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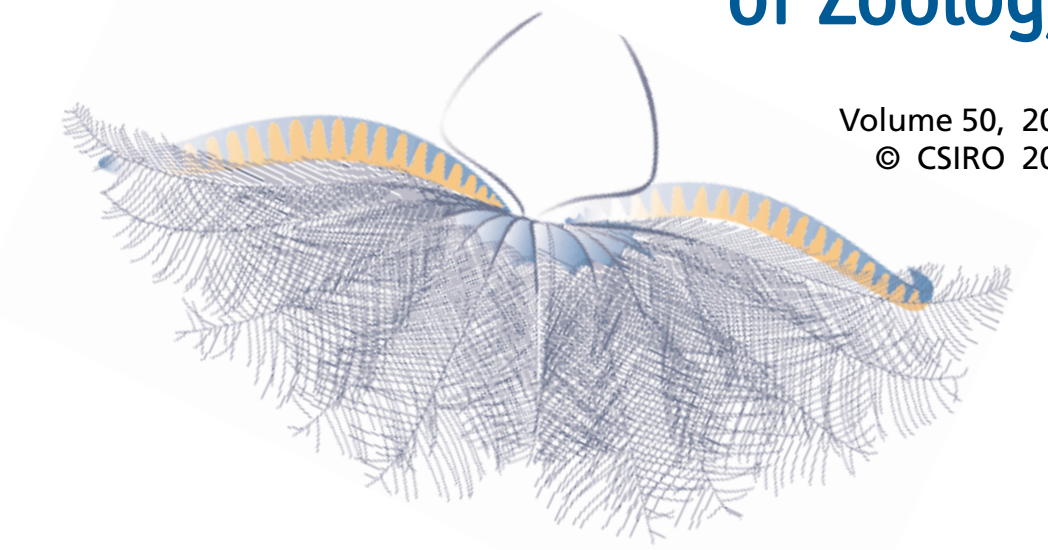

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A molecular phylogeny of rainbow skinks (Scincidae: *Carlia*): taxonomic and biogeographic implications

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Abstract

The phylogenetic relationships amongst 29 species of *Carlia* and *Lygisaurus* were estimated using a 726-base-pair segment of the protein-coding mitochondrial ND4 gene. Results do not support the recent resurrection of the genus *Lygisaurus*. Although most *Lygisaurus* species formed a single clade, this clade is nested within *Carlia* and includes *Carlia parrhasius*. Due to this new molecular evidence, and the paucity of diagnostic morphological characters separating the genera, *Lygisaurus* de Vis 1884 is re-synonymised with *Carlia* Gray 1845. Our analysis is also inconsistent with a previous suggestion that *Lygisaurus timlowi* should be removed to *Menetia*, a genus that is distantly related relative to outgroups used here. Intraspecific variation in *Carlia* is, in several instances, greater than interspecific distance. The most strikingly divergent lineages are found within *C. rubrigularis*, which appears to be paraphyletic, with southern populations more closely related to *C. rhomboidalis* than to northern populations of *C. rubrigularis*. The two *C. rubrigularis–C. rhomboidalis* lineages form part of a major polytomy at an intermediate level of divergence. Lack of resolution at this level, however, does not appear to be due to saturation or loss of phylogenetic signal. Rather, the polytomy probably reflects a period of relatively rapid diversification that occurred sometime during the Miocene.

Introduction

The genus *Carlia* represents an Australo-Papuan radiation of lizards, with 22 species occurring in Australia and at least two species groups occurring in Papua New Guinea and on neighbouring islands (a series of new Papuan species is currently being described by G. R. Zug, NMNH, Washington DC). The species are oviparous, actively foraging, diurnal, terrestrial skinks with well developed limbs. The genus is most notable for the generally marked sexual dichromatism (probably seasonally fluctuating) evident in most species, a phenomenon that is rare amongst Australian terrestrial skinks. Australian *Carlia* are primarily distributed in the tropical and subtropical part of the continent, with most species being found in Queensland, several in the Northern Territory and northern part of Western Australia, and only one species, *C. tetradactyla*, found in the southern half of the continent (Ingram and Covacevich 1989; Cogger 1996). *Carlia* species are found in diverse vegetation types, from tropical rainforest, to spinifex grasslands, tropical woodlands and open eucalypt forest to agricultural lands and suburban gardens. They are also found on diverse substrates, from the sandy soils in coastal and semi-arid areas to bare granite boulders (Ingram and Covacevich 1989, Cogger 1996). Despite the diversity of habitats they occupy, however, *Carlia* species are morphologically conservative relative to other congeneric lizard species (Storr 1974), making taxonomic diagnoses and the definition of phylogenetic relationships based on morphology alone difficult.

Carlia is allied with *Saproscincus*, *Lampropholis* and *Lygisaurus*, within the *Pseudomoia* group of lygosomine skinks (Greer 1989). These four genera can be distinguished by the presence of a ‘thumb-like’ projection at the base of the hemipenis,

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found in no other group of skinks (Greer 1989). This sub-group can be further divided into *Carlia* and *Lygisaurus* on the one hand and *Saproscincus* and *Lampropholis* on the other, primarily based on the loss of the fifth digit of the forelimb in *Carlia* and *Lygisaurus* (Greer 1989). *Lygisaurus* was resurrected from synonymy with *Carlia* in a relatively recent taxonomic revision of the genus (Ingram and Covacevich 1988). Species of *Lygisaurus* are smaller and generally lack the specific distinctive male breeding colours and scale carinations of *Carlia*. Not all species of *Carlia*, however, possess male breeding colours or scale carinations in adults and at least one species of *Lygisaurus* (*L. zuma* Couper, 1993) is sexually dichromatic. Apart from these characters, *Lygisaurus* can be distinguished from *Carlia* only by the number of premaxillary teeth and supradigital scales on the fourth toe (Ingram and Covacevich 1989).

Carlia is perhaps one of the more conspicuous and better studied Australian genera of skinks (e.g. Wilhoft 1963; Whittier and Martin 1992; Whittier 1993; Schneider *et al.* 1999; Stuart-Fox *et al.* 2001). In particular, recent interest has focused on the historical biogeography and evolution of two rainforest-associated species, *C. rubrigularis* and *C. rhomboidalis*. The former comprises two highly divergent lineages north and south of a well known biogeographic barrier known as the Black Mountain corridor (Schneider *et al.* 1999), whereas the latter species has only limited phylogeographic structure within its range in the rainforests of central-eastern Queensland (Stuart-Fox *et al.* 2001). Despite the substantial mtDNA divergence (>12% for cytochrome *b* sequences) between northern and southern *C. rubrigularis* and between these and *C. rhomboidalis*, there is negligible morphological divergence among these lineages (Schneider *et al.* 1999; DMS-F, unpublished data) other than for throat colour, which is red in *C. rubrigularis* and red and blue in *C. rhomboidalis* (Ingram and Covacevich 1989). However, within both species, there are substantial shifts in body size and shape between populations in closed *versus* open forest, suggesting the possibility of strong clinal selection and, perhaps parapatric speciation (Schneider *et al.* 1999).

Few studies of *Carlia* have looked at more than one species, and so far little is known about their phylogenetic relationships. The one exception, a morphological phylogeny of all currently recognised species of *Carlia*, resolves few relationships (G. R. Zug, unpublished). Interestingly, the morphological phylogeny found no synapomorphy differentiating the two *Lygisaurus* species included in the analysis from *Carlia* (G. R. Zug, unpublished), although the number of premaxillary teeth and supradigital scales on the fourth toe were not included in the character matrix. The purpose of this study, therefore, is to examine phylogenetic relationships amongst species of *Carlia*, including known divergent lineages, based on the mitochondrial protein-coding ND4 gene in order to investigate the speciation mechanisms and to provide a framework for comparative studies. In addition, several species of *Lygisaurus* are included in this phylogenetic analysis in order to assess whether the recent splitting of *Carlia* and *Lygisaurus* was warranted.

Methods

Laboratory procedures

Tissues were obtained from 50 individuals representing 29 species of the scincid genera *Carlia* (22 species) and *Lygisaurus* (7 species). Samples and localities are listed in the Appendix. Where possible, two individuals chosen to represent geographic extremes, or in the case of *C. rubrigularis*, known divergent mitochondrial lineages, were sequenced for each species. The protein-coding mitochondrial ND4 gene was chosen as the most appropriate for this study because it has a high amino acid substitution rate and has been shown to be of high phylogenetic utility (Arevalo *et al.* 1994; Russo *et al.* 1996; Zardoya and Meyer 1996). Total genomic DNA was extracted using a solution of 5% Chelex resin in ddH_2O and 5-µL proteinase K

Table 1. PCR and sequencing primers
Sequences are listed from 5′ to 3′ and correspond to the heavy strand of mtDNA. Reference positions follow the bovine sequence (Anderson *et al.*1982)

Primer name	Reference position	Sequence	Reference
ND4 light strand	11165–11196	CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC	Arevalo *et al.* 1994
t-Leu2 heavy strand	12086–12111	CAT TAC TTT TAC TTG GAT TTG CAC CA	Arevalo *et al.* 1994
DSF1light strand	11474–11493	ATA ATT GC(CT) CAC GG(CT) CTT AC	This study
Leu3 heavy strand	11991–12008	GAA TTA GCA GTT CTT T(AG)T G	This study

(10 μg mL^{-1}) (Walsh *et al.* 1991). Initially, the primers ND4 and Leu (Arevalo *et al.* 1994) were used to amplify a 945-bp fragment spanning the 3′ end of the gene. The heavy-strand primer, Leu, produced variable sequence quality so we designed two new internal primers, giving a 726-bp segment with 400 bp of overlapping sequence (Table 1). The PCR cycle profile was as follows: initial denaturation at 94°C for 1 min; 13 cycles of 94°C for 45 s, 46°C for 45 s, 72°C for 1 min 10 s; 20 cycles of 94°C for 45 s, 50°C for 45 s, 72°C for 1 min 10 s; final extension 72°C for 3 min. Each PCR was run with a negative control and each 25-μL reaction contained 5 pmol of each primer, 2.5 μL 10x Promega buffer, 0.475 μL 40 mM dNTPs, 3 μL 25 mM $MgCl_2$, 0.25 μL of 10X BSA (Bovine Albumin Serum); 0.125 μL Promega Taq polymerase and 4 μL of DNA extract. PCR product was gel purified and sequencing reactions were carried out according to standard ABI PRISM dye-deoxy terminator cycle sequencing protocols. Sequences have been deposited with the European Molecular Biology Laboratory nucleotide sequence database (Accession Nos AJ209504–53).

Phylogenetic analysis

Chromatographs were checked and sequences aligned using SeqEd 675 DNA Sequence Editor (Applied Biosystems Inc. 1990) and checked against translated amino acid sequence. Summary statistics, χ^2 test of homogeneity of base frequencies across taxa and phylogenetic analyses were computed in PAUP* (Swofford 2000). Bootstrap pseudo-resamplings were done in PHYLIP 3.5 (Felsenstein 1995) and then analysed in PAUP*.

Parsimony trees with and without bootstrapping (1000 resamplings) were derived using heuristic search with tree bisection reconnection (TBR) branch swapping and 10 random sequence additions. Maximum-likelihood (ML) analysis was conducted using model parameters optimised through an iterative procedure of successive searches and re-optimisations using subtree pruning regrafting (SPR) branch swapping (Swofford 2000). ML bootstrap analyses used 100 pseudo-resamplings generated in PHYLIP, fixed ML tree parameters and searches with SPR from a neighbour-joining starting tree. Relative-likelihood scores were assessed as parameters were added successively to the model according to the method of Posada and Crandall (1998).

We tested both for saturation of the data as well as for phylogenetic signal amongst all taxa and different subsets of taxa within the phylogeny, under different weighting schemes. Firstly, as an *a priori* test of saturation, we plotted the observed number of various classes of substitution against maximum-likelihood distance (calculated using the optimised GTR-g model). Secondly, to test for phylogenetic signal we calculated g1 statistics for subsets of taxa representing different levels of divergence (Huelsenbeck 1991; Hillis and Huelsenbeck 1992). In order to investigate levels of phylogenetic information for different parts of the phylogeny, but avoid confounding signal amongst different groups of taxa, g1 was calculated for seven subsets of taxa representing progressively greater amounts of divergence (see Lara *et al.* 1996) and also within the constraints of the maximum-likelihood/maximum-parsimony (MP) consensus tree (partly shown in Fig. 3). These were done for parsimony, weighted parsimony (tvs 4 : 1 and no 3rd positions) and with maximum likelihood with tree length distributions from 1000 random trees.

Tests of phylogenetic hypotheses

The phylogenetic hypothesis derived from this study was tested against five alternative hypotheses based on taxonomic and biogeographic groupings. The hypotheses are as follows: (1) *Carlia* and *Lygisaurus* as

reciprocally monophyletic sister groups; (2) *Lygisaurus* as a monophyletic group though it may be nested within *Carlia*; (3) *C. rubrigularis* and *C. rhomboidalis* as monophyletic sister groups; (4) *C. rubrigularis* and *C. rhomboidalis* individuals as a single monophyletic group; and (5) all three Papuan species as a monophyletic group. The tests are based on the likelihood ratio of the ML tree *versus* the best tree constrained to each of the alternative hypotheses. Constraint trees were generated in MacClade 3.07 (Maddison and Maddison 1995). We used the distribution of relative log-likelihood (lnL) to the ML tree among bootstrap (BS) pseudoreplicates as the basis for the likelihood ratio tests of Kishino and Hasegawa (1989) (KH) and Shimodaira and Hasegawa (1999) (SH), as described in Goldman *et al.* (2000). The distribution of relative lnLs was calculated from the PHYLIP-generated BS pseudoreplicates using fixed model parameters – the re-sampling estimated log-Likelihood approximation (Kishino and Hasegawa 1989). From these BS-relative lnLs, expected distributions of ΔlnL for the KH (two-tailed) and SH test statistics were calculated as described in Goldman *et al.* (2000). As the KH test is considered flawed, and there is some uncertainty as to what and how many trees are applicable to the SH test (Shimodaira and Hasegawa 1999; Goldman *et al.* 2000) we present the relative lnLs, the BS distribution values ($\delta^{(i)}$: Goldman *et al.* 2000) and the derived KH and SH statistics (Table 3).

Results

A total of 726 base pairs (242 codons) of the protein-coding ND4 gene were obtained and, of these, 376 were variable and 321 were phylogenetically informative. Mean base frequencies were not significantly different between taxa ($\chi^2 = 62.83$, d.f. = 99, $P = 0.998$) and were as follows: A = 33%, C = 27%, G = 12.5%, T = 27.5%. For variable sites only (376 out of 726) the base composition was less equal (A = 40.1%, = 28.1%, G = 9.0%, T = 22.8%) but stationarity cannot be rejected, $P = 0.07$.

Tests for saturation showed that both transitions (Fig. 1*A*) and synonymous changes (not shown) rapidly became saturated; however, the rate of increase for transversions (Fig. 1*A*) and non-synonymous changes (105 variable sites, 72 phylogenetically informative sites) remained linear, and therefore phylogenetically informative, at least for the distances of interest (relationships between *Carlia* and *Lygisaurus* species and between *Carlia* and close outgroups). Moreover, transversions at all three codon positions remained linear until the distant outgroups (Fig. 1*B*). Tests for phylogenetic signal based on random tree length distributions (Hillis and Huelsenbeck 1992) further showed that for all models of character-state change, excepting third-position transitions only, the data contain significant phylogenetic information ($P < 0.05$) at all levels of divergence except among outgroups. Within regions of low support in the MP/ML consensus tree (Fig. 3), g1 signal is low; however, applying weighted parsimony or ML analysis – modeling that is intended to enhance signal for saturated regions – increases g1 signal in these nodes (g1 MP = –0.17 *v.* ML = –0.68). Therefore, both the tree length distribution tests for phylogenetic signal and the plots of substitution event against ML distance show that for levels of divergence corresponding to the relationships between *Carlia* and *Lygisaurus* species, the ND4 gene segment is phylogenetically informative.

Phylogenetic analysis

The ML analysis used a GTR (general time reversible) model (Yang 1994) with gamma shape parameter. Optimal GTR model parameter estimates are given in Table 2. ML trees for GTR-Γ model and a simpler HKY-invariant sites model (not shown) differed in only 2 nodes, both within the polytomy in Fig. 3. Intraspecific maximum-likelihood distances were variable in the range of 0 to 0.22 (mean = 0.09), with the highest being the comparison between individuals from northern and southern populations of *C. rubrigularis*. The variability of intraspecific distances relative to interspecific distances is clearly visible in the ML phylogeny (Fig. 2). There are substantial distances between some individuals of the

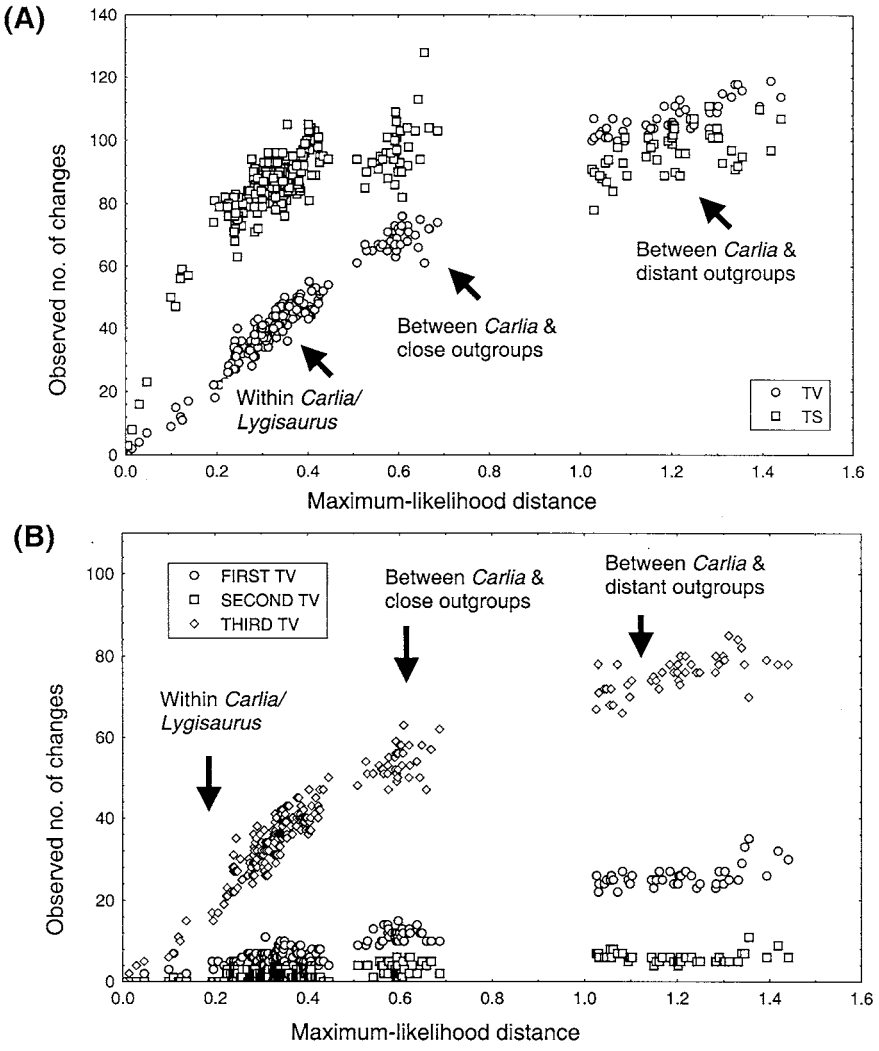

Fig. 1. Scatterplots of observed number of changes for different classes of substitution against maximum-likelihood distance: (*A*) transitions and transversions, (*B*) transversions at first, second and third positions. Close outgroups are represented by *Saproscincus* and *Lampropholis*, while distant outgroups are represented by *Mabuya* and *Sceloporus*. The latter two taxa were added to show level of divergence at which the ND4 gene becomes saturated.

same species, notably *C. amax* (0.21), *C. vivax* (0.20), *C. gracilis* (0.14), *C. schmeltzii* (0.14), *C. jarnoldae* (0.21), and *C. rubrigularis* (0.22), in some cases greater than distances between certain species such as *C. longipes* and *C. fusca* (0.11); and *C. rhomboidalis* and the southern *C. rubrigularis* (0.12). Interspecific distances range from 0.11 between *C. longipes* and *C. fusca* to 0.56 between *C. bicarinata* and *C. amax* (mean = 0.32).

Fig. 3 shows a consensus tree between the single most parsimonious tree from the MP analysis and the final ML tree, with bootstrap values. Both ML and MP trees showed strong

Table 2. Optimal GTR model parameter estimates for the ML analysis
Empirical base frequencies, gamma = 0.292 with four rate categories, remaining options default settings in PAUP*. T–G rate is normalised to 1.0

	A	C	G	T
A	–	3.71	26.98	4.81
C		–	0.56	35.63
G			–	1

support for the monophyly of species except *C. rubrigularis*, but low support and only moderate agreement for most other groupings. This is reflected in the lack of resolution amongst *Carlia* species, most of which form part of a single polytomy. Mean bootstrap

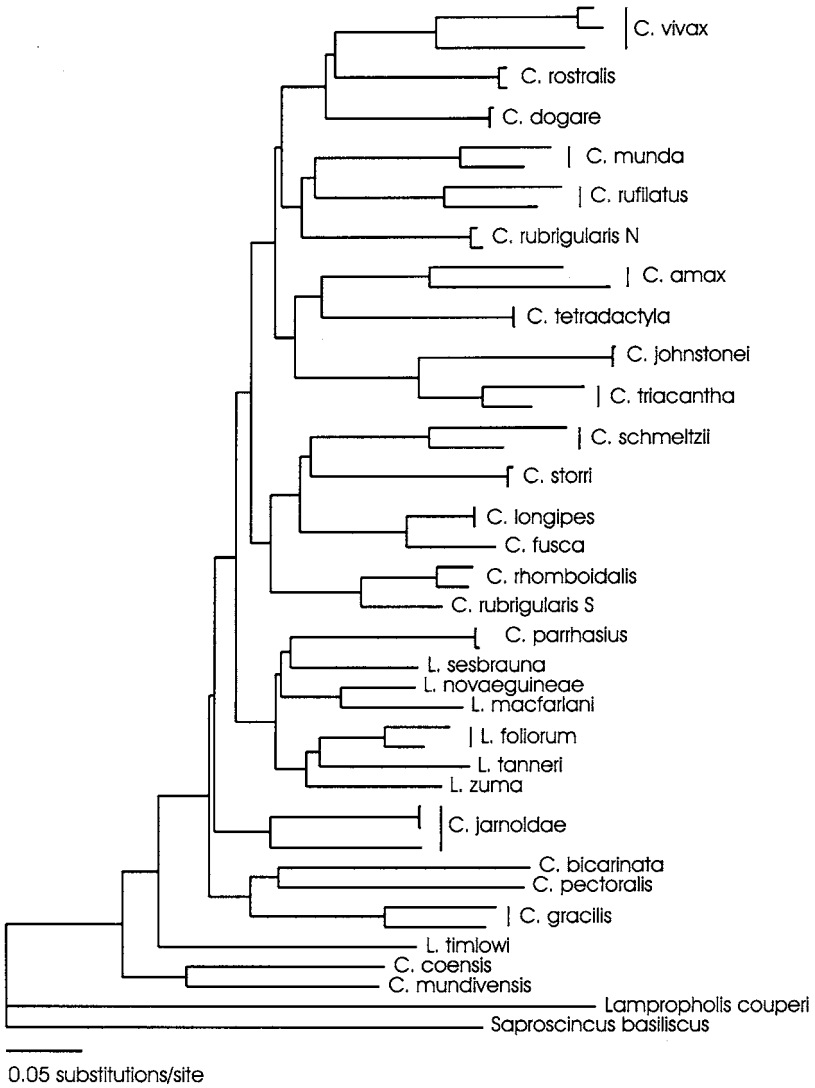

Fig. 2. Best maximum-likelihood tree with branch lengths shown proportionally.

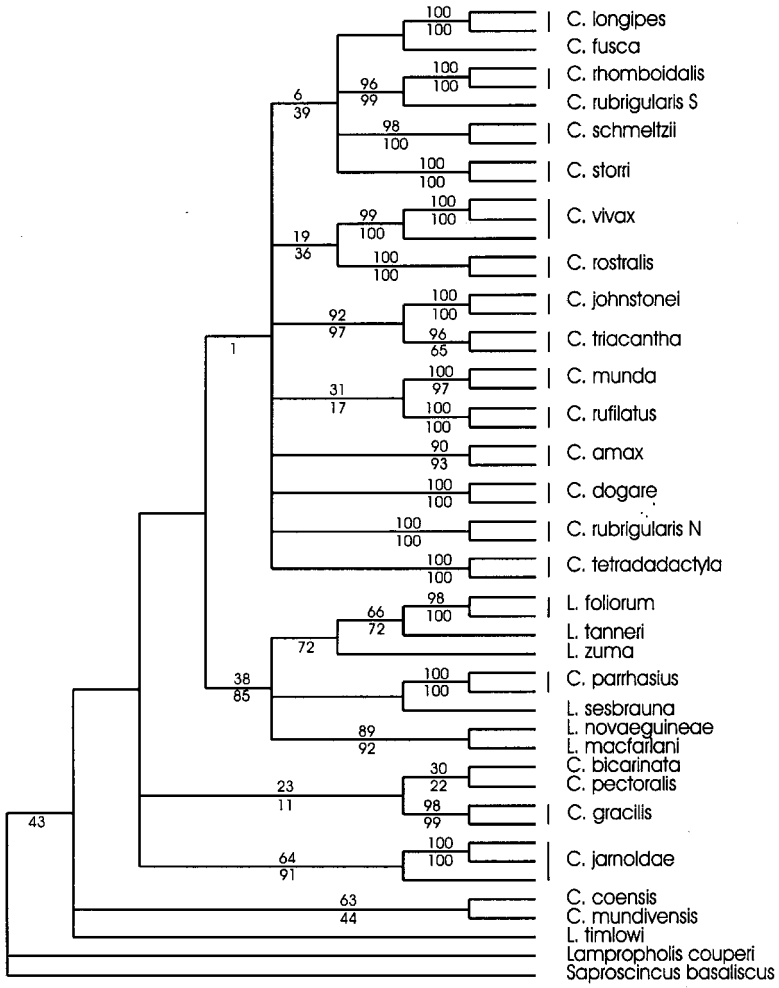

Fig. 3. Strict consensus of best maximum-parsimony and maximum-likelihood trees. Bootstrap values from MP analysis (1000 replicates) are given above branches, and those from ML analysis (100 replicates) below branches.

support for branches forming part of the large polytomy of *Carlia* species was 14.1 and 26 for the MP and ML trees respectively. Mean divergence of lineages forming part of the main polytomy is 0.30. Despite the general lack of resolution, there are a few moderately to strongly supported sister-species groupings that are consistent in the MP and ML analyses. *C. fusca* and *C. longipes* are sister species with high bootstrap support, as are *C. rhomboidalis* and the southern *C. rubrigularis*, *C. johnstonei* and *C. triacantha*, *L. foliorum* and *L. tanneri*, *L. novaeguineae* and *L. macfarlani*, *and C. coensis* and *C. mundivensis*. *C. munda* was also consistently the sister species to *C. rufilatus*, as was *C. vivax* to *C. rostralis* and *C. bicarinata* to *C. pectoralis*, although all with less than 50% bootstrap support.

Table 3. Likelihood ratio tests of phylogenetic hypotheses

Hypothesis	Δln Likelihood	Bootstrap frequency	*P* (KH)	*P* (SH)
Carlia & *Lygisaurus* reciprocal monophyly	50.5	0.01**	0.03*	0.05*
Lygisaurus monophyletic	32.8	0.03*	0.13	0.182
C. rubrigularis & *C. rhomboidalis* reciprocal monophyly	23.6	0.07	0.17	0.32
C. rubrigularis & *C. rhomboidalis* monophyletic	5.8	0.29	0.68	0.765
PNG species monophyletic	104.9	<0.01**	<0.01**	<0.01**

Tests of phylogenetic hypotheses

Species of *Lygisaurus*, except *L. timlowi*, form part of a moderately supported clade (38% in the MP bootstrap tree and 85% in the ML bootstrap tree) nested within *Carlia*, making *Carlia* paraphyletic. This phylogenetic hypothesis was significantly more likely than the alternative of the two genera being monophyletic sister groups using any test (Table 3). Forcing the monophyly of *Lygisaurus* only (Hypothesis 2) produced a better tree in only 3% of bootstrap resamplings but was not significantly less likely than the ML tree (KH test: $P = 0.13$). Similarly, forcing the monophyly of *C. rubrigularis sensu stricto* (Hypothesis 3) was not significantly worse while Hypothesis 4 (monophyly of the *C. rubrigularis/C. rhomboidalis* complex) produced better trees in a minority of bootstrap resamplings but was not rejected by the SH and two-tailed KH tests (Table 3). Forcing monophyly of the Papuan species results in a significantly worse tree using any test ($P < 0.01$ in all cases).

Discussion

Taxonomy

Our results do not support Ingram and Covacevich's (1988) resurrection of the genus *Lygisaurus*. Trees constrained to reflect current taxonomic designations (reciprocal monophyly of *Carlia* and *Lygisaurus*: Hypothesis 1, Table 3) were significantly worse using any test. Although all *Lygisaurus* species apart from *L. timlowi* clustered together, this group was clearly nested within *Carlia*. Moreover, *C. parrhasius* formed part of the group containing these *Lygisaurus* species. *C. parrhasius* is similar in size to *Lygisaurus*, the species of which are generally smaller than those of *Carlia* (Ingram and Covacevich 1988). However, it differs from the uniformly coloured *Lygisaurus* species in possessing a brown/black and white striped body and a bright red tail. Although no other *Carlia* possesses a brightly coloured tail, most species have striking breeding colours in males and/or have white stripes or dashes. For this reason, and because of its high supradigital scale count and presence of dorsal scale carinations, *C. parrhasius* was placed in *Carlia* rather than *Lygisaurus* (Couper *et al.* 1994).

In fact, there is some overlap between *Carlia* and *Lygisaurus* in almost all morphological characters used to differentiate the two genera, such as size, body scales, number of premaxillary teeth and male breeding colour. Table 4 lists the diagnostic characters used to separate *Carlia* and *Lygisaurus* and shows that there is overlap between the genera in all characters except perhaps the number of supradigital scales on the fourth toe (fewer than 10 in *Lygisaurus v.* 10 or more in *Carlia*). However, Couper and Covacevich (in press) have found that two of 55 *Lygisaurus* specimens examined had 10 supradigital scales on the fourth toe, suggesting that even this character cannot reliably differentiate the two genera.

Table 4. Diagnostic characters of *Carlia* and *Lygisaurus*
Data from Cogger (1996) and Ingram and Covacevich (1988, 1989)

Diagnostic character	*Carlia*	*Lygisaurus*
Size	Maximum SVL ranges from 34.5 mm (*C. parrhasius*)[A] to 70 mm (*C. rostralis*)	Maximum SVL ranges from 29 mm (*L. timlowi*) to 39 mm (*L. foliorum*, *L. aeratus*, *L. rococo*)
Body scales	Keeled or carinate in juveniles. Adults may have smooth or striate scales (*C. munda*, *C. tetradactyla*)	Striate body scales
Supradigital scales on the 4th toe	10 or more	Usually fewer than 10[B]
Number of premaxillary teeth	Usually 13[C]	Usually 15[C]
Male breeding colours	Usually red, orange, black or blue throats, red, orange or black dorsal and/or lateral markings, blue-green iridescence (*C. gracilis* and *C. triacantha*	Usually red or orange throat, dorsal surface patternless, iridescent grey-green or grey-orange-brown.

[A]Only six specimens of *C. parrhasius* have been measured (Couper *et al.* 1994; Stuart-Fox, unpublished data).
[B]A recent study of diagnostic characters for the genera of Australian skinks (Couper and Covacevich, in press) has found that two of 55 Lygisaurus specimens examined had 10 supradigital lamellae on the fourth toe.
[C]The degree of overlap between the two genera in this character is unclear because the number of premaxillary teeth present in each species is not given in the descriptions of Ingram and Covacevich (1988, 1989).

Furthermore, the genetic evidence presented here shows clearly that the two groups are not monophyletic. Although only six of the nine species of *Lygisaurus* are included here, inclusion of the remaining species is highly unlikely to alter relationships to the extent that *Lygisaurus* and *Carlia* become monophyletic. Therefore we re-synonymise *Lygisaurus* de Vis, 1884 with its senior synonym, *Carlia* Gray, 1845. *Carlia* thus comprises 31 Australian species, nine of which formerly belonged to *Lygisaurus*. A study of the systematics of the Papuan *Carlia* currently being undertaken by G. R. Zug (NMNH, Washington DC) describes a further eight species, bringing the number of species belonging to the genus to over 40.

Greer (1991) has argued that *Lygisaurus timlowi* should be included within the genus *Menetia*, to which it was originally assigned by Ingram (1977). Our analysis, however, places *L. timlowi* as a basal lineage clearly within the *Carlia–Lygisaurus* group, rather than amongst the outgroups. In his revision of the generic diagnosis of *L. timlowi*, Greer (1991) also described two new species, *Menetia koshlandae* and *M. sadlieri*, which he placed with *L. timlowi* in a subgroup within *Menetia*. These three species share the derived character of interparietal fused to the frontoparietals, which are fused (Greer 1991); this character is also shared by *C. rubrigularis*, *C. rhomboidalis* and some New Guinean species (Ingram and Covacevich 1989). The remaining *Menetia* species (*M. greyii* subgroup), which previously stood alone in the genus, share several characters not found in *L. timlowi* and the two new species. These characters include the uniquely derived character that had previously defined *Menetia*, namely two supraoculars obliquely aligned instead of three or four transversely aligned (Ingram and Covacevich 1988; Greer 1991). In addition, the *M. greyii* group is separated from the *L. timlowi* group by a reduced number of enlarged supraoculars,

fused premaxillae and a greater number of supradigital scales on the fourth toe (11–15 *v.* 9–10) (Greer 1991). This morphological division into subgroups within *Menetia*, combined with the new molecular evidence presented here, raises the possibility that *M. koshlandae* and *M. sadlieri* may belong with *L. timlowi* in the *Carlia–Lygisaurus* group or that the three species should be given separate generic status. The status of *M. koshlandae*, *M. sadlieri* and *L. timlowi* therefore requires further investigation.

Biogeography

Our results show that intraspecific variation in *Carlia* is, in several instances, greater than interspecific distance. The most strikingly divergent lineages are found within *C. rubrigularis*, which appears to be paraphyletic. Although the hypothesis that *C. rubrigularis* individuals are monophyletic could not be rejected (KH test, $P = 0.17$), it was more likely in only 7% of bootstrap resamplings. The maximum-likelihood distance (21%) between *C. rubrigularis* individuals from northern populations (Mt Lewis and Mossman Gorge) and the individual from the southern population (Seaview Range) is almost twice that between the southern individual and *C. rhomboidalis* (12%). Previous studies on the phylogeography of *C. rhomboidalis* and *C. rubrigularis* (Schneider *et al.* 1999; Stuart-Fox *et al.* 2001) based on a 350-bp fragment of the mitochondrial cytochrome *b* gene (40–50 individuals of each species), similarly revealed greater divergence (approximately 15% Kimura 2 parameter) between northern and southern *C. rubrigularis* than between southern *C. rubrigularis* and *C. rhomboidalis* (12–13%).

The main morphological character that separates *C. rubrigularis* and *C. rhomboidalis* is throat colour in breeding males. *C. rubrigularis* has a red throat, whereas *C. rhomboidalis* has a red and blue throat. Ingram and Covacevich (1989) suggest that the two species may be allopatric subspecies and recommend additional fieldwork to determine the role of throat colouration in mate recognition. Studies on morphological variation in *C. rubrigularis* have so far failed to find any identifiable morphological difference between northern and southern individuals (Schneider *et al.* 1999). The lack of morphological differentiation between northern and southern forms of *C. rubrigularis* is striking, given that the molecular distance between the two is as great as, or greater than, between other more morphologically divergent *Carlia* species. High levels of molecular divergence, in the absence of phenotypic differentiation among populations is a pattern that has been noted in three other lizard species (*Saltuarius cornutus*, *Gnypetoscincus queenslandiae* and *Carphodactylus laevis*) from the Wet Tropics rainforest of north-eastern Australia (Schneider and Moritz 1999).

Northern *C. rubrigularis* and southern *C. rubrigularis–C. rhomboidalis* lineages form part of a major polytomy that includes most *Carlia* species. Given the moderate size of our molecular data set, the unresolved nodes are likely to be fully dichotomous in reality but resemble a polytomy because the internal branches are so much shorter than the terminal branches (see Purvis and Garland 1993). Lack of resolution, however, does not appear to be due entirely to saturation or loss of phylogenetic signal. Phylogenetic resolution exists at both deeper and shallower levels within the phylogeny, and tests for saturation show that the data contain significant phylogenetic information at all levels of divergence except among outgroups. Therefore the main polytomy probably reflects a period of relatively rapid diversification that, judging by the mean level of divergence amongst these lineages (30%), took place sometime during the Miocene. Unfortunately, the large errors involved in estimating times of splitting events, coupled with relatively poor knowledge of Miocene

climatic history, prevent meaningful speculation about the circumstances that might have triggered these speciation events.

Although our molecular phylogeny of *Carlia* and *Lygisaurus* resolves relatively few relationships with any certainty, six sister-species relationships are identified. Bootstrap support for two of the pairs (*C. rufilatus–C. munda* and *C. rostralis–C. vivax*) is weak; however, they are consistently placed as sister taxa under any set of evolutionary assumptions (e.g. different optimality criteria, weighting schemes, ML models). These sister-species pairs show a classic geographic and ecological pattern: sympatric sister species occupy different habitats while allopatric sister species are more ecologically similar. In three cases (*C. johnstonei–C. triacantha*, *C. rufilatus–C. munda*, and *C. rostralis–C. vivax*) the former species of each pair occupies moister microhabitats and has a smaller distribution mostly or entirely within the range of the latter species. The remaining Australian sister-species pairs are ecologically very similar and their geographic ranges are separated by substantial distances (*C. coensis–C.mundivensis*, *L. tanneri–L. foliorum*), or abut but do not overlap (*C. rhomboidalis–C. rubrigularis* S). Further study of the ecology and history of these sister taxa, the sympatric and parapatric taxa in particular, may shed light on speciation mechanisms in this genus.

Finally, the three Papuan species included in our analysis are scattered throughout the tree, and forcing their monophyly results in a significantly worse tree. This is consistent with repeated migration between Australia and New Guinea during the multiple periods of connection between the two land masses (Kikkawa *et al.* 1981). Moreover, *Carlia* and *Lygisaurus* are found on the Torres Strait Islands between Papua New Guinea and Australia. The basal lineages, however, are Australian, and given that the subgroup containing the genera *Lampropholis*, *Saproscincus*, *Carlia* and *Lygisaurus* is predominantly Australian, it is likely that the Papuan members of the group are derived from Australian ancestors.

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Appendix. Materials examined

Institutional abbreviations are as follows: AM = Australian Museum; NTM = Northern Territory Museum; QM = Queensland Museum; SAM = South Australian Museum. No voucher specimens exist for samples lacking a museum registration number. Samples lacking vouchers have been used in previous phylogeographic studies on *C. pectoralis* (Moritz and Playford 1998), *C. rubrigularis* (Schneider *et al.* 1999), and *C. rhomboidalis* (Stuart-Fox *et al.* 2001) or were collected as part of ongoing ecological studies of *Carlia* species; numbers of samples collected for these studies precluded taking vouchers for each tissue sample

Carlia amax. SAMAR34243, Hell's Gate, Qld; AMSR123888, Weipa Plateau, WA.
Carlia bicarinata. AMSR122681 Moitaka, NCD PNG.
Carlia coensis. Peach Ck., McIllwraith Range, Qld.
Carlia dogare. QMJ62426 & QMJ62427, Cape Flattery, Qld.
Carlia fusca. AMSR122702,Waro SHP, PNG.
Carlia gracilis. SAMAR34125, Jabiru East, NT; NTMR21732, Litchfield NP, NT.
Carlia jarnoldae. QMJ46155, Morehead R., Qld; QMJ62732, Mt Abbot, Qld; QMJ62695, Mt Aberdeen, Qld.
Carlia johnstonei. AMSR123865 & AMSR123866, near Mitchell Falls, WA.
Carlia longipes. QMJ41352, near Cairns, Qld; QMJ62753, Lockerbie Scrub, Qld.
Carlia munda. AMSR123890, Mitchell Plateau, WA; NTMR16467, Bing Bong Stn, NT.
Carlia mundivensis. QMJ62700, Mt Aberdeen, Qld.
Carlia parrhasius. QMJ58680 & QMJ58681, Glennie Tableland, Qld.
Carlia pectoralis. QRFA434, Blackdown Tableland, Qld.
Carlia rhomboidalis. Magnetic Island, Qld; Boulder Ck, mid-east Qld.
Carlia rostralis. Big Crystal Ck, Paluma; AMSR114018, Townsville, Qld.
Carlia rubrigularis. Seaview Range, Qld; Mossman Gorge, Qld; Mt. Lewis, Qld.
Carlia rufilatus. NTMR17050, Dundee Downs Lodge, NT; NTMR21741, Litchfield NP, NT.
Carlia schmeltzii. Magnetic Island, Qld; QMJ62837, Mt Abbot, Qld.
Carlia storri. Big Crystal Ck, Paluma, Qld; Mission Beach, Qld.
Carlia tetradactyla. AMSR96641, Rylstone, NSW; AMSR111809, Euroa, Vic.
Carlia triacantha. Ewaninga, NT; NTMR20879, Jabiru airstrip, NT.
Carlia vivax. Boulder Ck Camp, mid-east Qld; south-east Qld; north-east NSW.
Lygisaurus foliorum. SAMAR33733, Denman Tip, NSW; QMR62701, Mt Aberdeen, Qld.
Lygisaurus macfarlani. NTMR20234, Maxwell Ck, Melville Island, NT.
Lygisaurus sesbrauna. AMSR94589, Captain Billy Ck, Cape York, Qld.
Lygisaurus tanneri. SAMAR32519, Mt Spec, Qld.
Lygisaurus timlowi. QMJ63955, Warro SF, Qld.
Lygisaurus zuma. QMJ62425, McIvor River Crossing, Qld.

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