Available online at www.sciencedirect.com

SCIENCE DIRECT®

Molecular Phylogenetics and Evolution 31 (2004) 618–629

MOLECULAR PHYLOGENETICS AND EVOLUTION

www.elsevier.com/locate/ympev

Shifting sands and shifty lizards: molecular phylogeny and biogeography of African flat lizards (*Platysaurus*)

Ian A.W. Scott,[a] J. Scott Keogh,[a,*] and Martin J. Whiting[b]

[a] *School of Botany and Zoology, Australian National University, Canberra, ACT, 0200, Australia*

[b] *Communication and Behaviour Research Group, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa*

Received 21 April 2003; revised 14 August 2003

Abstract

The African flat lizard genus *Platysaurus* is widely distributed on rock outcrops in southern Africa and is found both east and west of the Kalahari Desert and between major river drainage systems. We assembled a molecular phylogeny for the genus in order to test several biogeographic hypotheses. Sequence data were obtained from 29 specimens representing 14 taxa of *Platysaurus* that span the geographic range of the genus. We targeted a fragment of the mitochondrial genome comprising the 3′ half of the ND4 gene and most of the flanking tRNA-HSL cluster. The edited alignment comprised 864 characters, of which 479 (55%) were variable and 461 (96%) parsimony informative. Overall, the phylogeny was well resolved and supported by high bootstrap values. Four major clades were identified comprising two to seven species: *P. mitchelli* and *P. maculatus maculatus* from the north-eastern range of the genus; *P. broadleyi* and *P. capensis* from the western range; *P. imperator*, *P. torquatus*, and *P. intermedius rhodesianus*; *P. i. intermedius*, *P. monotropis*, *P. minor*, *P. i. nigrescens*, *P. lebomboensis*, *P. i. wilhelmi*, and *P. o. orientalis*. *Platysaurus* has been suggested to represent a recent adaptive radiation where rapid speciation was fuelled by population fragmentation brought on by vicariant events and possibly divergent sexual selection. The traditional explanation for the radiation of the genus is that the eastern migration of the Kalahari sands fragmented populations in the Plio-Pleistocene, resulting in conditions favorable for speciation. Our genetic data strongly suggests that many of the speciation events in *Platysaurus* already had occurred prior to the Plio-Pleistocene. Moreover, vicariant events associated with the formation of the major river systems played an additional role in the evolution and distribution of *Platysaurus* species. Our topology displays long internodes and long terminal branches, suggesting that the radiation is much older than previously believed.

Keywords: ND4; Phylogeography; Reptile; Sub-Saharan Africa; Kalahari Desert; Adaptive radiation

1. Introduction

The geological history of sub-Saharan Africa is highly complex and the timing of major geological changes has been the subject of considerable debate (Partridge, 1998; Stokes et al., 1997). However, it is generally agreed that there have been two important forces that have shaped the region and imposed strong selective forces on the evolutionary history of organisms. First, the sands of the Kalahari Desert have been moving slowly eastward resulting in regions of isolated rock outcrops creating an "island effect" for many organisms. The timing of this movement is controversial, but it is likely to be a relatively recent phenomenon, occurring during the Pliocene and Pleistocene (Thomas and Shaw, 1990, 1993; Thomas et al., 1997) and it also may have contributed to the high level of endemism in many groups of animals and plants. Second, southern Africa has a number of major river systems that could act as major biogeographic breaks for animals with poor dispersal abilities. Understanding these breaks has been complicated by the history of river flow direction (Dollar, 1998; Nugent, 1990, 1992; Thomas and Shaw, 1992). Each of these geological events is predicted to

---

[*] Corresponding author. Fax: +61-2-6125-5573.
E-mail address: Scott.Keogh@anu.edu.au (J. Scott Keogh).

1055-7903/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2003.08.010

have strongly influenced the biogeographic history of organisms through vicariant events. Testing the validity and effects of such scenarios can be difficult, but a full understanding of the phylogenetic relationships of organisms that span these regions can provide important insight into historical processes (Bauer, 1999).

While the geological history of sub-Saharan Africa is complex and not fully understood, another approach is to examine the current distribution of taxa in the geological area of interest in a phylogenetic framework. Ideally, the taxa or radiation used should have a current distribution that covers the geographic range where the geological activity occurred. The radiation also should be of an appropriate age; that is, the origin of the radiation should either predate or coincide with the geological activity. Radiations with multiple relict species distributed in the area of interest also offer the opportunity to specifically test more subtle hypotheses. Finally, animals that are habitat specific may have fewer opportunities to disperse and so their current distribution might better reflect past geological history. We chose the lizard genus *Platysaurus* because it met all of our criteria and represented an ideal system to begin to examine the complex biogeographic history of sub-Saharan Africa.

Platysaurus is a southern African endemic genus of cordylid lizard with a disjunct distribution consisting of several western species separated by more than 800 km from the remaining taxa in the east (Broadley, 1978). Although it is primarily a southern African radiation, one taxon occurs as far north as southern Tanzania (Fig. 1). All *Platysaurus* species are characterized by extreme sexual dichromatism in which males may be spectacularly colored while females are relatively uniform and conservatively colored (Branch and Whiting, 1997; Whiting et al., 2003). They are called flat lizards

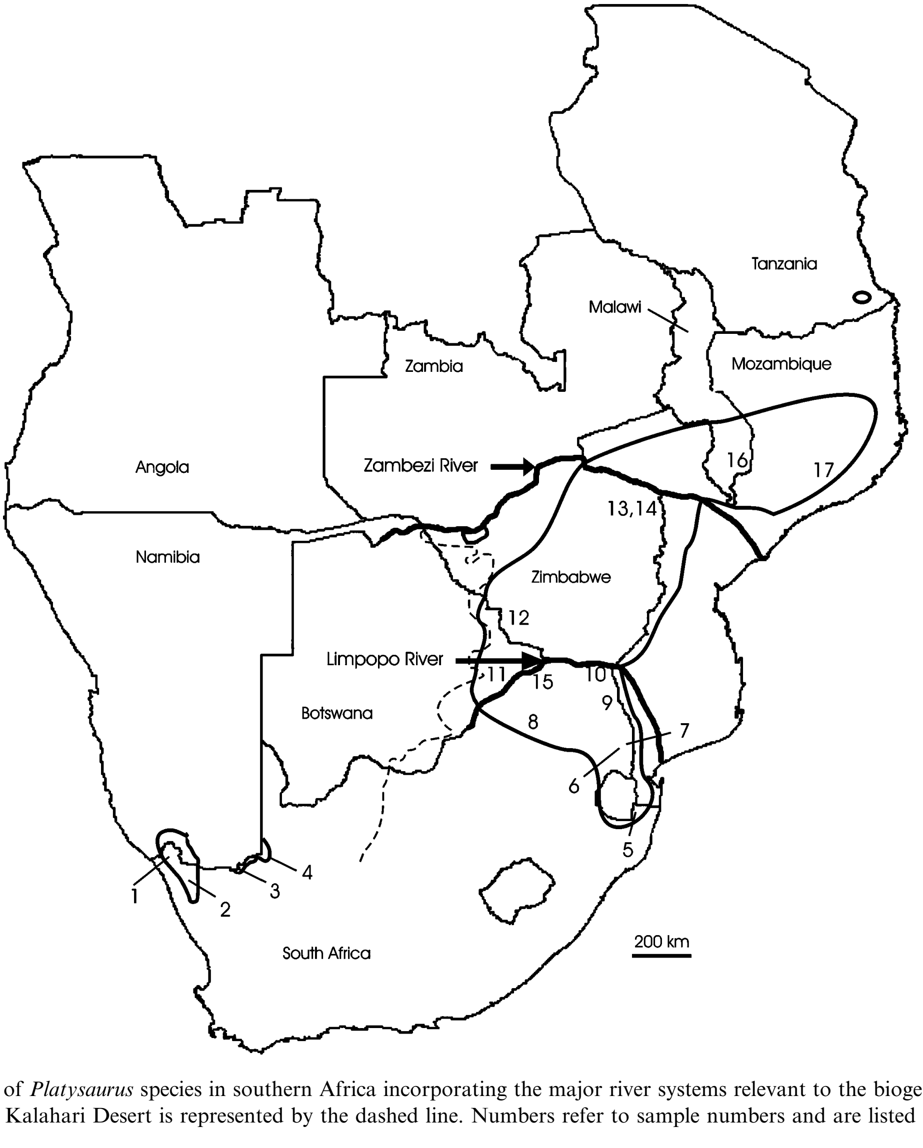

Fig. 1. The distribution of *Platysaurus* species in southern Africa incorporating the major river systems relevant to the biogeography of the genus. The eastern edge of the Kalahari Desert is represented by the dashed line. Numbers refer to sample numbers and are listed in Table 1.

because they are highly dorso-ventrally flattened rock specialists that use narrow crevices as retreat sites. Because they are such extreme rock specialists, they are thought to be poor dispersers (Branch and Whiting, 1997; Jacobsen, 1994). At present 15 species are recognized that comprise a total of 24 taxa (including subspecies). Past work has highlighted the complexity of the group and taxonomic relationships are far from resolved (Broadley, 1978; Jacobsen and Newbery, 1989; Jacobsen, 1994). This is in part due to their morphological conservatism as the primary differentiating characteristics among taxa are male color and subtle aspects of scutellation (Jacobsen, 1994), necessitating the use of molecular approaches to determine species relationships.

The two most recent taxonomic revisers believed that the current distribution of *Platysaurus* species is the direct result of recent biogeographic phenomena, and in particular the history of the Kalahari Desert (Broadley, 1978; Jacobsen, 1994). Broadley (1978) suggested that speciation in the genus has resulted directly from isolation of populations by the slow south-eastern movement of Kalahari sands during the Pliocene and Pleistocene. Jacobsen (1994) in describing three new taxa reinforced Broadley's suggestion and indicated that the number of relict populations in the north-western Transvaal might possibly be due to prolonged or more frequent episodes of isolation than were areas further to the east. Their hypotheses were based on the notion that the movement east of the Kalahari sands was a relatively recent phenomenon. While neither Broadley nor Jacobsen considered the phylogenetic history of the genus, their biogeographic hypotheses do make specific predictions about what the phylogeny of *Platysaurus* should look like if they are correct. Specifically, if the movement of the Kalahari sands has resulted in vicariant events, then this should be evident in the topology. Further, if speciation in the genus has been the result of a very recent adaptive radiation, then a molecular phylogeny would most likely comprise either a star phylogeny or a phylogeny with very short internodes. Alternatively, a phylogeny with long internodes would show that the splits between species are more ancient.

We had three major aims as we endeavoured to address these issues. First, we wanted to assemble a molecular phylogeny for *Platysaurus* comprising representative taxa from the full distribution of the genus and use our molecular data as the first independent evaluation of the morphologically based taxonomic schemes. Second, we wanted to test the hypothesis that the eastward movement of the Kalahari sands and the geological history of the major southern African rivers have resulted in vicariant events in the biogeographic history of *Platysaurus*. Third, we wanted to use the topology and the branch lengths from our molecular phylogeny and published geological data to test the specific hypothesis of Broadley (1978) and Jacobsen (1994) that *Platysaurus* represent a recent adaptive radiation.

2. Materials and methods

2.1. Samples

Because many *Platysaurus* species have restricted distributions and occur in remote or otherwise difficult to collect areas, we have strategically sampled taxa to emphasise distributional rather than taxonomic breadth. Sequence data were obtained from 29 specimens representing the following 14 taxa of *Platysaurus* (Table 1): *P. broadleyi*, *P. capensis*, *P. imperator*, *P. intermedius intermedius*, *P. i. nigrescens*, *P. i. rhodesianus*, *P. i. wilhelmi*, *P. lebomboensis*, *P. maculatus maculatus*, *P. minor*, *P. mitchelli*, *P. monotropis*, *P. orientalis orientalis*, and *P. torquatus*. These taxa represent the geographic range of the genus in southern Africa. *Cordylus peersi* and *C. polyzonus* were included for outgroup comparison.

2.2. DNA extraction, amplification, sequencing

Genomic DNA was extracted using a modified CTAB protocol. Approximately 100 ng of DNA was used as template for PCR amplification. We targeted an approximately 900 nbp fragment of the mitochondrial genome comprising the 3′ half of the ND4 gene and most of the flanking tRNA-HSL cluster with modified primers ND4I and Leu (Arevalo et al., 1994). This fragment has proven especially useful in phylogenetic studies of reptiles (Benabib et al., 1997; Forstner et al., 1998; Keogh et al., 2003; Kraus et al., 1996; Scott and Keogh, 2000). Sequencing was performed as described in Scott and Keogh (2000). Amplification primers also were used for sequencing. Some reverse (Leu) sequences were terminated prematurely so a third primer (Pl_ND4IV 5′-TCTTTAAAAGTAAGGRRYCTG-3′) was designed to provide sequence overlap with ND4I sequences. Sequence data were edited using Sequencher 3.0 (Gene Codes), and provisionally aligned using the default parameters of ClustalX (Thompson et al., 1994).

2.3. Phylogenetic analyses

We used maximum-likelihood (ML) to analyze the data. Our sequence data first were analyzed using the program Modeltest (Version 3.06, Posada and Crandall, 1998) to determine the most appropriate model of molecular evolution for our data. Parameter estimates and likelihood scores were obtained for each model using

Table 1
Locality data for specimens sequenced

Species	Map ref.	Museum number	Sample number	Locality
Cordylus peersi 1	2		MJW753	Kamieskroon, Northern Cape, RSA, 3017Bb
Cordylus peersi 2	2		MJW766	Kamieskroon, Northern Cape, RSA, 3017Bb
Cordylus polyzonus 1	2		MJW754	Kamieskroon, Northern Cape, RSA, 3017Bb
Cordylus polyzonus 2	2		MJW757	Kamieskroon, Northern Cape, RSA, 3017Bb
Platysaurus broadleyi 1	4		MJW570	Augrabies Falls National Park, Northern Cape, RSA
Platysaurus broadleyi 2	4		MJW587	Augrabies Falls National Park, Northern Cape, RSA
Platysaurus broadleyi 3	3		MJW755	Onseepkans, Northern Cape, RSA
Platysaurus capensis 1	1		MJW745	Tierhoek, Ploegberg, Richtersveld, Northern Cape, RSA, 28°37′59″S, 17°00′41″E
Platysaurus capensis 2	1		MJW746	Tierhoek, Ploegberg, Richtersveld, Northern Cape, RSA, 28°37′59″S, 17°00′41″E
Platysaurus capensis 3	1		MJW747	Tierhoek, Ploegberg, Richtersveld, Northern Cape, RSA, 28°37′59″S, 17°00′41″E
Platysaurus capensis 4	2	TM82664		Kamieskroon, Northern Cape, RSA, 3017Bb
Platysaurus imperator	13	NMZ1579		Near Avila Mission, Zimbabwe, 28°37′59″S, 17°00′41″E
Platysaurus intermedius intermedius	9	CAS209605		Harmony Farm, Hoedspruit District, Limpopo Province, RSA 24°18′27″S, 30°50′24″E
Platysaurus intermedius nigrescens 1	11		MJW675	Shoshong Hills, Botswana, 22°58′55″S, 26°22′80″E
Platysaurus intermedius nigrescens 2	11		MJW744	Shoshong Hills, Botswana, 22°58′55″S, 26°22′80″E
Platysaurus intermedius rhodesianus 1	10		AMB6129	Tshipise, Adventatus, Messina District, Limpopo Province, RSA, 22°36′22″S, 30°10′35″E
Platysaurus intermedius rhodesianus 2	12		MJW696	Matobo National Park campground, Zimbabwe
Platysaurus intermedius rhodesianus 3	12		MJW697	Matobo National Park campground, Zimbabwe
Platysaurus intermedius wilhelmi 1	7	TM82890		Hazeyview, Pilgrims Rest 2 District, Mpumalanga Province, RSA, 2431Cc
Platysaurus intermedius wilhelmi 2	7	TM82891		Hazeyview, Pilgrims Rest 2 District, Mpumalanga Province, RSA, 2431Cc
Platysaurus lebomboensis	5		AMB6070	Cecil Mack Pass, 5.5 km north Ingwavuna, Ingwavuna District, KwaZulu-Natal, 27°06′27″S, 31°58′39″E
Platysaurus maculatus maculatus 1	17		1	66 km SE Nampula, towards Moma, Mozambique
Platysaurus maculatus maculatus 2	17		2	66 km SE Nampula, towards Moma, Mozambique
Platysaurus minor 1	8	TM82892		Foothills of Blouberg Mountains, Sweethome Farm, Bochum District, Limpopo Province, RSA, 2328Bb
Platysaurus minor 2	8	TM82897		Foothills of Blouberg Mountains, Sweethome Farm, Bochum District, Limpopo Province, RSA, 2328Bb
Platysaurus mitchelli 1	16		MJW694	Mulanje Mountains, Malawi
Platysaurus mitchelli 2	16		MJW695	Mulanje Mountains, Malawi
Platysaurus monotropis 1	15		MJW700	Foothills of Blouberg Mountains, Sweethome Farm, Bochum District, Limpopo Province, RSA, 2328Bb
Platysaurus monotropis 2	15		MJW711	Foothills of Blouberg Mountains, Sweethome Farm, Bochum District, Limpopo Province, RSA, 2328Bb
Platysaurus orientalis 1	6	TM82882		Swadini, Blyde River, Mpumalanga Province, RSA, 2430Db
Platysaurus orientalis 2	6	TM82883		Swadini, Blyde River, Mpumalanga Province, RSA, 2430Db
Platysaurus torquatus 1	14	NMZ15786		Near Avila Mission, Zimbabwe, 17°30′51″S, 32°46′00″E
Platysaurus torquatus 2	14		MJW693	Near Avila Mission, Zimbabwe

Where sequences for two or more individuals were identical, only one individual was used in the phylogenetic analyses. The map reference refers to the numbers used in Fig. 1. RSA, Republic South Africa; TM, Transvaal Museum; NMZ, National Museum of Zimbabwe; CAS, California Academy of Sciences. *Cordylus peersi* and *Cordylus polyzonus* served as outgroups. Lizards without museum numbers were field sampled and not killed.

PAUP* (Swofford, 2000). When hypotheses were nested, log likelihood ratio tests were used to test null hypotheses about the DNA substitution model. Models which were not nested were compared with the Akaike information criterion (minimum theoretical information criterion, AIC). The optimal model identified by Modeltest was then used to perform a ML analysis using PAUP* with the heuristic search algorithm. To assess branch support, we preformed both a ML (100 "fast" stepwise pseudoreplicates using these parameters) and an unweighted parsimony (1000 pseudoreplicates) bootstrap analysis.

2.4. Parametric bootstrapping and hypothesis testing

In addition to generating a phylogeny, we also were interested in testing specific biogeographic and taxonomic hypotheses. A parametric bootstrap test involves obtaining score estimates for trees obtained first with and then without a topological constraint that reflects the hypothesised relationship of interest and then comparing this difference with a distribution of score differences generated from simulated data sets. Huelsenbeck et al. (1996) have advocated a likelihood framework for obtaining score estimates but this is computationally prohibitive (Goldman et al., 2000). We used the approach of Ruedi et al. (1998) that tests the difference in tree length scores for constrained and unconstrained MP trees. Simulated DNA sequence data sets were generated using Seq-Gen (Version 1.2.4, Rambaut and Grassly, 1997), which simulates the evolution of DNA sequences along a defined phylogeny under a defined model of the underlying substitution process. For any given hypothesis, a constraint topology was generated in MacClade 3.04 (Maddison and Maddison, 1992). This constraint was then used in PAUP* to search for the "best" alternate ML topology. This topology and its estimated parameters then were used to generate 100 simulated data sets. For each data set, heuristic searches (100 random addition replicates) were carried out first with and then without the specified constraint. The resulting cumulative frequency distribution curve of tree length differences was plotted and compared with the tree length difference (Δ) for the empirical constrained and unconstrained trees. All of the alternative taxonomic and biogeographic hypotheses we tested are outlined in Table 3.

3. Results

3.1. Phylogenetic analyses

The edited alignment comprised 864 characters, of which 479 (55%) were variable. Of these variable sites, 461 (96%) were parsimony informative (24% first, 11% second, 48% third, and 17% tRNA-HSL). Within the ingroup, 441 (51%) characters were variable of which 403 (91%) were parsimony informative (21.8% first, 8.7% second, 52.1% third, and 17.4% tRNA-HSL). Jukes and Cantor (1969) genetic distances are presented in Table 2. The total data set was significantly more structured than random indicating strong phylogenetic signal ($g_1 = -0.83$, $P \leqslant 0.01$; Hillis and Huelsenbeck, 1992). Following alignment, sequences were translated into amino acid sequences using the vertebrate mitochondrial genetic code. No premature stop codons were observed; therefore, we concluded that all sequences obtained were mitochondrial in origin.

The AIC from Modeltest supported the general time reversible (GTR) plus invariant sites (+I) plus gamma shape (+G) model as the best-fit substitution model for the data. The negative log likelihood score of this model was 7454.1309. When a ML search was done in PAUP* using the estimated parameters selected by AIC, the negative log likelihood score of the model was 7450.80919. This topology was then used to estimate the free parameters under the GTR + I + G model and returned a negative log likelihood score of 7450.71597 (Fig. 2). The estimated parameters were as follows: nucleotide frequencies (A) 0.333217, (C) 0.307909, (G) 0.106572, and (T) 0.252302; substitution rates ($A \leftrightarrow C$) 2.0907145, ($A \leftrightarrow G$) 8.5887777, ($A \leftrightarrow T$) 2.574601, ($C \leftrightarrow G$) 0.49081693, ($C \leftrightarrow T$) 11.837409, and ($G \leftrightarrow T$) 1.000000; proportion of invariant sites 0.364586; and gamma shape 1.336455.

Overall, the phylogeny is well resolved and supported by high bootstrap values (Fig. 2). Four major clades are identified comprising two to seven species. *Platysaurus mitchelli* and *P. maculatus maculatus* (our clade "D") from the northeastern range of the genus, form a well-supported sister group to the other species while the genetic divergence between the two species is still large (21.0–21.6%). *Platysaurus broadleyi* and *P. capensis* (our clade "A") from the western range form a very well supported sister clade to the remaining species in the eastern range. The two species are closely related with a genetic divergence of 8.6–10.4%. The remaining species comprise two clades. *Platysaurus imperator*, *P. torquatus*, and *P. intermedius rhodesianus* form a very well supported clade (our clade "B") with genetic distances between these three taxa ranging from 19.2–21.5%. *Platysaurus i. intermedius*, *P. monotropis*, *P. minor*, *P. i. nigrescens*, *P. lebomboensis*, *P. i. wilhelmi*, and *P. o. orientalis* together form a more weakly supported clade (our clade "C") with genetic distances ranging from 10.2% between *P. i. intermedius* and *P. monotropis*, to 21.1% between *P. i. intermedius* and *P. lebomboensis*. A summary of intra-clade and inter-clade genetic distances is presented in Table 4.

Table 2
Jukes–Cantor genetic distance matrix for taxa used in our phylogenetic analysis

	1	2	3	4	5	6	7	8	9	10
1 *C. peersi* 1	—									
2 *C. peersi* 2	.00117	—								
3 *C. polyzonus* 1	.26552	.26554	—							
4 *C. polyzonus* 2	.26548	.26550	.01301	—						
5 *P. broadleyi* 3	.38069	.38265	.37188	.37381	—					
6 *P. broadleyi* 1	.38068	.38264	.36999	.37578	.00587	—				
7 *P. capensis* 1	.38073	.38269	.36812	.37590	.08932	.08932	—			
8 *P. capensis* 2	.38267	.38464	.36618	.37394	.09196	.09196	.00234	—		
9 *P. capensis* 3	.38137	.38333	.36816	.37595	.08681	.08680	.00235	.00470	—	
10 *P. capensis* 4	.38851	.39049	.35851	.35851	.10406	.10406	.06989	.07246	.07001	—
11 *P. imperator*	.38677	.38678	.35810	.36766	.30635	.29760	.30287	.30287	.30346	.31569
12 *P. i. intermedius*	.33626	.33626	.32407	.33140	.27033	.26530	.25199	.25199	.25408	.26396
13 *P. i. nigrescens* 1	.35586	.35586	.32452	.33004	.25077	.24751	.23140	.23139	.23308	.24471
14 *P. i. rhodesianus* 2	.38675	.38675	.34312	.35249	.28224	.27378	.27374	.27712	.27423	.27927
15 *P. i. rhodesianus* 3	.38673	.38674	.34311	.35248	.28054	.27210	.27206	.27544	.27255	.27589
16 *P. i. rhodesianus* 1	.38479	.38479	.34130	.35065	.27549	.26710	.26707	.27042	.26756	.27424
17 *P. i. wilhelmi* 1	.34432	.34432	.33568	.34313	.28916	.28574	.26704	.26704	.26410	.27252
18 *P. i. wilhelmi* 2	.34245	.34245	.33569	.34313	.28744	.28402	.26537	.26537	.26419	.27083
19 *P. lebomboensis*	.33969	.33969	.33502	.34241	.29768	.29421	.27534	.27534	.27250	.29288
20 *P. m. maculatus* 1	.34198	.34197	.33369	.33733	.29601	.29601	.28729	.28729	.28950	.27568
21 *P. m. maculatus* 2	.34202	.34201	.33007	.33369	.29088	.29087	.28563	.28562	.28784	.27065
22 *P. minor* 1	.34583	.34583	.30284	.30642	.24714	.24552	.24224	.24547	.24429	.23938
23 *P. minor* 2	.34051	.34051	.30322	.30680	.24239	.24078	.23753	.24074	.23956	.23789
24 *P. mitchelli* 1	.34861	.34675	.31431	.32152	.28946	.28427	.28255	.28254	.28092	.27102
25 *P. mitchelli* 2	.34674	.34488	.31573	.32293	.28599	.28083	.27912	.27912	.27790	.27288
26 *P. monotropis* 1	.34982	.34982	.31918	.32283	.26705	.26372	.24562	.24725	.24768	.25259
27 *P. monotropis* 2	.34980	.34981	.32099	.32464	.26705	.26372	.24400	.24562	.24605	.25094
28 *P. o. orientalis* 1	.35178	.35178	.32103	.32470	.25720	.25556	.23765	.23765	.23808	.25101
29 *P. torquatus* 1	.38928	.38929	.34910	.36048	.30488	.29784	.29794	.30143	.29854	.29598
30 *P. torquatus* 2	.38731	.38732	.34910	.36049	.30487	.29783	.30142	.30493	.30203	.29946

	11	12	13	14	15	16	17	18	19	20
1 *C. peersi* 1										
2 *C. peersi* 2										
3 *C. polyzonus* 1										
4 *C. polyzonus* 2										
5 *P. broadleyi* 3										
6 *P. broadleyi* 1										
7 *P. capensis* 1										
8 *P. capensis* 2										
9 *P. capensis* 3										
10 *P. capensis* 4										
11 *P. imperator*	—									
12 *P. i. intermedius*	.23820	—								
13 *P. i. nigrescens* 1	.24892	.13301	—							
14 *P. i. rhodesianus* 2	.20536	.22671	.23582	—						
15 *P. i. rhodesianus* 3	.20383	.22513	.23422	.00233	—					
16 *P. i. rhodesianus* 1	.20386	.21576	.22789	.02972	.02851	—				
17 *P. i. wilhelmi* 1	.24480	.19540	.19942	.26276	.26110	.26110	—			
18 *P. i. wilhelmi* 2	.24318	.19388	.19956	.26111	.25945	.25945	.00117	—		
19 *P. lebomboensis*	.25822	.21077	.20270	.25325	.25160	.25323	.11718	.11582	—	
20 *P. m. maculatus* 1	.29198	.26425	.25804	.29670	.29496	.29846	.25596	.25431	.25763	—
21 *P. m. maculatus* 2	.28976	.26260	.25312	.29449	.29276	.29623	.25562	.25398	.25598	.00353
22 *P. minor* 1	.24681	.13152	.13681	.22434	.22276	.22594	.18270	.18121	.20068	.23961
23 *P. minor* 2	.24518	.13010	.13257	.22282	.22125	.22441	.17975	.17827	.19633	.23805
24 *P. mitchelli* 1	.28442	.26184	.24862	.25510	.25345	.26342	.26018	.26018	.25020	.21454
25 *P. mitchelli* 2	.28568	.26317	.25033	.25643	.25477	.26471	.26317	.26150	.24820	.21593
26 *P. monotropis* 1	.24845	.10270	.13819	.22909	.22750	.21341	.19169	.19019	.19628	.25269
27 *P. monotropis* 2	.25007	.10404	.13679	.23067	.22908	.21496	.19321	.19170	.19780	.25433
28 *P. o. orientalis* 1	.22728	.15266	.16244	.19619	.19467	.19165	.18699	.18549	.17970	.24627
29 *P. torquatus* 1	.19215	.24832	.24286	.21017	.20863	.21179	.24246	.24083	.25055	.30818
30 *P. torquatus* 2	.19519	.25159	.24285	.21328	.21173	.21491	.24245	.24082	.25055	.30817

Table 2 (*continued*)

	21	22	23	24	25	26	27	28	29	30
1 *C. peersi* 1										
2 *C. peersi* 2										
3 *C. polyzonus* 1										
4 *C. polyzonus* 2										
5 *P. broadleyi* 3										
6 *P. broadleyi* 1										
7 *P. capensis* 1										
8 *P. capensis* 2										
9 *P. capensis* 3										
10 *P. capensis* 4										
11 *P. imperator*										
12 *P. i. intermedius*										
13 *P. i. nigrescens* 1										
14 *P. i. rhodesianus* 2										
15 *P. i. rhodesianus* 3										
16 *P. i. rhodesianus* 1										
17 *P. i. wilhelmi* 1										
18 *P. i. wilhelmi* 2										
19 *P. lebomboensis*										
20 *P. m. maculatus* 1										
21 *P. m. maculatus* 2	—									
22 *P. minor* 1	.23801	—								
23 *P. minor* 2	.23646	.00352	—							
24 *P. mitchelli* 1	.20987	.25174	.25018	—						
25 *P. mitchelli* 2	.21126	.25312	.25157	.00117	—					
26 *P. monotropis* 1	.25109	.11721	.11450	.26348	.26482	—				
27 *P. monotropis* 2	.25273	.11858	.11586	.26514	.26649	.00117	—			
28 *P. o. orientalis* 1	.24590	.14501	.14077	.24387	.24516	.14358	.14500	—		
29 *P. torquatus* 1	.30281	.22959	.22796	.28080	.28031	.24414	.24577	.23117	—	
30 *P. torquatus* 2	.30281	.22641	.22478	.28079	.28031	.24413	.24576	.23435	.00236	—

Where sequences were identical, only one representative was included.

4. Discussion

We have produced a robust phylogeny for representative taxa for the southern African lizard genus *Platysaurus*. This is the first phylogeny for this group of lizards and the phylogenetic hypothesis we present has a number of important taxonomic and biogeographic implications. We tested a wide variety of alternative phylogenetic, taxonomic and biogeographic hypotheses with parametric bootstrap tests to determine where our tree was weak, where sound taxonomic decisions could be made, and the biogeographic history of *Platysaurus* (details in Table 3). Because many of the taxonomic questions also relate to the biogeography of *Platysaurus*, we outline the taxonomic implications of our phylogeny first, before discussing our biogeographic hypotheses.

4.1. Taxonomic implications

In our clade A, the multiple individuals of *P. capensis* we included in our ML analyses were not monophyletic. This result was further reinforced using parametric bootstrapping, where we were able to reject the alternative hypothesis of monophyly (Table 3). Our *P. capensis* 4 came from Kamieskroon in the Northern Cape, approximately 150 km from the Richtersveld population and the genetic divergence between the populations ranged from 6.9 to 7.2%. These populations also are morphologically divergent and allopatric, suggesting that there are two taxa (authors unpublished data). Our molecular data reinforce previous taxonomic work on this clade where based on color and morphometric data, *P. capensis* was split into two taxa, *P. capensis* and *P. broadleyi* (Branch and Whiting, 1997).

It is evident from our topology that the subspecies of *P. intermedius* that we have been able to include do not form a monophyletic group. There are several alternative hypotheses for relationships within what has been called the *intermedius* complex by Broadley (1978) and Jacobsen (1994). Two hypotheses pertaining to relationships among members of the *P. intermedius* group were postulated by Jacobsen (1994). The first suggests the presence of two groups, diagnosed by the appearance of enlarged neck scales, a western group, comprising *parvus*, *inopinus*, *monotropis*, *relictus*, and *P. i. nigrescens*, in which the enlarged neck scales are non-spinose, and an eastern group, comprising *P. i. intermedius*, *P. i. rhodesianus*, *P. i. natalensis*, *P. i. wilhelmi*, *P. lebomboensis*, *P. i. subniger*, and *P. i. nyasae*, which have spinose enlarged neck scales. While we do not have representatives of all these taxa, our data includes representatives of the two putative groupings. Our para-

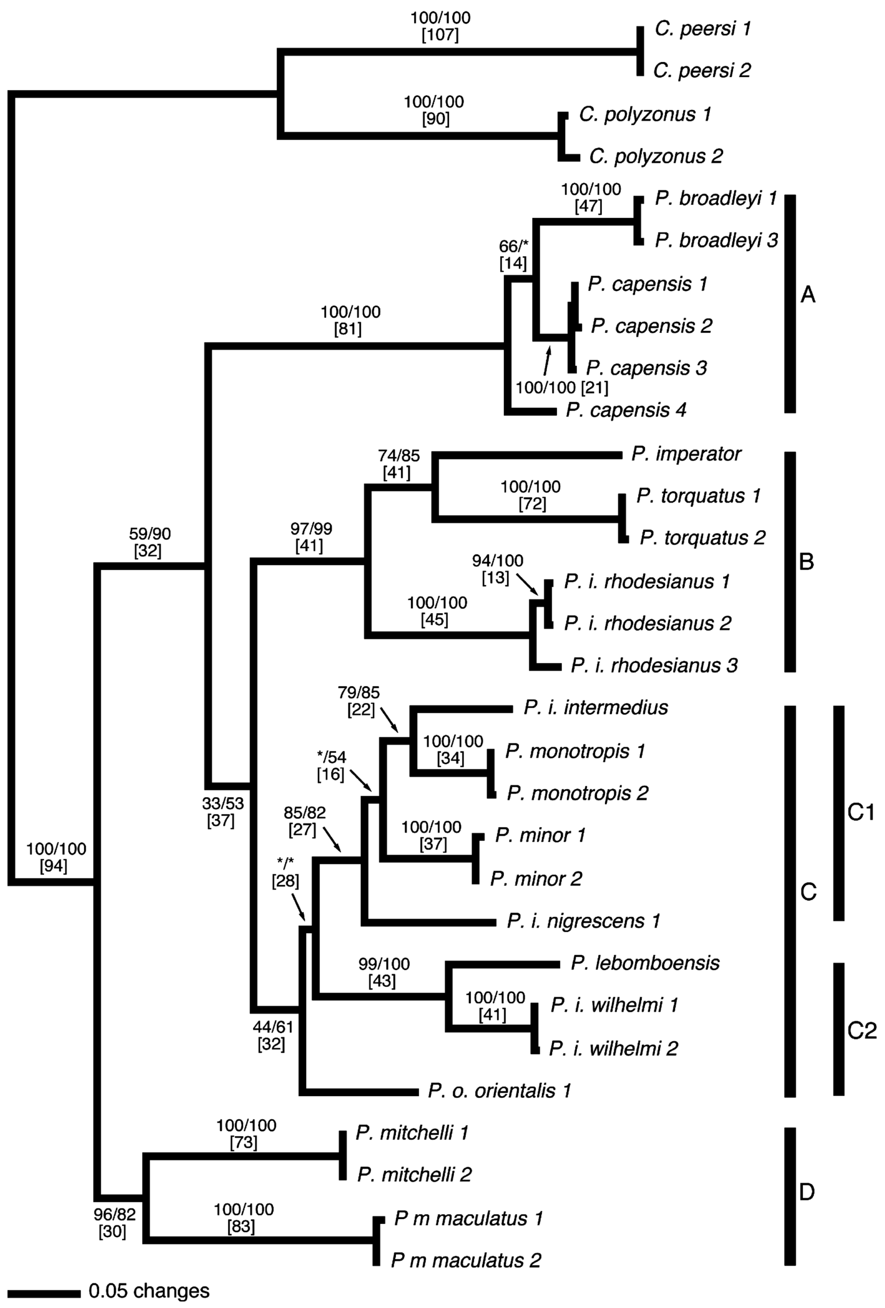

Fig. 2. Maximum-likelihood phylogram for the genus *Platysaurus*. Numbers in brackets are number of changes under parsimony. Numbers above brackets refer to bootstrap values for maximum-likelihood/parsimony.

metric bootstrap tests soundly reject the hypothesis that the appearance of enlarged neck scales in the *intermedius* group indicate relatedness (Table 3).

The second hypothesis postulated by Jacobsen (1994) suggests the existence of three groups within the *intermedius* complex. In this hypothesis, the western, non-spinose group remains intact and is referred to as the "*relictus*" group (*relictus*, *parvus*, *inopinus*, *monotropis*, and *nigrescens*). The eastern, spinose group is split in two: the "*intermedius*" group (*intermedius*, *rhodesianus*, *natalensis*, *subniger*, and *nyasae*), and the *"wilhelmi"* group (*wilhelmi* and *lebomboensis*. We have representatives from each of these postulated groups. Our parametric bootstrap tests soundly reject the hypothesis of reciprocal monophyly but *P. wilhelmi* and *P. lebomboensis* do form a well supported monophyletic group. Stemming from these results we also tested the a posteriori hypothesis that those taxa recognized as being members of the *intermedius* complex (sensu Jacobsen, 1994) form a monophyletic group. Representatives of this group in our data set include *P. i. intermedius*, *P. i. nigrescens*, *P. i. rhodesianus*, *P. i. wilhelmi*, *P. lebombo-*

Table 3
Results of parametric bootstrap tests of topologies performed using the modifications of Ruedi et al. (1998)

Constraint	Tree length	Attained *D*	P ($\alpha = 0.05$)
MP (unweighted)	1529	—	—
ML	1533	4	0.30
Alternative taxonomic hypotheses			
^{PRI}Monophyletic *P. capensis*	1534	5	0.01*
^{PRI}Eastern "spinose" vs western "non-spinose" "*intermedius*" sensu Jacobsen (1994): [(*rhodesianus*, *lebomboensis*, *intermedius*, *wilhelmi*), (*monotropis*, *nigrescens*)]	1592	63	<0.01*
^{PRI}Reciprocally monophyletic "*intermedius*," "*relictus*," and "*wilhelmensis*" groups sensu Jacobsen (1994): [(*rhodesianus*, *intermedius*), (*monotropis*, *nigrescens*), (*lebomboensis*, *wilhelmi*)]	1598	69	<0.01*
^{PRI}Monophyletic "*intermedius*" complex (Broadley, 1978; Jacobsen, 1994): (*rhodesianus*, *nigrescens*, *intermedius*, *lebomboensis*, *monotropis*, *wilhelmi*)	1580	52	<0.01*
Alternative topologies within Clade C			
((*minor*), (*intermedius*, *monotropis*, *nigrescens*))	1535	6	<0.01*
[(*orientalis*, *lebomboensis*, *wilhelmi*), (*intermedius*, *monotropis*, *nigrescens*, *minor*)]	1530	1	0.57
[(*orientalis*, *intermedius*, *monotropis*, *nigrescens*, *minor*), (*lebomboensis*, *wilhelmi*)]	1533	4	0.03*
(*orientalis*, B)	1553	24	0.01*
[(*lebomboensis*, *wilhelmi*), (A)]	1840	311	<0.01*
[(*lebomboensis*, *wilhelmi*), (B)]	1559	30	<0.01*
	1538	9	0.02*
Alternative biogeographic hypotheses			
^{PRI}[(A), (B, C1, C2, D)]			
[(A), (B)]	1536	7	0.02
[((A), (B)), ((C1, C2), D)]	1547	18	0.01*
[(A), (C1, C2)]	1540	11	0.01*
[((A), (C1, C2)), (B, D)]	1548	19	0.03*
[(A), (C1)]	1567	38	<0.01*
[(A), (C2)]	1564	35	<0.01*
[(A), (D)]	1538	9	0.04*
[(B), (D)]	1548	19	0.01*

Upper case letters refer to geographic groupings of *Platysaurus* taxa shown in Fig. 1 and described in the text. Hypotheses postulated a priori are indicated^{PRI}. All other hypotheses (except "ML") were postulated following investigation of nonparametric bootstrapped consensus parsimony and ML trees.

ensis, and *P. monotropis.* Our parametric bootstrap tests rejected the hypothesis that taxa attributed to the *intermedius* complex sensu Jacobsen (1994) form a monophyletic clade.

We also tested several alternative topologies within clade C to examine the position of *P. o. orientalis* and *P. i. nigrescens* (Table 3). We rejected all alternative topologies except one where *P. lebomboensis* and *P. wilhelmi* were sister to the rest of clade C. This is not surprising given the very short branch lengths and weak bootstrap support. Therefore the phylogenetic position of *P. o. orientalis* and the *P. lebomboensis*/*P. wilhelmi* clade within clade C is not well resolved.

4.2. Does Platysaurus represent a recent adaptive radiation?

Both Broadley (1978) and Jacobsen (1994) strongly supported the hypothesis that *Platysaurus* species represent a recent adaptive radiation where rapid speciation was fuelled by population fragmentation brought on by recent vicariant events and possibly divergent sexual selection. Our topology and the genetic distances between species do not support this hypothesis. If *Platysaurus* really did represent a recent adaptive radiation, we would expect to recover a poorly resolved or a star phylogeny due to the short time period over which the radiation occurred. Instead, we have recovered not only a well-resolved phylogeny, but also one with long internodes and long terminal branches. Genetic distance data also supports this notion. The *minimum* genetic distance between species is 6.9% between *P. broadleyi* and *P. capensis* and the majority of *Platysaurus* taxa differ by at least 23% and up to as much as 30%. One explanation for the genetic distances we have observed is that this fragment of mtDNA could be evolving very quickly. However, the mtDNA fragment we used also has been used extensively in other reptile groups and the genetic distances among *Platysaurus* species fall into the same range as species-level differences in other lizards (Benabib et al., 1997; Forstner et al., 1998; Scott and Keogh, 2000) and snakes (Feldman and Spicer, 2002; Kraus et al., 1996; Rodriguez-Robles et al., 2001; Wüster et al., 2002; Zamudio and Greene, 1997). Estimates of relative percent divergence against time for reptiles and other vertebrates range to as much as 2%

Table 4
Summary of the range of intra and inter-clade Jukes–Cantor genetic distances based on Fig. 2

Intra-clade genetic distances	
Clade A	.0868–.1041
Clade B	.2086–.2512
Clade C1	.1145–.1382
Clade C2	.1158–.1870
Clade D	.2099–.2615
Inter-clade genetic distances	
Clade A – Clade B	.2671–.3160
Clade A – Clade C	.2314–.2977
Clade A – Clade D	.2707–.2960
Clade B – Clade C	.1917–.2628
Clade B – Clade D	.2548–.3082
Clade C – Clade D	.2380–.2665

per million years (reviewed in Johns and Avise, 1998). Based on mtDNA phylogenies generated for other reptiles, Macey et al. (1999, 2001) estimate that there is approximately 1.3% sequence divergence per million years. Regardless of which is closer to reality, this evidence strongly suggests that *Platysaurus* does not represent a recent adaptive radiation (Table 4), but rather a group with a long and complex evolutionary and biogeographic history.

4.3. Have the eastward expansion of the Kalahari Desert and movement of the major river systems resulted in vicariant events in Platysaurus?

Our topology suggests that both the movement of the Kalahari sands and major river systems have resulted in vicariant events in *Platysaurus* and represent barriers to gene flow. The first major split among *Platysaurus* species is between our clade "D" (*P. mitchelli*, *P. m. maculatus*) and the other taxa. *Platysaurus mitchelli* and *P. m. maculatus* are distributed further north and east than any other *Platysaurus* taxa and are found in Mozambique and Malawi and recently as far north as southern Tanzania (*P. m. maculatus*). Importantly, both of these species are isolated from all other *Platysaurus* taxa by the Zambezi River system, suggesting that the Zambezi has represented a major biogeographic break, restricting gene flow. Our molecular topology agrees with Broadley (1978) who recognized *P. mitchelli* as the most "primitive" species based on both morphological and behavioural data and suggested that *Platysaurus* may have originated in south-eastern Africa. Only *P. torquatus* has populations on both sides of the Zambezi River, suggesting that there has been occasional gene flow across the river.

However, an alternative explanation is that ancestral *Platysaurus* were more widespread north of the Zambezi prior to the eastern movement of the Kalahari sands and our topology cannot rule out this possibility. Currently, *P. broadleyi* and *P. capensis* are the only species distributed in the western part of the range and they are isolated by at least 800 km from all other *Platysaurus* species by the Kalahari Desert (Broadley, 1978). However, they formed the sister group to our clades B and C, despite our clade D (*P. mitchelli* and *P. m. maculatus*) being geographically much closer to B and C. It certainly seems that the eastern expansion of the Kalahari sands has isolated *P. broadleyi* and *P. capensis*, however it is unclear whether the speciation events that lead to these two species were due to this vacariant event. The *P. broadleyi*/*P. capensis* clade is at the end of a long terminal branch and they are a minimum of 23.8% divergent from all other taxa, suggesting a long duration of isolation for the clade that may pre-date the eastern expansion of the Kalahari Desert. However, this is necessarily speculative because we lack accurate dates on the age of the Kalahari Desert, but our results are consistent with the high degree of endemismfs in the Richtersvelds herpetofauna (Bauer and Branch, 2003; Matthee and Flemming, 2002).

The remaining taxa distributed in the eastern part of the range (our clades B and C) represent the greatest species level diversity in the genus. Jacobsen (1994) in describing three new taxa reinforced Broadley (1978) suggestion that movement of Kalahari sands during the Plio-Pleistocene was responsible for the number of relict *Platysaurus* populations. Furthermore, Jacobsen indicated that the number of relict populations in northern South Africa might be due to prolonged or more frequent episodes of isolation than areas further to the east. We tested a variety of alternative biogeographic arrangements (Table 3) but were able to reject all alternatives. Again, our genetic distance data suggest that most or all of these speciation events occurred prior to the Plio-Pleistocene. The origin of the major river systems is certainly much older than the Plio-Pleistocene (Dollar, 1998; Nugent, 1990, 1992; Thomas and Shaw, 1992). Some *Platysaurus* species are entirely distributed between major systems (e.g., Limpopo and Zambezi River systems), suggesting that rivers may represent important barriers to gene flow. Several species are found on both sides of these rivers. For example, we have sequenced *Platysaurus i. rhodesianus* from both sides of the Limpopo River and demonstrated a genetic divergence of 3%, supporting the notion that rivers limit gene flow to some degree. Importantly, our results are broadly consistent with a similar study on *Pachydactylus* geckos (Bauer and Lamb, 2002).

The traditional explanation for the radiation of the genus is that the eastern migration of the Kalahari sands fragmented populations, resulting in conditions favourable for speciation. Our genetic data strongly suggest that many of the speciation events in *Platysaurus* already had occurred prior to the Plio-Pleistocene. The fragmentation effect of the Kalahari sands may have accelerated speciation, but probably did not cause it, as previously believed (Broadley, 1978; Jacobsen, 1994). Moreover, vicariant events associated with the formation of the major river systems played an additional role in the evolution and distribution of *Platysaurus* species.

Acknowledgments

This research was supported by an Australian Research Council Small grant to JSK and by grants to MJW from the University of Witwatersrand and the National Research Foundation of South Africa. We thank Aaron Bauer, Bill Branch, Don Broadley, Trip Lamb, and Jerry Theron for tissue samples and Christine Hayes for help in the lab. We thank Huxley Whiting, Oscar Keogh, and Rozi Scott for critical comments on the manuscript.

References

Benabib, M., Kjer, K.M., Sites, J.W., 1997. Mitochondrial DNA sequence-based phylogeny and the evolution of viviparity in the *Sceloporus scalaris* group (Reptilia, Squamata). Evolution 51, 1262–1275.

Arévalo, E., Davis, S.K., Sites, J., 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Syst. Biol. 43, 387–418.

Bauer, A.M., 1999. Evolutionary scenarios in the *Pachydactylus* Group geckos of southern Africa: new hypotheses. Afr. J. Herpetol. 48, 53–62.

Bauer, A.M., Branch, W.R., 2003. The herpetofauna of the Richterveld National Park and the adjacent northern Richtersveld, Northern Cape Province, Republic of South Africa. Herpetol. Nat. Hist. 8, 111–160.

Bauer, A.M., Lamb, T., 2002. Phylogenetic relationships among members of the *Pachydactylus capensis* group of southern African geckos. Afr. Zool. 37, 209–220.

Branch, W.R., Whiting, M.J., 1997. A new *Platysaurus* (Squamata: Cordylidae) from the Northern Cape Province, South Africa. Afr. J. Herpetol. 46, 124–136.

Broadley, D.G., 1978. A revision of the genus *Platysaurus* A. Smith (Sauria: Cordylidae). Occas. Pap. Natl. Mus. Rhod., B., Nat. sci. 6, 129–185.

Dollar, E.S.J., 1998. Palaeofluvial geomorphology in southern Africa: a review. Prog. Phys. Geog. 22, 325–349.

Feldman, C.R., Spicer, G.S., 2002. Mitochondrial variation in sharp-tailed snakes (*Contia tenuis*): evidence of a cryptic species. J. Herpetol. 36, 648–655.

Forstner, M.R.J., Dixon, J.R., Forstner, J.M., Davis, S.K., 1998. Apparent hybridization between *Cnemidophorus gularis* and *Cnemidophorus septemvittatus* from an area of sympatry in southwest Texas. J. Herpetol. 32, 418–425.

Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49, 652–670.

Hillis, D.M., Huelsenbeck, J.P., 1992. Signal, noise, and reliability in molecular phylogenetic analyses. J. Heredity 83, 189–195.

Huelsenbeck, J.P., Hillis, D.M., Nielsen, R., 1996. A likelihood-ratio test of monophyly. Syst. Biol. 45, 546–558.

Jacobsen, N.H.G., Newbery, R.E., 1989. The genus *Platysaurus* A. Smith 1844 in the Transvaal. J. Herp. Assoc. Africa 36, 51–63.

Jacobsen, N.H.G., 1994. The *Platysaurus intermedius* complex (Sauria: Cordylidae) in the Transvaal, South Africa, with descriptions of three new taxa. S. Afr. J. Zool. 29, 132–143.

Johns, G.C., Avise, J.C., 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome *b* gene. Mol. Biol. Evol. 15, 1481–1490.

Jukes, T.H., Cantor, C.R., 1969. Evolution of protein molecules. In: Munro, H.N. (Ed.), Mammalian Protein Metabolism. Academic Press, New York, pp. 21–132.

Keogh, J.S., Scott, I.A.W., Fitzgerald, M., Shine, R., 2003. Molecular phylogeny of the Australian venomous snake genus *Hoplocephalus* and conservation genetics of the threatened *H. stephensii*. Cons. Gen. 4, 57–65.

Kraus, F., Mink, D.G., Brown, W.M., 1996. Crotaline intergeneric relationships based on mitochondrial DNA sequence data. Copeia, 763–773.

Maddison, W.P., Maddison, D.R., 1992. MacClade: analysis of phylogeny and character evolution, Verson 3.04. Sinauer Associates, Sunderland, MA.

Macey, J.R., Wang, Y., Ananjeva, N.B., Larson, A., Papenfuss, T.J., 1999. Vicariant patterns in fragmentation among gekkonid lizards of the genus *Teratoscincus* produced by the Indian collision: a molecular phylogenetic perspective and an area cladogram for central Asia. Mol. Phylogenete. Evol. 12, 250–272.

Macey, J.R., Strasburg, J.L., Brisson, J.A., Vredenburg, V.T., Jennings, M., Larson, A., 2001. Molecular phylogenetics of western North American frogs of the *Rana boylii* species group. Mol. Phylogenet. Evol. 19, 131–143.

Matthee, C.A., Flemming, A.F., 2002. Population fragmentation in the southern rock agama, *Agama atra*: more evidence for vicariance in Southern Africa. Mol. Ecol. 11, 465–471.

Nugent, C., 1990. The Zambezi River: tectonism, climatic change and drainage evolution. Palaeogeogr. Palaeclimatol. Palaeoecol. 78, 55–69.

Nugent, C., 1992. The Zambezi River: tectonism, climatic change and drainage evolution – reply to discussion. Palaeogeogr. Palaeclimatol. Palaeoecol. 91, 183–196.

Partridge, T.C., 1998. Of diamonds, dinosaurs and diastrophism: 150 million years of landscape evolution in southern Africa. S. Afr. Tydskr. Geol. 101, 167–184.

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

Rambaut, A., Grassly, N.C., 1997. Seq-Gen: an application for the Mont-Carlo simulation of DNA sequence evolution along phylogenetic trees. Comp. Appl. Biosci. 13, 235–238.

Rodriguez-Robles, J.A., Stewart, G.R., Papenfuss, T.J., 2001. Mitochondrial DNA-based phylogeography of North American rubber boas, *Charina bottae*. Mol. Phylogenet. Evol. 18, 227–237.

Ruedi, M., Auberson, M., Sovalainen, V., 1998. Biogeography of Sulawesian shrews: testing for their origin with a parametric bootstrap on molecular data. Mol. Phylogenet. Evol. 9, 567–571.

Scott, I.A.W., Keogh, J.S., 2000. Conservation genetics of the endangered grassland earless dragon *Tympanocryptis pinguicolla* (Reptilia: Agamidae) in Southeastern Australia. Cons. Gen. 1, 357–363.

Stokes, S., Thomas, D.S.G., Washington, R., 1997. Multiple episodes of aridity in southern Africa since the last interglacial period. Nature 388, 154–158.

Swofford, D.L., 2000. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.

Thomas, D.S.G., Shaw, T.A., 1990. The deposition and development of the Kalahari group sediments, Central southern Africa. J. Afr. Earth Sci. 10, 187–197.

Thomas, D.S.G., Shaw, T.A., 1992. The Zambezi River: tectonism, climactic change and drainage evolution—is there really evidence for a catastrophic flood? A discussion. Palaeogeogr. Palaeclimatol. Palaeoecol. 91, 175–182.

Thomas, D.S.G., Shaw, T.A., 1993. The evolution and characteristics of the Kalahari, southern Africa. J. Arid Environ. 25, 97–108.

Thomas, D.S.G., Stokes, S., Shaw, P.A., 1997. Holocene Aeolian activity in the southwestern Kalahari Desert, southern Africa: significance and relationships to late-Pleistocene dune-building events. Holocene 7, 273–281.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1994. The CLUSTAL_Xwindows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Whiting, M.J., Nagy, K.A., Bateman, P.W., 2003. Evolution and maintainance of social status signaling badges: experimental manipulations in lizards. In: Fox, S.F., McCoy, J.K., Baird, T.A. (Eds.), Lizard Social Behavior. Johns Hopkins University Press, Baltimore, MD, pp. 47–82.

Wüster, W., Salomao, M.G., Quijada-Mascarenas, J.A., Thorpe, R.S., B.B.B.S.P., 2002. Origin and evolution of the South American pitviper fauna: evidence from mitochondrial DNA sequence analysis. In: Schuett, G.W., Höggren, M., Doughlas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Eagle Mountain, Utah, pp. 111–128.

Zamudio, K.R., Greene, H.W., 1997. Phylogeography of the bushmaster (*Lachesis muta*: Viperidae): implications for neotropical biogeography, systematics and conservation. Biol. J. Linn. Soc. 62, 421–442.