

Continental speciation in the tropics: contrasting biogeographic patterns of divergence in the *Uroplatus* leaf-tailed gecko radiation of Madagascar

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Although substantial recent advances have been made regarding phylogenetic inference, our understanding of the processes of speciation remains incomplete (Turelli, Barton & Coyne, 2001; Gavrillets, 2003; Butlin, 2004; Coyne & Orr, 2004; Doebeli *et al.*, 2005). This is especially the case concerning the dominant mode of speciation of continental biotas. In contrast to islands and archipelagos (where species are frequently isolated and segregated by obvious oceanic barriers to dispersal), continental regions induce a far greater degree of uncertainty about the extent of geographic isolation between populations. Post-speciation distribution shifts, driven by climate change, biotic interactions (e.g. competition), adaptation or other factors, have long

Abstract

A fundamental expectation of vicariance biogeography is for contemporary cladogenesis to produce spatial congruence between speciating sympatric clades. The *Uroplatus* leaf-tailed geckos represent one of most spectacular reptile radiations endemic to the continental island of Madagascar, and thus serve as an excellent group for examining patterns of continental speciation within this large and comparatively isolated tropical system. Here we present the first phylogeny that includes complete taxonomic sampling for the group, and is based on morphology and molecular (mitochondrial and nuclear DNA) data. This study includes all described species, and we also include data for eight new species. We find novel outgroup relationships for *Uroplatus* and find strongest support for *Paroedura* as its sister taxon. *Uroplatus* is estimated to have initially diverged during the mid-Tertiary in Madagascar, and includes two major speciose radiations exhibiting extensive spatial overlap and estimated contemporary periods of speciation. All sister species are either allopatric or parapatric. However, we found no evidence for biogeographic congruence between these sympatric clades, and dispersal events are prevalent in the dispersal–vicariance biogeographic analyses, which we estimate to date to the Miocene. One sister-species pair exhibits isolated distributions that we interpret as biogeographic relicts, and two sister-species pairs have parapatric distributions separated by elevation. Integrating ecological niche models with our phylogenetic results finds both conserved and divergent niches between sister species. We also found substantial intra-specific genetic variation, and for the three most widespread species, poor intra-specific predictive performance for ecological niche models across the latitudinal span of Madagascar. These latter results indicate the potential for intra-specific niche specialization along environmental gradients, and more generally, this study suggests a complex speciation history for this group in Madagascar, which appears to include multiple speciation processes.

been considered problematic for detecting speciation patterns (e.g. Cracraft, 1994; Barraclough & Nee, 2001; Losos & Glor, 2003). In addition, extinction of lineages represents another general problem for inferring speciation history, where the loss of species reduces the degree of biogeographic congruence recoverable between clades (Wiley, 1988; Cracraft, 1994; Barraclough & Nee, 2001; Crisci, Katinas & Posadas, 2003). Both spatial shifts and lineage losses degrade the biogeographic signatures associated with alternative modes of speciation, which are also used in phylogenetic comparative methods to infer modes of speciation (e.g. Lynch, 1989; Chesser & Zink, 1994; Barraclough, Vogler & Harvey, 1998; Dimmick *et al.*, 1999; Barraclough & Vogler,

2000; Johnson & Cicero, 2002; Bouchard, Brooks & Yeates, 2005; Lukhtanov *et al.*, 2005; Fitzpatrick & Turelli, 2006; Jiggins *et al.*, 2006).

Ideally, therefore, continental speciation studies should be preferably based on groups and regions that minimize problems of distribution shifts and lineage extinctions, and maximize speciation events for analysis. These selection criteria have received little discussion to date, although Coyne & Orr (2004, 172, 175) proposed that tropical groups offer the best conditions for comparative phylogenetic speciation (see also Moritz *et al.*, 2000), a conclusion supported by the results of Lessa, Cook & Patton (2003) who found little evidence for range expansion in the late Quaternary in tropical compared with temperate regions. The Indian Ocean continental island of Madagascar, in many respects, represents an ideal region to study speciation. The island is 1000 km in length, with a surface area of 586 000 km²; it is positioned almost entirely in the tropics, and has experienced relatively modest shifts in climate compared with many temperate regions (Burney, 2003; Wells, 2003); has been tectonically almost static, moving <8° latitude over the last 45 million years (Royer *et al.*, 1992; Smith, Smith & Funnell, 1994); and has been isolated (as an island) from all other land masses for 87–91 million years (Storey *et al.*, 1995; Torsvik *et al.*, 2000). Consequently, many of the radiations of species in Madagascar are endemic to the continental island, which, because of its size serves as a mini-continent. The biota also excludes many groups found on neighboring landmasses, thus resulting in a combination of spectacular taxonomic imbalance, endemism and species richness (Krause, Hartman & Wells, 1997; Yoder & Nowak, 2006).

Although recent phylogenetic analyses have included endemic taxa from Madagascar, a focus of most studies has centered on the origins of radiations in Madagascar via either Gondwanan vicariance or oceanic dispersal (reviewed in Yoder & Nowak, 2006). No phylogenetic studies we are aware of have specifically targeted the geographic speciation history of endemic clades within Madagascar. Consequently, processes driving species radiation within this island continent remain poorly explored, although patterns of regional endemism and phylogeographic structure have been described for some groups (e.g. Raxworthy & Nussbaum, 1997; Yoder *et al.*, 2000, 2005; Olsen, Goodman & Yoder, 2004; Pastorini, Thalmann & Martin, 2005; Wilme, Goodman & Ganzhorn, 2006).

The *Uroplatus* leaf-tailed geckos of Madagascar (family Gekkonidae) represent an ideal group to serve as a case study to explore speciation patterns and processes in Madagascar. We selected *Uroplatus* because of the following features of the genus: (1) it is monophyletic (never in dispute) and endemic to only Madagascar and its nearby islands (<10 km offshore); (2) it is inferred to have poor dispersal ability, as supported by its endemism to Madagascar, localized regional endemism within Madagascar, and habits; (3) its high species richness, with 20 species identified in this study; and (4) the availability of recently collected specimens and tissue samples associated with accurate

locality data. *Uroplatus* geckos are both arboreal and nocturnal, with species distributed in all regions of Madagascar with the exception of the most arid southern spiny forest, and the very highest montane regions above 2400 m (C. J. Raxworthy, pers. obs.).

Prior phylogenetic analyses that have included this genus are based on: (1) morphological data: two generic level analyses (Bauer, 1990; Kluge & Nussbaum, 1995), and one species level phylogeny that included six species (Bauer & Russell, 1989); (2) a fragment of 16S mtDNA molecular data that included seven species (Glaw *et al.*, 2006); and (3) two nuclear genes (RAG-1 and PDC), and two mitochondrial genes (ND2 and *cyt b*) that included 10 species (Greenbaum *et al.*, 2007). However, all these studies have been incomplete in their taxonomic sampling for the genus (due to combinations of known and unknown sampling gaps at the time of publication, and earlier taxonomies that included erroneous synonyms), and morphological and molecular data have not yet been included in a combined analysis.

Here, we present the first taxonomically comprehensive phylogeny for *Uroplatus*, combining molecular and morphological data, and we simultaneously use vicariance and dispersal methods, as well as species ecological niche models to explore the group's speciation history. We use: (1) multiple vicariance and dispersal methods to explore biogeographic patterns within the phylogeny; (2) sister-species ecological niche models projected into geographic space to explore the geography of speciation; and (3) intraspecific ecological niche models to infer potential intraspecific niche variation. Based on these results, we examine the support for alternative speciation scenarios for Madagascar, and compare these findings to other isolated continental gecko groups.

Materials and methods

Taxonomic sampling and characters

We selected nine Gekkonidae genera to serve as outgroups for our phylogenetic analyses of *Uroplatus* (Table 1, see Supplementary Material). Ingroup sampling of *Uroplatus* included all 12 described species, and the eight species currently being described (C. J. Raxworthy *et al.*, in prep.), with all represented by both molecular and morphological data except *Uroplatus malama* (morphology only).

A total of 59 morphological characters were scored for all species of *Uroplatus* and outgroups (see Supplementary Material). These characters included 20 potentially parsimony informative characters taken from Kluge & Nussbaum (1995), and 39 characters modified from Bauer & Russell (1989) or new to this study. Character descriptions, and the nexus file containing the taxon-character data matrix, are deposited in TreeBase.

We collected sequence data for two mitochondrial loci, 12S rRNA (~790 bp) and cytochrome *b* (*cyt b*, ~1066 bp) and the two nuclear loci, 18S rRNA (~855 bp) and brain-derived neurotrophic factor exon (BDNF, ~606 bp) (see Supplementary Material). Primer sequences are provided in

Table 1 Tissue sample sources with associated GenBank sequence numbers

Species/sample #	Catalogue #	Field #	Locality	GenBank #s for loci sequences			
				cyt <i>b</i>	12S	BDNF	18S
<i>Rhoptropus diporus</i>	AMB 5932	AMB 5932	Ugab River, Namibia	EU596690	EU596606	EU596840	EU596766
<i>Rhoptropella ocellata</i>	AMB 5982	AMB 5982	Kleinsee Res., S. Africa	EU596700	EU596619	EU596852	EU596773
<i>Phelsuma abbotti</i>	RAX187	RAX 187	Namoroka Reserve	EU596699	EU596618	EU596851	EU596772
<i>Phelsuma cepediana</i>	Pcep1	Pcep1	Midlands, Mauritius	AY221400	AY221294	NA	NA
<i>Phelsuma b. borbonica</i>	Pbor1	Pbor1	Route Takamaka, Réunion	AY221394	AY221288	NA	NA
<i>Phelsuma o. ornata</i>	Porn	Porn	Gabriel Island, Mauritius	AY221443	AY221312	NA	NA
<i>Phelsuma o. inexpectata</i>	Pinx1	Pinx1	Manapany, Réunion	AY221396	AY221290	NA	NA
<i>Phelsuma guentheri</i>	Pgue1	Pgue1	Round Island, Mauritius	AY221441	AY221310	NA	NA
<i>Phelsuma gigas</i>	Pgig2	Pgig2	Rodrigues	AY221399	AY221293	NA	NA
<i>Matoatoa brevipes</i>	RAX8357	RAX8357	Cap Ste. Marie	EU596696	EU596614	EU596848	XXXX
<i>Blaesodactylus boivini</i>	UMMZ 201501	RAN 38867	Ankarana Reserve	EU596691	EU596607	EU596841	EU596767
<i>Geckolepis maculata</i> (1)	AMNH R152997	RAX 2220	Antseva	EU596693	EU596610	EU596844	EU596769
<i>Geckolepis maculata</i> (2)	AMNH R152999	RAX 3932	Bezavona	EU596694	EU596611	EU596845	NA
<i>Paroedura oviceps</i> (1)	AMNH R153039	RAX 2223	Antseva	EU596697	EU596615	EU596849	EU596771
<i>Paroedura oviceps</i> (2)	AMNH R153055	RAX 3973	Bezavona	NA	EU596616	NA	EU596850
<i>Paroedura oviceps</i> (3)	AMNH R153064	RAX 6500	Irony	EU596698	EU596617	NA	NA
<i>Ebenavia inunguis</i> (1)	AMNH R152971	RAX 2243	Antsahabe	EU596692	EU596608	EU596842	EU596768
<i>Ebenavia inunguis</i> (2)	AMNH R152973	RAX 4028	Bezavona	NA	EU596609	EU596843	NA
<i>Lygodactylus madagascariensis</i> (1)	AMNH R152978	RAX 2283	Tsaratana Reserve	EU596695	EU596612	EU596846	EU596770
<i>Lygodactylus madagascariensis</i> (2)	AMNH R152985	RAX 4267	Salafaina	NA	EU596613	EU596847	NA
<i>Uroplatus pietschmanni</i> (1)	AMNH R152343	RAX 7152	Madagascar	EU596762	NA	NA	NA
<i>Uroplatus pietschmanni</i> (2)	AMCC 138142	RAX 7153	Madagascar	EU596763	EU596687	NA	EU596837
<i>Uroplatus alluaudi</i>	UMMZ 201641	RAN 38128	Montagne d'Ambre	EU596701	EU596620	EU596853	EU596774
<i>Uroplatus guentheri</i> (1)	AMNH R159684	RAX 9808	Tsaramandroso	EU596765	EU596689	EU596916	EU596839
<i>Uroplatus guentheri</i> (2)	AMNH R153371	RAX 7157	Madagascar	EU596764	EU596668	NA	EU596838
<i>Uroplatus malahelo</i> (1)	UMMZ 208502	RAN 40559	Ambatorongorongo	EU596738	EU596661	EU596892	EU596813
<i>Uroplatus malahelo</i> (2)	UMMZ 217087	RAN 51170	Analavelona	EU596739	EU596662	EU596893	EU596814
<i>Uroplatus malahelo</i> (3)	RAN 52578	RAN 52578	Andohahelo Reserve	EU596740	EU596663	EU596894	EU596815
<i>Uroplatus</i> sp. A (1)	UMMZ 208427	RAN 42092	Marojejy	EU596708	EU596628	EU596860	EU596781
<i>Uroplatus</i> sp. A (2)	AMCC 141773	RAX 3449	Betaolana	EU596706	EU596625	EU596857	EU596779
<i>Uroplatus</i> sp. A (3)	AMCC 141775	RAX 3487	Betaolana	NA	EU596626	EU596858	EU596780
<i>Uroplatus</i> sp. A (4)	AMCC 141777	RAX 3515	Betaolana	EU596707	EU596627	EU596859	EU596781
<i>Uroplatus</i> sp. A (5)	AMNH R152933	RAX 4706	Ankitsika	EU596709	EU596629	EU596861	EU596783
<i>Uroplatus</i> sp. A (6)	AMNH R152934	RAX 4877	Sorata	EU596710	EU596630	EU596862	EU596784
<i>Uroplatus</i> sp. A (7)	AMNH R152936	RAX 5012	Sorata	EU596711	EU596631	EU596863	EU596785
<i>Uroplatus</i> sp. B	UMMZ 208409	RAN 38058	Montagne d'Ambre	EU596713	EU596634	EU596865	EU596787
<i>Uroplatus</i> sp. C (1)	AMNH R152957	RAX 4029	Bezavona	EU596714	EU596635	EU596866	EU596788
<i>Uroplatus</i> sp. C (2)	AMNH R152958	RAX 4433	Salafaina	EU596715	EU596636	EU596867	EU596789
<i>Uroplatus</i> sp. D (1)	AMNH R152959	RAX 5176	Sorata	EU596716	EU596637	EU596868	EU596790
<i>Uroplatus</i> sp. D (2)	AMNH R152965	RAX 6740	Lohanandroranga	NA	EU596638	EU596869	EU596791
<i>Uroplatus</i> sp. E (1)	UMMZ 208428	RAN 43228	Tsaratana	EU596717	EU596639	EU596870	EU596792
<i>Uroplatus</i> sp. E (2)	AMNH R152961	RAX 5461	Tsaratana	EU596718	EU596640	EU596871	EU596793
<i>Uroplatus</i> sp. F (1)	AMNH R152390	RAX 3263	Tsaratana Reserve	NA	EU596632	NA	NA
<i>Uroplatus</i> sp. F (2)	AMNH R150376	RAX 4012	Madagascar	EU596712	EU596633	EU596864	EU596786
<i>Uroplatus ebenauai</i> (1)	UMMZ 208425	RAN 39053	Manongarivo	EU596704	EU596623	EU596855	EU596777
<i>Uroplatus ebenauai</i> (2)	UMMZ 208442	RAN 43644	Nosy Be	EU596703	EU596622	1082467	EU596776
<i>Uroplatus ebenauai</i> (3)	AMNH R152884	RAX 2222	Antseva,	EU596702	EU596621	EU596844	EU596775
<i>Uroplatus ebenauai</i> (4)	AMNH R152886	RAX 4476	Analalava	EU596705	EU596624	EU596856	EU596778
<i>Uroplatus phantasticus</i> (1)	UMMZ 196375	RAN 37535	Mantady	EU596741	EU596664	EU596895	EU596816
<i>Uroplatus phantasticus</i> (2)	UMMZ 208523	RAN 44586	Andringitra	EU596747	EU596670	EU596900	EU596821
<i>Uroplatus phantasticus</i> (3)	UMMZ 208528	RAN 45198	Zahamena	EU596746	EU596669	NA	NA
<i>Uroplatus phantasticus</i> (4)	AMNH R150377	RAX 4013	Madagascar	EU596742	EU596665	EU596896	EU596817
<i>Uroplatus phantasticus</i> (5)	AMNH R150378	RAX 4014	Madagascar	EU596743	EU596666	EU596897	EU596818
<i>Uroplatus phantasticus</i> (6)	AMNH R159685	RAX 8079	Betampona	EU596744	EU596667	EU596898	EU596819
<i>Uroplatus phantasticus</i> (7)	AMNH R159687	RAX 9399	Mandraka	EU596745	EU596668	EU596899	EU596820
<i>Uroplatus</i> sp. G	UMMZ 208526	RAN 42274	Marojejy	EU596748	EU596671	EU596901	EU596822

Table 1. Continued.

Species/sample #	Catalogue #	Field #	Locality	GenBank #s for loci sequences			
				cyt <i>b</i>	12S	BDNF	18S
<i>Uroplatus lineatus</i> (1)	UMMZ 208494	RAN 42243	Marojejy	EU596734	EU596657	EU596888	EU596809
<i>Uroplatus lineatus</i> (2)	UMMZ 208496	RAN 42559	Ankavanana River	EU596735	EU596658	EU596889	EU596810
<i>Uroplatus lineatus</i> (3)	AMNH R152906	RAX 3867	Bezavona	EU596733	EU596656	EU596887	EU596808
<i>Uroplatus lineatus</i> (4)	AMNH R150374	RAX 4010	Tamatave region	EU596736	EU596659	EU596890	EU596811
<i>Uroplatus lineatus</i> (5)	AMNH R150375	RAX 4011	Tamatave region	EU596737	EU596660	EU596891	EU596812
<i>Uroplatus fimbriatus</i> (1)	UMMZ 208450	RAN 38460	Montagne d'Ambre	EU596723	EU596646	EU596877	EU596799
<i>Uroplatus fimbriatus</i> (2)	UMMZ 208464	RAN 42239	Marojejy	EU596724	EU596647	EU596878	EU596800
<i>Uroplatus fimbriatus</i> (3)	UMMZ 208458	RAN 42560	Ankavanana River	EU596725	EU596648	EU596879	EU596801
<i>Uroplatus fimbriatus</i> (4)	AMNH R152890	RAX 3830	Bezavona	EU596719	EU596641	EU596872	EU596794
<i>Uroplatus fimbriatus</i> (5)	AMNH R150371	RAX 4007	Tamatave region	EU596726	EU596649	EU596880	EU596802
<i>Uroplatus fimbriatus</i> (6)	AMCC 103215	RAX 4327	Salafaina	EU596720	EU596642	EU596873	EU596795
<i>Uroplatus fimbriatus</i> (7)	AMNH R152894	RAX 4470	Analalava	EU596721	EU596643	EU596874	EU596796
<i>Uroplatus fimbriatus</i> (8)	AMNH R159700	RAX 7458	Betampona	NA	EU596644	EU596875	EU596797
<i>Uroplatus fimbriatus</i> (9)	AMNH R159708	RAX 9112	Andakibe	EU596722	EU596645	EU596876	EU596798
<i>Uroplatus henkeli</i> (1)	UMMZ 201576	RAN 39127	Manongarivo	EU596729	EU596652	EU596883	EU596805
<i>Uroplatus henkeli</i> (2)	UMMZ 208481	RAN 43676	Nosy Be	EU596728	EU596651	EU596882	EU596804
<i>Uroplatus henkeli</i> (3)	UMMZ 217077	RAN 54193	Bemaraha Reserve	EU596730	EU596653	EU596884	EU596806
<i>Uroplatus henkeli</i> (4)	AMNH R152902	RAX 2291	Tsaratana Reserve	EU596727	EU596650	EU596881	EU596803
<i>Uroplatus</i> sp. H (1)	UMMZ 201577	RAN 38929	Ankarana Reserve	EU596732	EU596655	EU596886	EU596807
<i>Uroplatus</i> sp. H (2)	AMNH R152969	RAX 4220	Analafiana	EU596731	EU596654	EU596885	NA
<i>Uroplatus sikorae</i> (1)	UMMZ 208504	RAN 38129	Montagne d'Ambre	EU596751	EU596674	EU596904	EU596825
<i>Uroplatus sikorae</i> (2)	UMMZ 208512	RAN 39721	Marojejy	EU596756	EU596680	EU596910	EU596757
<i>Uroplatus sikorae</i> (3)	AMNH R152910	RAX 2941	Tsaratana Reserve	EU596749	EU596672	EU596902	EU596823
<i>Uroplatus sikorae</i> (4)	AMNH R152912	RAX 3262	Tsaratana Reserve	EU596750	EU596673	EU596903	EU596824
<i>Uroplatus sikorae</i> (5)	AMCC 141782	RAX 3644	Betaolona	EU596756	EU596679	EU596909	EU596830
<i>Uroplatus sikorae</i> (6)	AMNH R152917	RAX 3937	Bezavona	EU596752	EU596675	EU596905	EU596826
<i>Uroplatus sikorae</i> (7)	AMNH R152920	RAX 4434	Salafaina	EU596753	EU596676	EU596906	EU596827
<i>Uroplatus sikorae</i> (8)	AMNH R152921	RAX 4577	Ankitsika	EU596755	EU596678	EU596908	EU596829
<i>Uroplatus sikorae</i> (9)	AMNH R152924	RAX 5260	Sorata	EU596754	EU596677	EU596907	EU596828
<i>Uroplatus sikorae</i> (10)	AMNH R152926	RAX 6704	Lohanandroranga	NA	EU596681	EU596911	EU596831
<i>Uroplatus sameiti</i> (1)	UMMZ 208515	RAN 45196	Zahamena	EU596761	NA	NA	EU596836
<i>Uroplatus sameiti</i> (2)	UMMZ 208516	RAN 45197	Zahamena	EU596760	EU596686	NA	NA
<i>Uroplatus sameiti</i> (3)	AMNH R152928	RAX 4483	Analalava	EU596758	EU596682	EU596912	EU596832
<i>Uroplatus sameiti</i> (4)	AMNH R159695	RAX 7700	Betampona	EU596759	EU596683	EU596913	EU596833
<i>Uroplatus sameiti</i> (5)	AMNH R159696	RAX 8954	Ambodiriana	NA	EU596684	EU596914	EU596834
<i>Uroplatus sameiti</i> (6)	RAX 9089	RAX 9089	Andakibe	NA	EU596685	EU596915	EU596835

Species sample numbers (for multiple species samples) correspond to those used in Figs 2 and 3. Catalogue number refers to the catalogued primary voucher at AMNH, UMMZ or Villanova University (AMB research collection). All localities (abbreviated) are in Madagascar, unless stated otherwise. Field catalogue series: AMB, Aaron M. Bauer; RAN, Ronald A. Nussbaum; RAX, Christopher J. Raxworthy.

Table 2. The nuclear 18S gene showed no length variation for the taxa examined, and therefore, alignment was straightforward. For BDNF and cyt *b*, the reading frame was verified visually in MacClade using translated amino acid sequences. For 12S, sequences were aligned with a squamate 12S secondary structure modified from the secondary structure of *Falco peregrinus* (Aves: Falconidae) (Mindell *et al.*, 1997). All sequences are deposited in GenBank (Table 1) and sequence alignments are deposited in TreeBase.

Phylogenetic analysis

Rhoptropus was used as the outgroup in all analyses. Data were analyzed using maximum parsimony (MP), maximum

likelihood (ML) and Bayesian inference (BI), with the latter two analyses restricted to only molecular data. MP analyses were performed using PAUP* Version 4.0 b10 (Swofford, 2002). ModelTest v3.07 (Posada & Crandall, 1998) was used to determine the appropriate model of sequence evolution for ML and BI analyses. Garli v0.942 (Zwickl, 2006) was used for all ML analyses. Bayesian posterior probabilities were calculated using the Metropolis-coupled Markov chain Monte Carlo sampling approach in MrBayes v3.01 (Huelssenbeck & Ronquist, 2001). The *Uroplatus* topologies found from MP (all data), and the ML and BI analyses (molecular data) were compared using the Shimodaira–Hasegawa (S–H) test (Shimodaira & Hasegawa, 1999) in PAUP*. For more detailed description of the analyses refer to Supplementary Material.

Table 2 Primer sequences used in this study

Locus	Primer	Sequence (5'–3')	Source	
cyt <i>b</i>	Lg 11	TAGTTGAATWACAACRGTGG	This study	
	Lg 98	CAAACATCTCCTCCTGATGAAA	This study	
	Lg 311	ACATCGGMCGAGGCCTTTATTA	This study	
	Lg 600	GGGTRGAGGAGCCGGTTTCGT	This study	
	L15710	CCMMCMCAYATCAARCCMGAATG	Sorenson <i>et al.</i> (1999)	
	Hg 421	ATGACTGTAGCGCCTCAGAA	This study	
	Hg 808	AAGCCAGAATGATACTTCCT	This study	
	Hg 1000	TCTACTGGTTGKCCWCCTASTCAGG	This study	
	H15149	GCCCTCAGAATGATATTTGCTCCTCA	Kocher <i>et al.</i> (1989)	
	H15563	GCGTATGCGAATAGGAAATA	H15712; Lee <i>et al.</i> (1997)	
	H16064	CTTCAGTTTTTGGTTTACAAGACC	Sorenson <i>et al.</i> (1999)	
	12S rRNA	tPhe	AAAGCACRGCCTGAAGATGC	Wiens, Reeder & Montes De Oca (1999)
		12e	GTRCGCTTACCWTGTTACGACT	Wiens <i>et al.</i> (1999)
		Lg 12s1	GAAACAACRTRTTTTGGTCCYAG	This study
12SD		ATCGATTATAGAACAGGCTCCTC	H1861; J. Groth, unpubl. data	
12SA		GGGATTAGATACCCCACTAT	Kocher <i>et al.</i> (1989)	
12SB		TGCAGAGGGTGACGGCGGTATGT	Kocher <i>et al.</i> (1989)	
18S rRNA	18S1F	TACCTGGTTGATCCTGCCAGTAG	Zilversmit <i>et al.</i> (2002)	
	18S5R	CTTGCCAAATGCTTTCCG	Zilversmit <i>et al.</i> (2002)	
BDNF	BDNF-F	GACCATCTTTTCTCCTACTATGGTTATTTTCATACTT	Leaché & McGuire (2006)	
	BDNF-R	CTATCTTCCCTTTTAATGGTCAGGTACAAAC	Leaché & McGuire (2006)	

Estimating divergence dates

We used three island origin dates and the phylogenetic results of Austin, Arnold & Jones (2004) to date the following four calibration points: (1) 8–11 MYBP for the formation of Rodrigues (Duncan & Storey, 1992) and divergence of *P. gigas* from the other included Mascarene *Phelsuma*; (2) 7–8 MYBP for the formation of Mauritius (Duncan & Storey, 1992) and divergence of *Phelsuma guentheri* from the other included Mascarene *Phelsuma* (excluding *P. gigas*); (3) 5 MYBP for the formation of Réunion Island (Gillot, Lefèvre & Nativel, 1994) and divergences of *Phelsuma b. borbonica* from *Phelsuma cepediana*, and *Phelsuma ornata ornata* from *Phelsuma o. inexpectata*. This Réunion origin date is older than the 2.1 MYBP estimated by McDougall (1971), which has been previously used to date *Phelsuma* divergences, but is concordant with Emerick & Duncan (1982; Fig. 1) who show Réunion to be approximately equidistant between Mauritius and the current hot-spot position. We also used one internal calibration date for *Uroplatus*, based on the volcanic formation of the Montagne d'Ambre massif. This sky island massif (see Raxworthy & Nussbaum, 1997) includes one endemic *Uroplatus* species, *U. sp. B*, which is restricted to higher elevation primary forest. We estimate the volcanic origin of Montagne d'Ambre at 14 MYBP using the consensus of the following sources: Emerick & Duncan (1982); Besaire (1972); Jenkins (1987) and Wells (2003).

BEAST v1.4.6 (Drummond & Rambaut, 2007) was used to estimate divergence times using a Bayesian Markov chain Monte Carlo method under a relaxed molecular clock (Drummond *et al.*, 2006). An uncorrelated lognormal

relaxed clock model was used in all analyses (see Supplementary Material). To evaluate assumptions that each island or massif was colonized by its endemic species soon after initial geological formation (see also de Queiroz, 2005), we conducted validation analyses using internal and external calibration dates separately to validate each other, and also compared these results with estimates based on all five calibration dates.

Vicariance and dispersal biogeography

Areas of endemism used in biogeographic analyses were based on Humbert's (1955) phytobiogeographic areas of Madagascar (see Supplementary Material). To explore biogeographic support for vicariance or dispersal from ancestral areas, we utilized dispersal–vicariance analysis (DIVA), as implemented in the program DIVA 1.1 (Ronquist, 1996, 1997), importing a fully resolved phylogeny, and using default options. To measure the degree of biogeographic congruence between sympatric clades within *Uroplatus* we implemented Brooks Parsimony Analysis (BPA) using PAUP*, with Deltran optimization and procedures as described by Brooks, van Veller & McLennan (2001) and Bouchard *et al.* (2005).

Species ecological niche models

We integrated phylogenetic results with species ecological niche modeling by projecting sister species models onto the Madagascar landscape. Ecological niche modeling can be used to test for niche conservatism or divergence between species, to provide evidence for allopatry, and to detect

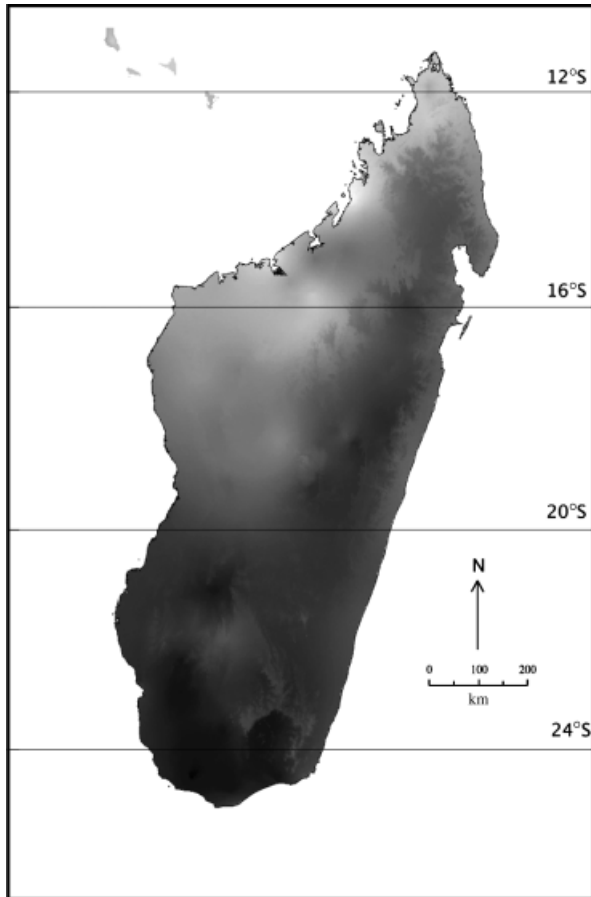


Figure 1 Seasonal temperature variation in Madagascar, showing less seasonal variation at lower latitudes. Shading is based on a linear scale of standard deviation of monthly means: white=minimum, black=maximum. Data from the WorldClim dataset (Hijmans *et al.*, 2005).

areas of endemism for biogeographic analyses (Raxworthy *et al.*, 2007; Kozak, Graham & Wiens, 2008). These models utilize associations between environmental variables and known species' occurrences (see Guisan & Thuiller, 2005; Elith *et al.*, 2006). We applied the maximum entropy method (Maxent; Phillips, Anderson & Schapire, 2006) with 20 environmental variables relating to temperature, precipitation and topography (for complete methodological details see Pearson *et al.*, 2007 and Supplementary Material). To investigate the potential for intra-specific niche variation across environmental gradients (Peterson & Holt, 2003), we divided occurrence records for the best sampled species (*Uroplatus henkeli*, *Uroplatus fimbriatus*, *Uroplatus sikorae*) equally between the north and south of Madagascar. We selected this north–south division of localities based upon the latitudinal gradient for variable (seasonal) temperatures that exists in Madagascar (Fig. 1). We tested the ability of ecological niche models built using one subset of occurrence localities to predict the other, and calculated statistical significance using an exact one-tailed binomial probability.

Results

Phylogenetic analyses

Uroplatus was inferred to be a monophyletic group in all analyses. The MP morphological analysis found 26 trees of 124 steps, with a consistency index (CI) of 0.573, and a retention index (RI) = 0.858 (Fig. 2). The strict consensus tree identified *Paroedura* as the sister taxon to a monophyletic *Uroplatus* clade. The *Uroplatus* stem polytomy included five lineages: *Uroplatus alluaudi*, *Uroplatus guentheri*, *Uroplatus malahelo* and two clades. The first clade included *Uroplatus pietschmanni* and the (as termed here) 'fimbriatus group' of *U. fimbriatus*, *U. sikorae*, *Uroplatus sameiti*, *U. henkeli*, *Uroplatus lineatus* and *U. sp. H*. The second clade included the (as termed here) 'ebenai group' of *Uroplatus ebenai*, *Uroplatus phantasticus*, *U. malama*, and *U. sps. A–G*. Bootstrap proportions (BP) for the *Paroedura–Uroplatus* sister relationship, monophyly of *Uroplatus*, and monophyly of the two species clades (as described above) was between 65 and 100%. However, many clades had <70% BP, which we consider as a threshold for strong support (see also Supplementary Material), and decay indices (DI) of 1–2.

The MP molecular analyses (excluding *U. malama*) found similar topologies to those described above (see Supplementary Material for detailed descriptions). A heuristic search using the combined molecular and morphology data found 24 equally parsimonious trees (TL = 10601, CI = 0.260, RI = 0.697). The monophyly of *Uroplatus* is well supported (BP = 100, DI = 51). Within *Uroplatus*, there is strong support for the 'ebenai' and 'fimbriatus' clades (BP = 95 and 80, respectively). The topology is described in more detail in the Supplementary Material and Fig. 3.

For the combined molecular dataset, the general time-reversible model (Yang, 1994), corrected for among-site rate variation using the discrete Γ distribution and a proportion of invariable sites (GTR + Γ + I) was significantly better than all simpler models (AIC, P -value < 0.001) and was used for the ML and BI analyses. The overall topology of the MP, ML and BI were very similar and the Garli ML phylogeny (with the highest likelihood value) is presented here (Fig. 3). The strict consensus MP tree based on these data (and the combined molecular and morphological data) is almost identical in tree topology except that *Matoatoa* forms part of a stem polytomy with the outgroups, the *U. guentheri*/*U. malahelo* clade is sister to the 'fimbriatus' clade, and *U. sp. F* is a stem lineage to *U. sps. A–E*. *Uroplatus malama* was found to be basal to all other 'ebenai' group species. The only notable difference between the ML and BI analyses were the relationships of *U. sp. F* within the ebenai group (Figs 3 and 4), and the outgroup relationships, with the BI analysis finding *Lygodactylus* as sister to *Uroplatus*. Comparing the MP trees and the ML and BI trees, using the S–H test, none of the topologies were found to be statistically significantly different from each other.

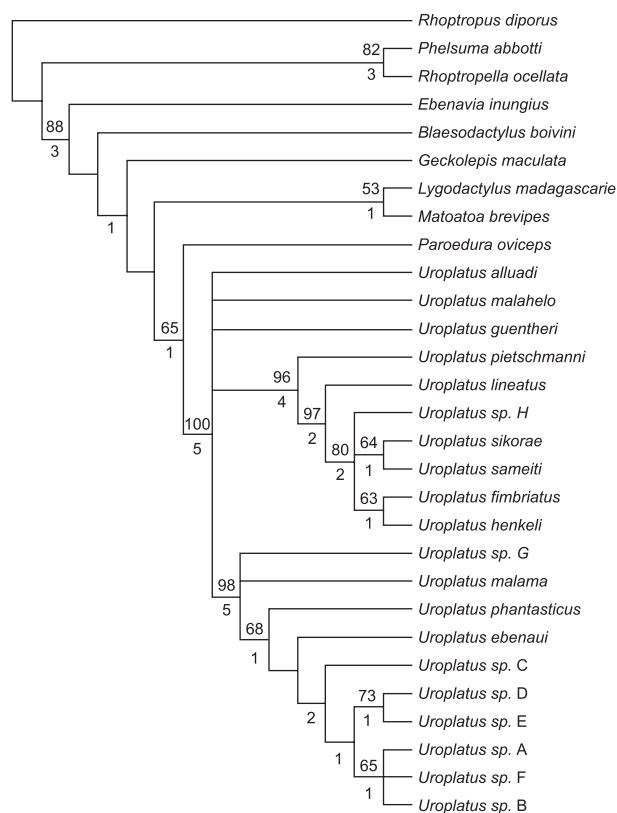


Figure 2 Strict consensus of 26 equally parsimonious trees (TL = 124, CI = 0.573) based on morphology. For all major branches, values above refer to bootstrap proportions (<50 not shown), and below refer to Bremer decay indices.

Divergence dates

The estimated mean divergence dates for nodes, including the upper and lower interval bounds for 95% of the highest posterior densities (HPDs) are shown in Table 3 and Fig. 4. When comparing the geological origin dates to the corresponding estimated lineage divergences (based on other calibrations), these dates all fell within the 95% HPD, with the exception of Réunion which is 1.8–3.9 MYBP older than the divergence of *P. o. inexpectata*, suggesting a more recent arrival for this taxon. This contrasts with the other island endemic *P. borbonica*, which has a contemporary estimated time of divergence with the formation of Réunion. When comparing the divergence dates estimated based on internal (Montagne d'Ambre) versus external (Mascarene Islands) calibrations, in all cases the estimates fell within the 95% HPD of the date estimated using the other calibration method. Based on these validation results, we consider the best estimate of divergence dates is obtained by using all calibration points. These estimates also exhibit the lowest range of 95% HPD (Table 3). The earliest estimated mean divergence within the *Uroplatus* clade is dated (with 95% HPD) at 31.7 (25.4–38.5) MYBP, and the youngest speciation event (*U. sameiti* and *U. sikorae*) dated at 8.6 MYBP (6.5–11.1 MYBP).

Biogeographic analyses

A fully resolved species cladogram based on the ML tree topology (with the addition of *U. malama*, based on the morphological phylogenetic results), was imported into DIVA, which yielded the optimized ancestral area distributions as depicted in Fig. 5. The ancestral distribution for the entire *Uroplatus* clade was optimized to be widespread across all regions except the high montane Tsaratanana region (which has a single dispersal event for *U. sp. E*); although widespread ancestral taxa represent a common result in DIVA analyses (Ronquist, 1997). The clade of seven species in the ebenauyi group (*U. phantasticus* to *U. sp. A*) was identified as having just a single area of origin in the Centre–North, while the species ancestral to the entire ebenauyi group had a distribution confined to the interior and north of Madagascar. For the fimbriatus group clade, all ancestral distributions below the clade including *U. henkeli*–*U. sikorae* were optimized to be widespread (with the exception of the Tsaratanana and Sambirano regions). Remarkably, the only support found for vicariance within the entire *Uroplatus* group was restricted to the *U. henkeli*, *U. sp. H*, *U. sameiti* and *U. sikorae* clade, where three ancestral areas split into smaller subset areas with not much sympatry between sister lineages. Utilizing primary BPA resulted in an area matrix including 39 syntaxa, of which 28 were parsimony informative. An exhaustive parsimony search found six equally parsimonious general area cladograms of 60 steps, with a CI of 0.5714 (excluding uninformative characters). The strict consensus of these six general area cladograms is shown in Fig. 6. The stem polytomy of five ancestral areas is indicative of the lack of biogeographic congruence that exists between these *Uroplatus* area clades. Only one clade was resolved for more than two areas: the areas Centre–Centre, Centre–North, East–North and West–North. The syntaxa providing the strongest support for this clade were from the *U. henkeli*, *U. sp. H*, *U. sameiti* and *U. sikorae* clade, as optimized by DIVA to support vicariance.

The phylogenetic results reveal strong support for five sister-species pairs (the sister relationship of *U. sp. F*–*U. phantasticus* has low support, see Fig. 3). All five have actual distributions that are allopatric or parapatric, although *U. sp. A* and *U. sp. D* have been found at the same massif (Lohanandroranga) separated by a narrow elevational band between 1760 and 1790 m. The sisters *U. pietschmanni* and *U. alluaudi* exhibit substantial geographic isolation, being separated from each other by about 400 km, and are highly localized in distribution (Fig. 7, Böhme & Schönecker, 2003; Andreone & Aprea, 2006). These apparent relict distributions appear to be indicative of substantial range contraction.

The distributions of the other sister-species pairs had sufficient localities for ecological niche models to be generated, as shown in Fig. 8. The *U. malahelo*–*U. guentheri* ecological niche models exhibit allopatry, reflecting clear niche divergence between these sister species. Both species also exhibit fragmented species ecological niche models,

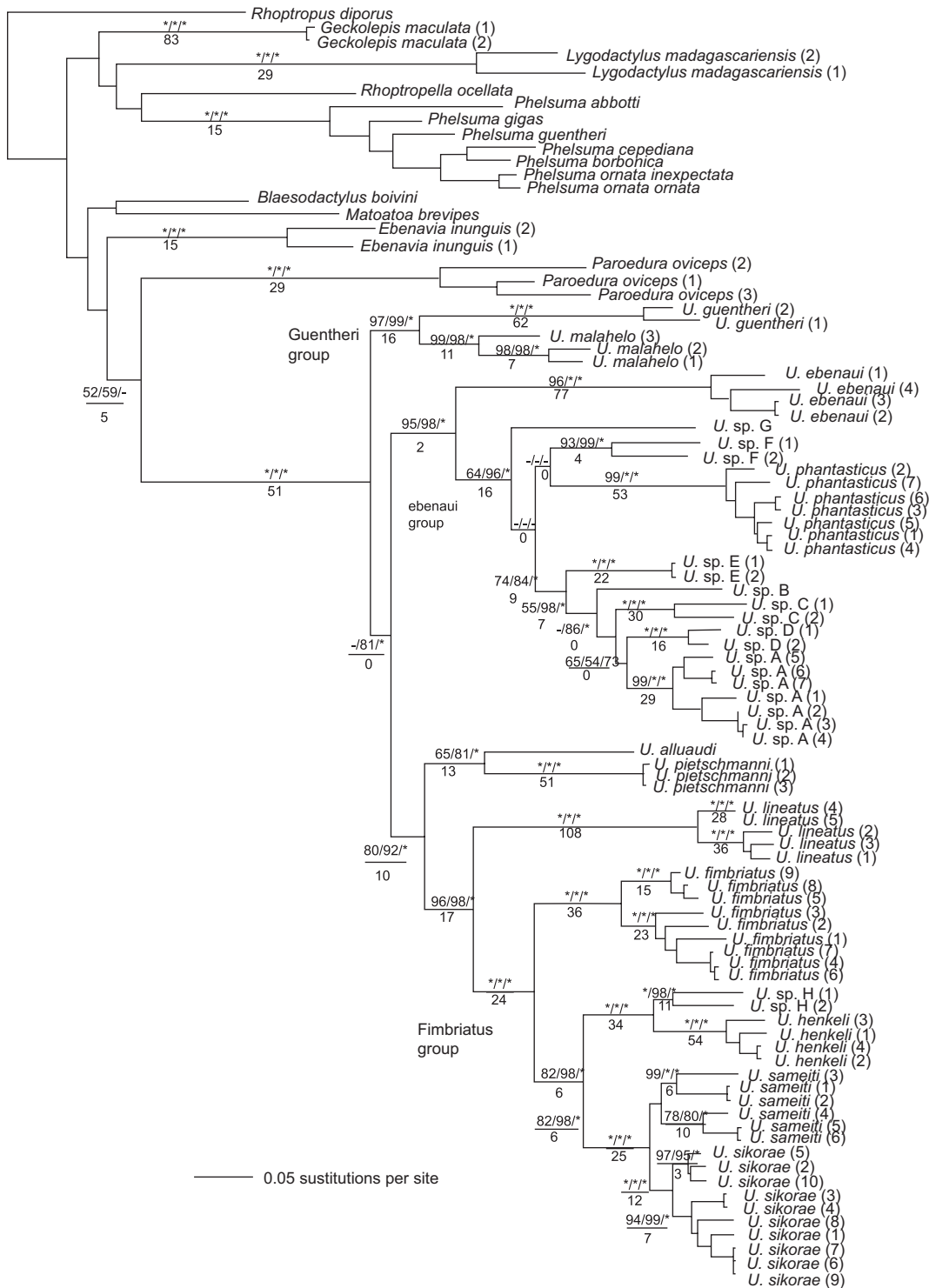


Figure 3 Combined (mt and nuc DNA) maximum-likelihood phylogeny under GTR + Γ + I model ($-\ln L = 48025.2092$, $\alpha = 0.71365$, proportion of invariable sites = 0.53976). For all major branches, values above branches refer to MP bootstrap proportions, ML bootstrap proportions and Bayesian posterior probabilities, respectively. *refer to 100, - refers to values < 50. Values below branches represent Bremer decay indices. Numbers correspond to the specimens listed in Table 1. MP, maximum parsimony; ML, maximum likelihood; mt, mitochondrial; nuc, nuclear.

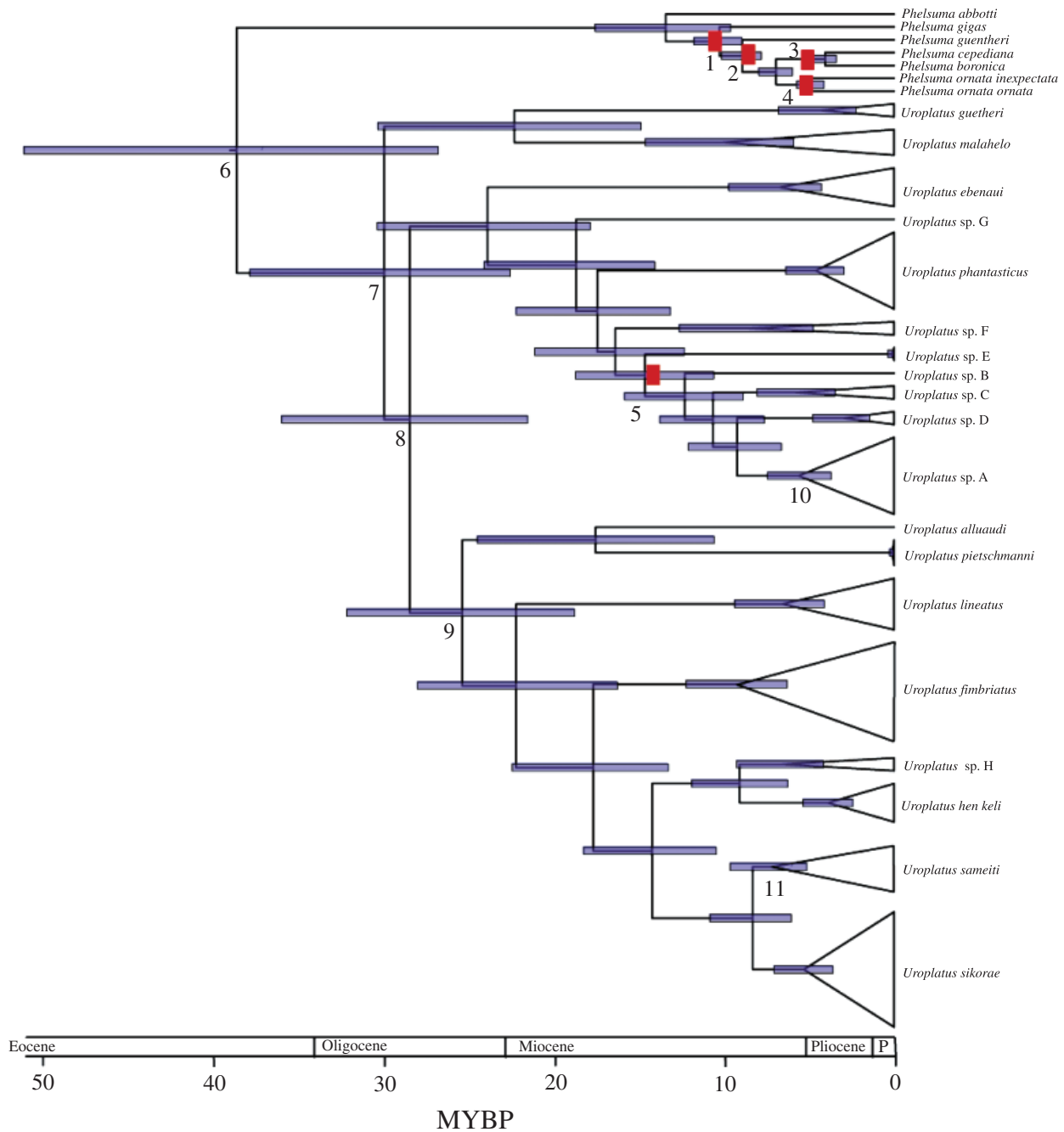


Figure 4 Node divergence time estimates for the major *Uroplatus* lineages (using BEAST, Drummond & Rambaut, 2007) based on the five geological calibration points (indicated by red squares). Horizontal bars represent the upper and lower interval bounds for 95% of the highest posterior densities (HPDs). Additional estimates for the numbered nodes are given in Table 3. P, Pleistocene.

with two major isolated populations in north-western and western Madagascar for *U. guentheri*, and multiple fragmented areas in the southern–central region for *U. malahelo*. The *U. sikorae*–*U. sameiti* ecological niche models have a parapatric distribution, with a narrow region of sympatry occurring between these species at mid-elevation. By contrast, the *U. henkeli*–*U. sp. H* models exhibit substantial

sympatry, although both species also occupy areas that are modeled as not being suitable for its sister, and these areas are asymmetrical, with a much larger fraction of the *U. henkeli* model not sympatric with its sister. The *U. sp. A*–*U. sp. D* ecological niche models also reveal substantial asymmetrical sympatry, with *U. sp. D* occupying a subset of environmental space occupied by *U. sp. A*. The *U. sp. D*

Table 3 The estimated mean divergence dates for numbered nodes shown in Fig. 4 based on alternative sets of calibration points

Node	Estimated node divergence		
	Calibrations utilized to estimate divergence time		
	Mt. d'Ambre	Mascarene Islands	Ambre and Mascarene
Nodes with geological calibrations			
(1) <i>Phelsuma gigas</i> (Rodrigues)	12.9 (8.8–17.0)	8–11	10.4 (9.0–11.8)
(2) <i>Phelsuma guentheri</i> (Mauritius)	11.1 (7.7–14.9)	7–8	9.0 (7.9–10.2)
(3) <i>Phelsuma o. inexpect.</i> (Réunion)	2.1 (1.1–3.2)	5	4.1 (3.4–4.7)
(4) <i>Phelsuma borbonica</i> (Réunion)	5.3 (3.3–7.6)	5	4.9 (4.1–5.7)
(5) <i>Uroplatus</i> sp. B (Mt. d'Ambre)	14	12.2 (8.9–15.9)	13.35 (11.2–15.5)
<i>Uroplatus</i> nodes without calibrations			
(6) <i>Uroplatus</i>	46.4 (32.9–61.1)	38.9 (27.3–51.1)	40.5 (29.7–52.5)
(7) gunetheri group	33.9 (25.8–43.1)	29.6 (22.0–37.2)	31.7 (25.4–38.5)
(8) ebenau group	32.5 (24.3–43.1)	28.0 (21.2–35.7)	30.0 (23.7–36.0)
(9) alluaudi/fimbriatus group	28.9 (21.6–36.6)	24.9 (18.7–31.7)	26.7 (21.2–32.6)
(10) <i>Uroplatus</i> sp. A	10.6 (8.1–13.2)	9.3 (6.4–12.1)	10.0 (7.8–12.1)
(11) <i>Uroplatus Sameiti</i>	9.2 (6.5–11.8)	8.1 (5.8–10.7)	8.6 (6.5–11.1)

Values in parentheses represent the upper and lower interval bounds for 95% of the HPDs (highest posterior densities). All dates millions of years before present (MYBP). Geological calibrations are shown in bold (see text). HPDs, highest posterior densities.

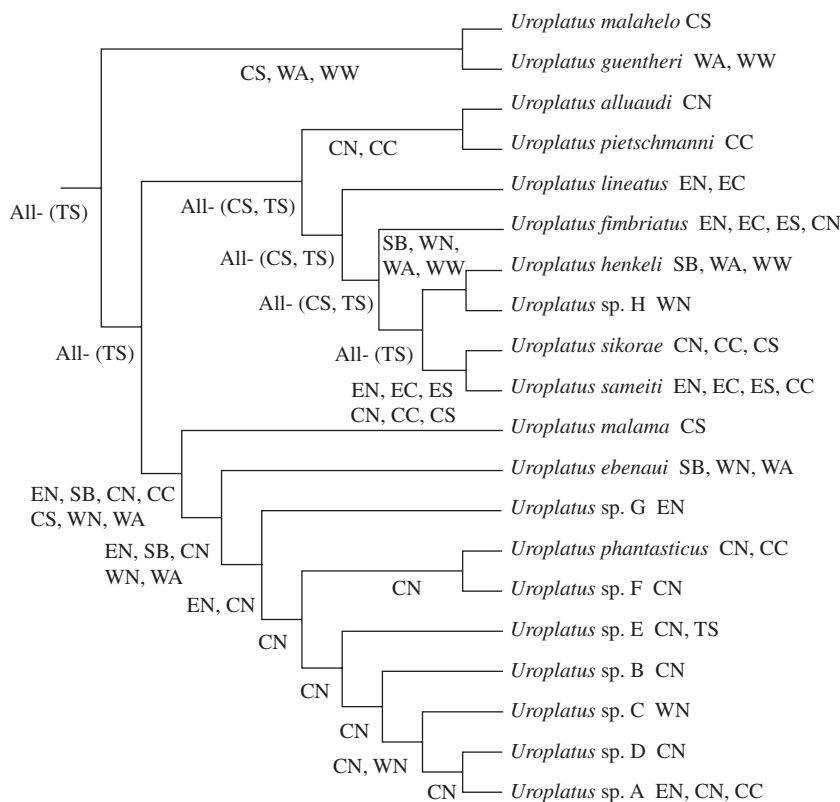


Figure 5 *Uroplatus* species-area cladogram, showing all the ancestral areas for nodes, as optimized by DIVA (Ronquist 1996, 1997). Area distributions for species are shown next to taxon labels. Area abbreviations (based on Humbert's, 1955 phytobiogeographic areas) are: EN, East-North; EC, East-Central; ES, East-South; SB, Sambirano; CN, Centre-North; CC, Centre-Centre; CS, Centre-South; TS, Tsaratanana (High Montane); WN, West-North, WA, West-Ambongo; WW, West-West; All, all areas except those shown in brackets.

ecological niche model also includes disjunct areas in central and southern Madagascar not known to be occupied by this species.

We also found substantial differences in environmental space between the southern and northern locality subsets for

the three species with the most extensive locality sample sizes (Fig. 9). Five of the six binomial intra-predictivity tests were not significant (north predicts south, and south predicts north, respectively): *U. fimbriatus*, 0 of 8 predicted (NS) and 4 of 7 predicted (NS); *U. henkeli*, 1 of 6 predicted

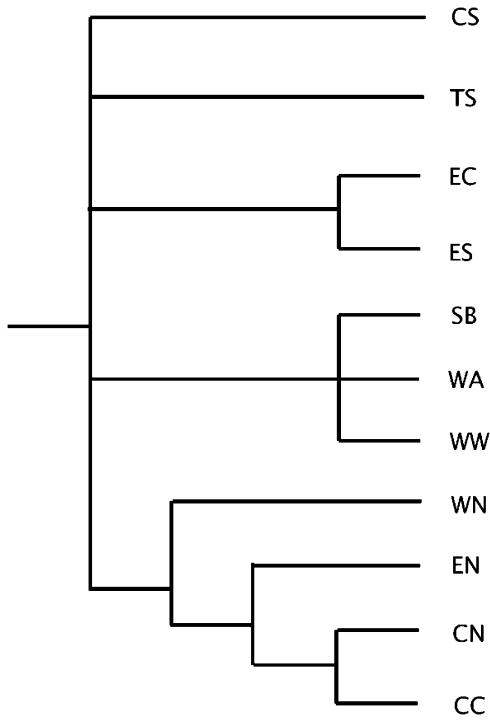


Figure 6 General area cladogram using primary Brooks Parsimony Analysis (BPA) based on the *Uroplatus* phylogeny and species distributions shown in Fig. 4. Area abbreviations as given in the Fig. 5 legend. Spatial incongruence between the *Uroplatus* clades results in a stem polytomy.



Figure 7 The only known localities for the sister species *Uroplatus alluaudi* and *Uroplatus pietschmanni*.

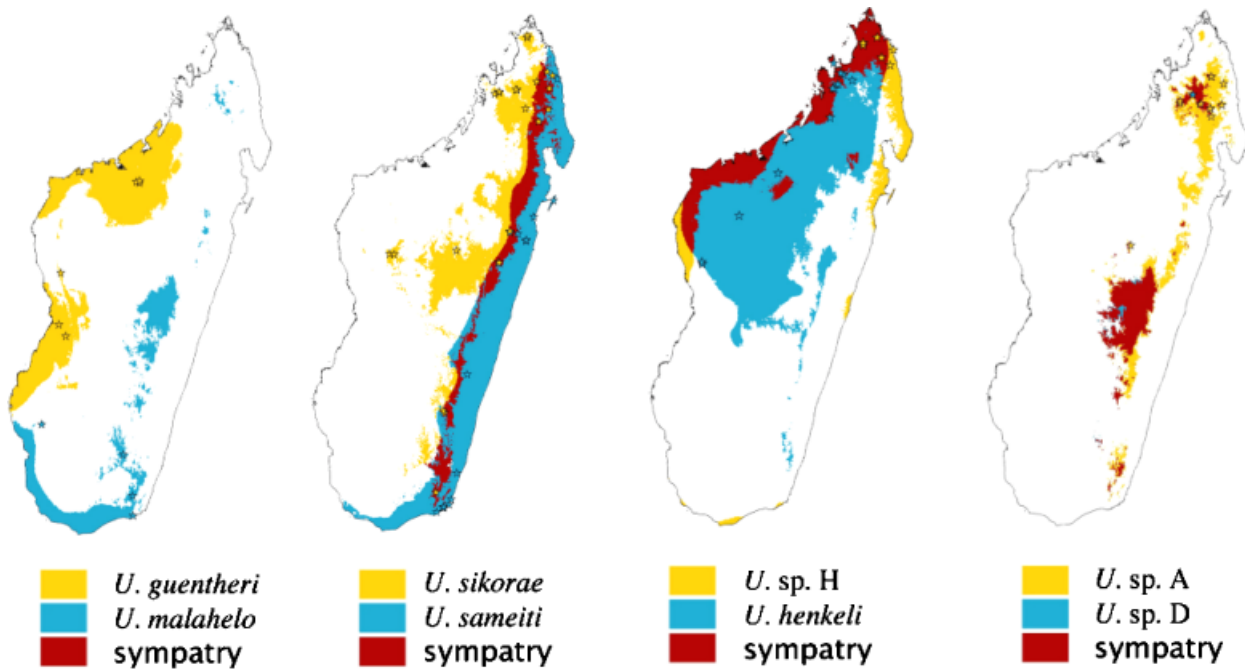


Figure 8 Ecological niche models for four sister-species pairs of *Uroplatus* (see Fig. 3). Areas of sympatry between species ecological niche models are shown in red.

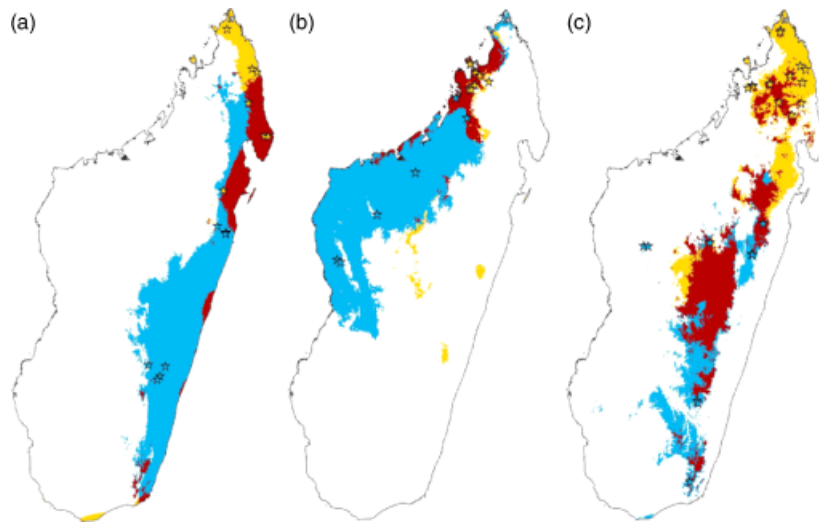


Figure 9 Intraspecific predictivity of ecological niche models based on northern and southern subsets of localities for (a) *Uroplatus fimbriatus*, (b) *Uroplatus henkeli* and (c) *Uroplatus sikorae*. Northern localities and modeled distributions are yellow, southern localities and modeled distributions are blue, and model overlap is red. In almost all cases, latitudinal subsets of localities were unable to predict substantial areas of the entire species distribution.

(NS) and 6 of 8 predicted ($P < 0.01$); and *U. sikorae*, 2 of 9 predicted (NS) and 4 of 14 (NS). For all three species, the southern (more temperate and seasonal) localities were consistently better at predicting northern localities, compared with the northern (more tropical) localities predicting southern localities.

Discussion

Origins and cladogenesis

Our phylogenetic results are largely congruent with the previous studies that included fewer species and populations. The morphological results reported for six species by Bauer & Russell (1989) are identical except that they found *U. alluaudi* sister to *U. guentheri*. Our phylogeny is also largely congruent with the 16S fragment neighbor joining tree presented by Glaw *et al.* (2006), except they report *U. henkeli* sister to a clade including *U. fimbriatus* and *U. sikorae*, and found *U. ebenau* sister to all other *Uroplatus*. The BI phylogeny reported by Greenbaum *et al.* (2007) for nine *Uroplatus* species is also largely congruent with our results, except that they found a sister relationship between *U. phantasticus* and a Tsaratanana lineage of *U. 'ebenau'*, and reported *Matoatoa* + *Afrogecko* sister to *Uroplatus*, when using *Paroedura* as the outgroup. However, a parsimony analysis (not shown) combining our *cyt b* data with those of Greenbaum *et al.* (2007), found the *Matoatoa brevipes* sequence (GenBank EF490751) falling inside the *U. fimbriatus* clade, indicating that this sequence was likely misidentified.

We find strongest support for *Paroedura* as the sister clade to *Uroplatus*, and the sister to both being *Ebenavia*. These results agree with the morphological results of Bauer (1990) who found these three genera as a monophyletic clade. Although not well supported, we also find *Matoatoa* and *Blaesodaactylus* forming the sister clade to *Uroplatus* + *Paroedura* + *Ebenavia*. A potential close relationship between these genera had been suggested by prior morpho-

logical analyses (see Kluge & Nussbaum, 1995; Nussbaum, Raxworthy & Pronk, 1998), and because these genera are endemic to the Indian Ocean (primarily Madagascar), they may thus represent another regional gekkonid species radiation with origins in Madagascar, similar to *Phelsuma* (Austin *et al.*, 2004).

The estimated 40.5 MYBP divergence date between *Uroplatus* and *Phelsuma*, and the deepest divergence within *Uroplatus* at 31.7 MYBP, support *Uroplatus* originating in Madagascar during the mid-Tertiary, long after Madagascar had become isolated from all other landmasses at around 87–91 MYBP (Storey *et al.*, 1995; Torsvik *et al.*, 2000). The three major *Uroplatus* lineages are estimated to have diverged during the Oligocene, with the youngest sister-species pairs having evolved during the Miocene, and all intraspecific divergence estimated to date from the Miocene onwards. Interestingly, these dates are similar to those estimated for the New Caledonia *Dierogecko* geckos radiation, with cladogenesis occurring from the mid-Tertiary onwards, and the most recent speciation event dated to 5.7 MYBP (Bauer *et al.*, 2006).

These divergence dates thus suggest that *Uroplatus* origin and speciation coincided with the period when Madagascar was becoming progressively wetter, when the island drifted northwards, emerging from the subtropical arid belt and entering into the humid 'Trade Wind' zone (Wells, 2003). Interestingly, the two species: *U. guentheri* and *U. malahelo*, that are descended from the stem lineage sister to all other *Uroplatus*, also occupy the most arid habitats that are known for this genus, in western and south-western Madagascar. If this evolutionary scenario is correct, this would mean that the ancestral *Uroplatus* species was adapted to more arid habitats, and that the development of more humid forests during the Oligocene and Miocene provided new evolutionary opportunities for specialization and species radiation.

The new *Uroplatus* species indicated here (to be formally described elsewhere) follow a common taxonomic trend in

Madagascar, where, as a result of better geographic sampling, many new species are continuing to be discovered (e.g. see Goodman & Bernstead, 2003; Goodman *et al.*, 2006). In particular, Madagascar's gekkonids exhibit a high degree of regional endemism, with relatively conservative morphology between closely related species, both of which suggests that this group will be rich in cryptic species diversity (Raxworthy, 2003; Raxworthy *et al.*, 2007). A very similar picture is emerging with the New Caledonia *Dierogecko* geckos, where cryptic species diversity is being discovered in association with allopatry and fine-scale endemism (Bauer *et al.*, 2006).

Nevertheless, because substantial mitochondrial variation is evident between all populations of *Uroplatus*, intraspecific geographic variation must also be carefully considered when identifying potential cryptic species, and spatial sampling must be adequate to draw conclusions. For example, we consider the recent description of *Uroplatus giganteus* by Glaw *et al.* (2006) based on four specimens from Montagne d'Ambre, as premature because of the limited sampling of *U. fimbriatus* localities that were included in that description. When we include *U. 'giganteus'* *cyt b* data from Greenbaum *et al.* (2007) (results not shown), or consider our own Montagne d'Ambre *U. fimbriatus* (1) as '*giganteus*', this renders *U. fimbriatus* paraphyletic, with the '*giganteus*' samples falling within a northern clade of *U. fimbriatus* (Fig. 3). Unfortunately, the *U. 'giganteus'* diagnostic morphological characters do not accurately diagnose the other specimens from this northern clade; and there is a possibility that this clade will include specimens from the *U. fimbriatus* neotype locality, Nosy Mangabe (see Bauer & Russell, 1989): one of our northern clade samples – *U. fimbriatus* (3) was collected just 50 km north-east of Nosy Mangabe.

Biogeographic incongruence

We found no evidence for either shared vicariant or shared dispersal events between the *Uroplatus* clades, despite this group including two species-rich radiations that speciated in the same region and at the same time period. This is a surprising result considering the biogeographic characteristics of Madagascar (see introduction) and the fundamental premise of vicariance biogeography, where speciation is assumed to be driven by shared vicariant events (e.g. Brooks & McLennan, 1991; Humphries & Parenti, 1999; Crisci *et al.*, 2003).

Our results suggest that the major source of this biogeographic incongruence concerns dispersal based on: (1) the DIVA ancestral area optimizations which require multiple dispersal events; (2) the extensive sympatry found between non-sister species and between sister clades for deeper tree nodes; and (3) the lack of sympatry between sister species (see also Barraclough & Vogler, 2000). However, it must also be considered that these inferred post-speciation range shifts would have accumulated over relatively long periods of time – with the youngest species estimated to have originated in the Miocene. Currently, almost nothing is known about the actual dispersal ability of *Uroplatus*, although all species

appear to be restricted to primary forests, suggesting that breaks in forest habitat would present barriers to dispersal. And oceanic dispersal appears to have never been successful for this group, which is unknown elsewhere in the Indian Ocean.

Another source of biogeographic incongruence: localized population extinction leading to distribution recession, is evident for one relict sister-species pair: *U. pietschmanni* and *U. alluaudi*, which are isolated from each other by 400 km (Fig. 7). These probable relict species also represent a stem lineage that is sister to a much more diverse species radiation (see also Stiasney & dePinna, 1994; Hoffman, 2005). In conclusion therefore, these biogeographic results indicate post-speciation range shifts occurring over long time periods within *Uroplatus*, which thus cautions against using current species distributions to infer the geography of speciation, especially for the deeper nodes (Losos & Glor, 2003).

Spatial relationships of sister species

Allopatric or parapatric sister species distributions have been used to support two main competing models of speciation: (1) classic allopatric speciation (requiring geographic isolation to facilitate genetic isolation), with or without subsequent secondary contact (e.g. Mayr, 1982; Johnson & Cicero, 2002; Hoskin *et al.*, 2005; Lukhtanov *et al.*, 2005); or (2) parapatric speciation (ecological speciation) along an environmental gradient (requiring disruptive selection and assortative mating to achieve genetic isolation) (e.g. Schneider, Smith & Moritz, 1999; Schluter, 2001; Via, 2001; Ogden & Thorpe, 2002; Doebeli & Dieckmann, 2003; Brown, 2004; Smith *et al.*, 2005; Jiggins *et al.*, 2006). The latter speciation model requires ecological niche evolution, but this may be absent or else otherwise evolve incidentally in allopatric speciation. Support for conservative niches (contra ecological speciation) has been recently reported (Peterson, Soberon & Sanchez-Cordero, 1999; Hoffman, 2005; Wiens & Graham, 2005; Kozak & Wiens, 2006; Yesson & Culham, 2006) but other studies have found considerable niche plasticity between sister species or closely related species (Losos *et al.*, 2003; Rice, Martínez-Meyer & Peterson, 2003; Graham *et al.*, 2004), or else no general relationship between phylogenetic similarity and niche similarity (Knouft *et al.*, 2006).

We find support for both conservative and divergent ecological niches between sister species (based on our ecological niche models). For the sister-species pairs: *U. sp. A* and *U. sp. D* and *U. henkeli* and *U. sp. H*, extensive sympatry is shown by their niche models (Fig. 8) indicating conservative niches, although neither pair is known to actually occur in sympatry. The known distributions of *U. henkeli* and *U. sp. H* are separated at their closest point in north-western Madagascar by an unsurveyed (and now mostly deforested) divide of 100 km, that includes the drainage of one of Madagascar's major rivers, the Mahavavy. Because both *U. henkeli* and *U. sp. H* are only known below 800 m elevation, this large river may represent a plausible historical barrier to dispersal, and thus provide support for classic allopatric speciation (see also Wiens & Graham,

2005). By contrast, the divergent ecological niche models and allopatric distributions of *U. guentheri* and *U. malahelo* best support allopatric speciation with subsequent incidental niche evolution.

However, the known distributions of *U. sikorae* and *U. sameiti*, and *U. sp. A* and *U. sp. D*, are parapatric, with both of these species pairs being almost perfectly separated by elevation at the same massifs, which is intriguing because there are no obvious historical barriers that might have isolated them. Similar elevational sister-species relationships have not yet been reported for other groups in Madagascar, but have been reported for riodinid butterflies, and these patterns have been considered as examples of vertical montane speciation and evidence for parapatric speciation across elevational gradients (Endler, 1977; Hall, 2005; although also see Coyne & Orr, 2004).

Intraspecific variation

The intraspecific mtDNA divergence we find within the species of *Uroplatus* (maximum uncorrected p distances: *cyt b* = 10.4–20.1%, and 12S = 4.3–9.6%) is generally much higher than has been reported for other gekkonid species (Carranza *et al.*, 2000; Austin *et al.*, 2004; Gübitz, Thorpe & Malhotra, 2005), although comparable to that reported for two species of Neotropical *Thecadactylus* geckos (Kronauer *et al.*, 2005; Bermann & Russell, 2007). Two types of landscape barriers to gene flow have previously been considered in Madagascar: major river drainages (Martin, 1972; Pastorini *et al.*, 2005), and watershed margins (Wilmé *et al.*, 2006). The latter have been proposed as isolating barriers during arid glacial periods of the Quaternary, when isolated mesic riverine refugia are hypothesized to have existed. Although intraspecific sampling is not sufficient to adequately compare these hypotheses against each other (additional samples are needed within watersheds), neither predicts the high levels of genetic divergence that we also find in the higher elevation species (*U. sikorae*, *U. malahelo*, *U. sp. A*, and *sp. D*). These species occupy areas bisected by much smaller rivers, and two are confined to single watershed areas of endemism (Fig. 3). We also find five species that exhibit asymmetrical haplotype trees which are suggestive of a possible relationship with latitude (see Supplementary Material).

These high levels of intraspecific genetic variation might also represent the product of local adaptation to environmental conditions. And, as demonstrated by the poor intrapredictivity that we found in the ecological niche models partitioned by latitude, the potential for intraspecific niche specialization also exists in Madagascar. Under this scenario, we would also expect niche specialization along elevational gradients, which provide even steeper temperature gradients than latitude in most regions of Madagascar, and which would be consistent with the parapatric elevational distributions of sister species that we find in some *Uroplatus* species. Unfortunately, intraspecific studies of genetic variation and fitness across elevational transects have not yet been conducted anywhere in the tropics (see Ghalambor

et al., 2006), but recent research in temperate North America has found genetically differentiated populations of salamanders based on elevation (Giordano, Ridenhour & Storfer, 2007), and weak population variation in fitness has been reported across the elevational range of two species of monkey-flowers (Angert & Schemske, 2005).

The considerable intraspecific genetic variation in *Uroplatus* might thus be indicative of ongoing speciation, either occurring *in situ* in parapatry along environmental gradients, or alternatively having developed from population isolation followed by secondary contact. The historical context for geographic isolation has not yet been well studied for Madagascar, but one promising future possibility will be to use ecological niche models with paleoclimatic landscapes to explore the potential of climate change driving species distribution shifts (e.g. see Hugall *et al.*, 2002).

Conclusions

We estimate the origins of the *Uroplatus* clade to date to the mid-Tertiary in Madagascar (which we here consider an island continent). Subsequently, the group has evolved to produce a species radiation of at least 20 species, all endemic to Madagascar, with most species also showing substantial intraspecific genetic variation. Biogeographic analyses failed to find congruent spatial patterns, despite the extensive sympatry of two contemporary species rich clades. The DIVA analysis finds support for terrestrial dispersal concerning this radiation of species, however, based on the estimated dates of divergence, this could date back to the Miocene. However, all sister species were found to be either allopatric or parapatric. Sister species ecological niche models provide examples of both conservative and divergent niches, and two pairs of species have parapatric distributions across elevational gradients. The high levels of intraspecific genetic variation, and the poor intra-predictive ecological niche modeling performance that we report, suggest the potential for intraspecific niche specialization within *Uroplatus* species distributed across environmental gradients in Madagascar.

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Supplementary material

The following material is available for this article online:

Appendix S1. Continental speciation in the tropics: contrasting biogeographic patterns of divergence in the *Uroplatus* leaf-tailed gecko radiation of Madagascar.

Figure S1. Haplotype trees based on mitochondrial data for five *Uroplatus* species. Terminals plotted to their geographic locality (see also Fig. 3 and Table 1).

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