

## Comparative phylogeography of three rainforest-restricted lizards from mid-east Queensland

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### Abstract

Several small isolates of rainforest situated on the central eastern coast of Australia are home to a rich herpetofauna, including four endemic species of leaf-tail geckos (*Phyllurus* spp.) and two skinks (*Eulamprus* spp.). To examine the extent and geographic pattern of historical subdivision among isolates, we assayed mtDNA variation in two species endemic to rainforests of this region (*Phyllurus ossa* and *Eulamprus amplus*) and, for comparison, a more widespread and less specialised lizard, *Carlia rhomboidalis*. There is a clear genetic signature of historical changes in population size and distribution in *P. ossa* that is consistent with Pleistocene (or earlier) rainforest contraction and subsequent expansion. Although more pronounced in the gecko, phylogeographic structure was congruent between *E. amplus* and *P. ossa*. In contrast to the saxicolous, rainforest-restricted *P. ossa* and *E. amplus*, the rainforest-generalist species, *C. rhomboidalis*, does not display strong geographic population structure. The differences in genetic population structure exhibited by the three species are consistent with species-specific differences in ecology.

### Introduction

Geographic isolation, especially that associated with contractions of habitat to mesic refugia under cool, dry periods of the Pleistocene, has been a prominent hypothesis to explain high species diversity and endemism in rainforest faunas (reviewed in Moritz *et al.* 2000). Within currently recognised species in the Australian wet tropics (WT), there is deep and geographically congruent genetic differentiation between areas identified as historical refugia by paleoclimatological modelling (Joseph *et al.* 1995; Schneider *et al.* 1998, 1999), but little, if any, corresponding divergence in morphology (Schneider and Moritz 1999; Schneider *et al.* 1999). An analogous observation was made for a species of frog restricted to wet forests of south-east Queensland (SEQ) (McGuigan *et al.* 1998). These observations have implications for understanding of speciation processes and also strategies for conservation (Moritz *et al.* 2000).

The rainforests of mid-east Queensland (MEQ) represent a component of the ‘mesotherm archipelago’ (Nix 1991) geographically intermediate between the rainforests of SEQ and WT. This region has a rich herpetofauna, including several endemic species of rainforest-restricted lizards (Covacevich and McDonald 1980; Moritz *et al.* 1997). Prominent among these are leaf-tailed geckos (*Phyllurus* spp.), which have undergone a limited radiation into four locally endemic species, perhaps due to a combination of geographic isolation among refugia and divergent selection (Couper *et al.* 1993, 2000). In addition, there are two endemic species of forest skinks (*Eulamprus amplus* and

*E. luteilateralis*) found in vine forests of this region, *E. amplus* being more geographically widespread and at lower altitudes than *E. luteilateralis* (Covacevich and McDonald 1980). Within *Phyllurus*, three species (*P. ossa*, *P. isis* and *P. nepthys*) occur allopatrically, or perhaps parapatrically, in small rainforest isolates in and around Eungella National Park (Fig. 1) and their combined distributions cover the same region as the distribution of *E. amplus*. A fourth species (*P. championae*) has been recently described from the Black Mountain/Cameron Creek region c.80 km to the south-east (Couper *et al.* 2000). The most widespread of the gecko species, *P. ossa*, occurs in only three areas: the Conway Range, Clarke Range and rainforest patches east of the Clarke Range. The sister species to *P. ossa*, *P. isis*, is endemic to the small rainforest isolates of Mt Blackwood and Mt Jukes. The third species occurring in the Eungella region, *P. nepthys*, is endemic to the Clarke Range. Although *P. ossa* also occurs on the lower eastern slopes of the Clarke Range, around St Helen's Gap, whether it is sympatric or parapatric with *P. nepthys* is unknown.

As the rainforests of MEQ are smaller in extent and generally lower in elevation than those of the WT or SEQ, they were probably more severely affected by historical climate fluctuations than their better studied northern or southern counterparts. This view is supported by the absence of several rainforest-restricted species of mammals and birds that are present in both WT and SEQ (e.g. the tiger quoll, *Dasyurus maculatus*; satin bowerbird, *Ptilonorhynchus violaceus*; yellow-throated scrubwren, *Sericornis citreogularis*; and pale-yellow robin, *Tregellasia capito*) (Winter and McDonald 1986; Low 1993). The absence of these taxa from MEQ suggests that they may have become extinct when rainforest refugia became too small to support viable populations (Joseph *et al.* 1993; Low 1993). Apart from such indirect evidence that rainforest in MEQ was reduced to relatively small mesic pockets at one or more times through the course of the Pleistocene, little is known of the biogeographic history within the region. However, just as the distribution of taxonomic diversity, particularly endemics, gives insight into possible refugia, so too can concordant geographic distributions of genetic diversity (Avice 2000; Hewitt 2000), particularly for rainforest-restricted species with low vagility. Thus, we expect that if severe Pleistocene rainforest contraction occurred in MEQ, patterns of mitochondrial DNA (mtDNA) variation should show indications of population isolation and historically reduced effective size in all taxa (Hewitt 1996, 2000; Schneider and Moritz 1999).

Here we analyse mtDNA phylogeography in two widespread species of lizards endemic to the rainforests of MEQ: the leaftail gecko, *Phyllurus ossa*, and the skink, *Eulamprus amplus*. We compare the intraspecific phylogeography of *E. amplus* with that of *P. ossa* and its sister species, *P. isis* (Couper *et al.* 2000). In addition, to evaluate the effects of rainforest restriction *versus* general geographic isolation, we compare the results for *P. ossa* and *E. amplus* to a corresponding mtDNA phylogeography for *Carlia rhomboidalis*, another skink found sympatrically with the former two species, but having a broader geographic range (mid-east Queensland north to Townsville) and occupying a broader range of habitats (Ingram and Covacevich 1989).

## Methods

### *Study species and sample sites*

Tissues were sampled non-destructively (approx. 5 mm of tail-tip) from individuals of each species, collected from 6–8 localities covering most of the major rainforest isolates in the region (Fig. 1), including type localities of two of the species (*P. ossa* and *E. amplus*). Sample sizes and localities are presented in Table 1. Although *P. ossa* has been found on the lower eastern slopes of the Clarke Range at St Helen's Gap (Queensland Museum registration Nos QMJ53426–28) we were unable to obtain any samples from this area.

**Table 1. Sampling localities and sample sizes**

For the sampling localities where *P. ossa* is replaced by a different species of leaf-tail gecko, the name of that species is given. Letters preceding locality names correspond to those in Fig. 1

Region	Locality	<i>P. ossa</i>	<i>E. amplus</i>	<i>C. rhomboidalis</i>
Mt Dryander	a. Mt Dryander	6	6	5
Conway Range	b. Brandy Ck	8	4	6
Clarke Range	c. Eungella	<i>P. nephtys</i>	6	13
	d. Finch Hatton	<i>P. nephtys</i>	10	10
Ossa region	f. Mt Pelion	3	7	3
	g. Ossa Creek	11	10	5
	e. Mt Charlton	4	5	5
Mt Blackwood	h. Mt Blackwood	<i>P. isis</i>	7	3
Total		34	55	50

#### DNA extraction, amplification and sequencing

Total genomic DNA was extracted using a solution of 5% chelex resin in ddH<sub>2</sub>O and 5 µL of 10 mg mL<sup>-1</sup> proteinase K (Walsh *et al.* 1991). Extractions were incubated overnight at 56°C, vortexed, then heated to 95°C, vortexed again, and finally spun at 13 000 rpm for 1 min before being stored at -20°C. Between 320 and 400 bp of the mitochondrial cytochrome-*b* gene was amplified using the primers Ph1 (Schneider and Moritz 1999) and MVZ04 (Kocher *et al.* 1989) for *P. ossa*; Trop 05 (Joseph *et al.* 1995) and Cyt-b2 (Kocher *et al.* 1989) for *E. amplus* and MVZ05 (Smith *et al.* 1992) and Cyt-b2 for *Carlia rhomboidalis* (PCR cycle profile: initial denaturation at 94°C for 1 min followed by 30 cycles of: 94°C for 45 s, 50°C for 45 s and 72°C for 1 min). Each PCR was run with a negative control and each 25-µL reaction contained 5 pmol of each primer, 2.5 µL of 10 × Promega buffer, 0.25 µL of 40 mM dNTPs, 2 µL of 25 mM MgCl<sub>2</sub>, 0.125 µL Promega Taq polymerase and 5 µL of DNA extract. Sequencing reactions were carried out according to standard dye-deoxy terminator cycle sequencing protocols. Sequences have been deposited with Genbank (Accession Nos AJ406168–304).

#### Analyses

Chromatographs were checked and sequences aligned using SeqEd 675 DNA sequence editor (Applied Biosystems Inc. 1990). Sequences were aligned by eye using translated amino acid sequence. Minimum-length unrooted trees based on absolute number of pairwise differences were generated using PAUP\* (Swofford 1996). Estimates of haplotype diversity (*h*) and nucleotide diversity ( $\pi$ ) within populations were calculated in Arlequin version 2.0 (Schneider *et al.* 1997) according to methods described in Tajima (1993). Levels of genetic subdivision were assessed using the AMOVA (Analysis of Molecular Variance: Excoffier *et al.* 1992) sub-program in Arlequin. AMOVA partitions variance into three components (between regions, within regions and within sites) that are used to compute fixation indices ( $\Phi$  statistics). Significance levels for the fixation indices were assessed using the permutation procedure described in Excoffier *et al.* (1992). Ten thousand permutations were employed. Sequence divergence between populations ( $d_{xy}$ ) for uncorrected pairwise differences were calculated using DnaSP version 2.0 (Rozas and Rozas 1997).

#### Results

Minimum-length unrooted trees were reconstructed for unique mtDNA haplotypes for all three species (Fig. 1). Phylogeographic structure in *E. amplus*, and *P. ossa* in combination with its sister species *P. isis* (sequence data from Couper *et al.* 2000), show broadly consistent patterns suggestive of persistent refugia in the Mt Dryander/Conway Range, Clarke Range and Mt Jukes/Mt Blackwood regions (Fig. 1). The phylogeographic patterns within *E. amplus* and *P. ossa* are dominated by divergence between the northern isolates (Mt Dryander + Conway Range) and the Clarke Range/Ossa region to the south. The southern coastal isolate of Mt Jukes/Mt Blackwood harbours another divergent

**Table 2. Divergence ( $d_{xy}$ ) between major rainforest blocks of mid-east Queensland and  $\Phi_{st}$  measures of population structuring**

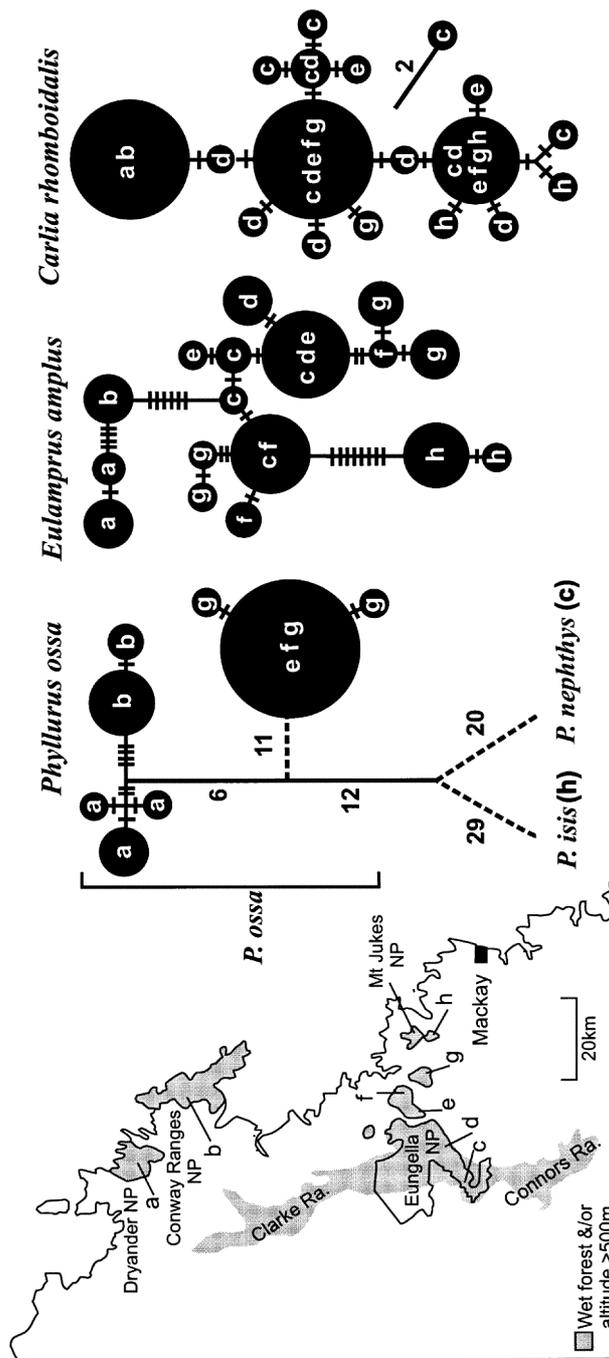
# denotes interspecific divergences in *Phyllurus*. Asterisks denote significance levels for  $\Phi_{st}$  values: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ . ‘Conway’ refers to the Mt Dryander/Conway Range area combined, ‘Clarke’ refers to sample sites in the Clarke Range (within Eungella National Park) and ‘Ossa’ refers to the rainforest isolates east of the Clarke Range

		<i>Phyllurus</i> spp.	<i>E. amplus</i>	<i>C. rhomboidalis</i>
$d_{xy}$	Conway – Clarke	13.9 ± 3.14#	2.5 ± 0.41	0.84 ± 0.1
	Conway – Ossa	5.78 ± 0.5	2.82 ± 0.41	0.88 ± 0.13
	Conway – Blackwood	12.23 ± 2.76#	4.34 ± 0.7	1.36 ± 0.33
	Clarke – Ossa	13.1 ± 2.62#	0.92 ± 0.12	0.48 ± 0.07
	Clarke – Blackwood	14.04 ± 7.02#	2.34 ± 0.31	0.79 ± 0.2
	Ossa – Blackwood	12.45 ± 2.5#	2.36 ± 0.27	0.61 ± 0.21
$\Phi_{st}$	Conway	0.93**	0.86**	0
	Clarke – Ossa	–	0.075*	–0.0065
	Conway – Clarke/Ossa	0.37**	0.20**	0.47**
	Conway – Blackwood	–	0.48**	0.93**
	Clarke/Ossa – Blackwood	–	0.33**	0.042
	Total	0.9**	0.75**	0.55*

lineage of *E. amplus* as well as *P. isis*, the sister species to *P. ossa*. Both *E. amplus* and *P. ossa* also exhibited phylogeographic structure between Mt Dryander and the Conway Range. While the patterns of phylogeographic structure in *Eulamprus* and *Phyllurus* are congruent, the depth of divergence is much greater in *P. ossa*. Estimates of nucleotide divergence between the northern (Conway Range/Mt Dryander) and southern (Clarke Range/Ossa region) isolates are *c.* 2–3% in *E. amplus* and 5–6% in *P. ossa*; divergence between Mt Jukes/Mt Blackwood and other areas ranges from *c.* 2–4% in the skink and is substantially higher (>12%) within *Phyllurus* (i.e. *P. ossa* *v.* *P. isis*) (Table 2). All pairwise comparisons of populations for both species exhibited substantial and significant subdivision, as did the values overall (*P. ossa*,  $\Phi_{st} = 0.93$ ,  $P < 0.05$ ; *E. amplus*,  $\Phi_{st} = 0.86$ ,  $P < 0.05$ ; Table 2).

In contrast to *E. amplus* and *P. ossa*, the rainforest generalist, *Carlia rhomboidalis*, shows much less overt phylogeographic structure (Fig. 1). There is no significant structure in the distribution of haplotypes among populations with the exception of the Mt Dryander/Conway Range sample, which contained a single unique haplotype (Fig. 1). This pattern of haplotype distribution results in significant overall geographic structure of mtDNA variation in *C. rhomboidalis* ( $\Phi_{st} = 0.551$ ,  $P < 0.05$ ), significant structure between the Mt Dryander/Conway Range and other populations, but no significant structure among the southern areas (Table 2).

For *E. amplus*, both nucleotide and haplotype diversity are high in the Clarke Range, Ossa region and the Mt Dryander/Conway Range region (Table 3). *P. ossa* also exhibits substantial diversity in the Mt Dryander/Conway Range area but has much lower diversity within the Ossa isolates. For both rainforest-restricted species, nucleotide diversity within the Mt Dryander/Conway Range area is inflated by internal phylogeographic structure. In contrast, *C. rhomboidalis* has zero diversity (11 individuals with identical sequences) within the northern isolates, but moderate diversity in each of the southern areas.



**Fig. 1.** Map showing sampling localities and minimum-length unrooted trees of mtDNA haplotypes for each species. Letters represent sample sites as follows: a, Mt Dryander; b, Conway Range; c, Eungella; d, Finch Hatton Gorge; e, Mt Pelton; f, Mt Charlton; g, Ossa Creek; h, Mt Blackwood. The size of the circles represents the frequency of haplotypes with the smallest equal to 1 and the largest equal to 16. Single-base substitutions are denoted by short cross-bars, and numbers next to dashed lines are the minimum number of substitutions between divergent groups.

**Table 3. Percentage mtDNA nucleotide diversity ( $\pi$ ) and haplotype diversity (h) within major rainforest blocks of mid-east Queensland based on absolute pairwise differences**

	<i>F. ossa</i> .		<i>E. amplus</i>		<i>C. rhomboidalis</i>	
	$\pi$	h	$\pi$	h	$\pi$	h
Mt Dryander	0.51 $\pm$ 0.21	0.6 $\pm$ 0.22	0.17 $\pm$ 0.18	0.53 $\pm$ 0.17	0	0
Conway Range (Brandy Ck)	0.12 $\pm$ 0.05	0.43 $\pm$ 0.17	0	0	0	0
Mt Dryander/ Conway Range	1.22 $\pm$ 0.01	0.76 $\pm$ 0.08	0.83 $\pm$ 0.12	0.71 $\pm$ 0.09	0	0
Clarke Range	–	–	0.44 $\pm$ 0.09	0.76 $\pm$ 0.08	0.53 $\pm$ 0.08	0.86 $\pm$ 0.06
Ossa region	0.06 $\pm$ 0.04	0.22 $\pm$ 0.12	0.98 $\pm$ 0.09	0.89 $\pm$ 0.06	0.42 $\pm$ 0.06	0.74 $\pm$ 0.09
Clarke Range & Ossa region	–	–	0.86 $\pm$ 0.52	0.87 $\pm$ 0.03	0.49 $\pm$ 0.32	0.82 $\pm$ 0.05
Mt Blackwood	–	–	0.09 $\pm$ 0.06	0.29 $\pm$ 0.19	0.54 $\pm$ 0.19	1.0 $\pm$ 0.27
Total	3.22 $\pm$ 1.59	0.71 $\pm$ 0.07	1.8 $\pm$ 0.97	0.92 $\pm$ 0.02	0.63 $\pm$ 0.39	0.85 $\pm$ 0.03

## Discussion

### *Phylogeography, cryptic diversity and ecology*

The pattern of phylogeography for two of the rainforest-restricted species examined here, *F. ossa* and *E. amplus* are broadly consistent, and this consistency holds when the comparison is extended to the southern coastal isolate (Mt Jukes/Mt Blackwood), which contains a divergent lineage of *E. amplus* as well as *F. isis*, the sister species to *F. ossa*. There is, however, substantial difference in the level of genetic divergence, this being higher in *F. ossa* than within *E. amplus*. Levels of diversity within major regions (Clarke Range, Ossa region and Mt Dryander/Conway Range) for both species are comparable to those within the two principal refugial areas of the Wet Tropics for rainforest-endemic lizard species such as *Gnypetoscincus queenslandiae* and *Carphodactylus laevis* (Schneider and Moritz 1999). In contrast to the two rainforest-restricted species, *Carlia rhomboidalis* does not display a strong genetic signature of long-term isolation and, with the possible exception of the Mt Dryander/Conway Range area, reduced historical population size. The low diversity in *C. rhomboidalis* from this area could be due to a severe bottleneck, a selective sweep or recent colonisation. Analyses of additional samples and genes are necessary to discriminate between these alternatives.

The different patterns of genetic variation within the three lizard species are consistent with what is known of their comparative ecology. *Carlia rhomboidalis* is a small, diurnal, actively foraging species that inhabits leaf-litter and sun patches within rainforest or rainforest edges (Ingram and Covacevich 1989; Cogger 1996). The species can be described as a rainforest generalist as it occupies a broad range of terrestrial microhabitats within rainforest and associated wet forest (Ingram and Covacevich 1989; Cogger 1996). In contrast, the second skink, *Eulamprus amplus*, is saxicolous and confined mostly to boulder-strewn areas within riparian vine thickets (Covacevich and McDonald 1980). It is a relatively large ambush predator, making limited excursions from its shelter. The gecko, *Phyllurus ossa*, is a nocturnal sit-and-wait predator, principally found on large granite boulders and sometimes tree buttresses in or near creek beds (Couper *et al.* 1993). The distributions of *E. amplus* and *F. ossa*, in particular, appear to be limited by the occurrence

of both rainforest and large boulders whereas *C. rhomboidalis* is not restricted to saxicolous habitats. Judging from locality data in museum collections, *C. rhomboidalis* is probably the least rainforest-restricted and exhibits the least habitat specificity. These natural history observations are reinforced by consideration of the broader ecology of the three genera: *Phyllurus* and *Eulamprus* have radiated only in relatively mesic environments while *Carlia* has repeatedly occupied drier habitats (Cogger 1996). Therefore in MEQ, as in the Wet Tropics (Schneider *et al.* 1999), species-specific ecology interacts with historical processes to shape current patterns of genetic diversity.

#### *Historical biogeography of mid-east Queensland*

The distribution of endemic species suggests that there existed three principal refugia in mid-east Queensland during times of rainforest contraction (Winter and McDonald 1986; Low 1993). The first, and most important, is thought to have been in the Clarke Range (in what is today Eungella National Park) and supports a number of endemic species including a leaf-tail gecko, *Phyllurus nepthys*, a spiny crayfish, *Euastacus eungella*, a skink, *Eulamprus luteilateralis*, and three frogs, *Taudactylus liemi* (Eungella tinkerfrog), *Taudactylus eungellensis* (Eungella dayfrog) and *Rheobatrachus vitellinus* (Northern gastric brooding frog) (Low 1993). The second and third refugia are thought to be the Mt Dryander/Conway Range and Mt Blackwood/Mt Jukes regions, both of which also support locally endemic species (Low 1993). The latter region covers the smallest area and has a lower elevation than either the Clarke Range or Mt Dryander and should therefore have been most severely affected by climate fluctuations. This conclusion is supported by our results: *Carlia rhomboidalis* has no haplotypes unique to Mt Blackwood while *Eulamprus amplus* has relatively high levels of nucleotide diversity in all areas sampled except Mt Blackwood, for which haplotype diversity was almost an order of magnitude smaller despite comparable sample sizes. We suggest that this reflects a greater long-term effective population size maintained within the principal refugia of Mt Dryander and the Clarke Range, but a severe or long-term population bottleneck at Mt Blackwood. Furthermore, long-term isolation and rainforest contraction at Mt Blackwood is consistent with allopatric speciation in the leaf-tail geckos as the endemic *P. isis* is found only in this small rainforest isolate (Couper *et al.* 1993, 2000).

Recent years have seen the discovery of many new vertebrate species from mid-east Queensland, particularly of herpetofauna. All four species of leaf-tail gecko from MEQ, two species of *Eulamprus* (including *E. amplus*), as well as several other endemic frog and skink species (*Saproscincus hannahae*, *Lygisaurus zuma*, *Taudactylus liemi*, *Taudactylus eungellensis*, and *Rheobatrachus vitellinus*), have been described over the past three decades (Winter and McDonald 1986; Couper *et al.* 1993; Low 1993; Couper and Keim 1998). Moreover, several rainforest isolates in the region remain poorly surveyed. The rainforests of the Mackay region, therefore, are likely to continue to yield new species and sampling from a greater range of rainforest isolates and elevated rocky outcrops may yet reveal the presence of cryptic diversity.

Although mid-east Queensland, and particularly Eungella National Park, has been acknowledged as one of the centres of Australian rainforest endemism (Joseph *et al.* 1993; Low 1993), our study highlights the need to recognise the conservation value not just of the major rainforest blocks, but also small fragments of dry rainforest. Our data demonstrate the long-term persistence of rainforest-restricted vertebrate populations in small patches such as Mt Blackwood, which might otherwise have been assumed to be ephemeral over evolutionary time. The unique lineages of leaf-tail gecko and forest skink found in

rainforest outside Eungella represent an important historical component of biodiversity (Moritz *et al.* 1997, 2000) and thus argue for the protection of these areas.

### Acknowledgments

We thank Michael Cunningham, Andrew Hugall, and Adnan Moussalli for support and critical readings of the manuscript as well as members of the Molecular Zoology Lab, Department of Zoology & Entomology, University of Queensland. This research was conducted with the financial support of the Co-operative Research Centre for Tropical Rainforest Ecology and Management and the Queensland Museum.

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Manuscript received 17 December 2000; accepted 17 April 2001