure 1 Maximum parsimony phylogram (strict consensus of 128 equally parsimonious trees) of all *Ptychadena* haplotypes sequenced for a fragment of the 16S rRNA gene. Symbols next to *P. mascareniensis* clades are those used for the samples sites in the map, which shows the approximate distribution area of *P. mascareniensis*, modified after the Global Amphibian Assessment records. Photographs show representative specimens of the different clades in life (Malagasy clade, specimen from Mantasoa; clade A, Aberdares, Kenya; clade B, Cameroon; clade C, Taï National Park, Ivory Coast; clade D, Kakamega, Kenya). Numbers m1–m8 in *P. mascareniensis* are haplotype numbers as used in Fig. 2. Numbers in parentheses after taxon names are the number of individuals sequenced for a particular haplotype. Numbers above branches are bootstrap support values in percent for maximum parsimony (2000 replicates) and maximum likelihood (100 replicates). Asterisks mark nodes that were supported by posterior probabilities > 95% in a Bayesian analysis.

haplotype (m1). The Seychellean specimen had an identical sequence to those from Mauritius, and this haplotype differed by only one substitution from the closest Malagasy one.

DISCUSSION

Cryptic species in *Ptychadena*

Our analysis identified a number of genetically highly divergent *Ptychadena* clades (pairwise divergences > 5%) that were morphologically identified as belonging to the same species. In all cases these together formed monophyletic groups,

indicating that, despite the considerable cryptic diversity, morphological characters are suitable to identify clades within the genus *Ptychadena*.

Mitochondrial genes are inherited only through the maternal line and recombination does in general not occur. Different discrete haplotype lineages can co-occur in the same species and even in the same population, and any hybridization event can lead to introgression of highly divergent haplotypes into a population (Avise, 2000). The usage of mitochondrial divergences as indicators for taxonomic distinctness is therefore controversial. However, so far, none of the known instances of intraspecific haplotype sharing in amphibians involved diver-
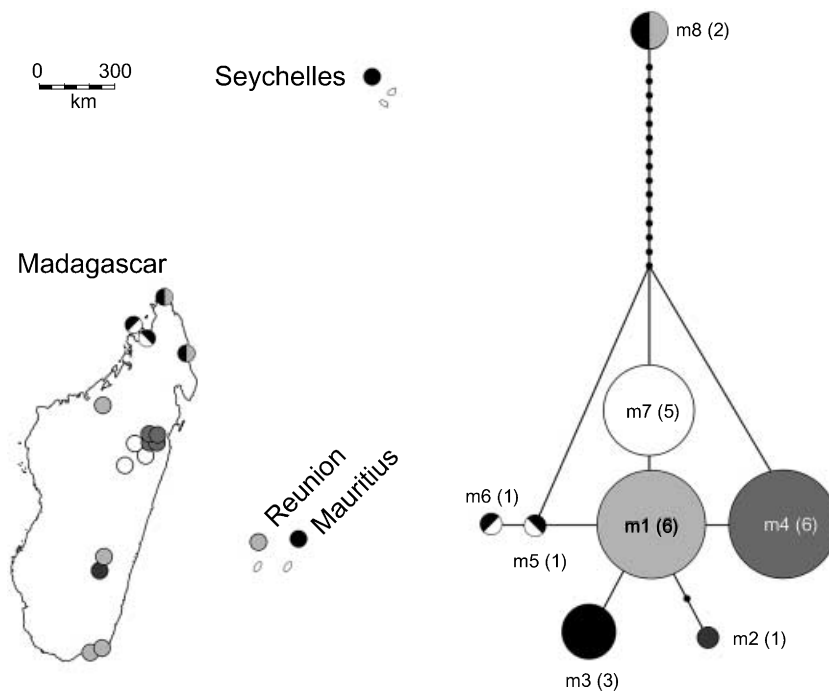
Figure 2 Haplotype network of *Ptychadena mascareniensis*, reconstructed using parsimony probability as implemented in TCS (right) and sample sites (left). The analysis was restricted to specimens of *P. mascareniensis* in a strict sense having uncorrected pairwise genetic divergences < 5% to those from the type locality (La Reunion). Each circle in the network corresponds to one observed haplotype (m1–m8), and size of circles is proportional to the number of individuals in which a haplotype was found (in parentheses). Small black dots represent hypothetical haplotypes needed to connect the network but not observed among the samples. The different grey shades and patterns of the haplotypes correspond to those used in the map.

gences as deep as those among the major intraspecific lineages of *Ptychadena*. Several Malagasy frog species, well diagnosed by morphological, ecological and bioacoustic characters, have been studied phylogeographically, and the differentiation in the 16S rRNA gene among conspecific populations never exceeded 2% (Vences *et al.*, 2002, 2003a; Vences & Glaw, 2002; Veith *et al.*, 2003a, b). Considering standard calibrations of the mitochondrial molecular clock (e.g. Caccone *et al.*, 1994; Veith *et al.*, 2003a), a pairwise divergence of 5% corresponds to a separation 20–5 Ma. We, therefore, suspect that most of the divergent lineages identified by us in *Ptychadena* actually are full species. However, to ascertain this hypothesis, and especially for any taxonomic conclusion, more detailed studies of morphology, bioacoustics and ecology are necessary.

According to this rationale, our data provide indications for the possible existence of five separate species currently subsumed under the name *P. mascareniensis*: besides the clade occurring at the type locality (Réunion) and on Madagascar, Mauritius and the Seychelles, this applies to the African clades A–D in Fig. 1. Of these, two are distributed in eastern and two in western Africa (Fig. 1). If future taxonomic studies corroborate their distinctness at the species (or subspecies) level, they may result to be undescribed taxa or to correspond to one or several of the available synonyms (Lamotte, 1967; Rödel, 2000). Judging from the origin of type specimens only, clade A almost certainly corresponds to *Rana nilotica* Seetzen, 1855 (from Egypt), while *Rana venusta* Werner, 1908 (from Sudan) may refer to clade A or D. *R. marchei* Rochebrune, 1885 (from Senegal) and *Rana (Ptychadena) mascareniensis hylaea* Schmidt and Inger, 1959 (from Mount Nimba, Liberia) may apply to clade C. A further name to be taken into account according to current synonymy (Amphibian Species of the World data base, http://research.amnh.org/cgi-bin/herpetology/amphibia, as of

15 June 2003) is *Rana savignyi* Jan, 1857, while the names *Rana idae* Steindachner, 1864 and *Rana nigrescens* Steindachner, 1864 refer to individuals from Madagascar and hence, apparently are correctly considered to be junior synonyms of *P. mascareniensis*. The name *P. bibroni*, in the past often used as subspecific name for West African *P. mascareniensis*, actually is the valid name for an unrelated species that previously was treated as *P. maccarthyensis* (Lamotte & Ohler, 1997).

Phylogeography of Malagasy, Mascarene and Seychellean *P. mascareniensis*

The haplotypes identified from the Mascarene and Seychelles islands were very similar or identical to the Malagasy ones. The specimens from the type locality Reunion had one of the most common haplotypes identified in Madagascar, making it almost certain that these frogs were introduced from Madagascar to Réunion (Probst, 1997). The introduction of *Ptychadena* from Madagascar to Mauritius has been documented as well (Staub, 1993) and we consider it probable that also the Seychellean populations were introduced. It is likely that a future wider sampling will provide evidence for the existence of the Mauritian/Seychellean haplotype (m3) in Madagascar.

On the contrary, it was most surprising to discover two deeply divergent haplotype lineages in Madagascar. The two specimens from northern and north-eastern Madagascar differed by 16–18 mutations (2.9–3.2%) from all other Malagasy and Indian Ocean specimens, while the remaining haplotypes from this area differed by a maximum of four mutations in the 16S rDNA fragment studied. Considering that intraspecific 16S rDNA divergences are usually not higher than 2% in Malagasy frogs (e.g. Vences *et al.*, 2003a), this high divergence could even indicate the presence of a second endemic species of *Ptychadena*

in Madagascar. Although we do not consider this possibility as likely, together with the geographical clustering of the other haplotypes and haplotype lineages from Madagascar, it provides one line of evidence indicating an autochthonous origin of the Malagasy *Ptychadena* populations.

Madagascar was colonized by humans only *c.* 2000 years ago (Dewar, 1997). Avise (2000), for mitochondrial sequences of *c.* 500 bp, expects about one nucleotide substitution to distinguish two matrilines that separated 100,000 years ago. Estimates from molecular clocks calibrated with ranid frogs (Veith *et al.*, 2003a) and salamanders (Caccone *et al.*, 1994) suggest that rates five to 10 times slower are to be expected in the 16S rRNA gene of amphibians. It is unlikely that the diversity of Malagasy *Ptychadena* haplotypes (six haplotypes with one to three substitutions, and one divergent haplotype with 16–18 substitutions) could arise within the last 2000 years in which humans are known to be present in Madagascar. This remains true even if we extend the time of human arrival to hypothetical periods of 10,000 or 50,000 years. To explain the presence of *Ptychadena* in Madagascar by human introduction would therefore require the assumption that a minimum of six separate matrilines have been introduced (i.e. six female specimens that all successfully reproduced or six clutches that all successfully developed).

Although frogs are occasionally consumed as food in Madagascar, this mostly refers to large species such as *Mantidactylus guttulatus*, *M. grandidieri*, *Boophis goudoti* and *Hoplobatrachus tigerinus* (Glaw & Vences, 1994). Only at a single occasion did we observe Malagasy people collecting *Ptychadena* for food (at Ambatolampy in the central highlands). Hence, there is no reason to assume that *Ptychadena* has intentionally been introduced to Madagascar for human consumption. We also consider repeated accidental introductions of this frog as very unlikely, because such introductions could then also be expected for any other of the widespread commensally African frogs, e.g. *Hyperolius* or *Bufo* spp.

A second line of evidence comes from the fact that the Malagasy *Ptychadena* haplotypes are highly divergent from all African mainland haplotypes that we sequenced. The large distribution area of this species complex, and the obvious existence of cryptic sibling species, poses difficulties to demonstrate the absence of the Malagasy haplotypes in Africa. However, the indications from our sampling make the hypothesis of multiple introductions even more unlikely. Actually, this would require the assumption that all separate introductions involved specimens from sites containing just those haplotypes and species not sampled by us. Furthermore, bioacoustic evidence indicates call differences between South African and Malagasy *P. mascareniensis*, although a comparison of the advertisement calls is difficult because of the existence of different call types (Glaw & Vences, 1994).

Recent transoceanic dispersals to Madagascar

Altogether we believe that there is sufficient evidence to assume that *P. mascareniensis*, in Madagascar, is of autochth-

onous origin. This is especially based on the high haplotype diversity found among Malagasy populations. However, because some crucial regions in Africa were not sampled, the African sister group of the Malagasy clade could not be reliably identified. As a consequence, the application of a molecular clock to the divergence between the Malagasy clade and its African sister group is not possible at present. The Malagasy lineage may have originated in the Pliocene or Pleistocene in the context of a major dispersal event that also included other Malagasy vertebrates considered as conspecific with African populations, such as the turtles *Pelomedusa subrufa* (Lacépède, 1788), *Pelusios castanoides* Hewitt, 1931 and *Pelusios subniger* (Lacépède, 1788), the crocodile *Crocodylus niloticus* Laurenti, 1768, and the house geckos *Hemidactylus mercatorius* Gray, 1842 and *H. platycephalus* Peters, 1854 (Glaw & Vences, 1994; Krause *et al.*, 1997).

Madagascar is separated from the African mainland by sea depths of more than 4000 m, and this separation dates back 130 Myr (Rabinowitz *et al.*, 1983). Hypotheses of Cenozoic land connections through the Davie fracture ridge (McCall, 1997) have been ruled out (Krause *et al.*, 1997, 1999). Thus the colonization by *Ptychadena* must have occurred by transoceanic dispersal. We have recently found morphological and genetic evidence (Vences *et al.*, 2003b) that the volcanic Comoros archipelago, located between Africa and Madagascar, harbors two endemic frog species of an otherwise exclusively Malagasy radiation which must have colonized these islands across the sea. Transoceanic dispersal has recently also been demonstrated in other groups thought to be unlikely to disperse over the sea, such as chameleons and terrestrial mammals (Raxworthy *et al.*, 2002; Yoder *et al.*, 2003), and it probably also has taken place in Malagasy and Seychellean hyperoliid frogs (Vences *et al.*, 2003b). Amphibian radiations in the western Indian Ocean region may, therefore, have relevantly been shaped by dispersal events, which stands in remarkable contrast to their low tolerance of salinity (Balinsky, 1981) and presumably low capacity of overseas dispersal (Inger & Voris, 2001; Brown & Guttman, 2002).

and Ivory Coast, to M.-O. Rödel, M. Veith and K. E. Linsenmair.

REFERENCES

Avise, J.C. (2000) *Phylogeography. The history and formation of species*. Harvard University Press, Cambridge.

Arnold, E.N. (2000) Using fossils and phylogenies to understand evolution of reptile communities on islands. *Isolated vertebrate communities in the tropics* (ed. by G. Rheinwald), pp. 309–324. Bonner Zoologische Monographien 46, Museum A. Koenig, Bonn.

Balinsky, J.B. (1981) Adaptation of nitrogen metabolism to hyperosmotic environment in amphibia. *Journal of Experimental Zoology*, **215**, 335–350.

Bentz, S., Leroy, S., du Preez, L., Mariaux, J., Vaucher, C. & Verneau, O. (2001) Origin and evolution of African *Polystoma* (Monogenea: Polystomatidae) assessed by molecular methods. *International Journal for Parasitology*, **31**, 697–705.

Blommers-Schlösser, R.M.A. & Blanc, C.P. (1991) Amphibiens (première partie). *Faune de Madagascar*, **75**, 1–379.

Brown, R.F. & Guttman, S.I. (2002) Phylogenetic systematics of the *Rana signata* complex of Philippine and Bornean stream frogs: reconsideration of Huxley's modification of Wallace's line at the Oriental-Australian faunal zone interface. *Biological Journal of the Linnean Society*, **76**, 393–461.

Caccone, A., Milinkovitch, M.C., Sbordoni, V. & Powell, J.R. (1994) Molecular biogeography: using the Corsica-Sardinia microplate disjunction to calibrate mitochondrial rDNA evolutionary rates in mountain newts (*Euproctus*). *Journal of Evolutionary Biology*, **7**, 227–245.

Clarke, B. (1981) Comparative osteology and evolutionary relationships in the African Raninae (Anura Ranidae). *Monitore zoologico italiano (n.s.)*, **15** (Suppl.), 285–331.

Channing, A. (2001) *Amphibians of Central and Southern Africa*. Cornell University Press, Ithaca.

Clement, X., Posada, D. & Crandall, K. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Dewar, R.E. (1997) Were People responsible for the extinction of Madagascar's subfossils, and how will we ever know? *Natural change and human impact in Madagascar* (ed. by S.M. Goodman and B.D. Patterson), pp. 364–377. Smithsonian Institution Press, Washington.

Dubois, A. (1992) Notes sur la classification des Ranidae (Amphibiens Anoures). *Bulletin mensuel de la Societé linnéenne de Lyon*, **61**, 305–352.

Duellman, W.E. & Trueb, L. (1986) *Biology of amphibians*. McGraw-Hill, New York.

Glaw, F. & Vences, M. (1994) *A fieldguide to the amphibians and reptiles of Madagascar, 2nd edition, including mammals and freshwater fish*. Vences and Glaw, Köln.

Hedges, S.B., Hass, C.A. & Maxson, L.R. (1992) Caribbean biogeography: molecular evidence for dispersal in West Indian terrestrial vertebrates. *Proceedings of the National Academy of Sciences of the USA*, **89**, 1909–1913.

Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Inger, R.F. & Voris, H.K. (2001) The biogeographical relations of the frogs and snakes of Sundaland. *Journal of Biogeography*, **28**, 863–891.

Kosuch, J., Vences, M., Dubois, A., Ohler, A. & Böhme, W. (2001) Out of Asia: mitochondrial DNA evidence for an Oriental origin of tiger frogs, genus *Hoplobatrachus*. *Molecular Phylogenetics and Evolution*, **21**, 398–407.

Krause, D.W., Hartman, J.H. & Wells, N.A. (1997) Late Cretaceous vertebrates from Madagascar. Implications for biotic changes in deep time. *Natural change and human impact in Madagascar* (ed. by S.M. Goodman and B.D. Patterson), pp. 3–43. Smithsonian Institution Press, Washington.

Krause, D.W., Rogers, R.R., Forster, C.A., Hartman, J.H., Buckley, G.A. & Sampson, S.D. (1999) The Late Cretaceous vertebrate fauna of Madagascar: implications for Gondwanan paleobiogeography. *GSA Today (A Publication of the Geological Society of America)*, **9**, 1–7.

Lamotte, M. (1967) Le probleme des *Ptychadena* (Fam. Ranidae) du groupe *mascareniensis* dans l'ouest africain. *Bulletin du Museum national d'Histoire naturelle, 2e ser.*, **39**, 647–656.

Lamotte, M. & Ohler, A. (1997) Redécouverte de syntypes de *Rana bibroni* Hallowell, 1845, désignation d'un lectotype et description d'une espèce nouvelle de *Ptychadena*(Amphibia, Anura). *Zoosystema*, **19**, 545–555.

McCall, R.A. (1997) Implications of recent geological investigations of the Mozambique Channel for the mammalian colonization of Madagascar. *Proceedings of the Royal Society of London B*, **264**, 663–665.

Nussbaum, R.A. (1984) Amphibians of the Seychelles. *Biogeography and ecology of the Seychelles Islands* (ed. by D.R. Stoddart), pp. 379–415. W. Junk Publisher, The Hague.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (1991) *The Simple Fool's Guide to PCR, Version 2.0*. Privately published document compiled by S. Palumbi. Department of Zoology, University of Hawaii, Honolulu.

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Probst, J.-M. (1997) *Animaux de la Réunion. Guide d'identification des oiseaux, mammifères, reptiles et amphibiens*. Azalées Éditions, Réunion.

Rabinowitz, P.D., Coffin, M.F. & Falvey, D. (1983) The separation of Madagascar and Africa. *Science*, **220**, 67–69.

Raxworthy, C.J., Forstner, M.R.J. & Nussbaum, R.A. (2002) Chameleon radiation by oceanic dispersal. *Nature*, **415**, 784–786.

Rödel, M.-O. (2000) *Herpetofauna of West Africa, Vol. I: amphibians of the West African savanna*. Chimaira, Frankfurt/M.

Staub, F. (1993) *Fauna of Mauritius and associated flora*. Précigraph Ltd, Mauritius.

Swofford, D.L. (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods), Version 4b10*. Sinauer Associates, Sunderland, MA.

Swofford, D.L., Olsen, G.J., Waddell, P.J. & Hillis, D.M. (1996) Phylogenetic inference. *Molecular systematics*, 2nd edition (ed. by D.M. Hillis, C. Moritz and B.K. Mable), pp. 407–514. Sinauer, Sunderland, MA.

Templeton, A.R., Crandall, K.A. & Sing, C.F. (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**, 619–633.

Veith, M., Kosuch, J. & Vences, M. (2003a) Climatic oscillationstriggered post-Messinian speciation of Western Palearctic brown frogs (Amphibia, Anura, Ranidae). *Molecular Phylogenetics and Evolution*, **26**, 310–327.

Veith, M., Schmidtler, J. F., Kosuch, J., Baran, I. & Seitz, A. (2003b) Palaeoclimatic changes explain Anatolian mountain frog evolution: a test for alternating vicariance and dispersal events. *Molecular Ecology*, **12**, 185–199.

Vences, M. & Glaw, F. (2002) Molecular phylogeography of *Boophis tephraeomystax*: a test case for east-west vicariance in Malagasy anurans (Amphibia, Anura, Mantellidae). *Spixiana*, **25**, 79–84.

Vences, M., Andreone, F., Glaw, F., Kosuch, J., Meyer, A., Schaefer, H.-C. & Veith, M. (2002) Exploring the potential of life-history key innovation: brook breeding in the radiation of the Malagasy treefrog genus *Boophis*. *Molecular Ecology*, **11**, 1453–1463.

Vences, M., Andreone, F., Glaw, F. & Randrianirina, J.E. (2003a) Molecular and bioacoustic divergence in *Mantidactylus granulatus* and *M. zavona* sp. n. (Anura: Mantellidae): bearings for the biogeography of northern Madagascar. *African Zoology*, **38**, 67–78.

Vences, M., Freyhof, J., Sonnenberg, R., Kosuch, J. & Veith, M. (2001) Reconciling fossils and molecules: Cenozoic divergence of cichlid fishes and the biogeography of Madagascar. *Journal of Biogeography*, **28**, 1091–1099.

Vences, M., Vieites, D., Glaw, F., Brinkmann, H., Kosuch, J., Veith, M. & Meyer, A. (2003b) Multiple overseas dispersal in amphibians. *Proceedings of the Royal Society of London B*, **270**, 2435–2442.

Yoder, A.D., Burns, M.M., Zehr, S., Delefosse, T., Veron, G., Goodman, S.M. & Flynn, J. (2003) Single origin of Malagasy Carnivora from an African ancestor. *Nature*, **421**, 734–737.

BIOSKETCHES

Miguel Vences is Assistant Professor and Curator at the Zoological Museum of the University of Amsterdam. Since his times as undergraduate, over 10 years ago, he has been working on the systematics and biogeography of the amphibians and reptiles of Madagascar.

Joachim Kosuch is working at the University of Mainz, focusing on molecular phylogeny and phylogeography of amphibians.

Mark-Oliver Rödel has a long-standing expertise on the amphibian and reptile fauna of western Africa, especially Ivory Coast.

Stefan Lötters is a *post-doc* in a monitoring project on East African amphibians of the Mainz University, Germany.

Alan Channing is Professor of Zoology at the University of Western Cape, South Africa, and a specialist of the amphibian fauna of southern and central Africa.

Frank Glaw is Curator for Herpetology at the Zoologische Staatssammlung München, and is a specialist of systematics and bioacoustics of Malagasy frogs.

Wolfgang Böhme is Professor and Curator for Herpetology at the Museum Koenig in Bonn, specialized on the African herpetofauna.