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Parallel adaptive radiations in arid and temperate Australia: molecular phylogeography and systematics of the *Egernia whitii* (Lacertilia: Scincidae) species group

DAVID G. CHAPPLE* and J. SCOTT KEOGH

School of Botany and Zoology, Australian National University, Canberra, Australian Capital Territory 0200, Australia

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It has been an enduring belief that increasing aridity combined with Pliocene-Pleistocene glacial cycles resulted in the formation of distinct arid zone and temperate zone faunas within Australia. We assembled a molecular phylogeny for the *Egernia whitii* species group, an endemic group of skinks that comprises representatives from arid and temperate Australia, in order to test several biogeographical hypotheses regarding the origin of the Australian arid zone fauna. Sequence data were obtained from ten of the 11 species within the species group, plus three other *Egernia* species and an outgroup (*Eulamprus heatwolei*). We targeted portions of the ND4 (696 base pairs) and 16S rRNA (500 bp) mitochondrial genes and the β-Fibrinogen 7th Intron nuclear gene (648 bp). The edited alignment comprised 1844 characters, of which 551 (30%) were variable and 382 (69%) were parsimony informative. We analysed the data using maximum likelihood and Bayesian techniques and produced a single optimal tree. Our phylogeny strongly supports two major clades within the species group, corresponding to temperate-adapted rock-dwelling species and arid-adapted obligate burrowing species. However, the phylogenetic affinities of *E. pulchra* were not resolved. Our topology indicates that the New South Wales population of *E. margaretae* is actually *E. whitii* and reveals that *E. margaretae margaretae* and *E. m. personata* are distinct species. There also appears to be a major phylogeographical break within *E. whitii* occurring in eastern Victoria. Although our data supported several previously proposed phylogenetic relationships, Shimodaira–Hasegawa tests soundly rejected several suggested affinities between certain species. The arid zone members of the *E. whitii* species group had been suggested to have originated as a result of multiple periods of colonization during the Pleistocene glaciation cycles. However, our genetic data suggest a single origin (presumably from a semiarid *E. multiscutata*-like ancestor) for the arid zone members of the group prior to the Plio-Pleistocene, probably during the late Miocene to early Pliocene. Our topology displays substantial sequence divergence between species with short internodes and long terminal branches, indicating rapid adaptive radiations within the arid and temperate zones. The presence of temperate-adapted species within more mesic refugia of the arid zone suggests that the necessary adaptations to aridity for colonizing the dry interior of the continent have not evolved since the initial period of adaptive radiation, despite the long evolutionary history of the species group. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **83**, 157–173.

ADDITIONAL KEYWORDS: 16S rRNA – β-fibrinogen – biogeography – mtDNA – ND4 – nuclear gene – phylogeny – skink – taxonomy.

INTRODUCTION

The biogeographical processes that led to the origin and differentiation of the Australian arid zone biota have been the subject of considerable interest and debate (reviewed in Barker & Greenslade, 1982; Cracraft, 1991; Matthews, 2000). The early to middle Tertiary (25–50 Mya) is generally recognized as the period when aridity started to develop in Australia, previously a continent characterized by extensive rain forest vegetation (Bowler, 1982; Truswell & Harris, 1982; Markgraf, McColone & Hope, 1995). Although there is some conjecture concerning the onset of aridity, it is generally agreed that the arid zone biotic and climatic region was established in Australia during

*Corresponding author. E-mail: david.chapple@anu.edu.au

the late Miocene (5–25 Mya) or Pliocene (2–5 Mya) (Bowler, 1982; Hope, 1982; Truswell & Harris, 1982; Crisp, Linder & Weston, 1995; Markgraf *et al*., 1995). Geological evidence indicates that the vegetation was intensely modified as a result of the increasing aridity, with wet forests being progressively more restricted to coastal regions as the continent became dominated by grasslands, scrublands, woodlands and more open vegetation (particularly *Eucalyptus* and *Acacia*; Bowler, 1982; Nix, 1982; Truswell & Harris, 1982; Williams, 1982; Markgraf *et al*., 1995). The increasing aridity of the continent is believed to have imposed strong selective forces on the evolutionary history of Australian organisms (Barker & Greenslade, 1982; Markgraf *et al*., 1995).

By the late Pliocene, climatic and glacial cycles had become increasingly marked on the Australian continent, with periodic oscillations in temperature, humidity, sea level and aridity (Keast, 1981; Truswell & Harris, 1982; Markgraf *et al*., 1995). Such cycles of glacial and interglacial periods are believed to have intensified during the early Quaternary (1.5–2 Mya), with climatic fluctuations characteristic of the Australian Pleistocene (0.01–1.8 Mya) environment (Keast, 1981; Bowler, 1982; Markgraf *et al*., 1995). Cyclic episodes in the expansion and contraction of arid zone habitat (e.g. scrubland, grassland) is believed to have gradually encroached on the wet forests and rain forests, resulting in the restriction of these more mesic habitats to predominantly coastal areas (Keast, 1981; Bowler, 1982; Markgraf *et al*., 1995). The distribution of fauna adapted to these peripheral mesic habitats presumably tracked the repeated expansion and contraction of their habitat during the Pleistocene, resulting in fragmentation and disjunctions of species (Keast, 1981; Ford, 1987; Markgraf *et al*., 1995). During periods of enhanced aridity, biogeographical barriers such as mountain ranges (e.g. Great Dividing Range, GDR) presumably isolated mesic-adapted fauna into refugial coastal areas (Keast, 1981; Markgraf *et al*., 1995; Schneider, Cunningham & Moritz, 1998). Isolated pockets of temperate-adapted organisms appear to have persisted within more humid microenvironments of central Australia created by mountain ranges such as the Central Ranges and the Flinders Ranges (Keast, 1981; Schodde, 1982; Cracraft, 1991). Consequently, increasing aridity since the mid-Tertiary and Pleistocene glacial cycles are predicted to have strongly influenced the biogeographical history of organisms through vicariance events (Keast, 1981; Bowler, 1982; Markgraf *et al*., 1995).

The perception that increased aridity and Pleistocene glacial cycles led to the differentiation of distinctive arid-adapted and temperate-adapted faunas has been a dominant biogeographical theme in Australia (Cracraft, 1991). Numerous vertebrate taxa are restricted to the arid interior, whilst many other taxa are confined to the peripheral areas (Cogger & Heatwole, 1981, 1984; Schodde, 1982; Cracraft, 1991). Hypotheses for the origins of the distinctive arid zone fauna generally rely on scenarios involving numerous independent colonizations from more temperate or coastal centres of diversity and radiation (Horton, 1972; reviewed in Cracraft, 1991). Speciation events resulting in the differentiation of the arid zone fauna are generally placed within the Pleistocene and correlated with glacial–interglacial cycles (Horton, 1972; Schodde, 1982; Heatwole & Taylor, 1987; reviewed in Cracraft, 1991). However, the validity of the arid–temperate dichotomy and the biogeographical mechanisms leading to the origin of the arid zone fauna has remained contentious (Cracraft, 1991). Testing the validity and effects of each of these biogeographical processes can be difficult, but examination of phylogenetic relationships of taxa that span the arid and temperate regions of Australia can provide insight into the putative historical processes. We chose the Scincid genus *Egernia*, and more specifically the *E. whitii* species group, as a system in which to examine the complex biogeographical history of arid Australia.

Except for *E. frerei*, which extends into New Guinea, *Egernia* is an endemic Australian genus of scincid lizards with widespread distributions throughout the continent, including the arid zone (Cogger, 2000; Chapple, 2003). The genus currently comprises 30 described species (Cogger, 2000; Chapple, 2003). *Egernia* is an ideal genus in which to examine the evolution and differentiation of the Australian arid zone fauna for several reasons. Most *Egernia* species have specific habitat requirements (e.g. burrow, hollow log, rock crevice) and exhibit limited dispersal (Chapple, 2003). Animals that are habitat specific may have fewer opportunities to disperse and so their current distribution might better reflect past geological history. Such habitat specificity is emphasized by the presence of several temperate-adapted species within mesic refugia in central Australia (e.g. *E. margaretae* Storr; Henzell, 1982).

Horton (1972) and Heatwole & Taylor (1987) hypothesized that the *Egernia* ancestor (suggested to be *Mabuya multifasciata*) entered Australia via a land bridge with New Guinea during the Miocene (Horton, 1972; Heatwole & Taylor, 1987). They suggested that the subsequent speciation and differentiation of *Egernia* into six species groupings (Storr, 1968, 1978; Horton, 1972) was concordant with Pleistocene glacial–interglacial cycles and fluctuations in the degree of aridity (Horton, 1972; Heatwole & Taylor, 1987). However, such scenarios were based largely on dispersal and pre-continental drift theories and there has been no rigorous examination of the origins of the *Egernia* genus. Although there is strong support for the mono-

phyly of the *Egernia* group (*Egernia*, *Tiliqua*, *Cyclodomorphus* and *Corucia* genera), karyotypic (Donnellan, 1991) and molecular evidence (Honda *et al*., 1999, 2000) indicates that the group does not have close phylogenetic affinities with Horton's (1972) proposed *Mabuya* ancestor. This contradiction strongly undermines the scenarios of Horton (1972) and Heatwole & Taylor (1987) and consequently there are few robust data relating to the origins of the *Egernia* genus and the six species groupings.

The *E. whitii* species group comprises 16 described taxa with 11 species, five with subspecies. Members of the *E. whitii* species group have distributions that span both the temperate and arid zone regions of the continent (Cogger, 2000). Species within the group can be broadly characterized as either rock-dwelling (six species: *E. whitii* Lacépède, *E. guthega* Donnellan *et al.* *E. margaretae*, *E. modesta* Storr, *E. montana* Donnellan *et al.* and *E. pulchra* Werner) or obligate burrowing species (five species: *E. inornata* Rosén, *E. kintorei* Stirling & Zietz, *E. multiscutata* Mitchell and Behrndt, *E. slateri* Storr and *E. striata* Sternfeld) (Chapple, 2003). The rock-dwelling species are predominantly temperate-adapted and tend to occur in more mesic or coastal environments and display diurnal activity patterns (Henzell, 1972, 1982; Chapple, 2003). In contrast, the obligate burrowing species are generally restricted to the arid or semiarid environments of the interior of the continent, except *E. multiscutata*, which also occurs in coastal areas and on offshore islands (Henzell, 1972, 1982; Chapple, 2003). These arid zone species exhibit several adaptations for living in dry conditions such as the construction of burrows and reduced rates of evaporative water loss compared to the more temperate members of the species group (Henzell, 1972, 1982). The obligate burrowers display restricted activity patterns (i.e. crepuscular or nocturnal; Henzell, 1972, 1982). The elliptical eyes of *Egernia striata*, and pupils intermediate between round and elliptical in *E. kintorei* enable these species to forage at night (Cogger, 2000; Pearson *et al*., 2001; Chapple, 2003).

Past work has highlighted the complex evolutionary history of the *E. whitii* species group and the taxonomic relationships therein are far from resolved (Mitchell, 1950; Storr, 1968, 1978; Horton, 1972; Donnellan *et al*., 2002; Fig. 1). Storr (1968) was the first to recognize the *E. whitii* species group and his initial work identified two major subgroupings within the group (*E. whitii* and *E. inornata* groups; Fig. 1A). However, Storr (1968) was unable to determine the phylogenetic affinities of *E. multiscutata* or *E. slateri*, suggesting that they possessed several characteristics of each subgrouping. Although Henzell (1972, 1982) did not formally propose any phylogeny for the species group, he suggested that *E. slateri* (derived from

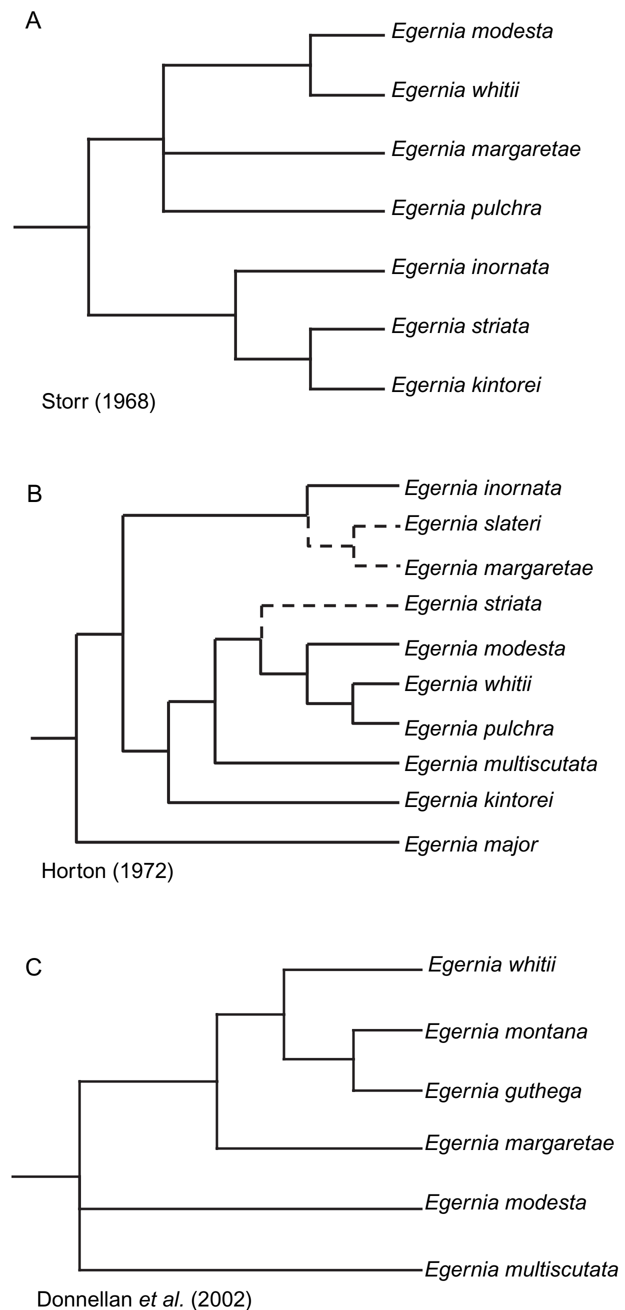

Figure 1. Previous phylogenetic hypotheses for the *Egernia whitii* species group. A, Storr's (1968) phylogeny based on morphological characters. The phylogenetic affinities of *E. slateri* and *E. multiscutata* were not resolved. B, Phylogenetic tree redrawn from Horton (1972) based on morphological data and distributional patterns. The dotted lines indicate areas of uncertain phylogenetic affinity. C, Phylogenetic tree by Donnellan *et al*. (2002) for the species from south-eastern Australia based on allozyme and morphological data. Note that the alpine species, *E. guthega* and *E. montana*, were considered to be *E. whitii* until they were described by Donnellan *et al*. (2002).

E. multiscutata) and *E. m. margaretae* (derived from *E. m. personata*) represented relict populations in central Australia (i.e. Central Ranges region) derived from temperate zone taxa.

Horton (1972) advocated a vastly different phylogeny for the *E. whitii* species group (Fig. 1B). He identified *E. inornata* and *E. whitii* as the two major lineages within the species group, which he believed originated as a species pair on either side of the GDR. However, the composition of the two subgroupings varied significantly to that proposed by Storr (1968). Horton (1972) recognized *E. modesta* as a distinct species (also see Milton, Hughes & Mather, 1983) and considered it to be a secondary movement of the *E. whitii* lineage through the GDR followed by isolation and adaptation to arid conditions. He proposed that *Egernia slateri* and *E. margaretae* originated from relict populations (either *E. whitii*, *E. inornata* or *E. kintorei*) isolated in relatively moist areas and mountains in the Central Ranges (Horton, 1972). Horton (1972) believed that temperate-adapted members of the species group colonized arid regions of the continent on multiple occasions during suitable periods of the Pleistocene glacial cycles. In contrast, Pianka (1972) proposed that the arid zone members of the species group arose from a single colonization followed by speciation within certain vegetation types of the arid zone region. More recently, Donnellan *et al*. (2002) completed a detailed revision of the *E. whitii* species group in south-eastern Australia, utilizing morphology and allozyme markers (Fig. 1C). Their taxonomic revision resulted in the description of two new alpine restricted taxa that were previously part of *E. whitii*. However, Donnellan *et al*. (2002) did not consider any species from the arid zone or western half of the continent and few evolutionary hypotheses were provided. Given the taxonomic uncertainty of the *E. whitii* species group, the use of molecular systematic approaches are necessary to resolve species relationships.

We had several major aims in the present study. First, we wanted to generate a molecular phylogeny for the *Egernia whitii* species group comprising representative taxa from the entire group (ten out of 11 species) and then utilize these molecular data (mtDNA and nuclear DNA sequences) to evaluate the previous morphologically based phylogenies and resolve several taxonomic issues within the species group. Recent analyses of DNA sequence data (S. Donnellan & M. Hutchinson, unpubl. data) have shown the *E. whitii* species group to be monophyletic. Secondly, we wanted to test whether the arid zone members of the species group arose as the result of a single colonization event or multiple episodes (Horton's 1972 hypothesis) of colonization by temperate-adapted species, and also whether the arid and temperate species constitute distinct and differentiated lineages within the species group. Finally, we wanted to use the molecular phylogeny to infer the origin and speciation of the arid zone species, and assess the putative impact of Plio-Pleistocene glaciation events on the biogeography of the members of the group.

MATERIAL AND METHODS

TAXONOMIC SAMPLING

We obtained tissue samples from representatives of ten (out of 11) species within the *Egernia whitii* species group (Table 1). The majority of the material was obtained from museum frozen tissue collections, although two tail clip samples obtained from live animals were included (Table 1). Tissue samples included in the study were selected after the completion of a finer-scale molecular phylogeny based solely on the ND4 mitochondrial gene (D. Chapple, S. Keogh & M. Hutchinson, unpubl. data), allowing special emphasis to be placed on the sampling of *E. whitii* and *E. margaretae*. No tissue samples were available for either subspecies of *E. slateri* (*E. s. slateri*, *E. s. virgata*), which is listed as critically endangered and has disappeared from many of its previously known localities (Chapple, 2003; G. Fyfe, pers. comm.). Representatives of both subspecies of *E. margaretae* (*E. m. margaretae*, *E. m. personata*) and *E. multiscutata* (*E. m. multiscutata*, *E. m. bos*) were included; however, only one subspecies of *E. pulchra* (*E. p. longicauda*) and *E. whitii* (*E. w. whitii*) were available for the study. Some of the *Egernia* species included as part of this study also were included in a broader phylogenetic study of relationships within the *Egernia* group (S. Donnellan & M. Hutchinson, unpubl. data). Based on that work, we chose three *Egernia* species (*E. saxatilis*, *E. major*, *Egernia* spp.) as outgroups and we also included a more distantly related (see Reeder, 2003) Australian *Sphenomorphus* group skink (*Eulamprus heatwolei*), and this species was used as the outgroup in all analyses (Table 1).

DNA EXTRACTION, AMPLIFICATION, SEQUENCING

Total genomic DNA was extracted from liver, toe or tail samples using a modified hexadecyl-trimethyl-ammoniumbromide (CTAB) protocol. For each sample we targeted portions of the mitochondrial genes 16S rRNA and ND4, which included the 3′ half of the ND4 gene and most of the tRNA cluster containing the Histidine, Serine and Leucine tRNA genes. We also targeted the β-fibrinogen 7th intron nuclear gene (BF) for each sample. These mitochondrial regions were targeted as work at comparable taxonomic levels in other squamate reptile groups has indicated useful levels of

Table 1. Museum registration numbers and locality data for taxa used in this study. Museum acronyms: ABTC, Australian Biological Tissue Collection; SAM, South Australian Museum, Adelaide; WAM, Western Australian Museum; ANWC, Australian National Wildlife Collection, CSIRO

Species	Museum tissue number	Voucher number	Locality
E. guthega (EW87)	ABTC 40951	SAMAR 37772	Guthega Village, NSW
E. inornata (EI20)	ABTC 38593	SAMAR 51383	22.5 km west-south-west of Haines Hill Simpson Desert, SA
E. inornata (EI55)	ABTC 35091	SAMAR 45573	3.5 km south-east of Inila Rockwater, SA
E. kintorei (EO6)	ABTC 67672		Pitlands survey, SA
E. margaretae margaretae (EA3)	ABTC 12624		Kings Creek Station, NT
E. m. margaretae (EA4)	ABTC 42404	SAMAR 42404	36.5 km east-south-east of Amata, SA
E. margaretae personata (EA1)	ABTC 53956	SAMAR 23267	Mt Remarkable NP, SA
E. m. personata (EA7)	ABTC 39287	SAMAR 52206	Near Angorichina Hostel, Alpana Station, SA
E. 'margaretae' (EA16)	ABTC 71454		Mutawintji NP, NSW
E. modesta (EM4)	ABTC 12411	SAMAR 39172	16 km west of Retreat, NSW
E. montana (EW81)	ABTC 16385	SAMAR 37767	Mt Gingera, ACT
E. multiscutata multiscutata (EU19)	ABTC 17379	SAMAR 38268	Greenly Island, SA
E. multiscutata bos (EU23)	ABTC 21716	WAMR 92010	6 km north of Eyre, WA
E. pulchra longicauda (EO3)	WAM 145186		Favourite Island, WA
E. striata (ES9)	ABTC 42030	SAMAR 48701	1.8 km north-north-west of Mt Lindsay, SA
E. whitii whitii (EW5)	ABTC 6960	AMSR 120848	Kanangra Walls, NSW
E. w. whitii (EW12)	ABTC 11488	AMSR 112344	15 km north of Bombala, NSW
E. w. whitii (EW18)	ABTC 16267	SAMAR 37783	Jindabyne, NSW
E. w. whitii (EW31)	ABTC 54276	SAMAR 23213	north-east of Mt Gambier, SA
E. w. whitii (EW32)	ABTC 54278	SAMAR 23485	Vivonne Bay, Kangaroo Island, SA
E. w. whitii (EW58)			Cannibal Creek Reserve, near Garfield, VIC
E. major (EO1)	ANWC 5298		Nana Creek, near Coffs Harbour, NSW
E. saxatilis (EO2)			Booroomba Rocks, ACT
Egernia spp. (EO4)	WAM 103114		Bungle Bungle NP, WA
Eulamprus heatwolei	ABTC 57494	SAMAR 40807	20.3 km north of Abercrombie River, NSW

variability (Benabib, Kjer & Sites, 1997; Keogh, 1998; Keogh, Shine & Donnellan, 1998; Scott & Keogh, 2000). The β-fibrinogen nuclear gene was targeted because recent studies have indicated that it provides similar resolution to that of mitochondrial genes in birds (Prychitko & Moore, 1997, 2000; Johnson & Clayton, 2000) and squamate reptiles (Giannasi, Malhotra & Thorpe, 2001). The inclusion of a nuclear gene also provides information from an independent gene and enables the production of a species tree rather than a gene tree (e.g. Moore, 1995; Nichols, 2001).

The primers used to amplify and sequence the ND4, 16S rRNA and β-fibrinogen genes are shown in Table 2. Reactions were 40 μL in total volume and contained ~100 ng template DNA, 4 μL 10× reaction buffer, 3 mM $MgCl_2$, 0.25 mM dNTPs, 10 pmol of each primer and 1 unit of Platinum *Taq*-polymerase (GibcoBRL, Life Technologies). This reaction was overlaid with 10 μL of mineral oil. PCR amplification of double-stranded product was carried out using a Corbett PC-960C cooled thermal cycler using a step-down cycling profile. Reactions were initially denatured at 94 °C for 5 min, followed by an annealing step at 70 °C for 15 s 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 70 °C for 15 s and extension at 72 °C for 1.5 min. The annealing temperature was then dropped by 5 °C in the next two rounds of cycling. This 'stepping down' in annealing temperature was repeated until a final annealing temperature of 35 °C was

Table 2. Oligonucleotide primers used in this study. The letters L and H refer to the light and heavy strands. Values in '3′ position' refer to the position of the 3′ base of the primer in the complete *Eumeces egregius* mtDNA sequence (Kumazawa & Nishida, 1999)

Gene	Primer Name	Sequence (5′-3′)	3′ Position	Source
16S rRNA	L2510	CGCCTGTTTATCAAAAACAT	1926	Palumbi (1996)
	H3056	CTCCGGTCTGAACTCAGATCACGTAGG	2452	Modified from Palumbi (1996)
ND4	ND4I	TGACTACCAAAAGCTCATGTAGAAGC	10796	Forstner, Davis & Arevalo (1995)
	tRNA-Leu	TACTTTTACTTGGATTTGCACCA	11691	Forstner *et al*. (1995)
	EgND4(L)	GGYTAYGGYATCATYCGAAT	10865	This study
	EgtRNA-Ser(H)	AGGKCGCRGAATTAGCAG	11600	This study
β-fibrinogen 7th intron	G375(F)	GACAGAGACAATGATGGA	NA	S. Donnellan & M. Hutchinson (unpubl. data)
	G376(R)	GTGAGGAATAATRCACAAAG	NA	S. Donnellan & M. Hutchinson (unpubl. data)

reached. The next 50 cycles then were performed with this annealing temperature. A final extension step at 72 °C was performed for 7 min. A slightly modified procedure was used to amplify the β-Fibrinogen gene with an annealing time of 20 s and 'stepping down' in 5 °C increments from 70 °C to a final annealing temperature of 55 °C (50 cycles at 55 °C).

PCR products were gel purified using the UltraClean 15 DNA Purification Kit (MO BIO Laboratories Inc.) following manufacturer's instructions. Following purification, products were directly sequenced with the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech). Reactions were carried out using half the amount of ET Terminator Sequencing Mix (4 μL) and 2 pmol of each amplification primer. Approximately 10–15 ng of purified PCR product was used as template. Cycle sequencing was carried out using the following profile for 50 cycles: 96 °C for 20 s, 50 °C for 15 s, 60 °C for 2 min. On completion of cycle 50, reactions were brought to 4 °C. Extension products then were removed from under the oil, placed in 1.5 mL tubes and the volume brought to 20 μL with deionized water. Dried extension products were resuspended in 1.5 μL of formamide loading dye. Sequences were electrophoresed on 5.2% denaturing polyacrylamide (Thermo-PAGE, Amresco) gels (36 cm well-to-read) and analysed on the ABI 377XL automated DNA sequencer.

Sequence data were edited using SEQUENCHER v.3.0 (Genes Codes Corporation), and provisionally aligned using the default parameters of CLUSTALX (Thompson *et al*., 1997). For ND4, aligned sequences were translated into amino acid sequences using the vertebrate mitochondrial genetic code. This was done to determine whether or not these data were truly mitochondrial in origin. As no premature stop codons were observed, we conclude that all sequences obtained are true mitochondrial copies. For β-Fibrinogen the nuclear gene sequences were aligned against sequences available for other members of the *Egernia* genus (S. Donnellan & M. Hutchinson, unpubl. data). Sequence data was deposited in GenBank under accession numbers AY520462–AY520533.

PHYLOGENETIC ANALYSES

Prior to phylogenetic analyses, we performed a partition homogeneity test in PAUP* v.4.0b10 (Swofford, 2002) to examine if the three individual data sets were heterogeneous with regard to phylogenetic signal. We could not reject the null hypothesis that the data were homogeneous ($P > 0.01$), and therefore all the results presented were based on analyses of the combined data set.

We used maximum-likelihood (ML) and Bayesian approaches to analyse the data. We used the objective criteria provided by the computer program MODELTEST v.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*.

We used the computer program MRBAYES (v.3.0b4; 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 getting trapped in local optima. We ran our analysis for a total of 500 000 generations and sampled the chain every 100 generations, resulting in 5000 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 4500 trees to estimate Bayesian posterior probabilities.

We used parsimony bootstrap values and Bayesian posterior probabilities to access branch support. It is computationally difficult and sometimes impossible to perform non-parametric bootstrap tests on large data sets with ML. Therefore, we performed a weighted parsimony bootstrap with the transition/transversion ratio of 4:1 (1000 bootstrap pseudoreplicates). This value approximates the empirical ratio generated from ML and Bayesian analyses. In addition to this, Bayesian analysis provided posterior probabilities for branches. The use of posterior probabilities to access branch support is still rather new (Holder & Lewis, 2003) and some issues have been raised with regard to how they compare to bootstrap values (Suzuki, Glasko & Nei, 2002; Alfaro, Zoller & Lutzoni, 2003; Douady *et al*., 2003). Nevertheless, they serve as an additional source of information on branch support and may represent a better estimate of phylogenetic accuracy (Wilcox *et al*., 2002; Reeder, 2003). As a rough guide, we consider branches supported by bootstrap values ≥70% (Hillis & Bull, 1993) and posterior probability values ≥95% (Wilcox *et al*., 2002) to be significantly supported by our data.

Hypothesis testing

We tested the significance of log-likelihood differences between our optimal ML/Bayesian tree (using the ML–ln L) and topologies representing various alternative hypotheses with the Shimodaira–Hasegawa test in PAUP* (Shimodaira & Hasegawa, 1999; see also Goldman, Anderson & Rodrigo, 2000) using full optimization and 1000 replicates. We tested a range of hypotheses regarding the systematics and biogeographical patterns of the *E. whitii* species group.

1. *Phylogenetic affinities of E. pulchra.* Recent speculation has indicated that *E. pulchra* may be the most ancestral member of the *E. whitii* species group (G. Shea, unpubl. data). The keeled scales of *E. pulchra* and its elongate skull suggests that it is either a member of the rock-dwelling subgroup or a sister taxon to the remainder of the *E. whitii* species group (Wilson & Knowles, 1988; G. Shea, unpubl, data; M. Hutchinson, pers, comm.,). We tested the predictions that *E. pulchra* is a member of the rock-dwelling subgroup or a sister taxon to the remainder of the species group.
2. *Taxonomic status of the New South Wales population of E. margaretae. Egernia margaretae* currently consists of three disjunct populations each of uncertain taxonomic status. Two of the populations are currently listed as subspecies (*E. m. margaretae*, Northern Territory/northern South Australia; *E. m. personata*, Flinders Ranges, South Australia), while the phylogenetic affinities of the third population (Mutawinjti NP, New South Wales) are uncertain (Storr, 1968; Foster, 1993; Swan & Foster, 2000). The validity of the two subspecies often has been questioned (Wilson & Knowles, 1988; Ehmann, 1992; G. Shea, unpubl. data) and Storr (1968) conceded that each might warrant species status when describing this taxon. In addition, the inability to definitively identify the New South Wales population as either of the two subspecies suggests that it may represent a new subspecies or a distinct species (NSW Recovery Plan, 2000). In order to resolve the taxonomic status of this disjunct population in New South Wales, we tested whether it is part of the *E. m. personata* or *E. m. margaretae* subspecies.
3. *Proposed phylogenetic relationships between the members of the species group.* We tested whether several of the previously proposed phylogenetic relationships within the species group were consistent with the phylogeny of the *E. whitii* species group recovered in the present study. We examined the hypothesis of Henzell (1972, 1982) that *E. m. margaretae* was a central Australian relict population that shared a common ancestor with *E. m. personata* by testing an alternative tree topology where both taxa represented sister species. Horton (1972) proposed several phylogenetic relationships between members of the species group that were not consistent with previous and subsequent authors (Fig. 1). We tested several aspects of his alternative topology, specifically the proposed close affinities between *E. inornata* and *E. margaretae*, *E. whitii* and *E. modesta*, *E. whitii* and *E. striata*, *E. whitii* and *E. multiscutata*, and *E. whitii* and *E. kintorei*.

RESULTS

The edited alignment comprised 1913 characters, of which 1844 were available for analyses after exclusion of several small unalignable regions in the 16S rRNA and BF data sets (696 ND4/tRNAs; 500 16S; 648 BF). Of the 1844 characters, 551 (30%) were variable (357 ND4/tRNAs; 115 16S rRNA; 79 BF) and of these vari-

Table 3. Jukes–Cantor (1969) distance matrix for taxa used in phylogenetic analyses (see Fig. 2)

		1	2	3	4	5	6	7	8	9	10
1	*Eulamprus heatwolei*	–									
2	*Egernia saxatilis* (EO2)	0.136	–								
3	*E. major* (EO1)	0.143	0.077	–							
4	*E. inornata* (EI20)	0.132	0.095	0.100	–						
5	*E. inornata* (EI55)	0.133	0.097	0.094	0.026	–					
6	*E. pulchra longicauda* (EO3)	0.143	0.095	0.091	0.078	0.077	–				
7	*Egernia* spp. (EO4)	0.147	0.091	0.094	0.120	0.109	0.111	–			
8	*E. kintorei* (EO6)	0.137	0.096	0.094	0.056	0.055	0.073	0.109	–		
9	*E. striata* (ES9)	0.141	0.098	0.100	0.057	0.057	0.078	0.116	0.043	–	
10	*E. montana* (EW81)	0.143	0.100	0.091	0.086	0.082	0.093	0.114	0.087	0.088	–
11	*E. guthega* (EW87)	0.132	0.088	0.091	0.081	0.081	0.089	0.107	0.087	0.084	0.074
12	*E. multiscutata bos* (EU23)	0.137	0.099	0.097	0.060	0.058	0.085	0.120	0.061	0.070	0.083
13	*E. m. multiscutata* (EU19)	0.141	0.092	0.100	0.067	0.061	0.087	0.115	0.066	0.073	0.088
14	*E. margaretae* NSW (EA16)	0.138	0.099	0.098	0.089	0.079	0.093	0.115	0.086	0.084	0.081
15	*E. margaretae margaretae* (EA4)	0.142	0.100	0.104	0.091	0.088	0.094	0.106	0.095	0.092	0.092
16	*E. m. personata* (EA7)	0.141	0.102	0.097	0.086	0.084	0.096	0.109	0.084	0.088	0.078
17	*E. m. personata* (EA1)	0.141	0.105	0.100	0.085	0.083	0.098	0.110	0.086	0.088	0.078
18	*E. m. margaretae* (EA3)	0.143	0.107	0.107	0.086	0.081	0.091	0.108	0.087	0.090	0.093
19	*E. whitii* (EW12)	0.144	0.101	0.101	0.084	0.081	0.093	0.118	0.085	0.085	0.077
20	*E. whitii* (EW58)	0.141	0.097	0.097	0.086	0.088	0.089	0.121	0.085	0.085	0.082
21	*E. whitii* (EW5)	0.145	0.107	0.103	0.088	0.083	0.099	0.116	0.088	0.088	0.077
22	*E. whitii* (EW32)	0.143	0.098	0.089	0.085	0.083	0.089	0.107	0.082	0.086	0.076
23	*E. whitii* (EW31)	0.139	0.091	0.095	0.088	0.081	0.092	0.113	0.086	0.086	0.080
24	*E. whitii* (EW18)	0.140	0.105	0.104	0.085	0.080	0.096	0.113	0.090	0.085	0.077
25	*E. modesta* (EM4)	0.151	0.114	0.109	0.096	0.090	0.100	0.129	0.096	0.094	0.097

able sites, 382 (69%) were informative under parsimony (282 ND4/tRNAs; 63 16S rRNA; 37 BF). Within the ingroup only, 461 characters were variable, of which 335 were informative under parsimony. Jukes & Cantor, 1969) genetic distances are presented in Table 3.

Both the hLRT and 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 and gave a –ln L = 10137.9346. The estimated parameters were as follows: nucleotide frequencies A = 0.3277, C = 0.2632, G = 0.1542, T = 0.2549; substitution rates A↔C 1.9926, A↔G 14.2298, A↔T 2.2796, C↔G 0.6186, C↔T 14.2298, G↔T 1.0000; proportion of invariant sites = 0.3931; gamma shape parameter = 0.2854. The Bayesian analysis produced parameter estimates that were very similar to those produced by MODELTEST.

The ML analysis in PAUP* using the above parameters and the Bayesian analysis both yielded the same single optimal tree (ML -ln L = 10337.07258, Bayesian –ln L was lower at 10358.1; Fig. 2). Bootstrap values and posterior probabilities were generally very high for most nodes, particularly the more terminal nodes. The parsimony topology differed only slightly from the ML and Bayesian tree and only at branches that were poorly supported by all analyses, so we base our discussion on Figure 2. There is strong support for two major clades within the *E. whitii* species group that correspond to the rock-dwellers (posterior probability 98%; *E. whitii*, *E. montana*, *E. guthega*, *E. m. personata*, *E. m. margaretae* and *E. modesta*) and obligate burrowers (bootstrap 82%, posterior probability 100%; *E. inornata*, *E. multiscutata*, *E. kintorei* and *E. striata*). The genetic distances separating members of rock-dwelling and obligate burrowing subgroups range from 7.9% to 10.1% (Table 3). However, although there was weak support (80% posterior probability) for *E. pulchra* being closely related to the obligate burrowing species, we were unable to rule out the possibility that it was part of the rock-dwelling clade or a sister species to the remainder of the species group (Table 4).

Although the rock-dwelling species (except for *E. pulchra*) were found to form a single clade within the species group, the phylogenetic relationships between the five species were not clearly resolved. The close relationship between the alpine species *E. guthega* and *E. montana* is confirmed with a posterior probability of 97% (genetic distance 7.4%) and the data confirm their distinctiveness from *E. whitii* (genetic distances 7.6–8.8%). There is strong sup-

11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
–														
0.080	–													
0.082	0.030	–												
0.085	0.093	0.096	–											
0.086	0.095	0.093	0.092	–										
0.079	0.086	0.092	0.082	0.094	–									
0.083	0.085	0.094	0.083	0.099	0.016	–								
0.090	0.090	0.094	0.094	0.074	0.091	0.098	–							
0.081	0.085	0.085	0.065	0.094	0.082	0.085	0.095	–						
0.083	0.084	0.087	0.039	0.099	0.079	0.082	0.099	0.055	–					
0.081	0.086	0.090	0.059	0.101	0.080	0.080	0.096	0.031	0.061	–				
0.078	0.083	0.090	0.043	0.095	0.082	0.084	0.091	0.065	0.037	0.061	–			
0.077	0.088	0.091	0.032	0.088	0.081	0.085	0.091	0.058	0.039	0.058	0.039	–		
0.082	0.085	0.083	0.063	0.094	0.082	0.081	0.091	0.032	0.062	0.032	0.065	0.062	–	
0.091	0.098	0.101	0.098	0.096	0.087	0.090	0.095	0.099	0.098	0.101	0.101	0.098	0.096	–

port for a subdivision (bootstrap and posterior probability values both 100%) within *E. whitii*, with a 'northern' subgroup (NSW populations) and a 'southern' subgroup (Victorian and South Australian populations). The genetic distances separating the 'northern' and 'southern' populations ranged from 5.5% to 6.5% (Table 3). The New South Wales population of *E. margaretae* formed part of the 'southern' subgroup of *E. whitii*, indicating that this disjunct population should be considered part of *E. whitii*. The hypotheses that the NSW population of *E. margaretae* had close phylogenetic affinities with either *E. m. margaretae* or *E. m. personata* were both rejected (Table 4; genetic distance 8.2–8.3%). There is no support for the conspecific status of the two subspecies of *E. margaretae* (genetic distances 9.1–9.9%), with both *E. m. personata* (bootstrap and posterior probability values both 100%) and *E. m. margaretae* (bootstrap value 98%, posterior probability 100%) forming well-supported clades. However, *E. m. margaretae* does appear to have a close phylogenetic affinity with *E. modesta*, supported by a posterior probability of 100%, although the two species are separated by genetic distances around 9.1–9.9%.

The species status of the four species within the obligate burrowing clade is supported by high bootstrap values and posterior probabilities, although the phylogenetic relationships between each of the four species remains poorly resolved. However, the close relationship between *E. kintorei* and *E. striata* is confirmed with a bootstrap value of 95%, a posterior probability of 100% and a genetic distance of only 4.3%.

Although our molecular data suggest that *E. m. margaretae* and *E. m. personata* should be considered distinct species, we were unable to reject the hypothesis of Henzell (1972, 1982) that these species represent sister taxa (Table 4). The majority of the alternative topologies proposed by Horton (1972) were strongly rejected (Table 4). However, Horton's (1972) suggested close phylogenetic affinity between *E. whitii* and *E. modesta* could not be rejected (Table 4).

DISCUSSION

We have produced the first detailed and well-supported phylogeny for the *Egernia whitii* species group. Our phylogeny has several important taxonomic and biogeographical implications and we have utilized it to

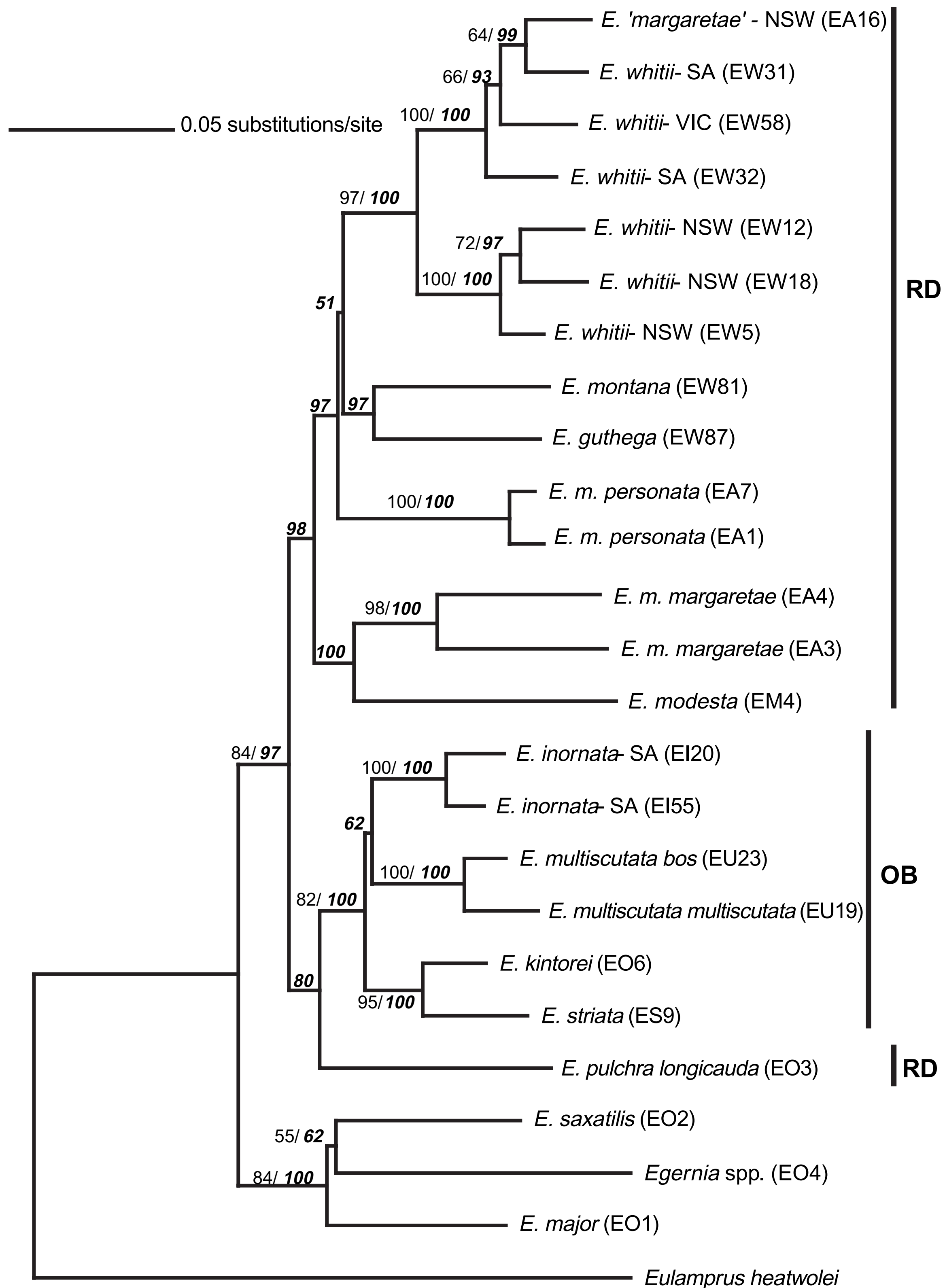

Figure 2. Maximum likelihood tree for the *Egernia whitii* species group, based on the combined ND4, 16S rRNA and β-fibrinogen data sets (1844 bp). Parsimony bootstrap values are shown in plain-text and Bayesian posterior probabilities are shown in bold. Rock-dwelling (RD) and obligate burrowing (OB) species are identified in the phylogeny.

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Table 4. Results of Shimodaira–Hasegawa tests of alternative sister group relationships and topologies proposed by Henzell (1972, 1982) and Horton (1972) (see Fig. 1). A significant *P*-value (<0.05) indicates that the alternative topology is significantly different from the maximum likelihood (ML) tree

Alternative topology	–ln L	Difference in –ln L	*P*-value
Optimal tree	10337.07		
E. pulchra + rock-dwelling subgroup	10343.73	6.66	0.817
E. pulchra + remainder of the *E. whitii* species group	10338.58	1.51	0.924
NSW *E. margaretae* + *E. m. personata*	10448.27	111.20	0.001
NSW *E. margaretae* + *E. m. margaretae*	10469.53	132.46	<0.001
E. m. margaretae + *E. m. personata*	10350.40	13.33	0.612
E. m. margaretae + *E. inornata*	10424.99	87.92	0.001
E. whitii + *E. modesta*	10348.71	11.64	0.654
E. whitii + *E. striata*	10436.09	99.02	<0.001
E. whitii + *E. multiscutata*	10404.65	67.57	0.008
E. whitii + *E. kintorei*	10436.23	99.16	<0.001

examine a number of phylogenetic, taxonomic and biogeographical hypotheses. Because many of the taxonomic questions also relate to the biogeography of the *E. whitii* species group, we outline the taxonomic implications of our phylogeny first, before discussing our biogeographical hypotheses.

Taxonomic implications

The major result of our analysis is the strong support for two distinct clades within the *E. whitii* species group. The rock-dwelling subgroup (*E. whitii*, *E. montana*, *E. guthega*, *E. m. margaretae*, *E. m. personata* and *E. modesta*) and the obligate burrowing subgroup (*E. inornata*, *E. striata*, *E. kintorei* and *E. multiscutata*) form well-supported monophyletic clades within the species group. The molecular phylogenetic work of S. Donnellan & M. Hutchinson (unpubl. data), although only considering selective representative taxa within the species group, also found support for the rock-dwelling–obligate burrowing dichotomy within the species group. Although not included in our study, *E. slateri* is presumably a member of the obligate burrowing subgroup. Despite the strong support for two major clades within the species group, we were unable to determine the phylogenetic affinities of *E. pulchra*. Morphological evidence appears to favour its recognition as a sister species to the remainder of the species group or as part of the rock-dwelling group (Wilson & Knowles, 1988; G. Shea, unpubl. data; M. Hutchinson, pers. comm.).

The broad division of the species group into rock-dwelling and obligate burrowing lineages, sometimes termed the *E. whitii* and *E. inornata* groups, has been a consistent feature in many of the proposed phylogenies for the group (Storr, 1968; Henzell, 1972, 1982; Horton, 1972; Ehmann, 1992; Chapple, 2003; Fig. 1). Indeed, species within each subgroup share numerous morphological, behavioural and ecological traits, which are divergent from the other subgroup (Chapple, 2003). Rock-dwelling species tend to occur in more mesic or coastal environments and display diurnal activity patterns (Henzell, 1972, 1982; Chapple, 2003). In contrast, the obligate burrowing species generally inhabit arid or semiarid environments and are either crepuscular or nocturnal (Henzell, 1972, 1982; Chapple, 2003). Members of the obligate burrowing group exhibit several adaptations for living in arid environments such as reduced levels of evaporative water loss and reduced exposure (i.e. utilization of burrow, crepuscularity, nocturnality; Henzell, 1972, 1982), while *E. striata* and *E. kintorei* have evolved elliptical eyes characteristic of nocturnal species (Chapple, 2003). Because the arid-adapted members of the species group form a monophyletic clade, it appears to indicate that such adaptations to aridity arose only once within the group rather than evolving independently on multiple occasions. The close affinity between the temperate to semiarid *E. multiscutata* (Henzell, 1972, 1982) and the more arid-adapted members of the obligate burrowing clade may indicate that the arid zone was colonized by a temperate or semiarid *E. multiscutata*-like ancestor.

The phylogenetic relationships between *E. pulchra* and the other members of the *E. whitii* species group remain unresolved. *Egernia pulchra*, a rock-dwelling species (Chapple, 2003), is part of the obligate burrowing subgroup. Tests of alternative topologies were unable to rule out the possibility that *E. pulchra* may be part of rock-dwelling subgroup or a sister taxon to the remainder of the *E. whitii* species group (Table 4). Morphological evidence suggests that *E. pulchra* is the most ancestral member of the species group, perhaps representing a sister taxon to the group. Indeed,

the keeled scales of *E. pulchra* and its elongate skull are supportive of it representing a sister taxon (Wilson & Knowles, 1988; G. Shea, unpubl. data; M. Hutchinson, pers. comm.). Alternatively, *E. pulchra* also shares numerous characteristics with members of the rock-dwelling subgroup and may be part of this subgrouping. However, it is clear that *E. pulchra* is closely aligned with the *E. whitii* species group.

It is clear from our topology that the New South Wales population of '*E. margaretae*' does not form a monophyletic group with either subspecies. This population is restricted to about seven individuals that have been observed over four consecutive years within a single gorge in Mutawintji National Park in the west of the state (Foster, 1993; NSW Recovery Plan, 2000; Swan & Foster, 2000). The population is disjunct from the distributions of all members of the rock-dwelling subgroup, and the failure of specimens to key out as either subspecies of *E. margaretae* lead to its uncertain taxonomic status (NSW Recovery Plan, 2000). The population was initially identified tentatively as *E. m. personata* on the basis of a photograph of a single individual (Foster, 1993). After the examination of two voucher specimens and subsequent fieldwork the population was believed to be *E. margaretae* (Swan & Foster, 2000). We were able to strongly reject the suggestion that the NSW population of '*E. margaretae*' belonged to either *E. m. margaretae* or *E. m. personata*. Rather, our data suggest that this disjunct population is part of *E. whitii*. More specifically, the NSW '*E. margaretae*' groups out as part of the southern clade of *E. whitii* because it is most closely related to populations from South Australia and Victoria.

The validity of the two subspecies of *E. margaretae*, *E. m. margaretae* (Northern Territory/northern South Australia) and *E. m. personata* (Flinders Ranges, South Australia), has long been questioned (Wilson & Knowles, 1988; Ehmann, 1992; G. Shea, unpubl. data). Indeed, in describing the subspecies Storr (1968) conceded that each might warrant specific status because several shared characteristics could have evolved independently in response to similar environments (e.g. rocky habitats in semiarid mountain ranges). Although both subspecies exhibit a close phylogenetic affinity (Fig. 2, Table 4), our analyses clearly indicate that *E. m. margaretae* and *E. m. personata* should be considered separate species.

Although we were unable to assess the validity of the two recognized subspecies within *E. whitii*, as a consequence of the lack of samples from *E. w. moniligera*, our analyses identified a significant and well-supported phylogeographical break within *E. whitii*. The phylogenetic subdivision occurs in eastern Victoria; however, a large sampling gap in this region in the present study precludes extensive comment on the significance of this phylogeographical structuring. Interestingly, Donnellan *et al.* (2002) found similar evidence for differentiation between the northern (NSW, ACT) and southern (Victorian, South Australian) populations of *E. whitii* in allozyme markers, although a similar sampling gap in eastern Victoria made it difficult to assess the significance of this divergence. We discuss elsewhere the results of more intensive sampling on either side of the eastern Victoria phylogeographical break and the biogeographical implications (D. Chapple, S. Keogh & M. Hutchinson, unpubl. data).

Historically, the majority of the members of the rock-dwelling subgroup were considered to be part of the *E. whitii* superspecies (e.g. Storr, 1968). However, detailed taxonomic work in the last few decades has resulted in the separation and description of several taxa previously regarded as *E. whitii*. Our analyses confirm the validity of *E. modesta* (Horton, 1972; Milton *et al.*, 1983) and the alpine species *E. guthega* and *E. montana* (Donnellan *et al.*, 2002) as distinct species. The phylogenetic relationships proposed by Donnellan *et al.* (2002) for the temperate species within south-eastern Australia are congruent with the topology in our study (Figs 1, 2). Likewise, the close phylogenetic affinity found between the desert burrowing species *E. inornata*, *E. striata* and *E. inornata* supports the previous suggestions of Storr (1968) and Pianka (1972). However, all sister group relationships postulated by Horton (1972) were strongly rejected by our data, except for the proposed close affinity between *E. modesta* and *E. whitii* (Fig. 1; Table 4).

The composition of the *E. whitii* species group, as originally conceived by Storr (1968), has been substantially modified during the last few decades. Although numerous taxonomic issues were resolved in the present study, several additional issues remain unresolved. The taxonomic status of the subspecies within *E. whitii*, *E. multiscutata*, *E. pulchra* and *E. slateri* remains problematic (Storr, 1968; Donnellan *et al.,* 2002). Likewise, the phylogenetic affinities of *E. slateri* and an isolated population of *E. modesta* in western New South Wales (Wilson & Swan, 2003) remains unresolved. However, future molecular phylogenetic work should enable resolution of many of these taxonomic issues.

Biogeographical implications

The belief that increasing aridity in Australia combined with Pleistocene glacial–interglacial cycles resulted in the development of two distinct faunas, an arid zone fauna and one in the more moist peripheral areas of the continent, has been an enduring biogeographical theme (Cracraft, 1991). This arid zone–peripheral zone dichotomy has been invoked for rep-

tiles (Cogger & Heatwole, 1981, 1984), birds (Schodde, 1982) and mammals (Hope, 1982). Although numerous vertebrate taxa are restricted to the arid interior, and while many other groups are confined to the moist peripheral areas of Australia, there is also evidence for vicariance events and subsequent differentiation within each region (Cracraft, 1991). For example, there is little support for a single arid zone fauna; rather the interior of the continent comprises numerous biogeographical regions and divergent faunas that are closely aligned with particular vegetation types (Keast, 1981; Pianka, 1981, 1984). Although the dichotomy may oversimplify the complexity of the biogeographical patterns observed in Australia (Cracraft, 1991), the distinction between arid zone and temperate or peripheral taxa appears to be a real phenomenon in many vertebrate groups (Cogger & Heatwole, 1981, 1984; Hope, 1982; Schodde, 1982).

Explanations for how vertebrate taxa differentiated into separate temperate and arid zone faunas have been proposed by several authors (e.g. Hope, 1982; Schodde, 1982), although the proposed mechanisms (i.e. aridity, glaciation cycles) and timing of faunal partitioning remain controversial (Cracraft, 1991). Our analysis strongly supports an arid zone–temperate zone dichotomy within the *E. whitii* species group. The genetic distance between the arid-adapted and temperate-adapted members of the *E. whitii* species group is 7.9–10.1%. Using a rough mitochondrial calibration of 1.3–2% sequence divergence per million years (Brown, George & Wilson, 1979; Wilson *et al*., 1985; Zamudio & Greene, 1997), this disparity suggests that the two clades within the species group separated during the late Miocene to early Pliocene, approximately 4–8 Mya. Indeed, the level of divergence (7.9–10.1%) alone is fairly strong evidence for a pre-Pleistocene origin of this species group. Although this estimated time of divergence predates the putative Pleistocene adaptive radiation for this group (e.g. Horton, 1972), it is supported by fossil evidence indicating that several extant species of *Egernia* (e.g. *E. frerei*, *E. hosmeri*, *E. major*, *E. striolata* group), and the closely related genus *Tiliqua*, had already differentiated by the early Miocene to early Pliocene (Hutchinson, 1992; Shea & Hutchinson, 1992; Mackness & Hutchinson, 2000). Taken together, this evidence strongly suggests that the *Egernia* genus and the *E. whitii* species group represent taxa with long and complex evolutionary and biogeographical histories.

Recent studies of the Australian herpetofauna have indicated deep phylogeographical divergences in some taxa, suggesting that several groups represent late Tertiary (Miocene, Pliocene) radiations (McGuigan *et al*., 1998; James & Moritz, 2000; Schäuble & Moritz, 2001). A similar pattern is evident in *Egernia*. The genetic distances between species in the *E. whitii* species group range between 4.3 and 10.1% (Table 4), suggesting that the group was shaped predominantly as a result of pre-Pleistocene vicariance events. Even the 'northern' and 'southern' populations of *E. whitii* exhibit sequence divergence of 5.5–6.5%, indicating that the deep phylogenetic break occurred prior to the Pleistocene. Many members of the species group exhibit substantial sequence divergence, suggesting that the majority of the species evolved soon after the differentiation of the group into obligate burrowing and rock-dwelling forms. This may indicate that the group has been characterized by an initial period of rapid radiation, followed by an extended period of relatively minimal morphological and phylogenetic divergence. The short internodes and long terminal branches, coupled with the conserved morphological characteristics within the group (e.g. Storr, 1968; Milton *et al*., 1983; Donnellan *et al*., 2002), provide support for this evolutionary scenario.

Several biogeographical mechanisms may be responsible for the distinct arid- and temperate-adapted lineages within the *E. whitii* species group. Horton (1972) proposed that *E. whitii* and *E. inornata* originated as a species pair on either side of the GDR, which subsequently differentiated into arid- and temperate-adapted forms during the Pleistocene aridity cycles. Although the vicariance event appears to be pre-Pleistocene in age, the separation of the two clades is roughly concordant with a major period of uplift in the GDR during the middle–late Miocene (Keast, 1981; Taylor, 1994). However, it is unclear whether such geological activity, presumably combined with a period of extensive aridity, resulted in the observed divergence between the arid and temperate clades. Interestingly, the split between *E. whitii* and the recently described *E. montana* and *E. guthega*, which are confined to the alpine regions of the GDR in south-eastern Australia, represents a more recent origin in the early Pliocene (genetic distance 7.6–8.8%).

The transition in vegetation from wet forests towards more open, and arid-adapted, grasslands and woodlands during the early Pliocene (Bowler, 1982; Truswell & Harris, 1982; Markgraf *et al*., 1995) is another possible selective force responsible for the differentiation of the *E. whitii* species group. Indeed, the early Pliocene represents a critical period for the origin of arid-adapted mammals in central Australia and the restriction of wetter forest taxa to the periphery of the continent, suggesting substantial floral modification during this time (Hope, 1982). Ecological and climatic factors appear to strongly influence the distribution of species within the *E. whitii* species group (Henzell, 1972, 1982). Henzell (1972, 1982) demonstrated that the capacity for members of the species group to adapt to aridity is constrained by their inability to restrict the rate of evaporative water loss below

a certain threshold and limit exposure to dehydrating conditions. Because both evaporative water loss and exposure patterns can differ markedly between temperate and semiarid populations of a single species (Henzell, 1972, 1982), a period of rapidly increasing aridity may have resulted in a vicariance event separating the *E. whitii* group ancestor into arid and temperate adapted forms which eventually differentiated into the obligate burrowing and rock-dwelling subgroupings, respectively.

As a result of the cyclic expansion and contraction of arid zone and more mesic habitats during the Pleistocene, arid zone faunas are generally believed to have arisen via repeated and independent colonizations from more peripheral regions of diversity (Cracraft, 1991). It is clear from our topology that the arid zone members of the *E. whitii* group arose from a single colonization by a presumably temperate-adapted ancestor. This finding plainly contradicts the hypothesis of Horton (1972) that the arid zone species within the *E. whitii* species group were the result of multiple colonizations of interior regions of the continent by temperate species during periods of reduced aridity. Our analysis implies that the members of the obligate burrowing clade are monophyletic and arose as a result of vicariance events within the arid interior itself (e.g. Pianka, 1972). Given the apparent long and complex evolutionary and biogeographical history of the *E. whitii* species group, it is surprising that arid-adapted taxa have evolved only once within the group. However, those adaptations required to successfully colonize the dry regions of the continent appear to have evolved infrequently within the group even in the presence of a relevant selective force.

Several temperate-adapted species of *Egernia* persist within the arid zone in more humid and wet refugia, usually in mountain ranges, that offer relief from the dry conditions of the interior (Henzell, 1972, 1982; Pianka, 1972). Perhaps the mostly widely known refugial area within arid Australia is the Central Ranges, where numerous species of vertebrate persist in relic populations (Pianka, 1972; Keast, 1981; Cracraft, 1991). The distribution of *Egernia m. margaretae* is restricted to the Central Ranges region (Cogger, 2000). Likewise, *E. m. personata* is restricted to rocky and moist habitats within the semiarid Flinders Ranges of South Australia (Cogger, 2000). *Egernia whitii* (Mutawintji NP) and *E. modesta* (Yathong NR) also have disjunct populations in more mesic rocky habitats in arid western New South Wales (Swan & Foster, 2000; Wilson & Swan, 2003). Henzell (1972, 1982) demonstrated that all four species are temperate-adapted and appear to persist within the less arid mountainous or rocky regions of the dry interior. The presence of such relict populations suggests that each species was once more widely distributed before becoming restricted to more mesic areas during periods of aridity. Each species appears to have split from their nearest relative during the Pliocene–early Pleistocene, with substantial genetic distances between *E. m. margaretae* and *E. m. personata* (9.1–9.9%), and between the Mutawintji *E. whitii* and the remainder of the southern *E. whitii* clade (3.2–4.3%). It appears that aridity may have increased too rapidly for these species to have evolved adequate adaptations to the drier conditions (Henzell, 1982), although it remains unclear why such adaptations have not arisen during their long evolutionary history within these refugial habitats. It has been suggested previously that *E. whitii* and *E. modesta*, which occur sympatrically in parts of their distribution, are closely related sister taxa (Storr, 1968; Horton, 1972; Milton *et al*., 1983). However, our data indicate that these two species are more distantly related than previously believed (genetic distance 9.6–10.1%). The isolated population of *E. modesta* in western New South Wales and the close affinity of *E. modesta* with the arid zone relic *E. m. margaretae* supports Horton's (1972) proposal that this species evolved on the western side of the GDR before re-colonizing the east coast.

Our topology indicates that the semiarid, and obligate burrowing, *E. multiscutata* is a sister species to the three most arid-adapted species within the group (*E. inornata*, *E. striata*, *E. kintorei*) which are confined solely to the sandy deserts of the interior of the continent. Although *E. multiscutata* occurs predominantly in coastal areas and on offshore islands, its range includes numerous populations in semiarid to arid habitats (Cogger, 2000; Chapple, 2003). *Egernia multiscutata* appears to be morphologically and geographically intermediate between the members of the obligate burrowing group and the rock-dwelling group (G. Shea, unpubl. data), suggesting that the arid zone members of the group originated from an *E. multiscutata*-like ancestor. Although it seems plausible that a semiarid species such as *E. multiscutata* could have given rise to the arid-adapted members of the species group, it is equally plausible that *E. multiscutata* represents a secondarily derived temperate species evolved from an arid-adapted ancestor.

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