



## A phylogeny of the enigmatic Madagascan geckos of the genus *Uroplatus* (Squamata: Gekkonidae)

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### Abstract

Since its discovery in the 17th century, the morphological peculiarities of the gekkonid lizard genus *Uroplatus* have generated a great deal of attention. A large number of skeletal, integumentary and visceral features are autapomorphic for the genus and some of the more well-known members of the group possess such aberrant characteristics that a separate family was once recognized to accommodate them. Recent phylogenetic analyses confirm that *Uroplatus* is a typical gekkonid gecko, but the specific affinities of the genus, as well as its intrageneric relationships have remained unresolved. Both nuclear (RAG-1 and PDC) and mitochondrial (ND2 and *cyt b*) genes (~3.2 Kb) were sequenced for 10 of 13 recognized species of *Uroplatus*, as well as two Madagascan and mainland African outgroups. The large-bodied forms of *Uroplatus* (*U. fimbriatus*, *U. giganteus*, *U. henkeli*, and *U. sikorae*) form a monophyletic group, and the small-bodied, short-tailed species are also monophyletic (*U. ebenau*i and *U. phantasticus*). *Uroplatus alluaudi* + *U. pietschmanni* comprise another distinct clade, whereas *U. lineatus* was weakly supported as the sister taxon of the large-bodied clade and *U. guentheri* was sister to all other members of the genus. Our phylogenetic hypothesis based on combined DNA sequence data is mostly congruent with previous hypotheses based on morphological data. Based on a larger, more inclusive dataset, the closest relatives of *Uroplatus* are mainland African *Afrogecko* and Madagascan *Matoatoa*, suggesting that the diverse Malagasy gecko fauna does not comprise a single evolutionary lineage. A high diversity of new taxa (either representing synonyms to resurrect or undescribed species), morphologically similar either to *U. ebenau*i/*phantasticus* or to *U. henkeli/sikorae*, was apparent from our data. Many of these genetically highly divergent lineages originated from localities in northern Madagascar, which indicates this region as the possible center of diversity and endemism for several subgroups of *Uroplatus*.

**Key words:** *Uroplatus*, Gekkonidae, Madagascar, molecular phylogeny, phosducin, systematics

### Introduction

Lizards of the genus *Uroplatus* are among the most morphologically distinctive of all geckos and are perhaps, next to chameleons, the most widely recognized of the endemic lizard radiations of Madagascar. Larger species of *Uroplatus* are distinguished among geckos in having the largest number of marginal teeth (highest among all living amniotes), and other rare apomorphic character states within the Gekkota include multiple inscriptional ribs, restriction of autotomy planes, and finger-like diverticula of the lungs (Siebenrock 1893; Werner 1912; Bauer and Russell 1989). Numerous uniquely derived features, such as basal paraphalanges that are associated with the control and support of the extensive interdigital webbing (Russell and Bauer 1988) and a diversity of features of the laryngeotracheal system (Tiedemann 1818; Meckel 1819; Rittenhouse *et al.*

1997, 2000) provide strong support for the monophyly of the genus. The peculiarities and relationships of *Uroplatus* have long been of interest to herpetologists and more pages were devoted to this genus in the 19<sup>th</sup> and early 20<sup>th</sup> centuries than to any other tropical gecko (Bauer and Russell 1989).

The possession of these various unique character states led to interpretations of the intermediacy of *Uroplatus* between geckos and chameleons (Fürbringer 1900), and the recognition of the family Uroplatidae by Boulenger (1884) and later Camp (1923). Most early workers on the group, however (Mocquard 1909; Angel 1929; Smith 1933; Wellborn 1933), demonstrated the affinities of *Uroplatus* were indeed with the Gekkonidae. This was verified by several pre-cladistic analyses (Underwood 1954; Kluge 1967), although more specific relationships of *Uroplatus* within the Gekkonidae remained unclear.

Underwood (1954) based on external digital anatomy and the lack of preanal pores considered *Ptyodactylus* as the closest relative of *Uroplatus*. Russell (1972) placed *Uroplatus* in his *Phyllodactylus* group, along with *Ebenavia* and *Ptyodactylus* and the taxa now allocated to *Paroedura*, *Urocotyledon*, *Asaccus*, *Christinus*, *Phyllodactylus*, *Euleptes*, *Afrogecko*, *Goggia*, *Cryptactites*, *Dixonius*, *Haemodracon* and *Matoatoa*, but this was a phenetic assemblage based on digital morphology and was not intended as an explicit hypothesis about relationships. Kluge (1983) included *Uroplatus* in his Gekkonini but within this broad group proposed no specific relationships. Joger (1985) noted that *Uroplatus* shared the derived condition of unpaired nasal bones with *Ebenavia*, *Homopholis* (including *Blaesodactylus*), *Ailuronyx*, *Phelsuma* and *Lygodactylus*. Bauer (1990) and Kluge and Nussbaum (1995) subsequently examined the relationships of Afro-Malagasy geckos at the generic level and, although retrieving different hypotheses of relationship, each suggested that the closest relatives of *Uroplatus* were other members of a Madagascan regional radiation. The former author found its closest affinities to *Paroedura* and *Ebenavia*, whereas the latter workers hypothesized that *Uroplatus* is the sister group to *Urocotyledon* within a larger clade that also includes *Ebenavia*, *Blaesodactylus* and *Paroedura*.

Bauer and Russell (1989) provided the first explicit phylogeny for the six species of *Uroplatus* recognized at that time, based on a small set of 13 morphological characters. They recognized *U. fimbriatus* (Schneider, 1797), and *U. sikorae* Boettger, 1913 as sister taxa, with these subtended sequentially by *U. lineatus* Duméril and Bibron, 1836 and *U. ebenau*i Boettger, 1878. The most generalized morphologies characterized *U. alluaudi* Mocquard, 1894 and *U. guentheri* Mocquard, 1908 and these forms were retrieved as sister taxa to each other and as the sister group to remaining *Uroplatus*. Subsequently, the accumulation of a great deal of additional material, as well as significant observations both in the field and in captivity have resulted in the resurrection of *U. phantasticus* Boulenger, 1888 from the synonymy of *U. ebenau*i (Böhme and Henkel 1995) and the description of several new taxa: *U. henkeli* (Böhme and Ibisch 1990), *U. sikorae sameiti* (Böhme and Ibisch 1990), *U. malahelo* (Nussbaum and Raxworthy 1994), *U. malama* (Nussbaum and Raxworthy 1995), *U. pietschmanni* (Böhle and Schönecker 2003), and *U. giganteus* (Glaw *et al.* 2006).

Glaw and Vences (1994) and Nussbaum and Raxworthy (1995) identified four species groups within *Uroplatus*. The *U. alluaudi* group (*U. alluaudi*, *U. malahelo*, *U. guentheri*), the *U. ebenau*i group (*U. ebenau*i, *U. phantasticus*, *U. malama*), the *U. fimbriatus* group (*U. fimbriatus*, *U. henkeli*, *U. sikorae*) and the monotypic *U. lineatus* group. Böhme and Ibisch (1990) concluded that *U. henkeli* was closer to *U. fimbriatus* than to *U. sikorae* and suggested that there would be additional cryptic taxa described in the genus. Böhle and Schönecker (2003) were unable to assign *U. pietschmanni* to any of these groups because it exhibits a mosaic of morphological features compatible with no single species group. Glaw *et al.* (2006) described *U. giganteus* which they presumed to be the sister species to *U. fimbriatus* *sensu stricto*. They also presented a neighbor-joining tree based on 16S rRNA sequence data (Fig. 1). This was primarily used to demonstrate the distinctiveness of their new species from *U. fimbriatus*, but also retrieved a weakly supported broader pattern of intrageneric relationships (*U. ebenau*i (*U. guentheri* (*U. lineatus* (*U. henkeli* (*U. sikorae* (*U. fimbriatus*, *U. giganteus*))))).

**TABLE 1.** List of samples used in this study, sample locality (M = Madagascar, SA = South Africa), museum voucher specimen (if available), and GenBank accession numbers for each gene. Collection abbreviations: CAS = California Academy of Sciences, FGMV = Frank Glaw and Miguel Vences Field Series, UADBA = Université d'Antananarivo, Département de Biologie Animale, ZCMV = Zoological Collection of Miguel Vences, ZFMK = Zoologisches Forschungsmuseum Alexander Koenig, ZMA = Zoological Museum, Amsterdam, ZSM = Zoologische Staatssammlung, München.

Sample	Locality	Museum No.	GenBank Accession Numbers				
			ND2	cyt <i>b</i>	RAG-1	PDC	
<i>Afrogecko porphyreus</i>	SA: Cape Hangklip, Western Cape Province	CAS 206995	EF490776	EF490750	EF490723	EF490697	
<i>Matoatoa brevipes</i>	M: no specific locality	FGMV 2002.2237	EF490777	EF490751	EF490724	EF490698	
<i>Paroedura androyensis</i>	M: no specific locality	ZCMV 2483 (deposited in UADBA)	EF490774	EF490748	EF490721	EF490695	
<i>Paroedura karstophila</i>	M: Ankarafantsika	ZSM 484/2001	EF490775	EF490749	EF490722	EF490696	
<i>Uroplatus alluaudi</i>	M: Montagne d' Ambre	ZSM 251/2004	EF490793	EF490766	EF490740	EF490713	
<i>Uroplatus ebenau</i>	M: Manongarivo	ZSM 856/2003	EF490788	EF490762	EF490735	EF490709	
<i>Uroplatus "ebenau"</i>	M: Montagne d' Ambre	ZSM 322/2004	EF490786	EF490760	EF490733	EF490707	
<i>Uroplatus "ebenau"</i>	M: Marojejy	ZSM 79/2005	EF490787	EF490761	EF490734	EF490708	
<i>Uroplatus "ebenau"</i>	M: Tsaratanana	FGMV 2001.F10	EF490789	EF490763	EF490736	EF490710	
<i>Uroplatus fimbriatus</i>	M: Nosy Mangabe	ZSM 56/2005	EF490792	—	EF490739	EF490712	
<i>Uroplatus cf. giganteus</i>	M: Marojejy	ZSM 55/2005	EF490790	EF490764	EF490737	—	
<i>Uroplatus giganteus</i>	M: Montagne d' Ambre	ZSM 267/2004	EF490791	EF490765	EF490738	EF490711	
<i>Uroplatus guentheri</i>	M: Ankarafantsika	ZSM 476/2001	EF490778	EF490752	EF490725	EF490699	
<i>Uroplatus henkeli</i>	M: Bemaraha	ZSM 113/2006	EF490794	EF490767	EF490741	EF490714	
<i>Uroplatus henkeli</i>	M: Nosy Be	FGMV 2000.C1 (tissue only, no voucher)	EF490796	EF490769	EF490743	EF490716	

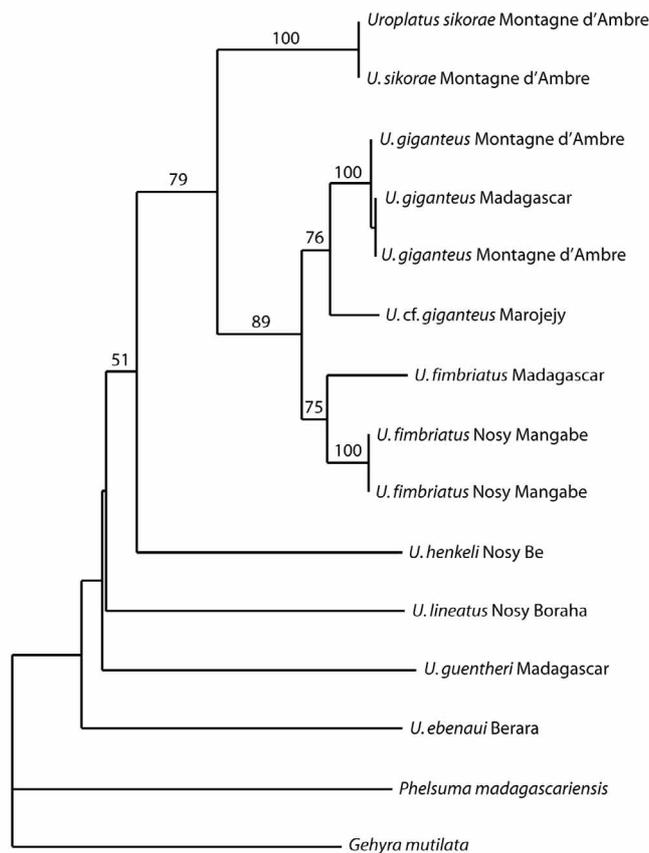
to be continued.

TABLE 1 (continued).

Sample	Locality	Museum No.	GenBank Accession Numbers			
			ND2	cyt <i>b</i>	RAG-1	PDC
<i>Uroplatus henkeli</i>	M: Montagne des Français	FGMV 2000.312 (deposited in UADBA)	EF490795	EF490768	EF490742	EF490715
<i>Uroplatus henkeli</i>	M: Ankarana	ZSM 889/2003	EF490797	EF490770	EF490744	EF490717
<i>Uroplatus lineatus</i>	M: no specific locality	ZSM uncatalogued	EF490779	EF490753	EF490726	EF490700
<i>Uroplatus lineatus</i>	M: no specific locality	ZCMV 2486 (deposited in UADBA)	EF490780	EF490754	EF490727	EF490701
<i>Uroplatus phantasticus</i>	M: Fierenana	ZSM 212/2002	EF490798	EF490771	EF490745	EF490718
<i>Uroplatus phantasticus</i>	M: Ranomafana	FGMV 2002.640 (deposited in ZFMK)	EF490799	EF490772	EF490746	EF490719
<i>Uroplatus phantasticus</i>	M: Vohidrazana	ZMA 19620	EF490800	EF490773	EF490747	EF490720
<i>Uroplatus pietschmanni</i>	M: no specific locality	ZCMV 2482 (deposited in UADBA)	EF490781	EF490755	EF490728	EF490702
<i>Uroplatus sikorae</i>	M: Andasibe	ZSM 920/2003	EF490782	EF490756	EF490729	EF490703
<i>Uroplatus sikorae</i>	M: Ranomafana	ZSM 690/2003	EF490784	EF490758	EF490731	EF490705
<i>Uroplatus sikorae</i>	M: Montagne d'Ambre	ZSM 264/2004	EF490783	EF490757	EF490730	EF490704
<i>Uroplatus sikorae</i>	M: Manongarivo	FGMV 2002.783 (deposited in UADBA)	EF490785	EF490759	EF490732	EF490706

**TABLE 2.** List of primers used in this study.

Primer	Gene	Reference	Sequence
CytbF700	cyt b	Bauer <i>et al.</i> (2007)	5'-CTTCCAACACCAAYCAAACATCTCAGCATGAT-GAAA-3'
CytbR700	cyt b	Bauer <i>et al.</i> (2007)	5'-ACTGTAGCCCCTCAGAATGATATTTGTCTCA-3'
PHOF2	PDC	Bauer <i>et al.</i> (2007)	5'-AGATGAGCATGCAGGAGTATGA-3'
PHOR1	PDC	Bauer <i>et al.</i> (2007)	5'-TCCACATCCACAGCAAAAACTCCT-3'
L4437b	Met tRNA	Macey <i>et al.</i> (1997)	5'-AAGCAGTTGGGCCCATACC-3'
L5002	ND2	Macey <i>et al.</i> (1997)	5'-AACCAAACCCAACTACGAAAAAT-3'
ND2f101	ND2	this study	5'-CAAACACAAACCCGRAAAAT-3'
ND2r102	ND2	this study	5'-CAGCCTAGGTGGGCGATTG-3'
Trpr3a	Trp tRNA	this study	5'- TTTAGGGCTTTGAAGGC-3'
H5934a	COI	Macey <i>et al.</i> (1997)	5'- AGRGTGCCAATGTCCTTTGTGRTT-3'
R13	RAG-1	Groth & Barrowclough (1999)	5'- TCTGAATGGAAATTCAAGCTGTT-3'
R18	RAG-1	Groth & Barrowclough (1999)	5'-GATGCTGCCTCGGTCGGCCACCTTT-3'
RAG1f700	RAG-1	Bauer <i>et al.</i> (2007)	5'-GGAGACATGGACACAATCCATCCTAC-3'
RAG1r700	RAG-1	Bauer <i>et al.</i> (2007)	5'-TTTGTACTGAGATGGATCTTTTTGCA-3'



**FIGURE 1.** Neighbor-joining tree and maximum likelihood support values (numbers above branches) of *Uroplatus* relationships from Glaw *et al.* (2006).

Herein, we use a combination of mitochondrial and nuclear DNA sequence data to examine the phylogenetic relationships of 10 of the 13 recognized species of *Uroplatus*. Only the relatively recently described taxa *U. malahelo* and *U. malama*, known from only a few specimens (Pearson *et al.* 2007), and *U. sameiti*, recently elevated from subspecific status within *U. sikorae* (Pearson *et al.* 2007), were not available for inclusion.

## Material and methods

Twenty-three samples of *Uroplatus* representing 10 of 13 currently recognized species were included in this study (Table 1). Four outgroup taxa were selected on the basis of an on-going broader-scale phylogenetic study of all gekkotan lizards (A. M. Bauer, T. R. Jackman and E. Greenbaum, unpubl. data). Based on a preliminary phylogeny of approximately 100 gekkotan genera, we identified the probable sister group of *Uroplatus* as a clade of leaf-toed geckos including the Madagascan *Matoatoa* and southern African *Afrogecko*. Both of these outgroup genera were formerly included in *Phyllodactylus*, which is now restricted to Neotropical leaf-toed geckos (Bauer *et al.* 1997). Although these two outgroup taxa have been considered closely allied (Mocquard 1900a, 1900b; Dixon and Kroll 1974), neither has previously been postulated to be part of the immediate sister group of *Uroplatus*. Two species of the Malagasy-Comoran genus *Paroedura* were also included as outgroups. Although there is no evidence for their close relationship to *Uroplatus*, both may be members of a much more inclusive Afro-Malagasy clade of gekkonids (Bauer 1990).

Genomic DNA was isolated from 95–100% ethanol-preserved tail, liver, or forelimb samples with the Qiagen DNeasy tissue kit (Valencia, CA, USA). We used double-stranded PCR to amplify 3244 aligned bases of mitochondrial (ND2, 1474 bp; *cyt b*, 307 bp) and nuclear (RAG-1, 1068 bp; PDC, 395 bp) gene sequence data with eight different pairs of published primers (Table 2). We also used the following specific internal primers: ND2f101, ND2r102, L5002, TrpR3a, RAG1f700, and RAG1r700 (Table 2).

Amplification of 25 µl PCR reactions were executed on an Eppendorf Mastercycler gradient thermocycler. Amplification of genomic DNA occurred with an initial denaturation step of 95°C for 2 min, followed by denaturation at 95°C for 35 s, annealing at 50°C for 35 s, and extension at 72°C for 95 s with 4 seconds added to the extension per cycle for 32 cycles for mitochondrial DNA and 34 cycles for nuclear DNA. When necessary, annealing temperatures were adjusted to increase or decrease specificity on a case by case basis, and products were visualized with 1.5% agarose gel electrophoresis. Target products were purified with AMPure magnetic bead solution (Agencourt Bioscience) and sequenced with either the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) or the DYEnamic™ ET Dye Terminator Kit (GE Healthcare). Sequencing reactions were purified with CleanSeq magnetic bead solution (Agencourt Bioscience) 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, and 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.

Phylogenetic relationships among the samples were assessed with maximum parsimony, maximum 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) to assess node support in resulting topologies. The Akaike Information Criterion (Posada and Buckley 2004) was used 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. In

the ML analysis (implemented in PAUP\* 4.0b10), the GTR +  $\Gamma$  + I model was used with the most parsimonious tree to estimate the parameters, and the same conditions as the parsimony search were used to find the ML tree.

Partitioned Bayesian analyses were conducted with MrBayes 3.1 (Ronquist and Huelsenbeck 2003) with default priors. Separate models for each gene and codon position of protein-coding genes were estimated (Brandley *et al.* 2005). A total of 10 partitions were made: RAG-1, 3 codons; PDC, 3 codons, ND2 and *cyt b*, 3 codon positions; and mitochondrial tRNAs. Analyses were initiated with random starting trees and run for 2,000,000 generations; Markov chains were sampled every 100 generations. Convergence was checked by plotting likelihood scores against generation, and 112 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). Both analyses ended with the standard deviation of split frequencies less than 0.01 (0.002 for both).

## Results

Results of the Bayesian inference (BI) analysis of *Uroplatus* relationships are shown in Fig. 2. Both parsimony and likelihood analyses resulted in a single tree with the same topology as the Bayesian inference tree. For the parsimony analysis, there were 1673 variable characters, 1284 of which were parsimony-informative. The maximum parsimony tree was 6473 steps. The likelihood score of the optimal ML tree was  $-\ln L$  31,323.59.

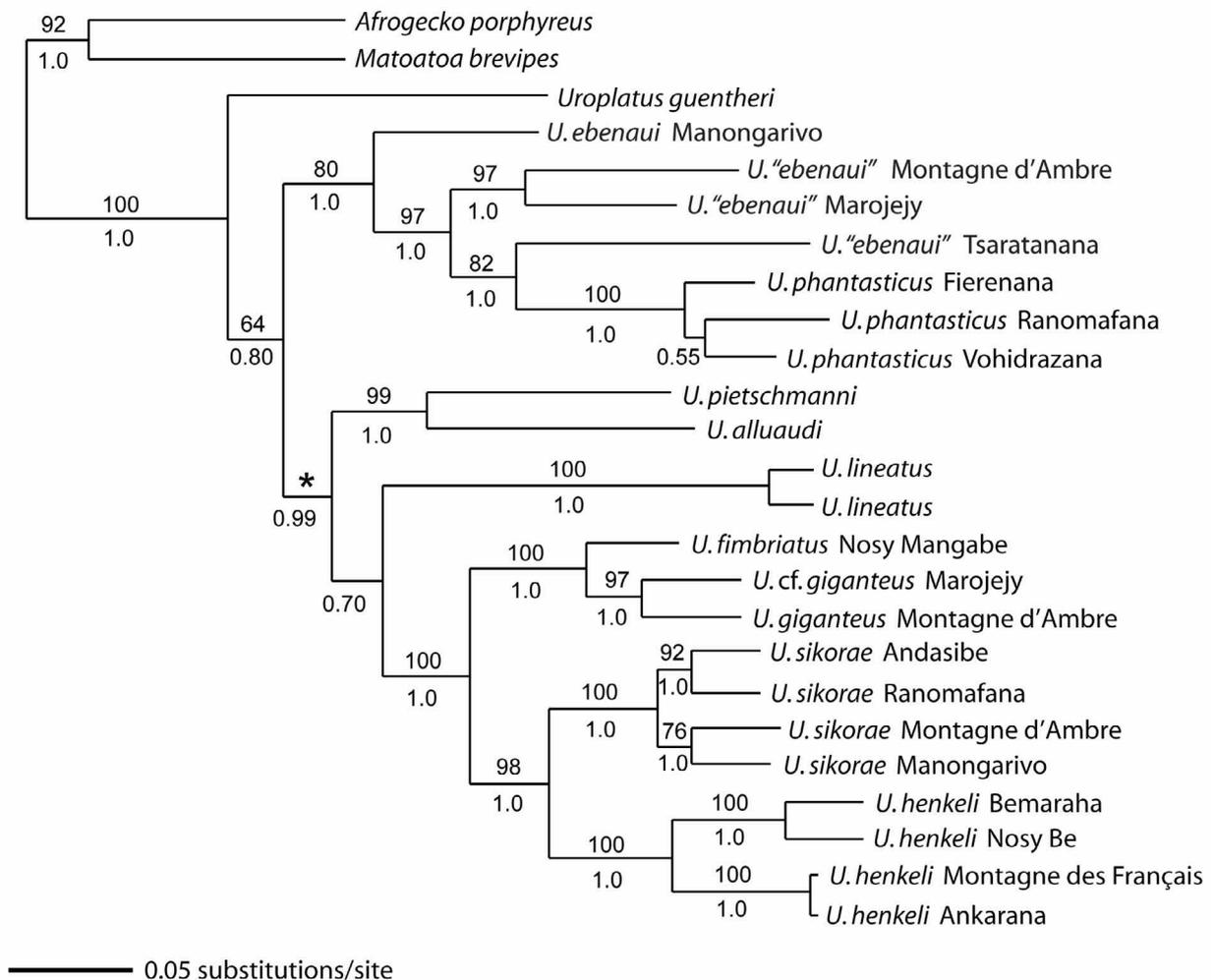
In *U. ebenau*i from Manongarivo, there is a short tandem repeat of the tRNAs and the light strand replication origin in the WANCY region, downstream from the ND2 gene. In this sample, part of the Asparagine tRNA, the light strand replication origin, and part of the Cysteine tRNA are repeated so that the gene order in the Manongarivo sample is  $WAN_pO_LC_pNO_LCY$ . ( $N_p$  is a partial Arginine tRNA and  $C_p$  is a partial Cysteine tRNA) as opposed to the normal gene order of  $WANO_LCY$ , where  $O_L$  is the light strand replication origin. The duplicated section that includes the partial, presumably non-functional, tRNAs was not included in the phylogenetic analyses.

The genus *Uroplatus* is strongly supported as monophyletic in all analyses, and the monophyly of all species except *U. ebenau*i is well-supported by bootstrap support  $\geq 97\%$  or a posterior probability of 1.0. All analyses retrieve the large-bodied forms of *Uroplatus* (*U. fimbriatus*, *U. giganteus*, *U. henkeli*, and *U. sikorae*) as a monophyletic group, and the small-bodied, short-tailed species are also monophyletic (*U. ebenau*i and *U. phantasticus*). Three additional lineages include *Uroplatus guentheri*, *U. lineatus*, and *U. alluaudi* + *U. pietschmanni*. The MP tree is not fully resolved, but is entirely compatible with the ML and BI trees. In the BI tree a subclade including the larger-bodied species of *Uroplatus*, *U. lineatus*, as well as *U. alluaudi* and *U. pietschmanni*, is also supported by an amino acid deletion in RAG-1. *Uroplatus ebenau*i is paraphyletic with respect to *U. phantasticus* and branch lengths suggest that divergences between the “*ebenau*i” populations sampled are comparable to those between congeneric species pairs. Uncorrected ND2 p distances of our *U. ebenau*i” specimens range from 22.3–36.3%.

## Discussion

That the monophyly of *Uroplatus* is strongly supported comes as no surprise, given the many derived features of the genus (Bauer and Russell 1989). Although results are preliminary (Bauer, Jackman and Greenbaum, unpublished) *Uroplatus*, as well as the two outgroups included in this study, *Afrogecko* and *Matoatoa*, seem to be part of a much larger Afro-Malagasy radiation of geckos. Within this radiation, species divergences are

deeper within *Uroplatus* than within any other genus, suggesting a relatively great age for the lineage as a whole, commensurate with the accumulation of the host of striking autapomorphies that characterize the genus.



**FIGURE 2.** Bayesian inference (BI) tree of *Uroplatus* relationships. Numbers above branches are MP bootstrap values; those below branches are posterior probability support values. The asterisk indicates a shared amino acid deletion in the RAG-1 gene. Two outgroups (*Paroedura androyensis* and *P. karstophila*) that were used to construct the tree are not shown here.

The previously identified large-bodied *fimbriatus* and small-bodied, short-tailed *ebenai* groups (Glaw and Vences 1994; Nussbaum and Raxworthy 1995) are strongly supported by our analyses, but the *alluaudi* group receives no support (MP) or is polyphyletic (BI). Except for the placement of *U. alluaudi*, our preferred molecular tree is congruent with the morphologically based phylogeny of Bauer and Russell (1989). *Uroplatus guentheri*, *U. alluaudi*, *U. malahelo* and *U. pietschmanni* have previously been interpreted as relatively primitive species within the genus based on their lack of the highly derived morphological features that characterize the other species groups (Bauer and Russell 1989; Nussbaum and Raxworthy 1994). Our data suggest that the similarity of these forms to one another is symplesiomorphic rather than indicative of close relationship. The affinities of *U. malahelo* remain uncertain, but Nussbaum and Raxworthy (1994) believed that it was more similar, and perhaps more closely related, to *U. alluaudi* than to *U. guentheri*.

Specimens currently recognized as *U. ebenai* do not constitute a monophyletic group, and likely include several cryptic species. Although Bauer and Russell (1989) accepted the synonymy of *U. phantasticus* with *U.*

*ebenau*, subsequent accumulation of material of small-bodied *Uroplatus* has clearly revealed not only the distinctness of these two forms (Böhme and Henkel 1995), but the existence of several other members of the *ebenau* group (Nussbaum and Raxworthy 1995). Our samples of *U. "ebenau"* include no topotypical material, but a specimen from Manongarivo is both geographically proximate and morphologically similar to those from Nosy Be and we tentatively consider it representative of "true" *U. ebenau*. Each of the other *U. "ebenau"* specimens in our samples (Montagne d'Ambre, Marojejy, Tsaratanana) is highly genetically distinct from the typical form and from each other (22.3–36.3% uncorrected p ND2 sequence divergence) and all show closer affinities to *U. phantasticus* than to *U. ebenau* itself. Each of these is undoubtedly representative of a different new species. However, we are aware that other such distinctive populations are also known and believe that the description of these new forms should be part of a thorough revision of the *ebenau* group, which is beyond the scope of this paper. Such a revision should also clarify the status of the existing names *U. boettgeri* Fischer, 1883 and *U. schneideri* Lambertson, 1913, which have variously been synonymized with or resurrected from *U. ebenau* by past authors (Angel 1929, 1931, 1942; Bauer and Russell 1989). All of the three probable new species of the *U. ebenau* complex were collected in localities in northern Madagascar. *Uroplatus ebenau* itself, from Nosy Be and possibly Manongarivo, too, occurs in northern Madagascar. In contrast, only two species of this complex have so far been found in the vast rainforests of central eastern and south-eastern Madagascar (*U. phantasticus* and *U. malama*). Although additional species of this complex are to be expected from throughout Madagascar, the available data may suggest that northern Madagascar plays the role of a center of diversity and endemism for the small-bodied *Uroplatus* as it does for dwarf chameleons of the genus *Brookesia* (Raxworthy and Nussbaum 1995).

Among the large-bodied *Uroplatus* (*fimbriatus* group), our results support the recent recognition of specimens from far northern Madagascar as a distinctive species allied to *U. fimbriatus*, *U. giganteus* (Glaw *et al.* 2006). Like Glaw *et al.* (2006) we found that a specimen from Marojejy was moderately divergent (13.2% uncorrected ND2 p distance) from topotypical *U. giganteus* from Montagne d'Ambre and believe that this population may also warrant specific status. Our results, however, strongly support the sister species relationship of *U. sikorae* and *U. henkeli*. This is in contrast to the relationships suggested by the neighbor-joining tree of Glaw *et al.* (2006), which found support for the clustering of *U. sikorae* with the *U. fimbriatus/U. giganteus* clade and to the conclusions of Böhme and Ibsch (1990), who stated that *U. henkeli* was more closely related to *U. fimbriatus* than to *U. sikorae*. Within *U. henkeli*, specimens from Bemaraha and Nosy Be form one subclade, whereas those from Montagne des Français and Ankarana constitute another. The divergence between these two lineages is greater than that between *U. giganteus* and *U. fimbriatus* and suggests that one or more cryptic taxa may be subsumed within *U. henkeli* as currently conceived. Again, it is interesting that many of the highly divergent specimens were collected in northern Madagascar and also that *U. henkeli* and *U. giganteus* occur in this area. Divergences within the *U. sikorae* clade are not as pronounced but the status of members of this clade are, nonetheless, in need of reevaluation (Pearson *et al.* 2007).

The phylogenetic position of *Uroplatus lineatus* is not well supported in any of the analyses performed. However, we regard the amino acid deletion that this species shares with the large-bodied forms and the *alluaudi-pietschmanni* clade as strong evidence for its inclusion in this larger group (Fig. 2). Its distinctive body form, as well as the possession of autapomorphies, such as hardened hemibacula in the hemipenes (Rösler and Böhme 2006), suggest that *U. lineatus* has had a long independent history within this clade.

The phylogenetic hypotheses presented here largely corroborate earlier morphologically founded views of intrageneric affinities within *Uroplatus*. Likewise, they underscore the conclusions of many previous authors that diversity within the genus remains under appreciated (Böhme and Henkel 1995; Nussbaum and Raxworthy 1995; Svatek and van Duin 2001; Glaw *et al.* 2006).

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