

Molecular phylogenetic relationships among species of the Malagasy-Comoran gecko genus *Paroedura* (Squamata: Gekkonidae)

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Abstract

We use ~3100 bp of mitochondrial (ND2, ND4) and nuclear (RAG1, phosducin) DNA sequence data to recover phylogenetic relationships among 14 of the 16 recognized taxa of the lizard genus *Paroedura* as well as two undescribed forms. These geckos are endemic to Madagascar and the Comores and are popularly kept and bred by herpetoculturalists. The closest relative of *Paroedura* is another Indian Ocean leaf-toed gecko, *Ebenavia*. Both Bayesian inference and maximum parsimony strongly support the monophyly of two major clades within *Paroedura* that conflict with existing species group assignments based on scale characteristics. Our well-resolved tree elucidates a biogeographic pattern in which eastern *Paroedura* are most basal and western and south-western species form a monophyletic group. Our data demonstrate the phylogenetic utility of phosducin, a novel marker in squamate phylogenetics, at the intrageneric level.

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1. Introduction

Lizards of the genus *Paroedura* are the most speciose group of nocturnal geckos in Madagascar and the Comores (Glaw and Vences, 1994; Nussbaum and Raxworthy, 2000; Bauer, 2003; Meirte, 2004) and even the most rare and restricted forms are widely kept and bred in captivity by herpetoculturalists (Daoues and Girard, 2004). More common and widespread forms have been bred in captivity for many generations (Rösler, 1986; Manwaring, 1992) and have even become model organisms for studies of sexual behavior (Brillet, 1991, 1993) and thermoregulation (Blumberg et al., 2002).

The genus was initially described by Günther (1879) on the basis of the Comoran endemic *P. sanctijohannis*. It was subsequently synonymized with the widespread leaf-toed

gecko genus *Phyllodactylus* until resurrected by Dixon and Kroll (1974), who identified a suite of features diagnosing the group from other leaf-toed forms, including: coossification of the skin to the underlying skull, fused nasal bones, paired parietal bones, imperforate stapes and absence of the second epibranchial element of the hyoid apparatus. Dixon and Kroll (1974) recognized nine species in *Paroedura* and an additional six species were described by Nussbaum and Raxworthy (1994b, 2000), who also synonymized *P. guibae* Dixon and Kroll, 1974 with *P. bastardi* (Mocquard, 1909). Glaw et al. (2001) described a fifteenth species. The status of the subspecies *P. bastardi ibityensis*, described by Rösler and Krüger (1998), remains unresolved.

Since the resurrection of *Paroedura* its monophyly has been accepted, either explicitly or implicitly, by all subsequent workers (Kluge, 1983; Bauer, 1990; Kluge and Nussbaum, 1995; Bauer et al., 1997; Nussbaum and Raxworthy, 2000). Higher order relationships of *Paroedura* have not been resolved, but preliminary analyses at the generic level

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have resulted in alternative hypotheses of relationship, each supported by a small number of morphological characters. Bauer (1990) suggested that *Paroedura* was most closely related to *Ebenavia* and *Uroplatus* and that this group, in turn, was part of a larger Afro-Malagasy clade that also included *Ailuroonyx*, *Geckolepis*, *Homopholis* (including *Blaesodactylus*), *Lygodactylus* and *Urocotyledon*. Kluge and Nussbaum (1995) considered *Paroedura* as part of a clade including *Urocotyledon*, *Uroplatus*, *Ebenavia* and *Blaesodactylus*, although their expansion of the ingroup to include several non-Afro-Malagasy taxa resulted in the placement of *Paroedura* near the base of their tree.

Mocquard (1909) identified two species groups within *Paroedura* on the basis of the condition of nostril-rostral contact. This feature was also used by Angel (1942) and Guibé (1956) in their keys to the members of the genus. In one group the rostral is excluded from the nostril by a supranasal scale and in the other there is nostril-rostral contact. The members of the former group, called the *picta*-Group by Nussbaum and Raxworthy (2000) are chiefly distributed in the more xeric regions of southern and southwestern Madagascar, whereas those of the *sanctijohannis*-Group occur in west-central, northern, and eastern Madagascar (and the Comores) and occupy more mesic habitats, including rainforest. The *picta*-Group includes *P. picta* (Peters 1854), *P. bastardi* (Mocquard, 1909), *P. androyensis* (Grandidier 1867), *P. maingoka* Nussbaum and Raxworthy, 2000 and *P. vahiny* Nussbaum and Raxworthy, 2000. The *sanctijohannis*-Group includes *P. sanctijohannis* Günther, 1879, *P. gracilis* (Boulenger 1896), *P. homalorhina* (Angel, 1942), *P. oviceps* (Boettger 1881), *P. stumpffi* (Boettger 1879), *P. masobe* Nussbaum and Raxworthy, 1994, *P. karstophila* Nussbaum and Raxworthy, 2000, *P. tanjaka* Nussbaum and Raxworthy, 2000 and *P. vazimba* Nussbaum and Raxworthy, 2000.

Nussbaum and Raxworthy (2000) did not state if they believed that the two groups were reciprocally monophyletic, but noted that the biogeographic correlates of the nostril-rostral character were supported by the five new species they described. However, the more recently described *P. lohatsara*, possesses the *picta* scalation condition, but occurs on the Montagne des Français in the far north of Madagascar (Glaw et al., 2001).

Nussbaum and Raxworthy (2000) suggested that *P. karstophila* and *P. vazimba* were most similar to one another and were probable sister species and that *P. stumpffi* and *P. sanctijohannis* were each others' closest relatives. They further predicted that these four taxa would prove to be members of a single monophyletic group. They also remarked that *P. tanjaka* and *P. bastardi* are superficially similar. Likewise, they suggested that *P. vahiny* and *P. androyensis* were sister taxa on the basis of their shared possession of multicarinate scales, a derived character state within *Paroedura*. Glaw et al. (2001) found the greatest phenetic similarity between *P. lohatsara* and *P. stumpffi*, despite the fact that the two differed in the condition of nostril-rostral contact.

We here analyze the phylogenetic relationships among species of *Paroedura* based on a multi-gene data set containing mitochondrial and nuclear DNA sequences representing 14 of the 16 recognized species and subspecies of *Paroedura* as well as two undescribed species. We use our data to evaluate the monophyly of the two previously recognized species groups identified on the basis of nostril-rostral contact and to assess the hypothesis that *Paroedura* species fall into *sanctijohannis* (mesic) and *picta* (xeric) clades. We further use our data set to evaluate the phylogenetic utility of phosducin, a novel nuclear marker in squamate phylogenetics.

2. Materials and methods

2.1. Sampling

Twenty-five samples of *Paroedura* were collected in Madagascar and the Comores or, in two cases, obtained from captive specimens (Table 1). Outgroup taxa were chosen on the basis of a broader multi-locus study of gekkotan relationships (Bauer, Jackman and Greenbaum, unpubl. data) that incorporated representatives of 95 genera, including all Afro-Malagasy gecko genera. The only gekkonid genera not included were two South American genera (*Bogertia* and *Gymnodactylus*) and representatives of the Asian taxa *Alsophylax*, *Altigecko*, *Siwaligecko* and *Indogecko*, all of which are probably members of a larger clade of Palearctic bent-toed geckos which was represented by other taxa in our sampling. As a result, the Malagasy-Comoran genus *Ebenavia* was unambiguously identified as the closest relative to *Paroedura* and we employed *Ebenavia inunguis* as the outgroup in our analysis.

2.2. DNA extraction, amplification and sequencing

Genomic DNA was isolated from the tail, liver, or forelimb of tissue samples preserved in 95–100% ethanol with the Qiagen DNeasy tissue kit (Valencia, CA, USA). We used double-stranded PCR to amplify 3489 aligned bases of eight mitochondrial (ND2, partial ND4, and six tRNAs: Trp, Ala, Asn, Cys, His, Ser) and two nuclear (RAG1 and phosducin) genes using the primers listed in Table 2. Although RAG1 has been a widely used nuclear marker in squamate phylogenetics (Townsend et al., 2004; Noonan and Chippindale, 2006), phosducin has been employed only in two studies (Bauer et al., 2007; Greenbaum et al., 2007) and has not been discussed in detail. In vertebrates, phosducin (PDC) is a member of a well-studied photophosphorylation cascade. It is expressed in high levels in retinal photoreceptor and pineal cells (Lee et al., 2004). In addition to light-activated dephosphorylation in photoreceptor cells (Kobayashi et al., 2002; Lee et al., 2004), phosducin also has a role as a transcriptional activator (Zhu and Craft, 2000). For the known sequences in amniotes, the number of amino acids varies from 244 in *Mus musculus* to 249 in *Phelsuma madagascariensis*. *Pan troglodytes* has

Table 1

List of samples used in this study, sample locality (M, Madagascar; C, Comores), museum voucher specimen (if available), and GenBank accession numbers for each gene

Sample	Museum No.	Locality	GenBank Accession numbers			
			ND2	ND4	RAG1	PDC
<i>Ebenavia inunguis</i>	ZSM 81/2005	M: Ste. Luce	EF536191	EF536215	EF536143	EF536167
<i>Paroedura androyensis</i>	UADBA uncatalogued (ZCMV 2483)	M: locality unknown	EF490774	EF536218	EF490721	EF490695
<i>Paroedura bastardi</i>	UADBA uncatalogued (FGZC 313)	M: Tolagnaro	EF536210	EF536236	EF536162	EF536186
<i>Paroedura bastardi</i>	ZSM 180/2004	M: Tranoroa	EF536211	EF536237	EF536163	EF536187
<i>Paroedura gracilis</i>	ZSM 82/2005	M: Nosy Mangabe	EF536194	EF536219	EF536146	EF536170
<i>Paroedura gracilis</i>	UADBA uncatalogued (FGZC 2795)	M: Marojejy	EF536209	EF536235	EF536161	EF536185
<i>Paroedura homalorhina</i>	ZSM 345/2004	M: Ankarana	EF536214	EF536240	EF536166	EF536190
<i>Paroedura</i> sp. n. 1	ZSM 342/2004	M: Montagne des Français	EF536213	EF536239	EF536165	EF536189
<i>Paroedura</i> sp. n. 1	Captive specimen (to be catalogued in ZSM)	M: Montagne des Français	EF536212	EF536238	EF536164	EF536188
<i>Paroedura ibityensis</i>	ZSM 396/2000	M: Mt. Ibity	EF536206	EF536232	EF536158	EF536182
<i>Paroedura lohatsara</i>	UADBA uncatalogued (FGMV 2002.936)	M: Montagne des Français	EF536203	EF536228	EF536155	EF536179
<i>Paroedura karstophila</i>	ZSM 484/2001	M: Ankarafantsika	EF490775	EF536229	EF490722	EF490696
<i>Paroedura masobe</i>	JFBM18	captive specimen	EF536193	EF536217	EF536145	EF536169
<i>Paroedura masobe</i>	JS15	captive specimen	EF536192	EF536216	EF536144	EF536168
<i>Paroedura oviceps</i>	ZSM 83/2005	M: Nosy Be	EF536207	EF536233	EF536159	EF536183
<i>Paroedura oviceps</i>	ZSM 336/2004	M: Montagne d'Ambre	EF536208	EF536234	EF536160	EF536184
<i>Paroedura picta</i>	Uncatalogued specimen (WRB 3)	M: Toliari Sands Project, Toliara region	EF536198	EF536223	EF536150	EF536174
<i>Paroedura picta</i>	ZSM 85/2005	M: Tranomaro	EF536196	EF536221	EF536148	EF536172
<i>Paroedura picta</i>	Voucher not collected (tissue sample FGMV 2002.B1)	M: Berenty	EF536197	EF536222	EF536149	EF536173
<i>Paroedura sanctijohannis</i>	ZSM 318/2002	C: La Grille, Grande Comore	EF536204	EF536230	EF536156	EF536180
<i>Paroedura sanctijohannis</i>	ZSM 709/2000	C: La Grille, Grande Comore	EF536205	EF536231	EF536157	EF536181
<i>Paroedura stumpffi</i>	UADBA uncatalogued (tissue sample FGMV 2001.F46)	M: Nosy Be	EF536202	EF536227	EF536154	EF536178
<i>Paroedura tanjaka</i>	ZSM 40/2006	M: Tsingy de Bemaraha, near Antranopasasy	EF536200	EF536225	EF536152	EF536176
<i>Paroedura</i> sp. n. 2	ZSM 163/2006	M: Tsingy de Bemaraha, Andafiabe	EF536199	EF536224	EF536151	EF536175
<i>Paroedura tanjaka</i>	Uncatalogued specimen (tissue sample FGMV DNA 331)	M: locality unknown	EF536201	EF536226	EF536153	EF536177
<i>Paroedura vazimba</i>	ZSM 481/2001	M: Ankarafantsika	EF536195	EF536220	EF536147	EF536171

Abbreviations: FGMV, Frank Glaw and Miguel Vences Field Series; FGZC, Frank Glaw Zoological Collection; JFBM, James Ford Bell Museum, University of Minnesota; JS, Jay Sommers; UADBA, Université d'Antananarivo, Département de Biologie Animale; WRB, William Roy Branch (Bayworld, Port Elizabeth Museum); ZCMV, Zoological Collection of Miguel Vences; ZSM, Zoologische Staatssammlung, München.

Table 2
Primers used in this study

Primer	Gene	Reference	Sequence
ND4f11	ND4	This study	5'-GCAAATACAAACTAYGAACG-3'
Leur1	Leu tRNA	Arevalo et al. (1994)	5'-CATTACTTTTTTACTTGGATTTGCACCA-3'
PHOF2	Phosducin	Bauer et al. (2007)	5'-AGATGAGCATGCAGGAGTATGA-3'
PHOR1	Phosducin	Bauer et al. (2007)	5'-TCCACATCCACAGCAAAAACTCCT-3'
L4437b	Met tRNA	Macey et al. (1997)	5'-AAGCAGTTGGGCCCATACC-3'
L5002	ND2	Macey et al. (1997)	5'-AACCAAAACCAACTACGAAAAAT-3'
ND2f101	ND2	Greenbaum et al. (2007)	5'-CAAACACAAACCCGRAAAAT-3'
ND2r102	ND2	Greenbaum et al. (2007)	5'-CAGCCTAGGTGGGCGATTG-3'
Trpr3a	Trp tRNA	Greenbaum et al. (2007)	5'-TTTAGGGCTTTGAAGGC-3'
H5934a	COI	Arevalo et al. (1994)	5'-AGRGTGCCAATGTCTTTGTGRIT-3'
R13	RAG1	Groth and Barrowclough (1999)	5'-TCTGAATGGAAATTCAAGCTGTT-3'
R18	RAG1	Groth and Barrowclough (1999)	5'-GATGCTGCCTCGGTCCGCCACCTTT-3'
RAG1 F700	RAG1	Bauer et al. (2007)	5'-GGAGACATGGACACAATCCATCCTAC-3'
RAG1 R700	RAG1	Bauer et al. (2007)	5'-TTTGTACTGAGATGGATCTTTTTCGA-3'

an additional 167 amino acids at the amino end of the protein, but is otherwise 99% similar to the *Homo sapiens* phosducin. In all amniotes except primates, phosducin has three exons; the last begins in the same place in all amniotes. Our primers match the cAMP-dependent protein kinase (PKA) recognition site at the 5' end (Kobayashi et al., 2002), and a conserved region thought to be important for transcriptional regulation at the 3' end (Zhu and Craft, 2000). Both the primer sites and the resulting phosducin sequences reported here are all part of the third exon.

Amplification of 25 µl PCR reactions were executed on an Eppendorf Mastercycler gradient thermocycler. Amplification of genomic DNA began with an initial denaturation for 2 min at 95 °C followed by 95 °C for 35 s, annealing at 50 °C for 35 s, and extension at 72 °C for 150 s with 4 s added to the extension per cycle for 32 cycles for mitochondrial DNA and 34 cycles for nuclear DNA. When needed, annealing temperatures were adjusted to increase or decrease specificity on a case by case basis. Products were visualized with 1.5% agarose gel electrophoresis. Target products were purified with AMPure magnetic bead solution (Agencourt Bioscience, Beverly, MA, USA) and sequenced with either the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) or the DYEnamic™ ET Dye Terminator Kit (GE Healthcare, Piscataway, NJ, USA). Sequencing reactions were purified with CleanSeq magnetic bead solution (Agencourt Bioscience, Beverly, MA, USA) and analyzed with an ABI 3700 automated sequencer. The accuracy of sequences was ensured by incorporating negative controls and sequencing complementary strands. Sequences were aligned by eye in the computer program SeqMan Pro (DNASTAR Inc., Madison, WI, USA), and all four protein-coding genes were translated to amino acids with MacClade (Maddison and Maddison, 1992) to confirm conservation of the amino acid reading frame and check for premature stop codons. The alignment of all sequences is available from TreeBase (<http://www.treebase.org>) as a Nexus file (study Accession No. S1891 and matrix Accession No. M3474).

2.3. Phylogenetic analysis

Phylogenetic relationships among the samples were assessed with parsimony, likelihood, and Bayesian optimality criteria. Maximum parsimony (MP) analyses were conducted in PAUP* 4.0b10 (Swofford, 2002). The heuristic search algorithm was used with the following conditions: 25 random addition replicates, accelerated character transformation (ACCTRAN), tree bisection-reconnection (TBR) branch swapping, zero-length branches collapsed to yield polytomies, and gaps treated as missing data. Each base position was treated as an unordered character with four alternate states. We used nonparametric bootstraps (1000 pseudoreplicates unless stated otherwise) to assess node support in resulting topologies with TBR branch swapping and 5 random addition replicates per pseudoreplicate. Strict consensus trees were calculated when several equally parsimonious trees resulted from MP searches.

We used the Akaike Information Criterion (AIC) in ModelTest 3.06 (Posada and Crandall, 1998) to find the model of evolution that best fit the data for subsequent Maximum Likelihood (ML) and Bayesian inference (BI) analyses. All genes were pooled to determine the best model for ML analyses, but separate models for each gene were run for BI. Separate models for each gene and codon position of protein-coding genes were estimated (Brandley et al., 2005). ML analyses with empirical base frequencies (obtained in ModelTest) were performed in PAUP* with a neighbor-joining starting tree. As with MP, the nonparametric bootstrap was used to assess the stability of internal nodes in the resulting phylogenies.

Partitioned Bayesian analyses were conducted with MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) with default priors. Analyses were initiated with random starting trees and run for 2,000,000 generations; Markov chains were sampled every 1000 generations. Convergence was checked by plotting likelihood scores against generation, and 114 trees were discarded as “burn in.” Two separate analyses with two independent chains were executed to check for convergence of log-likelihoods in stationarity (Huelsenbeck and

Ronquist, 2001). Phosducin was also analyzed separately with ML using a general-time-reversible model with invariant sites and a gamma distribution all estimated from the MP tree of the phosducin data. A likelihood nonparametric bootstrap analysis with 1000 replicates was then used to assess support for the phosducin gene alone.

3. Results

Results of the Bayesian inference analysis of *Paroedura* relationships are shown in Fig. 1. The likelihood analyses resulted in a single tree with the same topology as the

Bayesian inference tree. For the parsimony analysis, there were 5 equally parsimonious trees. The combined data set had 1652 variable characters, 1277 of which were parsimony-informative. The mitochondrial genes had a much higher proportion of parsimony informative sites than did the nuclear genes, but the consistency index (CI) of the two nuclear genes was higher than that of either ND2 or ND4 and their associated tRNAs (Table 3). The CI of phosducin (0.747) was only marginally lower than that for RAG1 (0.811). The maximum parsimony tree was 5685 steps. The likelihood score of the optimal ML tree was $-\ln L$ 28842.63.

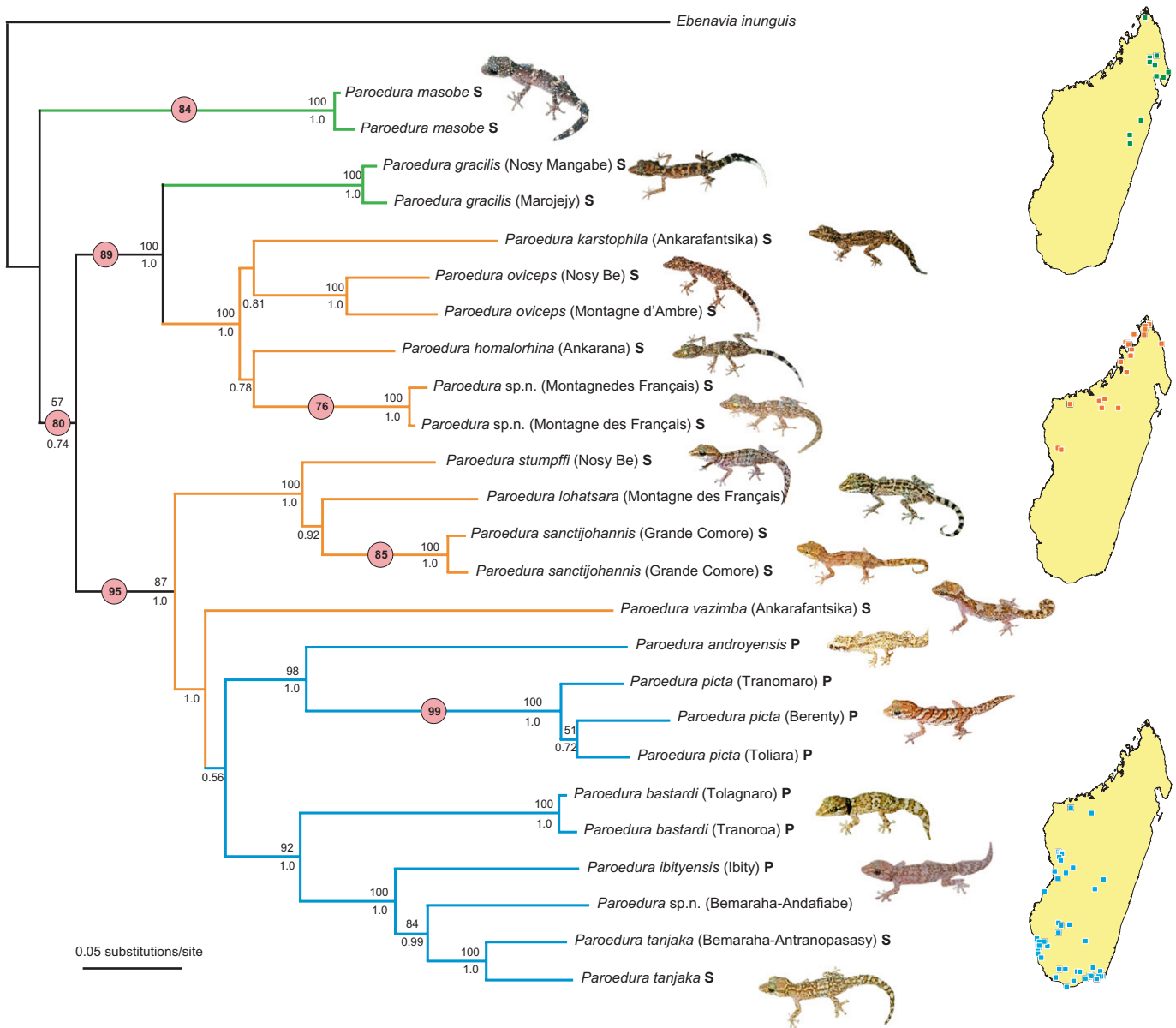


Fig. 1. Bayesian inference (BI) tree of *Paroedura* relationships. Numbers above branches are MP bootstrap values; those below branches are Bayesian posterior probability support values. Circled numbers on branches indicate ML bootstrap support from phosducin alone. Colors of branches represent general distribution of species: green—eastern and north-eastern Madagascar; orange—north-western and northern Madagascar; blue—western Madagascar; points on the maps show collection localities for all species occurring in each of the respective geographic regions based on published sources and the authors' unpublished data. The letters P and S following the species names and localities denote the *picta* and *sanctijohannis* groups, respectively, as defined by Nussbaum and Raxworthy (2000).

Table 3
Comparison of relative informativeness and consistency of genes used in this study

	Phosducin	RAG1	ND2	ND4
Percent parsimony informative characters	7.6	9.5	60.1	57.8
CI on combined Bayesian tree	0.747	0.811	0.546	0.454

The combined RAG1, Phosducin, ND2, ND4 and tRNA data set, analyzed using Bayesian inference yields a well-supported pattern of relationships. Based on a higher order analysis of gekkotan relationships (see Section 2), the Malagasy-Comoran leaf-toed gecko genus *Ebenavia* is well supported as the sister group of *Paroedura*. These two genera share an amino acid insertion in RAG1 that seems to be unique relative to other squamates. *Paragehyra* is weakly supported as the next most distant outgroup. Broader relationships using these genes alone were not well supported, but a large group of chiefly Afro-Malagasy genera are included in the next most inclusive clade retrieved.

The likelihood analysis of phosducin alone revealed seven nodes that had nonparametric bootstrap values over 70% (Fig. 1). There were no bootstrap values over 70% in the phosducin analysis that conflicted with the combined analysis. Support was high for the three deepest nodes of our combined analysis, and the remaining support was for monophyly of particular species.

There are two well-supported (pp 1.0) subclades of *Paroedura*. The first includes *P. gracilis*, *P. karstophila*, *P. oviceps*, *P. homalorhina*, and an undescribed species from Montagne des Français, whereas the second includes *P. stumpffi*, *P. lohatsara*, *P. sanctijohannis*, *P. tanjaka*, *P. ibityensis*, *P. bastardi*, *P. vazimba*, *P. androyensis*, *P. picta*, and an undescribed species from Andafiabe in the Tsingy de Bemaraha. *Paroedura masobe* was retrieved as the sister taxon of these two subclades, but this placement was poorly supported (pp 0.74). Relationships within each of the subclades of *Paroedura* are well-supported (pp 1.00), except for the sister species relationship between *P. sanctijohannis* and *P. lohatsara* (pp 0.92), and between *P. karstophila* and *P. oviceps* (pp 0.81), and the sister group relationship of the *picta* + *androyensis* clade to the clade including *P. bastardi*, *P. tanjaka*, and *P. ibityensis* (pp 0.56). The new species from Montagne des Français receives weak support as the sister to *P. homalorhina*. The strict consensus of the parsimony trees only conflicts with Bayesian and likelihood analyses in the position of *P. karstophila* which, in the MP analysis is weakly supported (61% bootstrap support) as the sister to the new species from Montagne des Français.

4. Discussion

4.1. Phosducin as a phylogenetic marker in gekkonids

In this analysis phosducin provided strong support for basal relationships within *Paroedura* (Fig. 1). Further, the

CI for phosducin on the combined Bayesian tree was much higher than that for the two mitochondrial genes and roughly comparable to that for RAG1 (Table 3), which has been widely used in squamate phylogenetics and has been regarded as a valuable marker for both interspecific and higher order relationships in geckos (Bauer and Lamb, 2005) and other lizards and snakes (Townsend et al., 2004; Vidal and Hedges, 2004; Noonan and Chippindale, 2006). Phosducin has also proved to be comparably informative in phylogenetic analyses of geckos of the genera *Cnemaspis* (Bauer et al., 2007) and *Uroplatus* (Greenbaum et al., 2007). The small size (395 bp) and concomitant ease and cheapness of sequencing the phosducin fragment, combined with its empirical consistency with RAG1 derived results (in *Paroedura* the mitochondrial genes do not provide strong support for basal relationships), suggest a more general utility for this gene in vertebrate phylogenetics.

4.2. Phylogenetic relationships of *Paroedura*

Nussbaum and Raxworthy (1998) suggested that *Ebenavia* might be related to *Cryptactites* (as *Phyllodactylus*), but did not explicitly hypothesize any suprageneric sister-group relationships and previous considerations of *Paragehyra* (Nussbaum and Raxworthy, 1994a; Kluge and Nussbaum, 1995) were unable to suggest close relatives of that genus. Our results strongly support *Ebenavia* as the immediate sister group to *Paroedura* and suggest that these genera are only remotely related to *Cryptactites* and other mainland African leaf-toed geckos.

Our results contradict the hypothesis that the *picta* and *sanctijohannis* groups are monophyletic. Although the majority of species in which the rostral is excluded from the nostril (*picta*-Group) are members of a single clade, there are species in that clade that exhibit the alternative condition (*P. tanjaka*, *P. sp. n. 2*) and others that exhibit the *picta* condition, but occur in other clades (*P. lohatsara*). However, several clades retrieved in our analyses are supported by other morphological and chromatic characters. The life-coloration of adult *Paroedura* species is known (Fig. 1), and due to intensive field surveys and captive breeding, even the juvenile (or at least subadult) coloration of most species (*masobe*, *gracilis*, *homalorhina*, sp. n. 1, *stumpffi*, *lohatsara*, *sanctijohannis*, *androyensis*, *picta*, *maingoka*, *bastardi*, *ibityensis*, and *tanjaka*) has been documented. All these species, except the most basal—*P. masobe*, in which the newly hatched juveniles strongly resemble the adults in color and pattern, are characterized by juveniles with more colorful and contrasting patterns than the adults. This suggests that the distinct juvenile coloration may be a synapomorphy of the remaining *Paroedura* species. A comparably distinct juvenile coloration is unknown from any other Malagasy gekkonid genus (although juvenile and adult coloration can also vary somewhat in the genus *Phelsuma*). The two eastern rainforest species, *masobe* and *gracilis*, share a blackish dorsal ground color and even a blackish iris, but apparently do not repre-

sent a monophyletic group. Another rather basal clade contains *gracilis*, *karstophila*, *oviceps*, *homalorhina* and sp. n. 1. This clade is characterized by a more-or-less laterally compressed and comparatively smooth original tail which usually terminates in a thin white tip at least several millimeters in length. A similar tail shape, including the white tip, can also occur in the outgroup genus *Ebenavia*, but the white tail tip is absent from *P. masobe* and all other *Paroedura* species, whereas a laterally compressed original tail is shared with the most basal species, *P. masobe*. The tail shape of the remaining *Paroedura* clade (including *stumpffi*, *sanctijohannis*, *lohatsara*, *vazimba*, *androyensis*, *picta*, *bastardi*, *ibityensis*, sp. n. 2, and *tanjaka*) is either more-or-less round in cross-section or even dorsoventrally compressed (e.g., *ibityensis*) and usually colorful (with yellow or orange) in juveniles. The clade including *bastardi*, *ibityensis*, sp. n. 2, and *tanjaka* is characterized by a synapomorphic juvenile coloration including only three light dorsal bands between the fore- and hindlimbs instead of four in the other *Paroedura* (except the entirely black *P. gracilis*). The distribution of these and several other character states indicates a relatively high congruence of genetic and non-molecular data.

Most previous ad hoc hypotheses of relationship within *Paroedura* are supported by our results. The phenetic similarity of both *P. sanctijohannis* and *P. lohatsara* to *P. stumpffi* noted by Nussbaum and Raxworthy (2000) and Glaw et al. (2001) is a reflection of phylogeny as is the former authors' pairing of *P. tanjaka* and *P. bastardi*. Similar results were obtained by Yang (2001) in an unpublished M.S. thesis from San Diego State University, using only mitochondrial data. On the other hand, Nussbaum and Raxworthy's (2000) prediction that *P. stumpffi* + *P. sanctijohannis* would form a monophyletic group with *P. androyensis* (and *P. vahiny*) is not corroborated. *Paroedura ibityensis*, previously considered as a subspecies of *P. bastardi*, is significantly different from the nominate form of this species and is more closely related to *P. tanjaka* than to *P. bastardi*. On this basis, as well as a suite of diagnostic morphological features (Rösler, 1997; Rösler and Krüger, 1998), it should be regarded as specifically distinct.

4.3. Biogeography

The relationships among members of the genus *Paroedura* do not correspond to the convenient wet-dry biogeographic division implied by the *picta* and *sanctijohannis* groups earlier recognized. Nonetheless, some biogeographic patterning is obvious from our tree and is symbolized by colors of branches (Fig. 1). The most basal species, *Paroedura masobe*, is endemic to a small area in the central-eastern part of Madagascar, and the second species distributed in eastern and north-eastern rainforests, *P. gracilis*, occupies a relatively basal position as well. The sister clade of *P. gracilis* contains mostly species distributed in the far north or Sambirano region (*P. homalorhina*, *P. oviceps*, and an undescribed species from Montagne des Français), and

in the north-west (*P. karstophila*). The two basal lineages of the monophyletic group that contains all other *Paroedura* species also show a distribution in the north and north-west. The monophyletic group of the remaining species contains only species distributed in the arid west (and the central highlands—*P. ibityensis*). The Comoran species is nested among species from the north and the Sambirano region, which is geographically closest to the Comores, confirming a trend of relationships between the Comoran endemic reptile populations and those occurring in these regions of Madagascar (Raselimanana and Vences, 2003; Sound et al., 2006; Rocha et al., 2007) and indicating recurring overseas dispersal from northern Madagascar to these islands.

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