Substantial genetic substructuring in southeastern and alpine Australia revealed by molecular phylogeography of the *Egernia whitii* (Lacertilia: Scincidae) species group

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Abstract

Palaeoclimatic events and biogeographical processes since the mid-Tertiary are believed to have strongly influenced the evolution and distribution of the terrestrial vertebrate fauna of southeastern Australia. We examined the phylogeography of the temperate-adapted members of the *Egernia whitii* species group, a group of skinks that comprise both widespread low- to mid-elevation (*E. whitii*) and montane-restricted species (*Egernia guthega*, *Egernia montana*), in order to obtain important insights into the influence of past biogeographical processes on the herpetofauna of southeastern Australia. Sequence data were obtained from all six temperate-adapted species within the *E. whitii* species group, and specifically from across the distributional ranges of *E. whitii*, *E. guthega* and *E. montana*. We targeted a fragment of the *ND4* mitochondrial gene (696 bp) and analysed the data using maximum likelihood and Bayesian methods. Our data reveal a deep phylogeographical break in the east Gippsland region of Victoria between 'northern' (Queensland, New South Wales, Australian Capital Territory) and 'southern' (Victoria, Tasmania, South Australia) populations of *E. whitii*. This divergence appears to have occurred during the late Miocene–Pliocene, with the Gippsland basin possibly forming a geographical barrier to dispersal. Substantial structuring within both the 'northern' and the 'southern' clades is consistent with the effects of Plio–Pleistocene glacial-interglacial cycles. Pleistocene glacial cycles also appear to have shaped the phylogeographical patterns observed in the alpine species, *E. guthega* and *E. montana*. We used our results to examine the biogeographical process that led to the origin and subsequent diversification of the lowland and alpine herpetofauna of southeastern Australia.

Keywords: biogeography, Gippsland Basin, glaciation, lizard, mtDNA, *ND4*

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Introduction

The evolution and distribution of the terrestrial vertebrate fauna in southeastern Australia has been strongly influenced by several palaeoclimatic events and by the biogeographical processes that have prevailed since the late Tertiary (Hope 1982; Frakes *et al*. 1987; Cracraft 1991; Markgraf *et al*. 1995). Geological evidence indicates that the Miocene–Pliocene boundary (5 Ma) marked the commencement of a substantial transition in the vegetation of southeastern Australia, with the previously widespread forests and rainforests being replaced with more open vegetation and sclerophyllous woodlands comprising a mosaic of *Eucalyptus* and *Acacia* by the end of the Pliocene (2 Ma) (Bowler 1982; Markgraf *et al*. 1995; Gallagher *et al*. 2001, 2003). During this period, the climate fluctuated between warm-wet and cool-dry, although there was an overall cooling-drying trend evident during the Pliocene (Bowler 1982; Frakes *et al*. 1987; Gallagher *et al*. 2003). Such biogeographical processes presumably imposed strong selective forces on the evolutionary histories of the resident temperate-adapted vertebrate fauna (Markgraf *et al*. 1995). Evidence of marine incursions in several regions along the southern Australian coastal line (Bowler 1982; Frakes *et al*. 1987) and regional tectonic

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uplift (Dickinson *et al*. 2002) may have contributed to habitat fragmentation during this period, promoting divergence between isolated populations.

During the late Pliocene to early Pleistocene (0.7–2 Ma), climatic oscillations intensified resulting in rapid fluctuation between cool-dry and warm-wet conditions across southeastern Australia (Bowler 1982; Frakes *et al*. 1987; Markgraf *et al*. 1995). At least 20 cycles are believed to have occurred in southeastern Australia during the Quaternary, at approximately 100 000 years rotations, although both the frequency and amplitude of these glacial-interglacial cycles increased during the late Pleistocene (0.7–0.01 Ma; Bowler 1982; Frakes *et al*. 1987; Markgraf *et al*. 1995). Drastic fluctuations in sea level during this time resulted in the periodic flooding of coastal and inland basins (e.g. Murray basin, Gippsland basin) across southern Australia (Frakes *et al*. 1987; Wilson & Allen 1987; Holdgate *et al*. 2003). Temperatures during glacial maxima were up to 9 °C cooler than at present (Hesse *et al*. 2004), with climatic oscillations driving the repeated expansion, contraction and fragmentation of temperate habitats in southeastern Australia (Bowler 1982; Markgraf *et al*. 1995).

Such environmental instability would have dramatically impacted upon the temperate-adapted vertebrate fauna, especially taxa within the montane regions in the southeast of the continent. Although full glacial conditions were evident only in the most elevated regions of the Great Dividing Range (GDR; Kosciusko region), periglacial conditions presided throughout most environments above 1000 m during Pleistocene glacial maxima (Frakes *et al*. 1987; Barrows *et al*. 2001). It is believed that the depression of alpine tree lines by as much as 1000 m during glacial periods led to the repeated extinctions of montane taxa, or their persistence in refugial habitats such as protected gullies and valleys until warmer conditions during interglacial periods enabled the tree line to revert to its initial elevation (Singh & Geissler 1985; Frakes *et al*. 1987; Green & Osborne 1994). The continual fragmentation and reconnection of montane vegetation during Quaternary glacial cycles has been shown to dramatically influence the evolutionary histories of many taxa (Hewitt 2000; Knowles 2000; Masta 2000).

Surprisingly little is known about the historical biogeography of the vertebrate fauna of southeastern Australia, despite the relatively detailed palaeoecological records available for this region. Most early authors believed that the biogeographical processes responsible for the evolution and diversification of the herpetofauna in eastern Australia occurred during the Pleistocene (e.g. Horton 1972, 1984; Cogger & Heatwole 1981). Recent molecular studies on the herpetofauna of eastern Australia have revealed deep phylogenetic splits both within and between taxa, indicative of late Tertiary (Miocene–Pliocene) divergences, although there are indications that biogeographical events during the Pleistocene have had an important role in shaping intraspecific phylogeographical patterns (McGuigan *et al*. 1998; Schneider *et al*. 1998; James & Moritz 2000; Schäuble & Moritz 2001; Stuart-Fox *et al*. 2001; Keogh *et al*. 2003). Few studies have examined the influence of late Tertiary and Pleistocene biogeographical events on the phylogeography of the montane and lowland herpetofauna of southeastern Australia. However, the temperate-adapted skinks of the *Egernia whitii* species group have the potential to provide important insights into the phylogeographical patterns in southeastern Australia, as it comprises both widespread low- to mid-elevation species and montane-restricted species.

The *E. whitii* species group comprises 12 species of medium-sized skinks (Chapple 2003; Wilson & Swan 2003) and represents a monophyletic lineage within the endemic Australian genus, *Egernia* (Donnellan *et al*., unpublished). Five species have distributions confined to the southeast of the continent (*E. whitii*, *Egernia guthega*, *Egernia montana*, *Egernia modesta*, *Egernia margaretae personata*), while *E. m. margaretae* is an arid-relic that has a range that is restricted to the more mesic areas in the central ranges of Australia (Wilson & Swan 2003; Fig. 1). All six species live in rocky microhabitats within open woodland, heathland and grasslands (Chapple 2003). These species are adapted to temperate habitats and appear to have a limited capacity to inhabit arid environments as a result of their inability to minimize their rate of evaporative water loss below a certain threshold or reduce their exposure to dehydrating conditions (Henzell 1972, 1982). It has been demonstrated that *E. whitii* is highly susceptible to habitat fragmentation (Mac Nally & Brown 2001; Jellinek *et al*. 2004), therefore it might be expected that the cyclic expansion and contraction of temperate vegetation in southeastern Australia would have profoundly influenced the evolution and historical distributions of these lizards. Chapple & Keogh (2004) recently suggested that the *E. whitii* species group originated in the Miocene, with the divergence of arid-adapted and temperate-adapted taxa within the group primarily occurring during the late Miocene to early Pliocene. Given that the *E. whitii* species group appears to have its origins in the late Tertiary, it enables examination of the impact of both the late Miocene–Pliocene and the Pleistocene climatic events on the phylogenetic history of these temperate-adapted species.

Here we examine the mitochondrial DNA (mtDNA) sequence (*ND4*) divergence of the temperate-adapted members of the *E. whitii* species group in southeastern Australia in order to investigate the effects of palaeoclimatic events on the phylogeography of the herpetofauna of this region. Using a larger data set comprising mtDNA and nuclear DNA sequence, Chapple & Keogh (2004) demonstrated that these species formed a single clade within the lineage and examined in detail the interspecies relationships, therefore the primary focus in the present

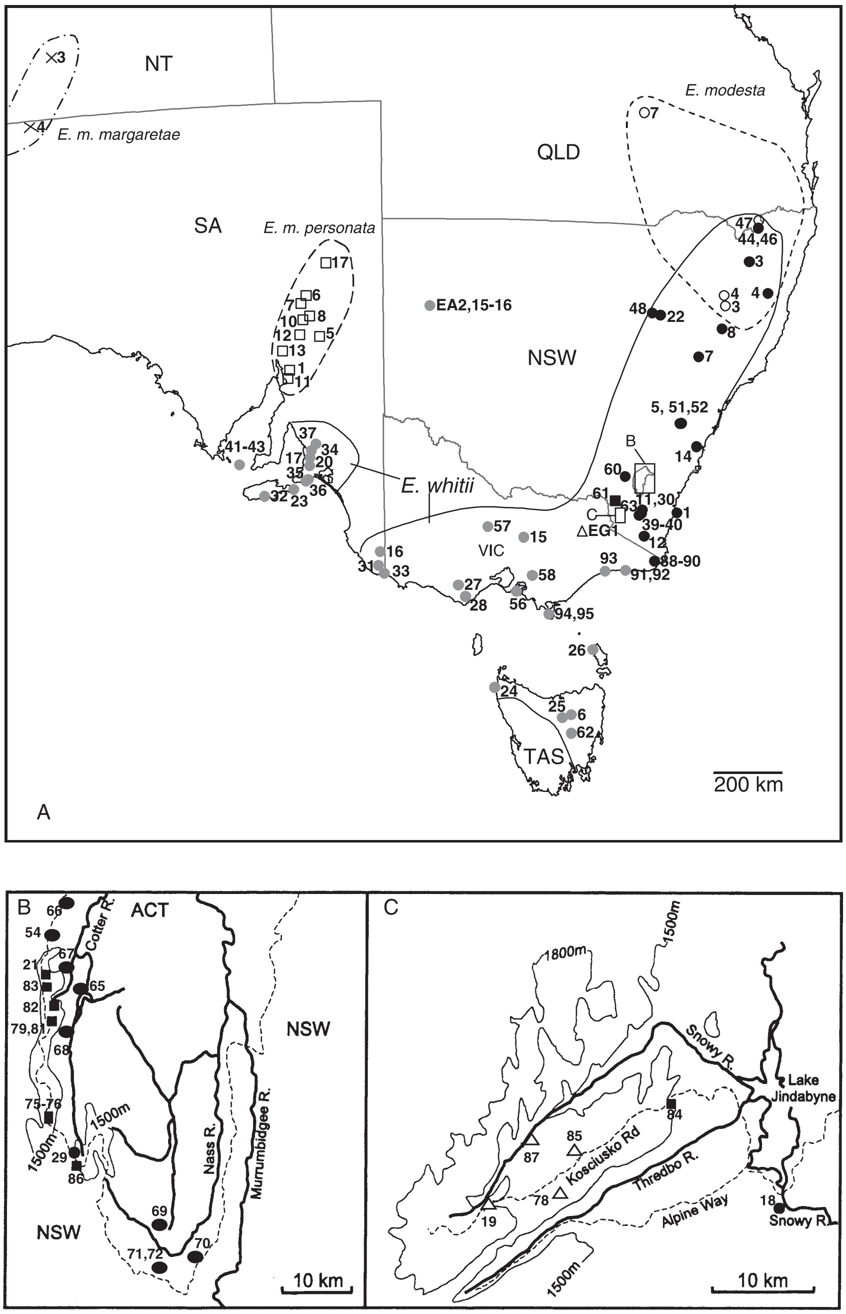

Fig. 1 Map showing the collecting localities of the tissue samples from the temperate-adapted members of the *Egernia whitii* species group presented in Appendix. (A) Locality of tissue samples for 'northern' *E. whitii* (●), 'southern' *E. whitii* (grey ●), *Egernia guthega* (△), *Egernia montana* (■), *Egernia modesta* (○), *E. margaretae margaretae* (+) and *E. m. personata* (□). (B) Inset for samples from the Australian Capital Territory. (C) Inset for samples from the Snowy Mountains region of New South Wales. Species distributions have been adapted from Wilson & Swan (2003). Note that the distribution of *E. guthega* is restricted to the Snowy Mountains region of Inset C and one locality in Victoria on the Bogong High Plains, while *E. montana* is restricted to the regions shown in Inset B & C and the alpine regions of Victoria (Victorian distribution not shown). Numbers refer to the tissue codes listed in Appendix. State codes are: NT, Northern Territory; SA, South Australia; QLD, Queensland; NSW, New South Wales; ACT, Australian Capital Territory; VIC, Victoria; TAS, Tasmania.

study is to examine intraspecific phylogeographical patterns. We focus specifically on the phylogeography of the widespread low- to mid-elevation *E. whitii* (sea level to 1600 m) and the montane specialists, *E. montana* (900–1800 m) and *E. guthega* (1600–1940 m) (Fig. 1). In particular, we conducted more fine-scale sampling of *E. whitii* populations in eastern Victoria, the region where Chapple & Keogh (2004) identified a substantial and well-supported phylogeographical break between 'northern' and 'southern' populations, in order to assess its biogeographical significance.

Materials and methods

Taxonomic sampling

We obtained tissue samples from all five temperate-adapted species within the *Egernia whitii* species group in southeastern Australia, as well as from *Egernia margaretae margaretae*, which occurs in central Australia (Appendix; Fig. 1). Recent evidence suggests that *E. m. margaretae* and *Egernia margaretae personata* are distinct species (Chapple & Keogh 2004), therefore we consider them separately here.

Likewise, Chapple & Keogh (2004) indicated that the disjunct New South Wales (NSW) population of '*Egernia margaretae*' is actually *E. whitii*, and we follow this designation in the present study. Samples were obtained primarily from museum frozen tissue collections, although several additional field-collection samples were included (Appendix). We obtained samples that encompassed the entire range of *E. whitii* (67 samples), *Egernia guthega* (five samples), *E. m. margaretae* (two samples) and *E. m. personata* (10 samples) (Appendix, Fig. 1). However, we were only able to obtain samples from portions of the distribution of *E. montana* (10 samples; entire range except Victoria) and *Egernia modesta* (four samples; limited sampling across its range) (Appendix, Fig. 1). All tissue samples of *E. whitii* were from the nominate form as no sample was available from the Sydney region subspecies *Egernia whitii moniligera* (Cogger 2000; Donnellan *et al*. 2002). Two *Egernia* species (*E. saxatilis* and *E. major*) were included as outgroups, as was the distantly related (see Reeder 2003) Australian sphenomorphus group skink, *Eulamprus heatwolei* (Appendix).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from liver, toe or tail samples using a modified hexadecyl-trimethyl-ammonium bromide (CTAB) protocol. For each sample, we targeted approximately 700 base pair (bp) DNA fragment of the mitochondrial genome, which included the 3′ half of the *ND4* gene and most of the tRNA cluster containing the histidine and serine tRNA genes. This region was targeted because work at comparable taxonomic levels in other squamate reptile groups has indicated useful levels of variability (Forstner *et al*. 1995; Scott & Keogh 2000; Keogh *et al*. 2003; Chapple *et al*. 2004).

The primers used to amplify this region were *EgND4(L)*, *EgtRNA-Ser(H)* (Chapple & Keogh 2004), *ND4* and *Leu* (Forstner *et al*. 1995). Sequencing was performed as described in Chapple & Keogh (2004). Sequence data were edited using SEQUENCHER version 3.0 (Genes Codes Coporation), and provisionally aligned using the default parameters of CLUSTAL_X (Thompson *et al*. 1997) and refined by eye. Aligned sequences were translated into amino acid sequences using the vertebrate mitochondrial genetic code. This was performed 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) and Bayesian approaches to analyse the data. We used the objective criteria provided by the computer program MODELTEST version 3.06 (Posada & Crandall 1998) with the hierarchical likelihood ratio test (hLRT) to select the most appropriate model of molecular evolution for our 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* (Swofford 2002).

We used the computer program MRBAYES (version 3.0b4; Huelsenbeck & Ronquist 2001) for our Bayesian analyses. Using the identical data set as our ML analyses, the general time reversible (GTR) plus gamma distribution plus 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 1 million generations and sampled the chain every 100 generations, resulting in 10 000 sampled trees. Log-likelihood values reached a plateau after approximately 100 000 generations (1000 sampled trees), so we discarded the first 2000 trees as the burn-in phase and used the last 8000 trees to estimate Bayesian posterior probabilities.

We used the bootstrap values and Bayesian posterior probabilities to assess branch support. Our data set was too large to do ML bootstraps so we performed a weighted parsimony bootstrap using the observed transitions/transversion (ti/tv) ratio of 4:1 with 1000 pseudoreplicates. Additional 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 *et al*. 2002; Alfaro *et al*. 2003; Douady *et al*. 2003), but 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 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.

To infer the approximate timing for several divergences, we used a rough calibration of 1.3–2% mitochondrial sequence divergence per million years (Myr). The lower limit was derived from Zamudio & Greene's (1997) estimate based on viper (*Lachesis muta*) mtDNA. The upper limit of 2% per Ma was based on Brown *et al*.'s (1979) molecular clock from primate mtDNA using a calibration point of 5 Ma. Although our mtDNA data set includes the *ND4* sequence data from the temperate-adapted species contained in Chapple & Keogh's (2004) study, the estimated divergence times may differ slightly in the present study as it is based solely on *ND4* sequence divergence rather than the combined genetic distances presented in Chapple & Keogh (2004).

Results

The edited alignment comprised 696 characters and of these, 360 (52%) were variable and of these variable sites, 305 (44%) were informative under parsimony. Within the ingroup only, 333 characters were variable of which 292 were informative under parsimony.

The hLRT from MODELTEST supported the GTR plus invariant sites (+I) plus gamma shape (+G) model as the best-fit substitution model for the data and gave a –ln L = 7594.3213. The estimated parameters were as follows: nucleotide frequencies A = 0.3590, C = 0.3311, G = 0.0971, T = 0.2129; substitution rates A↔C 0.2964, A↔G 11.6736, A↔T 0.6403, C↔G 0.1348, C↔T 6.4085, G↔T 1.0000; proportion of invariant sites = 0.4080; gamma shape parameter = 0.9379. The Bayesian analysis produced parameter estimates that were very similar to those produced by MODELTEST.

The ML analysis in PAUP* using the previously mentioned parameters and the Bayesian analysis both yielded almost identical optimal trees (ML –ln L = 7561.1133, Bayesian –ln L was higher at 7689.04; Fig. 2, ML tree shown). Figure 2 shows a conservative tree with weak branches collapsed. Geographic structure is evident in *Egernia whitii*, *Egernia montana*, *Egernia guthega*, *Egernia modesta* and *Egernia margaretae personata*. The presence of two clades is strongly supported in *E. montana* (bootstrap value 100%, posterior probability 95–100%), with clade 1 comprised of populations from the Australian Capital Territory (ACT) and surrounding areas of NSW and clade 2 comprised of populations in the Snowy Mountains region of NSW (Table 1, Fig. 2). Similarly, two well-supported clades were identified within *E. guthega* (bootstrap value 87%, posterior probability 92%), with clade 1 corresponding to populations from the NSW Snowy Mountains region and clade 2 representing a population of *E. guthega* from the Bogong High Plains, its only known locality in Victoria (Table 1, Fig. 2). There is also weak support (bootstrap values 54–61%, posterior probability 90%) for phylogeographical structure between the NSW and Queensland populations of *E. modesta*, although intensive sampling across the species range was not conducted (Table 1, Fig. 2). Within *E. m. personata* there is relatively less phylogeographical structure, with only the Freeling Heights population (EA17) grouping outside the remainder of the range (bootstrap value 91%, posterior probability 61%) (Table 1, Fig. 2).

Our data reveal a deep phylogeographical break within *E. whitii* between 'northern' (i.e. QLD, NSW, ACT, extreme SE Victoria) and 'southern' populations (i.e. remainder Victoria, Tasmania, South Australia). The subdivision between the 'northern' and 'southern' populations of *E. whitii* occurs in the east Gippsland region of Victoria, between Genoa Peak (near Mallacoota) and Cape Conran (Fig. 3). Genetic divergence within each subgrouping is between 5.5% and 8.5% for the 'northern' populations and between 5.2% and 8.7% for the 'southern' populations (Table 1). Divergence between populations on either side of the phylogeographical break is 12.3–12.6% (Table 1), although genetic distances between the two subgroupings range from 11.5% to 16.0%. This divergence is substantial considering that Genoa Peak and Cape Conran are only 80 km apart (Figs 1 and 3). In addition to revealing a substantial phylogeographical break within *E. whitii* between the 'northern' and 'southern' populations, several clades are also evident within each of these subgroupings (Table 1; Fig. 3). Six clades are identified within the 'northern' subgroup and seven clades are identified within the 'southern' subgroup, although the relationships between clades are not resolved (Table 1; Fig. 3). Genetic divergences within each clade are in the range 0–6.8% (Table 1).

Table 1 Jukes–Cantor (Jukes & Cantor 1969) genetic distances for specific clade comparisons, and within the species/clades identified in Fig. 2. The 'southern' vs. 'northern' *Egernia whitii* comparison is based on the divergence between populations immediately on either side of the phylogeographical break in east Gippsland. NA, clade only contained one sample

Species/clade	Genetic distance range
'Northern' vs. 'Southern' *E. whitii*	0.123–0.126
'Northern' *E. whitii*	
Clade 1	0.000–0.026
Clade 2	0.000–0.009
Clade 3	0.032–0.061
Clade 4	0.007
Clade 5	0.000–0.041
Clade 6	0.006
Between *E. whitii* 'northern' clades	0.055–0.085
'Southern' *E. whitii*	
Clade 1	0.000–0.068
Clade 2	0.004–0.022
Clade 3	0.007–0.043
Clade 4	0.000–0.006
Clade 5	0.001–0.009
Clade 6	0.040
Clade 7	NA
Between *E. whitii* 'southern' clades	0.052–0.087
E. montana	
Clade 1	0.000–0.054
Clade 2	0.049
Clade 1 vs. Clade 2	0.048–0.054
E. guthega	
Clade 1	0.000–0.016
Clade 2	NA
Clade 1 vs. Clade 2	0.022–0.023
E. m. personata	
Clade 1	0.004–0.034
Clade 2	NA
E. m. margaretae	0.171
E. modesta	
Clade 1	0.035
Clade 2	0.019

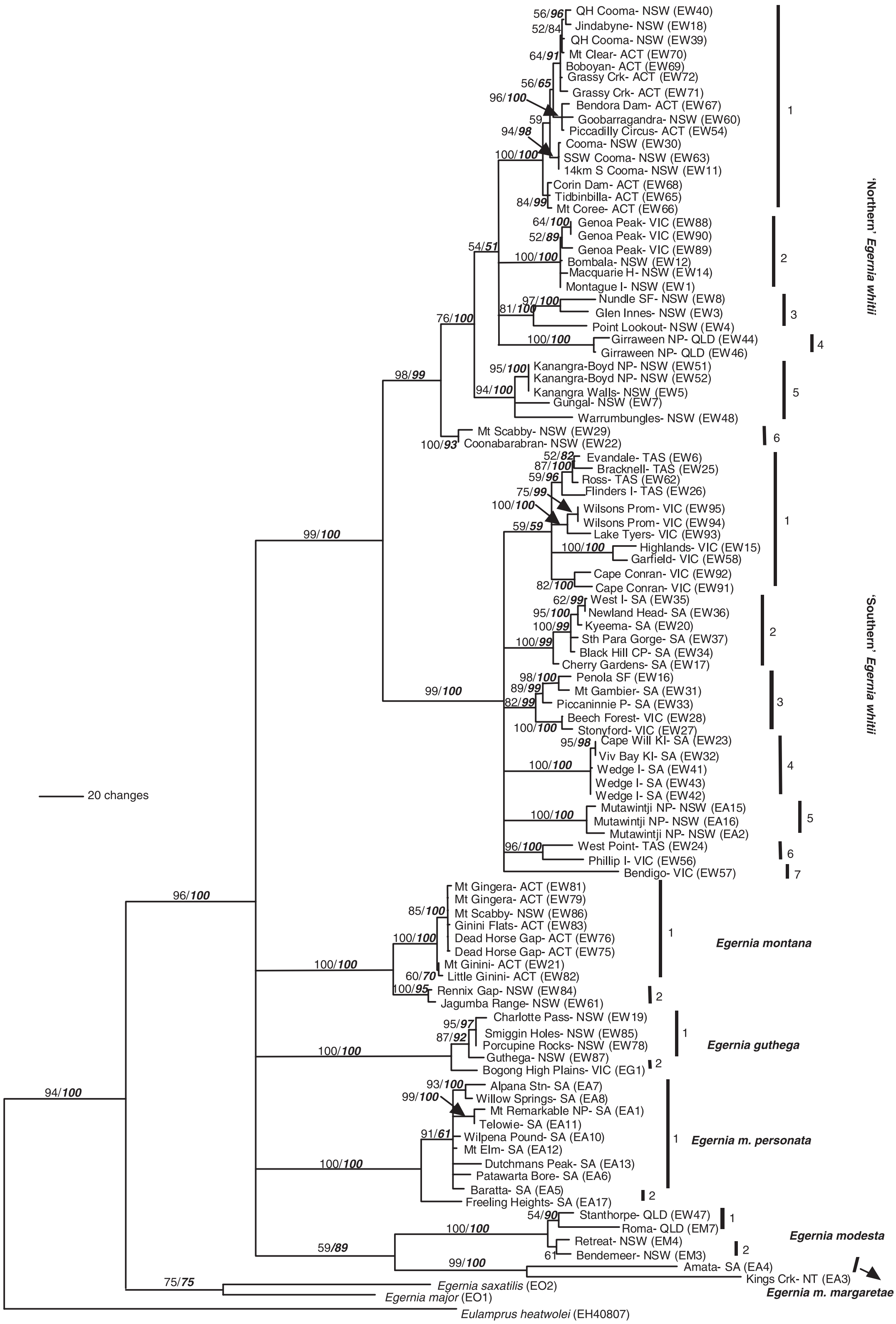

Fig. 2 Conservative phylogram from maximum-likelihood analyses for the temperate-adapted members of the *Egernia whitii* species group based on 696 base pairs of the *ND4* mitochondrial gene. Parsimony bootstrap values are shown in plain text and Bayesian posterior probabilities are shown in bold. See Figs 1 and 3 and Tables 1 and 2 for information regarding the clades identified in *Egernia guthega*, *Egernia modesta*, *Egernia margaretae personata*, *Egernia montana*, 'northern' *E. whitii* and 'southern' *E. whitii*.

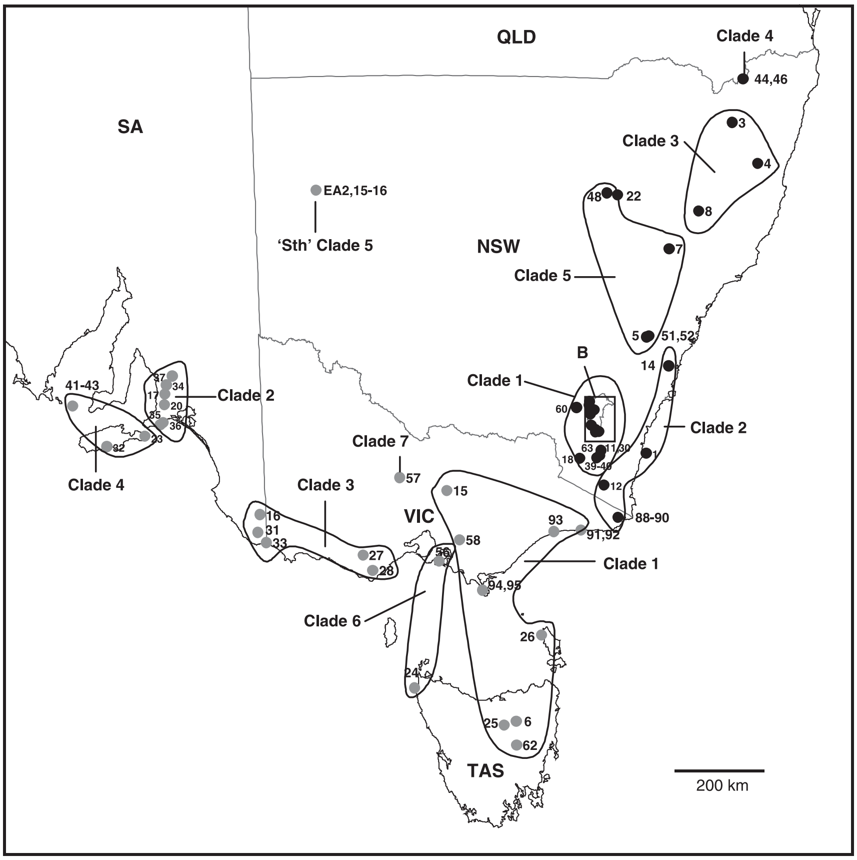

Fig. 3 Map showing the distribution of the clades identified in Fig. 2 for the 'northern' *Egernia whitii* (●) and 'southern' *E. whitii* (●) subgroups. 'Northern' clade 6 is not shown, but comprises EW22 and EW29. Refer to Fig. 1 (B) for the sample localities within inset B. Numbers refer to the tissue codes listed in Appendix. State codes are: SA, South Australia; QLD, Queensland; NSW, New South Wales; ACT, Australian Capital Territory; VIC, Victoria; TAS, Tasmania.

Discussion

We have produced a phylogeny for the temperate rock-dwelling skinks of the *Egernia whitii* species group in southeastern Australia, which appears to have several important biogeographical implications. Our study has highlighted a substantial phylogenetic break within *E. whitii* and identified phylogeographical splits within the alpine species, *Egernia guthega* and *Egernia montana*. The following discussion highlights the main features relating to the phylogeography of *E. whitii*, *E. guthega* and *E. montana*, specifically examining (i) the significance of the major phylogeographical break within *E. whitii* in eastern Victoria, (ii) the possible biogeographical processes driving the divergence between populations within the 'northern' and 'southern' *E. whitii* subgroups, and (iii) the possible scenarios that led to the origin and subsequent diversification of *E. guthega* and *E. montana* in the Australian alpine zone.

Major phylogenetic break within E. whitii *in eastern Victoria*

The dominant feature of our phylogeny is a deep phylogenetic split between the 'northern' and the 'southern' populations of *E. whitii*. Specifically, we have identified a substantial genetic break between Genoa Peak and Cape Conran in the east Gippland region of Victoria, which are separated by a distance of approximately 80 km. Although such deep phylogenetic divergences generally result from historical isolation as a result of geographical or environmental barriers to gene flow and dispersal (Avise 2000), a recent simulation study has indicated that such phylogeographical breaks can arise in the absence of barriers to gene flow. The likelihood of divergence occurring without barriers to gene flow is enhanced in taxa with restricted dispersal and small population sizes (Irwin 2002). However, Irwin (2002) suggested that in instances where concordant phylogeographical breaks were evident in multiple independent genetic markers and across several codistributed taxa, deep phylogeographical divergences are more likely to be the result of restricted gene flow as a result of geographical or environmental barriers rather than neutral divergence. In succeeding discussions we highlight that the deep phylogeographical break within *E. whitii* located in eastern Victoria (Gippsland) is corroborated by (i) multiple independent genetic markers (i.e. mtDNA, nuclear DNA, allozymes; Donnellan *et al*. 2002; Chapple & Keogh 2004); (ii) mtDNA divergences in other taxa (e.g. Schäuble & Moritz 2001); and (iii) current distributional patterns of the herpetofauna of southeastern Australia.

The phylogeographical break in eastern Victoria was initially found by Donnellan *et al*. (2002) in a recent study of *E. whitii* that utilized allozymes. Donnellan *et al*. (2002) found a fixed difference between Jindabyne (New South Wales; our sample EW18, Appendix) and Stonyford (Victoria; EW27); however, they were unable to determine the location of the split because of the absence of samples from eastern Victoria. A concordant phylogenetic break was identified in the phylogenetic analyses of both mtDNA (*ND4*, *16S rRNA*) and nuclear DNA (β-Fibrinogen 7th intron) data sets conducted by Chapple & Keogh (2004). Interestingly, Schäuble & Moritz (2001) found a substantial phylogenetic break in the spotted grass frog (*Limnodynastes tasmaniensis*), and the related striped marsh frog (*Limnodynastes peronii*), in the east Gippsland region of Victoria; although, once again the exact location and significance of this split could not be determined because of the absence of detailed sampling in the region.

East Gippsland generally has not been recognized as a significant biogeographical region (e.g. Cracraft 1991) and few current geographical barriers are apparent. Consequently, the broad congruence in the positioning of phylogeographical breaks in *E. whitii* (Donnellan *et al*. 2002; Chapple & Keogh 2004; present study), *L. tasmaniensis* and *L. peronii* (Schäuble & Moritz 2001) is intriguing. However, the east Gippsland fauna represents a southern continuation of the east coast fauna (e.g. Cracraft 1991) and the boundary between east Gippsland and west Gippsland (generally taken as the Thomson River) represents a significant area of faunal interchange in the Australian herpetofauna (Littlejohn & Rawlinson 1971). The distributions of some species span this boundary, while the distributions of species such as *Litoria citropa* (Donnellan *et al*. 1999), *Litoria phyllochroa*, *Litoria aurea*, *Litoria littlejohni*, *Heleioporus australiacus*, *Physignathus lesueurii* (Rawlinson 1971), *Cyclodomorphus michaeli* (Shea 1995) and *Eulamprus heatwolei* (Gippsland populations; Hutchinson & Rawlinson 1995) terminate abruptly in this region. In contrast, *E. whitii* appears to be continuously distributed across southern Victoria (Wilson & Swan 2003).

Despite the current absence of substantial geographical barriers in eastern Victoria and the seemingly continuous distribution of *E. whitii* across this region, the concordance of phylogeographical breaks and evidence for faunal interchange in this area suggest that geographical barriers might have existed in the Gippsland region. Indeed, geological evidence strongly indicates that major geographical barriers have been periodically present in this area since the mid-Tertiary. Specifically, the Gippsland basin in southeastern Victoria represents one of the major Mesozoic-Tertiary sedimentary basins in southern Australia (Frakes *et al*. 1987; Gallagher *et al*. 2001, 2003). At present the Gippsland basin is a low lying coastal plain, but this region was subject to substantial periods of marine incursion during the Eocene, Oligocene to early Miocene (10–32 Ma), and late Miocene to early Pliocene (Frakes *et al*. 1987; Unmack 2001). Chapple & Keogh (2004) suggested that the split between 'northern' and 'southern' *E. whitii* populations occurred during the late Miocene to early Pliocene (2.75–5 Ma), possibly coinciding with the most recent major marine incursion. Although the estimated divergence time based solely on *ND4* (present study) suggests an earlier split (6.1–9.6 Ma), it is still consistent with the most recent episode of marine incursion during the late Miocene.

Inundation of the Gippsland coastal strip had the potential to cause severe habitat fragmentation and barriers to gene flow in the resident herpetofauna, as the region is bordered to the west by sections of the southern uplands and to the north by the eastern highlands region of the GDR (Hodgson 2001; Dickinson *et al*. 2002). There is also geological evidence of significant tectonic activity in the Gippsland basin region near the Miocene–Pliocene boundary (Dickinson *et al*. 2002). Periodic minor marine incursions in this area during the Pleistocene glacial-interglacial cycles (Wilson & Allen 1987; Holdgate *et al*. 2003) might have isolated *E. whitii* populations in southeastern NSW from those in the west Gippsland region. The present-day Gippsland lakes were formed during the late Pleistocene to Holocene by drainage from six major rivers originating in the eastern highlands, when an extensive protective coastal sand barrier system was created (Hodgson 2001; Holdgate *et al*. 2003). It is possible that the stability provided by this coastal barrier system, in conjunction with the absence of major sea level changes during the last 5000 years (Hooley *et al*. 1980), enabled more recent dispersal across this region resulting in secondary contact between the 'northern' and 'southern' *E. whitii* clades. Indeed, *E. whitii* appears to be relatively uncommon and only patchily distributed across east Gippsland (Atlas of Victorian Wildlife data Victorian Department of Sustainability and Environment), inhabiting 'atypical' sandy coastal heathland habitats rather than its more typical associations with rocky outcrops (Chapple 2003; D. Chapple, personal observation). Clearly, future study is needed in order to evaluate the biogeographical processes that led to the substantial phylogeographical structure in *E. whitii*. Our documentation of a possible major biogeographical barrier provides a hypothesis-testing framework (e.g. Irwin 2002) in which to conduct more fine-scale sampling of *E. whitii* in eastern Victoria and comparative phylogeographical studies using several vertebrate and invertebrate taxa.

Given the level of genetic divergence between the 'northern' and 'southern' lineages of *E. whitii*, each may warrant recognition as subspecies or distinct species. However, further morphological and molecular work is required to determine whether taxonomic designation as subspecies or species is most appropriate as there are some incomplete and conflicting data. The allozyme sampling

(Donnellan *et al*. 2002) did not include specimens from the Sydney type locality of *moniligera*, the name usually applied to eastern NSW populations, but the mtDNA indicates that all NSW populations are monophyletic. Although there are many areas of agreement between the mtDNA data and other data, the mtDNA pattern is at odds with the patterns of variation seen in other data within *E. whitii*. Donnellan *et al*. (2002) found that the most distinctive populations were those from Tasmania and concluded that there was therefore little support for recognizing a primary split between southern and eastern populations as the conventionally recognized subspecies, *E. whitii whitii* and *E. w. moniligera*, respectively (Cogger 2000). The mtDNA data revives interest in this possibility, but suggests that the position of the break between the eastern and the southern subspecies is much further south. The mtDNA data also differ from the allozyme data in finding no support for a distinctive Tasmanian population. Consequently, the problems relating to the incongruence between the mtDNA patterns and other data need to be resolved before any recommendation regarding the taxonomic status of the 'northern' and 'southern' lineages of *E. whitii* can be made.

Phylogeographical patterns within the 'northern' and 'southern' Egernia whitii *lineages*

Recent studies have demonstrated that *E. whitii* is particularly susceptible to habitat fragmentation (Mac Nally & Brown 2001; Jellinek *et al*. 2004). As a temperate-adapted species (Henzell 1972, 1982), the distribution of *E. whitii* presumably tracked the expansion and contraction of its habitat in southeastern Australia. The genetic distances between clades within both 'northern' and 'southern' *E. whitii* lineages (*c*. 5–8%) are indicative of divergence during the Pliocene, while phylogeographical structure within clades (genetic distances 0–6.8%) is consistent with late Pliocene–Pleistocene glacial-interglacial cycles. Our analyses revealed six clades within the 'northern' *E. whitii* lineage and seven in the 'southern' lineage, although several only had weak support (e.g. 'southern' clade 1). While we sampled extensively across the range of *E. whitii*, our sampling within most populations and regions is relatively spare. In addition, the relationships among most clades are unresolved, therefore we only briefly highlight the distributions of the major clades within *E. whitii*.

Within the 'northern' lineage there is an interesting pattern with separate inland and coastal clades in both northern New South Wales (clade 5 vs. clade 3) and southern NSW/ACT (clade 7 vs. clade 2; Fig. 3). The inland clades appear to be confined to the more mountainous regions along the GDR, while populations in coastal clades are generally located to the east of the GDR. This pattern suggests that the GDR has historically been an important geographical barrier, possibly accentuating the effects of habitat fragmentation during Pleistocene glacial-interglacial cycles. In addition, Queensland populations of *E. whitii* appeared to be distinct from those in NSW and ACT.

Our analyses suggest that more complex patterns of substructuring are present within the 'southern' lineage of *E. whitii*. Populations from eastern Victoria form part of a weakly supported clade with those from eastern Tasmania (clade 1; Fig. 3). This is in contrast to Donnellan *et al*. (2002) who found a fixed difference between the Tasmanian populations and the remainder of the range. Populations in western Tasmania (West Point) appear to be more closely related to a population on Phillip Island in Victoria (clade 6) than to those in the rest of Tasmania, possibly suggesting the historical presence of multiple land bridges across Bass Strait. *E. whitii* from western Victoria and southeastern South Australia formed a single clade (clade 3). Within South Australia, the geographically proximate populations around the Adelaide region (clade 2) and Kangaroo Island and surrounding islands (clade 4) both formed distinctive clades. The Mutawintji NP population of *E. whitii* formed a single clade within the 'southern' lineage. Rocky gorges within Mutawintji NP provide wetter and more mesic environments compared to the surrounding semiarid habitats (Foster 1993; Swan & Foster 2000). The presence of a population of *E. whitii* within a disjunct refugial habitat, separated from the nearest 'southern' population by approximately 600 km, indicates that the distribution of *E. whitii* was previously more widespread and suggests that Pleistocene glacial-interglacial cycles may have substantially influenced its current distribution.

Phylogeography of E. guthega *and* E. montana *in alpine Australia*

The genetic structuring evident within the alpine species, *E. guthega* and *E. montana*, presumably reflects some important historical patterns within the Australian alpine zone. Before the early to mid-Tertiary, the area that is now the alpine zone, was dominated by extensive rainforest resulting from the relatively warm climate of the area (reviewed in Green & Osborne 1994). Although the geological activity that resulted in the uplift of the southern highlands is believed to have commenced in the mid-Tertiary, the development of true alpine flora and fauna was delayed until at least the late Miocene because of a combination of unsuitable climate and altitude (Green & Osborne 1994). The rapid cooling and increasing aridity of the southeast of the continent during the late Miocene to Pliocene (2–5 Ma) is believed to have resulted in the evolution of the subalpine and alpine-adapted biota (Galloway & Kemp 1984; Green & Osborne 1994). This period coincides with the suggested timing of the split between the low- to mid-elevation *E. whitii* and the alpine specialists, *E. guthega* and *E. montana* (Chapple & Keogh 2004).

Pleistocene glacial cycles are believed to have strongly impacted the alpine fauna of southeastern Australia (Green & Osborne 1994). Repeated depression of the tree line created vast montane regions that were devoid of forest vegetation, resulting in periodic local extinctions and restricting many alpine taxa to well-vegetated refugia in gulleys and valleys (Frakes *et al*. 1987; Green & Osborne 1994). However, it is also possible that populations tracked the depression of alpine habitats and became more, not less continuous (e.g. Knowles 2000), only to become separated again as temperatures increased in the Holocene. Given that the current distributions of *E. montana* and *E. guthega* encompass the Mount Kosciusko area, which is the only region to experience full glaciation during the Pleistocene (Barrows *et al*. 2001), it is not surprising that we found substantial phylogeographical structure within both species. The phylogeographical structure evident in *E. guthega* between populations in NSW and Victoria (2.2% genetic divergence; 1.1–1.7 Ma), and between ACT and NSW populations in *E. montana* (4.8–5.4%; 2.4–4.1 Ma) is consistent with Pliocene and Pleistocene glacial-interglacial cycles. Both species occur in small, isolated populations on disjunct mountaintops within the alpine region of southeastern Australia (Donnellan *et al*. 2002). The current distribution of both species within suitable montane 'islands' surrounded by a 'sea' of lower altitude woodlands is similar to the 'sky island' distributions reported in a range of other taxa (e.g. Knowles 2000; Masta 2000). Alpine species with such distributions are not only predicted to have limited gene flow between populations because of their isolation, but also have elevated phylogeographical divergences as a result of genetic drift (Knowles 2000; Masta 2000). Similar 'sky island' distributions appear to be present in other Australian alpine lizards, including *Eulamprus kosciuskoi* (Wilson & Swan 2003), *Pseudemoia* (Hutchinson & Donnellan 1992) and *Niveoscincus* (*Niveoscincus greeni*, *Niveoscincus microlepidotus*, *Niveoscincus orocryptus*; Hutchinson & Schwaner 1991; Melville & Swain 2000).

The historical dissociations and disjunctions of *E. guthega* and *E. montana* indicates that both species may be susceptible to future climatic changes and other threatening processes. Clemann (2002) identified a range of threatening processes (e.g. development of ski resorts, introduced predators/competitors/livestock, global warming) for the Australian alpine herpetofauna and indicated that the current restricted distributions and rarity of *E. guthega* and *E. montana* warrants consideration of conservation listing for both species in the near future. However, more detailed consideration of both species, with the inclusion of *E. montana* samples from Victoria, may be required before such conservation management decisions can be made.

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This research is part of David Chapple's doctoral project on the evolutionary ecology and molecular phylogenetics of White's skink (*Egernia whitii*) and the other members of the *Egernia whitii* species group. Scott Keogh splits his own research time between molecular phylogenetics and molecular and behavioural ecology. He and the members of his group mostly work on reptiles, frogs and fish. Mark Hutchinson is curator of herpetology at the South Australian Museum and his research is focused on the systematics, biogeography and natural history of the Australian herpetofauna.

Appendix

Museum registration numbers, GenBank Accession nos and locality data for taxa used in this study

Species	Museum tissue no.	Voucher no.	GenBank Accession no.	Locality
Egernia guthega (EW19)	ABTC 16273	SAMAR 37781	AY612995	Charlotte Pass Kosciusko NP, NSW
Egernia guthega (EW78)			AY612997	Porcupine Rocks Kosciusko NP, NSW
Egernia guthega (EW85)	ABTC 16271	SAMAR 37779	AY612996	Smiggin Holes Kosciusko NP, NSW
Egernia guthega (EW87)	ABTC 40951	SAMAR 37772	AY520479	Guthega Village, NSW
Egernia guthega (EG1)			AY612998	Ruined Castle, Bogong High Plains, VIC
Egernia margaretae margaretae (EA3)	ABTC 12624		AY520486	Kings Creek Station, NT
Egernia m. margaretae (EA4)	ABTC 42404	SAMAR 51590	AY520483	36.5 km ESE Amata, SA
Egernia margaretae personata (EA1)	ABTC 53956	SAMAR 23267	AY520485	Mount Remarkable NP, SA
Egernia m. personata (EA5)	ABTC 36718	SAMAR 48206	AY613006	6 km NW Baratta, SA
Egernia m. personata (EA6)	ABTC 39279	SAMAR 52174	AY613004	3.2 km S Patawarta Bore Narrina Station, SA
Egernia m. personata (EA7)	ABTC 39287	SAMAR 52206	AY520484	Near Angorichina Hostel Alpana Station, SA
Egernia m. personata (EA8)	ABTC 39386	SAMAR 52286	AY613005	4.3 km ENE Willow Springs HS, SA
Egernia m. personata (EA10)	ABTC 70400	SAMAR 53248	AY612999	Wilpena Pound, SA
Egernia m. personata (EA11)	ABTC 70482	SAMAR 53284	AY613003	4.7 km NE Telowie, SA
Egernia m. personata (EA12)	ABTC 70519	SAMAR 53082	AY613001	5 km E Mount Elm, SA
Egernia m. personata (EA13)	ABTC 70566	SAMAR 53113	AY613002	1.9 km N Dutchmans Peak, SA
Egernia m. personata (EA17)	ABTC 74064	SAMAR 52989	AY613000	4.6 km NE Freeling Heights, Arkaroola, SA
Egernia modesta (EM3)	ABTC 11454	AMSR 106896	AY613068	13 km W Bendemeer, NSW
Egernia modesta (EM4)	ABTC 12411	SAMAR 39172	AY520493	16 km W Retreat, NSW
Egernia modesta (EM7)	QMR 78542		AY613069	Alicker Station, NW Roma, QLD
Egernia modesta (EW47)			AY613067	Texas Road, Stanthorpe, QLD
Egernia montana (EW21)	ABTC 16386	SAMAR 37769	AY612990	Mount Ginini, ACT
Egernia montana (EW61)			AY612988	Jagumba Range, Kosciusko NP, NSW
Egernia montana (EW75-76)	ANWC 5930, ANWC 5935		AY612994, AY612993	Dead Horse Gap, Bimberi Range, ACT
Egernia montana (EW79, 81)	ABTC 16385	SAMAR 37767	AY612986, AY520470	Mount Gingera, ACT
Egernia montana (EW82)	ABTC 16387	SAMAR 37771	AY612991	Little Ginini, ACT
Egernia montana (EW83)	ABTC 16388	SAMAR 37770	AY612992	Ginini Flats, ACT
Egernia montana (EW84)	ABTC 17272	SAMAR 37809	AY612987	Rennix Gap, NSW
Egernia montana (EW86)	ABTC 40949	SAMAR 37773	AY612989	Mount Scabby, NSW
Egernia whitii (EA2, 15, 16)	ABTC 58207, ABTC 71453, ABTC 71454	SAMAR 45513	AY613008, AY613007, AY520482	Homestead Gorge, Mutawintji NP, NSW
Egernia whitii whitii (EW1)	ABTC 1167	AMSR 130072	AY613035	Montague Island, NSW
Egernia w. whitii (EW3)	ABTC 3789	SAMAR 33640	AY613058	38 km E Glen Innes, NSW
Egernia w. whitii (EW4)	ABTC 3796	SAMAR 33641	AY613039	Point Lookout, NSW
Egernia w. whitii (EW5)	ABTC 6960	AMSR 120848	AY520489	Kanangra Walls, NSW
Egernia w. whitii (EW6)	ABTC 11472	AMSR 106837	AY613032	5 km E Evandale, TAS
Egernia w. whitii (EW7)	ABTC 11473	AMSR 106897	AY613016	1 km W Gungal, NSW
Egernia w. whitii (EW8)	ABTC 11474		AY613021	Nundle SF, NSW
Egernia w. whitii (EW11)	ABTC 11486	AMSR 106838	AY613052	14 km S Cooma, NSW
Egernia w. whitii (EW12)	ABTC 11488	AMSR 112344	AY520487	15 km N Bombala, NSW
Egernia w. whitii (EW14)	ABTC 11491	AMSR 95717	AY613031	Macquarie Hill, NSW
Egernia w. whitii (EW15)	ABTC 12874	SAMAR 44075	AY613027	6.3 km N Highlands, VIC
Egernia w. whitii (EW16)	ABTC 14282	SAMAR 33294	AY613011	Penola SF, SA
Egernia w. whitii (EW17)	ABTC 14779	SAMAR 32962	AY613030	Cherry Gardens, SA
Egernia w. whitii (EW18)	ABTC 16267	SAMAR 37783	AY520492	Jindabyne, NSW
Egernia w. whitii (EW20)	ABTC 16336	SAMAR 34521	AY613043	5 km E Kyeema, SA
Egernia w. whitii (EW22)	ABTC 16656	SAMAR 34757	AY613057	16 km W Coonabarabran, NSW
Egernia w. whitii (EW23)	ABTC 16692	SAMAR 34783	AY613041	Cape Willoughby, Kangaroo Island, SA
Egernia w. whitii (EW24)	ABTC 23053		AY613017	West Point, TAS

Appendix *Continued*

Species	Museum tissue no.	Voucher no.	GenBank Accession no.	Locality
Egernia w. whitii (EW25)	ABTC 23054		AY613040	4 km S Bracknell, TAS
Egernia w. whitii (EW26)	ABTC 23602	NMVD 62228	AY613056	Mount Tanner, Flinders Island, TAS
Egernia w. whitii (EW27)	ABTC 40844	NMVD 62061	AY613055	5 km W Stonyford, VIC
Egernia w. whitii (EW28)	ABTC 40886	NMVD 62064	AY613023	2 km E Beech Forest, VIC
Egernia w. whitii (EW29)	ABTC 40945		AY613045	Mount Scabby, NSW
Egernia w. whitii (EW30)	ABTC 40976		AY613014	Cooma, NSW
Egernia w. whitii (EW31)	ABTC 54276	SAMAR 23213	AY520491	NE of Mount Gambier, SA
Egernia w. whitii (EW32)	ABTC 54278	SAMAR 23485	AY520490	Vivonne Bay, Kangaroo Island, SA
Egernia w. whitii (EW33)	ABTC 54286	SAMAR 23928	AY613028	Piccaninnie Ponds CP, SA
Egernia w. whitii (EW34)	ABTC 57791	SAMAR 43152	AY613053	Black Hill CP, SA
Egernia w. whitii (EW35)	ABTC 58049	SAMAR 45135	AY613015	West Island, SA
Egernia w. whitii (EW36)	ABTC 58695	SAMAR 49869	AY613022	Newland Head, SA
Egernia w. whitii (EW37)	ABTC 68812	SAMAR 52628	AY613013	South Para Gorge, SA
Egernia w. whitii (EW39–40)			AY613025, AY613034	Quartz Hill, 11 km S Cooma, NSW
Egernia w. whitii (EW41–43)			AY613020, AY613047, AY613024	Wedge Island, SA
Egernia w. whitii (EW44)			AY613037	Underground Creek Walk, Girraween NP, QLD
Egernia w. whitii (EW46)			AY613051	Roberts Walk, Girraween NP, QLD
Egernia w. whitii (EW48)			AY613050	Grand High Tops Walk, Warrumbungles NP, NSW
Egernia w. whitii (EW51, 52)			AY613018–9	Kanangra-Boyd NP Campground, NSW
Egernia w. whitii (EW54)			AY613046	Piccadilly Circus, Namadgi NP, ACT
Egernia w. whitii (EW56)			AY613054	Summerlands, Phillip Islands, VIC
Egernia w. whitii (EW57)			AY613049	Mandurang State Forest near Bendigo, VIC
Egernia w. whitii (EW58)			AY520488	Cannibal Creek Reserve, Garfield, VIC
Egernia w. whitii (EW60)			AY613038	Goobarragandra River, Brindabella NP, NSW
Egernia w. whitii (EW62)	ANWC 5624		AY613044	Ellenthorpe Station, 16 km W Ross, TAS
Egernia w. whitii (EW63)	ANWC 5940		AY613036	19 km SSW Cooma, NSW
Egernia w. whitii (EW65)			AY613026	Tidbinbilla NR, Scenic Lookout, ACT
Egernia w. whitii (EW66)			AY613029	Mount Coree Summit, Namadgi NP, ACT
Egernia w. whitii (EW67)			AY613010	Bendora Dam, ACT
Egernia w. whitii (EW68)			AY613009	Corin Dam, ACT
Egernia w. whitii (EW69)			AY613012	Boboyan Homestead Ruins, Namadgi NP, ACT
Egernia w. whitii (EW70)			AY613033	Mount Clear Summit, Namadgi NP, ACT
Egernia w. whitii (EW71, 72)			AY613042, AY613048	Grassy Creek Firetrail, Namadgi NP, ACT
Egernia w. whitii (EW88–90)			AY613061-2, AY613064	Genoa Peak Lookout, Croajingolong NP, VIC
Egernia w. whitii (EW91, 92)			AY613060, AY613066	Sunset Peak, Cape Conran CP, VIC
Egernia w. whitii (EW93)			AY613059	Red Bluff Carpark, Lake Tyers CP, VIC
Egernia w. whitii (EW94, 95)			AY613063, AY613065	Tidal Overlook, Wilsons Promotory NP, VIC
Egernia major (EO1)	ANWC 5298		AY520464	Nana Creek, near Coffs Harbour, NSW
Egernia saxatilis (EO2)			AY520463	Booroomba Rocks, ACT
Eulamprus heatwolei (EH40807)	ABTC 57494	SAMAR 40807	AY520462	20.3 km N Abercrombie River, NSW

Museum acronyms as follows: ABTC, Australian Biological Tissue Collection; SAM, South Australian Museum, Adelaide; WAM, Western Australian Museum; ANWC, Australian National Wildlife Collection, CSIRO; QM, Queensland Museum.