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Molecular phylogeography of Rosenberg's goanna (Reptilia:Varanidae: *Varanus rosenbergi*) and its conservation status in New South Wales

Abstract Rosenberg's goanna, *Varanus rosenbergi*, is listed as 'Vulnerable' in New South Wales, but it is not currently listed nationally in Australia as large populations exist on Kangaroo Island in South Australia. There are several significant morphological and behavioural differences between populations in NSW and those in the rest of Australia and some researchers believe these differences may be great enough to warrant separate taxonomic status. To investigate the magnitude and nature of these differences with an independent data set, we sequenced a 867 base pair DNA fragment of the mitochondrial genome that includes half of the ND4 gene and three tRNA genes. We obtained sequence data from 30 individuals of *V. rosenbergi* drawn from across its range in southern Australia, and also included representatives of two other *Varanus* species, *V. gouldii* and *V. varius*. Thirteen mitochondrial haplotypes were found among the 30 *V. rosenbergi* samples. Phylogenetic signal in the data sets was strong and a variety of phylogenetic analyses of the data all resulted in the same single fully resolved tree. The differences observed between populations of *V. rosenbergi* are not considered great enough to support the description of new taxa, but are sufficient to clearly define five evolutionary significant units, one of which is comprised of the threatened NSW/ACT populations.

Key words monitor lizard, goanna, *Varanus*, Varanidae, molecular phylogeny, ND4, populations, conservation status

Introduction

The Family Varanidae, commonly referred to as monitor lizards or goannas, contains the largest living lizards in the world. Varanids are found throughout Africa, the Middle East, Asia and Australia, but more than half of the 50-plus species of varanids occur in Australia. Varanids are of special interest to evolutionary biologists due to their sister-group relationship to snakes (Lee, 1997; Vidal & Hedges, 2004), but they also are of significant conservation concern (Phillips & Packard, 1994; Ciofi *et al*., 1999). Several species of varanids are harvested from the wild in large numbers for the reptile leather industry (e.g. *V. salvator* in Indonesia; Shine *et al*., 1996, 1998 and *V. niloticus* in Africa; de Buffrenil & Hemery, 2002). Populations of many other varanid species have declined due to the loss of suitable habitat, and this has been especially true for the larger species (Phillips & Packard, 1994). Unfortunately, there has been very little published research on varanid conservation and the only published research on the conservation implications of population-level genetic differences is a microsatellite based study conducted on *Varanus komodoensis* (Ciofi & Bruford, 1999; Ciofi *et al*., 1999).

Varanus rosenbergi occurs across southern Australia in three apparently disjunct regions on the mainland and on islands of various sizes off the coast of South Australia (Fig. 1). It is believed that its occurrence on some or all of the Sir Joseph Banks Islands was the result of introduction by humans for the purposes of controlling tiger snake populations (Mirtschin & Jenkins, 1985). Originally described by Mertens (1957) as a subspecies of *V. gouldii*, *V. rosenbergi* was elevated to full species status by Storr (1980) based on colour pattern differences and a lack of interbreeding with *V. gouldii gouldii* where they are sympatric in southern Western Australia. *Varanus rosenbergi* is listed as 'Vulnerable' in New South Wales under the *Threatened Species Conservation Act 1995* and while in large numbers on some offshore islands, there is concern for its status in other areas on the mainland. Moreover, the disjunct distribution and differences in body size, colouration and behaviour among the three main centres of distribution is a source of conservation concern due to possible unresolved

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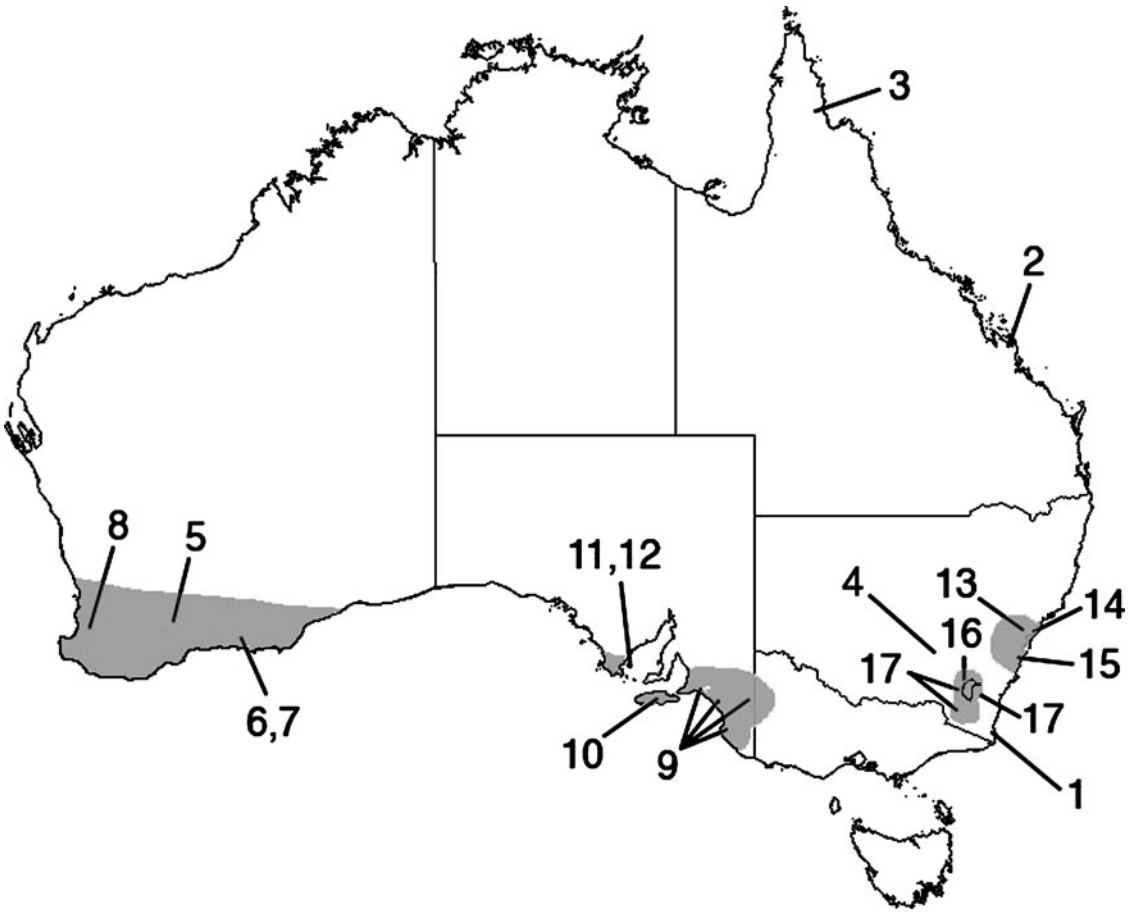

Figure 1 Map of the distribution of *V. rosenbergi* (shaded area). Numbers correspond to haplotype numbers in Tables 1 and 2 and Figure 2.

taxonomic issues (Green & King, 1978; Storr, 1980). *Varanus rosenbergi* on Kangaroo Island reach about twice the size of those in Western Australia and those in NSW/ACT reach about twice the size of those on Kangaroo Island and are among the largest lizards in the world. Populations are strongly sexually dimorphic on Kangaroo Island and in Western Australia where males reach approximately twice the body weight of females and aggressive behaviour regularly occurs between males during the breeding season (King & Green, 1999). This does not appear to be the case in southeastern Australia with males and females reaching similar adult body weights (WS, personal observation).

A substantial amount of research has been conducted on the home ranges, breeding biology, energetics and diet of this species on Kangaroo Island (Green & King, 1978; King & Green 1979; King, 1980; Green *et al*., 1991; Christian & Weavers, 1994; Green *et al*., 1999), but little is known about this species in other parts of the range. What we do know is that across its distribution, *V. rosenbergi* generally shelters in burrows, rock outcrops and hollow logs (King & Green, 1999). Breeding occurs in late summer and eggs are deposited in active termite mounds where they incubate until the following spring (Green *et al*., 1999).

The lack of basic information on populations other than Kangaroo Island has made the initiation of conservation programmes difficult. Results of studies carried out on Kangaroo Island are being used to make management decisions regarding the threatened populations in NSW. However, the morphological and behavioural differences between these two areas are significant and, in the absence of molecular genetic data on the relationship among *V. rosenbergi* populations, cast doubt on the applicability of extrapolating results of Kangaroo Island studies. In this study, we use mtDNA nucleotide sequence data to construct a species-level phylogeny for *V. rosenbergi* populations across the entire distribution of the species and discuss the conservation implications.

Materials and methods

Taxon sampling and characters

A total of 30 samples of *V. rosenbergi* from across Australia were obtained for analysis. The majority of the material came from frozen tissue collections, although several blood samples obtained from live animals in NSW were also included (Table 1). We used existing phylogenetic hypotheses for varanids to select appropriate outgroups. There have been several approaches used to establish a phylogeny of the varanids including the use of hemipenial morphology (Böhme, 1988), lung morphology (Becker *et al*., 1989), chromosomal morphology (King & King, 1975), chromosomal and microcomplement fixation data (King, 1990; Baverstock *et al*., 1993) and

Species	Museum No	Locality	Haplotype	GenBank #
V. varius	ANWC R6172	Pambula, NSW	1	DQ329284
V. varius	ANWC R5477	Shoalwater Bay, QLD	2	DQ329285
V. gouldii	ANWC R5243	McIlwraith Range Cape York, QLD	3	DQ329286
V. gouldii	ANWC R6167	Rankin Springs, NSW	4	DQ329287
V. rosenbergi	WAM R132913	Lilly McCarthy Rock, WA	5	DQ329288
V. rosenbergi	SAM R22943	Coomalbidgup, WA	6	DQ329289
V. rosenbergi	SAM R22944	Coomalbidgup, WA	7	DQ329290
V. rosenbergi	ABTC 10433	Collie, WA	8	DQ329291
V. rosenbergi	ABTC 10434	Collie, WA	8	DQ329292
V. rosenbergi	SAM R47041	15 km NE of Kingston, SE SA	9	DQ329293
V. rosenbergi	SAM R45139	South boundary Messent CP, SA	9	DQ329294
V. rosenbergi	SAM R23531	Coorong NP, SA	9	DQ329295
V. rosenbergi	ABTC 71461	Ngarkat CP, SA	9	DQ329296
V. rosenbergi	ABTC 71462	Ngarkat CP, SA	9	DQ329297
V. rosenbergi	ANWC R5149	Kangaroo Island, SA	10	DQ329298
V. rosenbergi	ANWC R5152	Kangaroo Island, SA	10	DQ329299
V. rosenbergi	ANWC R5151	Kangaroo Island, SA	10	DQ329300
V. rosenbergi	SAM R23427	Kangaroo Island, SA	10	DQ329301
V. rosenbergi	SAM R30488	Taylors Is, SA	11	DQ329302
V. rosenbergi	SAM R30487	Taylors Is, SA	12	DQ329303
V. rosenbergi	SAM R27483	Thistle Is, SA	11	DQ329304
V. rosenbergi	SAM R27482	Thistle Is, SA	12	DQ329305
V. rosenbergi	ABTC 55391	Reevesby Is, SA	12	DQ329306
V. rosenbergi	ABTC 55391	Reevesby Is, SA	12	DQ329307
V. rosenbergi	SAM R30491	Spilsby Is, SA	12	DQ329308
V. rosenbergi	SAM R30492	Spilsby Is, SA	12	DQ329309
V. rosenbergi	SAM R123331	Kulnura, NSW	13	DQ329310
V. rosenbergi	AM R143983	Mangrove Mtn Wollombi Rd, NSW	14	DQ329311
V. rosenbergi	AM R152794	Heathcote NR Woronora Dam Rd., NSW	15	DQ329312
V. rosenbergi		Ginninderra Falls, NSW	16	DQ329313
V. rosenbergi		Googong, NSW	17	DQ329314
V. rosenbergi		Googong, NSW	17	DQ329315
V. rosenbergi		Gooberragandera River	17	DQ329316
V. rosenbergi		Khancoban, NSW	17	DQ329317

Table 1 Summary of specimens sampled and their locality information. *Varanus varius* was used as the outgroup in phylogenetic analyses. SAM = South Australian Museum, ANWC = Australian National Wildlife Collection, ABTC = Australian Biological Tissue Collection. Only unvouchered blood samples were available for the last five animals listed. The haplotype identification number corresponds to those in Table 2 and Figure 1.

mitochondrial DNA sequencing (Fuller *et al*., 1998; Ast, 2001). The most broadly accepted of these is that published by Ast (2001), but only Baverstock *et al*. (1993) included *V. rosenbergi* in their analyses. They placed *V. rosenbergi* as sister to *V. gouldii*, which is always placed within a group also comprising *V. panoptes*, *V. giganteus*, *V. mertensi* and *V. spenceri* (Fuller *et al*., 1998; Ast, 2001). Therefore, we included two samples of *V. gouldii* to represent a member of the same clade and two samples of *V. varius* to represent a more distantly related clade.

DNA extraction and PCR

Total genomic DNA was extracted using a modified hexadecyltrimethyl-ammoniumbromide (CTAB) protocol. Approximately 100 ng of genomic DNA was used as template for PCR amplification experiments. For each sample, we targeted an approximately 900 base pair (bp) DNA fragment of the mitochondrial genome that included the 3' half of the ND4 gene and most of the tRNA cluster containing the histidine, serine and leucine tRNA genes. This region was targeted because work at comparable taxonomic levels in other squamate reptile groups indicated useful levels of variability (Kraus *et al*., 1996; Benabib *et al*., 1997; Forstner *et al*., 1998; Keogh *et al*., 2003; Chapple & Keogh, 2004; Chapple *et al*., 2005).

The target fragment was amplified using a modified primer pair ND4, TGACTACCAAAAGCTCATGTAGAAGC and tRNA-leu, TRCTTTTACTTGGATTTGCACCA (Arevalo *et al*., 1994; Forstner *et al*., 1995). Reactions were 40 μl in volume and contained 10 pmol of each primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM MgCl2, 0.25 mM dNTPs and 2 units of *Taq*-polymerase (*Amplitaq* DNA polymerase,

Perkin-Elmer). PCR amplification was done using a step-down cycling profile on a Corbett PC-960C cooled thermal cycler. Reactions were initially denatured at 94 °C for 5 min, followed by an annealing step at 65 °C for 30s and extension at 72 °C for 1.5 min. This was followed by a further round of denaturation at 94 °C for 30 s, annealing at 65 °C for 30 s and extension at 72 °C for 1.5 min. The annealing temperature was then dropped by 5 °C in the next 2 rounds of cycling. This 'stepping-down' in annealing temperature was repeated until a final annealing temperature of 40 °C was reached. The next 30 cycles then were performed with this annealing temperature. A final extension step at 72 °C was done for 7 min before reactions were cooled to 4 °C.

DNA sequencing

PCR products were gel purified using the BRESA clean kit (Geneworks) following manufacturer's instructions. Following template purification and quantification, products were directly sequenced with the ABI PRISM™ BigDye Terminator Cycle Sequencing Ready Reaction Kit. Reactions were done using half the amount of Ready Reaction Premix and 1.6 pmol of each amplification primer. Approximately 10–15 ng of purified PCR product was used as template. Cycle sequencing was done using either of the following profiles for 30 cycles: (1) 96 °C for 30 s, 50 °C for 15 s, 60 °C for 4 min, or (2) 96 °C for 30 s, 60 °C for 4 min. Ramping was set for 1 °C/s. On completion of cycle reactions were brought to 4 °C. Extension products were removed from under the oil, placed in 1.5 ml tubes and the volume brought to 20 μl with deionised water. Dried extension products were resuspended in 3–4 μl of loading dye. Labelled DNA fragments were electrophoresed on 5.2% denaturing polyacrylamide (PAGE-PLUS, Amresco) gels (36 cm well-to-read) for 8–10 hours on an ABI 377XL™ automated DNA sequencer.

Sequence editing and analysis

Sequence data were edited using Sequencher 3.0 (Gene Codes Corporation), and provisionally aligned using the default parameters of ClustalX (Thompson *et al*., 1994). Aligned sequences were translated into amino acid sequences using the vertebrate mitochondrial genetic code to determine if these data were truly mitochondrial in origin. No premature stop codons were observed, therefore, we conclude that all sequences obtained are true mitochondrial copies.

Phylogenetic analyses

We used maximum-likelihood (ML), parsimony and Bayesian approaches to analyse the data. We used the objective criteria provided by the computer program ModelTest 3.06 (Posada & Crandall, 1998) with both the Hierarchical Likelihood Ratio Test (hLRT) and the Akaike Information Criterion (AIC) to select the most appropriate model of molecular evolution for our combined data. We used the ModelTest estimates of the empirical nucleotide frequencies, substitution rates, gamma distribution, and proportion of invariant sites (I) in our ML analyses implemented in PAUP*4.0b10 (Swofford, 2002).

Data analysed using parsimony criteria were weighted in a variety of ways to evaluate any influence on tree topology; unweighted, or a ti/tv ratio of 2, 5, 10 or transversion only were used. The actual ti/tv ratio was estimated from the data via maximum likelihood. The ti/tv ratios used for parsimony analyses were designed to approximate and flank the actual ratio. Parsimony analyses were conducted using PAUP*4.0b10 (Swofford, 2002).

We used the computer program MrBayes v3.0b4 (Huelsenbeck & Ronquist, 2001) for our Bayesian analyses. Using the identical data set as our ML analyses, the General Time Reversible (GTR) + gamma distribution + proportion of invariant sites parameters were all estimated from the data during the run. We used the default value of four Markov chains per run and also ran the full analysis five times to make sure overall tree-space was very well sampled and to avoid being trapped in local optima. We ran our analysis for a total 2 000 000 generations and sampled the chain every 100 generations, resulting in 20 000 sampled trees. Log-likelihood values reached a plateau after approximately 50 000 generations (500 sampled trees), so we discarded the first 500 trees as the burn-in phase and used the last 19 500 trees to estimate Bayesian posterior probabilities.

We performed a non-parametric maximum likelihood bootstrap analysis (500 bootstrap pseudoreplicates) to assess branch support. Additionally to this, Bayesian analysis provided posterior probabilities for branches, which serve as an additional source of information on branch support and may represent a better estimate of phylogenetic accuracy (Wilcox *et al*., 2002). We consider branches supported by bootstrap values greater than or equal to 70% (Hillis & Bull, 1993) and posterior probability values greater than or equal to 95% (Wilcox *et al*., 2002) to be significantly supported by our data.

Results

The edited multiple alignment is 867 nbp in length, comprising 700 nbp of the 3' end of ND4 and 167 nbp of the HSL tRNA cluster, including complete sequences of tRNAs histidine and serine and partial sequence of tRNA-leucine. The alignment commences at a third codon position and includes 289 each of first, second and third positions. A total of 242 sites were variable and 178 informative under parsimony. Fifty-two substitutions (21% of all substitutions) occurred at first positions of which 40 (77%) were parsimony informative. Thirty-three substitutions occurred at second positions (14% of all substitutions) of which 25 (76%) were parsimony informative and 157 (65%) substitutions occurred at third positions of which 113 (72%) were parsimony informative.

Both the hLRT and the AIC from Modeltest supported the (HKY) plus gamma shape (+G) model as the best-fit substitution model for the data and gave a $-\ln L = 2537.8950$. The estimated parameters were as follows: nucleotide frequencies A = 0.3195, C = 0.3366, G = 0.0953, T = 0.2492; Transition/Transversion ratio = 8.4453; proportion of invariant sites = 0; gamma shape parameter = 0.1920. The Bayesian analysis produced parameter estimates that were very similar to those produced by ModelTest. Uncorrected and corrected interspecific genetic distances among the genotypes sampled are presented in Table 2.

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1		0.0636	0.4174	0.5648	0.4939	0.4988	0.5059	0.5189	0.4814	0.4936	0.5096	0.5219	0.5801	0.5530	0.5589	0.5589	0.5526
2	0.0515		0.4681	0.5744	0.5099	0.5147	0.5219	0.5420	0.4701	0.4933	0.4976	0.5095	0.5541	0.5280	0.5338	0.5338	0.5277
3	0.1578	0.1753		0.2302	0.2366	0.2404	0.2471	0.2541	0.2403	0.2469	0.2321	0.2383	0.2627	0.2489	0.2527	0.2527	0.2489
4	0.1892	0.1958	0.1281		0.2617	0.2658	0.2583	0.2578	0.2731	0.2889	0.2849	0.2924	0.3178	0.3185	0.3228	0.3228	0.3183
5	0.1860	0.1943	0.1343	0.1351		0.0038	0.0065	0.0108	0.0409	0.0449	0.0557	0.0582	0.0816	0.0756	0.0735	0.0779	0.0757
6	0.1876	0.1959	0.1359	0.1367	0.0038		0.0026	0.0066	0.0428	0.0468	0.0537	0.0561	0.0793	0.0734	0.0713	0.0756	0.0734
7	0.1876	0.1959	0.1374	0.1351	0.0064	0.0026		0.0065	0.0432	0.0473	0.0543	0.0567	0.0741	0.0742	0.0721	0.0764	0.0742
8	0.1893	0.1992	0.1389	0.1336	0.0103	0.0064	0.0064		0.0448	0.0489	0.0519	0.0543	0.0713	0.0714	0.0693	0.0735	0.0714
9	0.1828	0.1828	0.1359	0.1398	0.0352	0.0366	0.0366	0.0379		0.0149	0.0269	0.0288	0.0328	0.0289	0.0306	0.0306	0.0289
10	0.1845	0.1877	0.1375	0.1444	0.0379	0.0393	0.0393	0.0406	0.0142		0.0287	0.0305	0.0418	0.0376	0.0393	0.0393	0.0376
11	0.1910	0.1910	0.1329	0.1444	0.0460	0.0446	0.0446	0.0433	0.0246	0.0260		0.0013	0.0449	0.0406	0.0424	0.0424	0.0406
12	0.1927	0.1927	0.1344	0.1460	0.0474	0.0460	0.0460	0.0447	0.0260	0.0273	0.0013		0.0472	0.0427	0.0445	0.0445	0.0427
13	0.2023	0.1991	0.1403	0.1536	0.0610	0.0597	0.0569	0.0555	0.0286	0.0352	0.0379	0.0393		0.0025	0.0065	0.0065	0.0052
14	0.1990	0.1958	0.1372	0.1536	0.0583	0.0569	0.0569	0.0555	0.0260	0.0326	0.0352	0.0366	0.0025		0.0038	0.0038	0.0025
15	0.2007	0.1974	0.1388	0.1552	0.0569	0.0555	0.0555	0.0542	0.0273	0.0339	0.0366	0.0379	0.0064	0.0038		0.0025	0.0012
16	0.2007	0.1974	0.1388	0.1552	0.0597	0.0583	0.0583	0.0569	0.0273	0.0339	0.0366	0.0379	0.0064	0.0038	0.0025		0.0012
17	0.1990	0.1958	0.1372	0.1536	0.0583	0.0569	0.0569	0.0556	0.0260	0.0326	0.0352	0.0366	0.0051	0.0025	0.0013	0.0013	

Table 2 Jukes–Cantor intraspecific genetic distances (lower matrix) and likelihood corrected distances (upper matrix) for *Varanus rosenbergi*. Haplotype numbers correspond to those in Table 1.

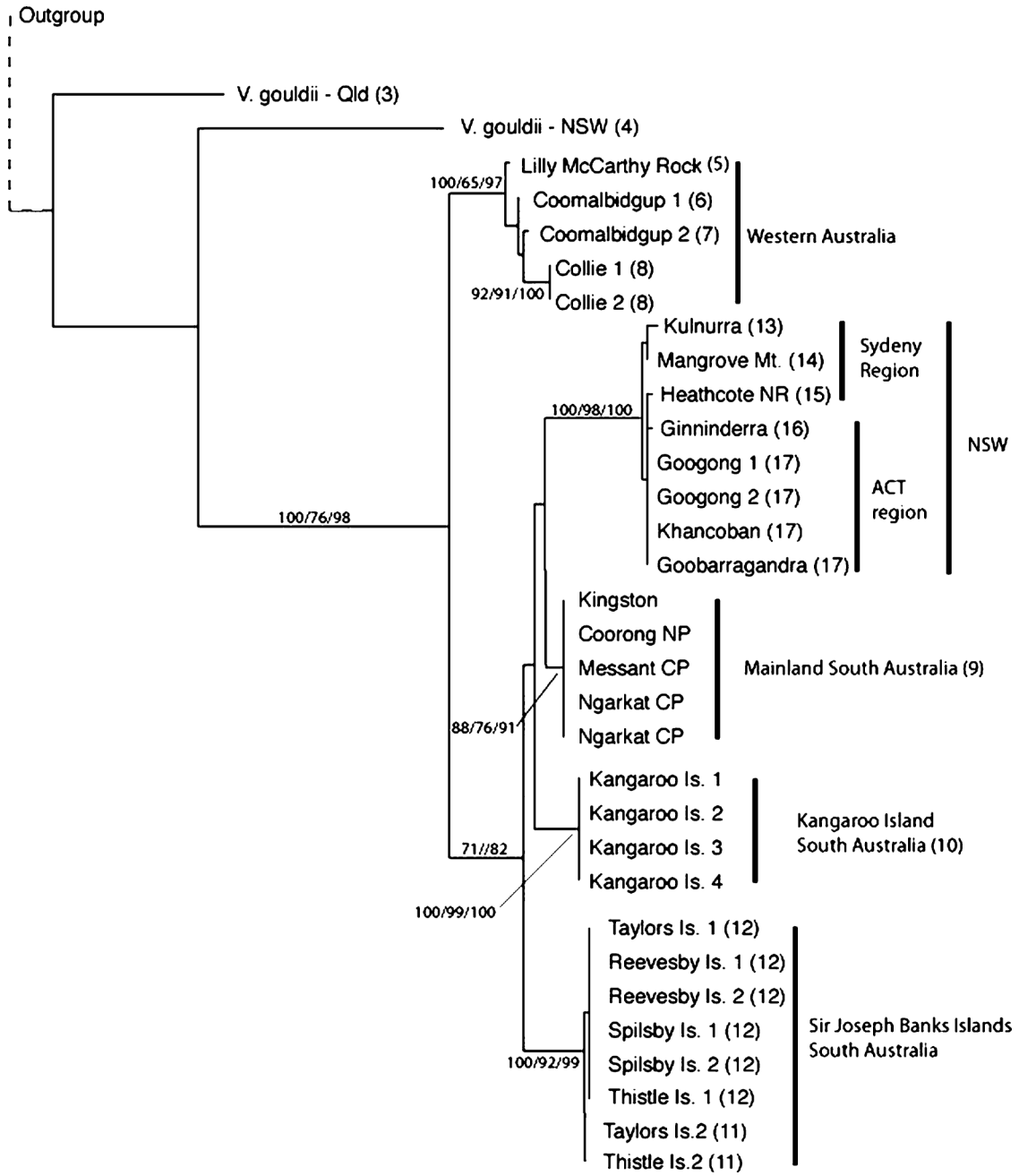

Figure 2 Likelihood tree constructed using HKY + G model. The numbers at each node correspond to parsimony bootstrap/likelihood bootstrap/bayesian posterior probabilities respectively. Numbers in parentheses after the locality names correspond to the haplotype numbers listed in Tables 1 and 2 and shown in Figure 1.

The ML analysis in PAUP* using the above parameters, parsimony and the Bayesian analyses all yielded the same single optimal tree (Fig. 2) and bootstrap support for all major branches is high. There are five well supported monophyletic clades across the distribution of *V. rosenbergi* based on these mitochondrial data: Western Australia, the Sir Joseph Banks Islands, Kangaroo Island, mainland South Australia and NSW (Fig. 1). Substantial mitochondrial divergence between the major populations reflects the disjunct distribution of *V. rosenbergi*. The Western Australian populations differed from South Australia by between 4.1 and 5.8% likelihood corrected genetic distance and from NSW by as much as 8.2% while NSW and SA populations differed from one another by between 2.9 and 4.7% (see Table 1). This compares with 6.4% sequence divergence observed between the two *V. varius* samples and 23% between the two *V. gouldii* samples. Given the difficulty in discriminating between *V. gouldii* and *V. panoptes* in north Queensland it is possible that a misidentification occurred and ANWC R5243 is actually *V. panoptes*.

Discussion

Our phylogenetic data raise two important issues regarding the conservation of *Varanus rosenbergi*. First, given that

differences found between sampled *V. rosenbergi* populations are not much greater than those found between *V. varius* populations, there is no reason, based on these data, to conclude that *V. rosenbergi* contains more than one species based on an evolutionary species concept. The levels of genetic divergence among populations also correspond to within-species levels of divergence in several other reptile groups based on the same gene (Kraus *et al*., 1996; Benabib *et al*., 1997; Forstner *et al*., 1998; Keogh *et al*., 2003; Chapple & Keogh, 2004; Chapple *et al*., 2005). There is no evidence of reproductive incompatibility and there is anecdotal evidence which argues against raising these populations to specific status. For example, captive *V. rosenbergi* from Kangaroo Island are known to reach similar size to those from eastern Australia (B. Green, pers. comm.), indicating that resources or population density may be responsible for some of the morphological differences rather than underlying genetic divergence. These facts lead us to conclude that more information would be required before the elevation of these populations to species status could be justified. Second, *Varanus rosenbergi* may be divided into five monophyletic groups that correspond to well-defined geographic areas; southwest Western Australia, mainland South Australia, the Sir Joseph Banks Islands, Kangaroo Island and the ACT/NSW populations (Fig. 1). We first consider the phylogenetic and biogeographical implications of our phylogeny and then turn to conservation implications.

The distribution of *V. rosenbergi* appears to follow the distribution of woodland and mallee in far southern Australia with the larger breaks in distribution being across areas of arid scrubland (Nullarbor plain) and of grassland and forest (eastern Victoria, southern NSW). This pattern fits the classic Bassian distribution, which is also seen in tiger snakes (Keogh *et al*., 2005) and some scincid lizards (Cogger & Heatwole, 1981), but the biogeography of southern Australia is surprisingly poorly understood. Many species of plants and animals display a Bassian distribution where they occur in the southern moist temperate regions of the south-west of Western Australia and in south-eastern Australia, but do not occur in arid regions in between (Cogger & Heatwole, 1981; Peck, 2001). There are also many species pairs where one of the sister taxa occurs in the south-west and the other in the south-east (Cogger & Heatwole, 1981; Littlejohn, 1981). Cogger and Heatwole (1981) did not find the classic Bassian distribution pattern in varanids, but it is unlikely that, at the time, they were aware of the occurrence of *V. rosenbergi* in NSW and the ACT. This Bassian distribution suggests that the species was probably continuously distributed across southern Australia when a mesic corridor existed connecting southeastern and southwestern Australia (Bowler, 1982; Horton, 1984). The separation time between these major areas of distribution probably coincided with changes in broad vegetation types across southern Australia during ice age and aridity cycles over the last 17 000 years (Horton, 1984; Heatwole, 1987).

The suggestion that *V. rosenbergi* were introduced to some or all of the Sir Joseph Banks Islands (Mirtschin & Jenkins, 1985) is neither confirmed nor discredited by this study. Unfortunately no specimens were available from the Eyre Peninsula in South Australia, which would be the most likely mainland source for introductions. Thistle Island was cited as the source for the Reevesby Island population and the two islands do share a haplotype (Table 1). A fine scale population genetics study would be required to resolve the origins and movements of animals between the various islands and between the islands and the mainland. This issue is also of some conservation concern as the presence of *V. rosenbergi* on Reevesby Island may have contributed to the extinction of the Vulnerable Greater Stick Nest Rat, *Leporillus conditor* on that island (Mirtschin, 1982) and may hamper efforts to reintroduce the species there.

Our phylogeny supports the view that the current disjunct distribution is a reflection of pre-European distribution of woodland and mallee. Based on the criteria set out by Moritz (1994) our phylogeny would support the recognition of each of the five biogeographically discrete *V. rosenbergi* clades as 'evolutionarily significant units' (ESUs). However, the criteria for exchangeability set out by Crandall *et al*. (2000) would identify three ESUs based on genetic differentiation, morphological differences and a lack of gene flow: Western Australia, South Australia and NSW/ACT. We believe that, in this case, the use of the exchangeability criteria does not sufficiently capture the structured variation within South Australia, and that this variation is significant and worthy of recognition. We therefore support the recognition of five ESUs as defined by Moritz (1994). The identification of the ACT/NSW populations as a distinct ESU justifies the state listing as Vulnerable and the allocation of conservation resources for this species in the eastern part of its distribution. The ESU status also provides for the possibility of federal listing under the *Environment Protection and Biodiversity Act 1999*. There is concern that other mainland populations are declining and this is certainly the case in some parts of South Australia (P. Riscmiller, pers. comm.). The results of our phylogenetic analysis suggest that more attention should be paid to the conservation status of the species in the other major areas of distribution. *Varanus rosenbergi* appears to be secure on Kangaroo Island, but ongoing construction of tarmac roads is having localised effects on population size and, almost certainly, on the demographics of populations. There is little information on the populations on the Sir Joseph Banks Islands, but anecdotally, they also appear secure.

Our results also have broader implications for the conservation status of keystone species. In many of the areas where *V. rosenbergi* occurs it is the largest native terrestrial predator. The conservation of large predators is an effective way to use conservation resources as large predators require significant areas of habitat for maintenance of viable populations, thereby acting as umbrellas for the conservation of other species. Also, the absence of predators can have dramatic effects on the ecology of the community from which they have been extirpated. Studies of community ecology following the extirpation and reintroduction of Grey wolves in several different regions of North America have demonstrated how the removal of a single predator from a system can dramatically alter the entire ecological balance due to changes in distribution, behaviour and density of prey items and the resulting changes in grazing pressure (Peinetti *et al*., 2002; Beschta, 2003; Ripple & Beschta,

2003; White *et al*., 2003) and to changes in resources available to other species (Wilmers *et al*., 2003). The changes brought about by the removal of wolves were readily observable because of their dramatic nature, but it is likely that the removal of a large predator like *V. rosenbergi* could have equally devastating effects on community function.

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