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Molecular Phylogeny of the Australian Frog Genera *Crinia, Geocrinia,* and Allied Taxa (Anura: Myobatrachidae)

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We present a mitochondrial gene tree for representative species of all the genera in the subfamily Myobatrachinae, with special emphasis on *Crinia* and *Geocrinia*. This group has been the subject of a number of long-standing taxonomic and phylogenetic debates. Our phylogeny is based on data from approximately 780 bp of 12S rRNA and 676 bp of ND2, and resolves a number of these problems. We confirm that the morphologically highly derived monotypic genera *Metacrinia, Myobatrachus,* and *Arenophryne* are closely related, and that *Pseudophryne* forms the sister group to these genera. *Uperoleia* and the recently described genus *Spicospina* are also part of this clade. Our data show that *Assa* and *Geocrinia* are reciprocally monophyletic and together they form a well-supported clade. *Geocrinia* is monophyletic and the phylogenetic relationships with the genus are fully resolved with two major species groups identified: *G. leai, G. victoriana,* and *G. laevis;* and *G. rosea, G. alba,* and *G. vitellina* (we were unable to sample *G. lutea*). We confirm that *Taudactylus* forms the sister group to the other myobatrachine genera, but our data are equivocal on the phylogenetic position of *Paracrinia*. The phylogenetic relationships among *Crinia* species are well resolved with strong support for a number of distinct monophyletic clades, but more data are required to resolve relationships among these major *Crinia* clades. *Crinia tasmaniensis* and *Bryobatrachus nimbus* form the sister clade to the rest of *Crinia*. Due to the lack of generic level synapomorphies for a *Bryobatrachus* that includes *C. tasmaniensis,* we synonymize *Bryobatrachus* with *Crinia*. *Crinia georgiana* does not form a clade distinct from other *Crinia* species and so our data do not support recognition of the genus *Ranidella* for other *Crinia* species. *Crinia subinsignifera, C. pseudinsignifera,* and *C. insignifera* are extremely closely related despite differences in male advertisement call. A preliminary investigation of phylogeographic substructure within *C. signifera* revealed significant divergence between samples from across the range of this species. © 2001 Academic Press

Key Words: **mitochondrial DNA; 12S rRNA; ND2; amphibian; frog; Myobatrachidae; Australia; New Guinea; Pacific.**

INTRODUCTION

Approximately 57% of the 211 known frog species in Australia are allocated to the Family Myobatrachidae. While there is some disagreement about the monophyly of the Myobatrachidae (Tyler *et al.*, 1981; Ford and Cannatella, 1993; Hay *et al.*, 1995; Ruvinsky and Maxson, 1996), there is general consensus that the Australian and New Guinea species are each others' closest relatives and most workers recognize two myobatrachid subfamilies, the Myobatrachinae with 12 genera and 71 species and the Limnodynastinae with nine genera and 47 species (Parker, 1940; Lynch, 1971; Tyler *et al.*, 1981; Farris *et al.*, 1982). The recognition of a third subfamily, Rheobatrachinae, comprising only the genus *Rheobatrachus* (Heyer and Liem, 1976; Davies and Burton, 1982) is contentious (Daugherty and Maxson, 1982; Farris *et al.*, 1982; Hutchinson and Maxson, 1987; Ford and Cannatella, 1993).

Phylogenetic studies of *Crinia* and other myobatrachine genera began with the phenetic morphological work of Blake (1973). Heyer and Liem (1976) produced a phylogeny of all Australian myobatrachine genera based on 40 morphological and ecological characters, but reanalysis of a subset of these same characters with different methods resulted in some fundamentally different hypotheses of relationships (Farris *et al.*, 1982). Several immunological distance studies produced alternative rather than corroborating phylogenies (Daugherty and Maxson, 1982; Maxson and Roberts, 1985; Maxson, 1992; see Fig. 1). Despite the incongruence, some clear patterns emerged, such as support for the distinctiveness of the genera *Paracrinia, Assa, Geocrinia,* and *Taudactylus.*

Only the monotypic genera *Arenophryne* and *Myoba-*

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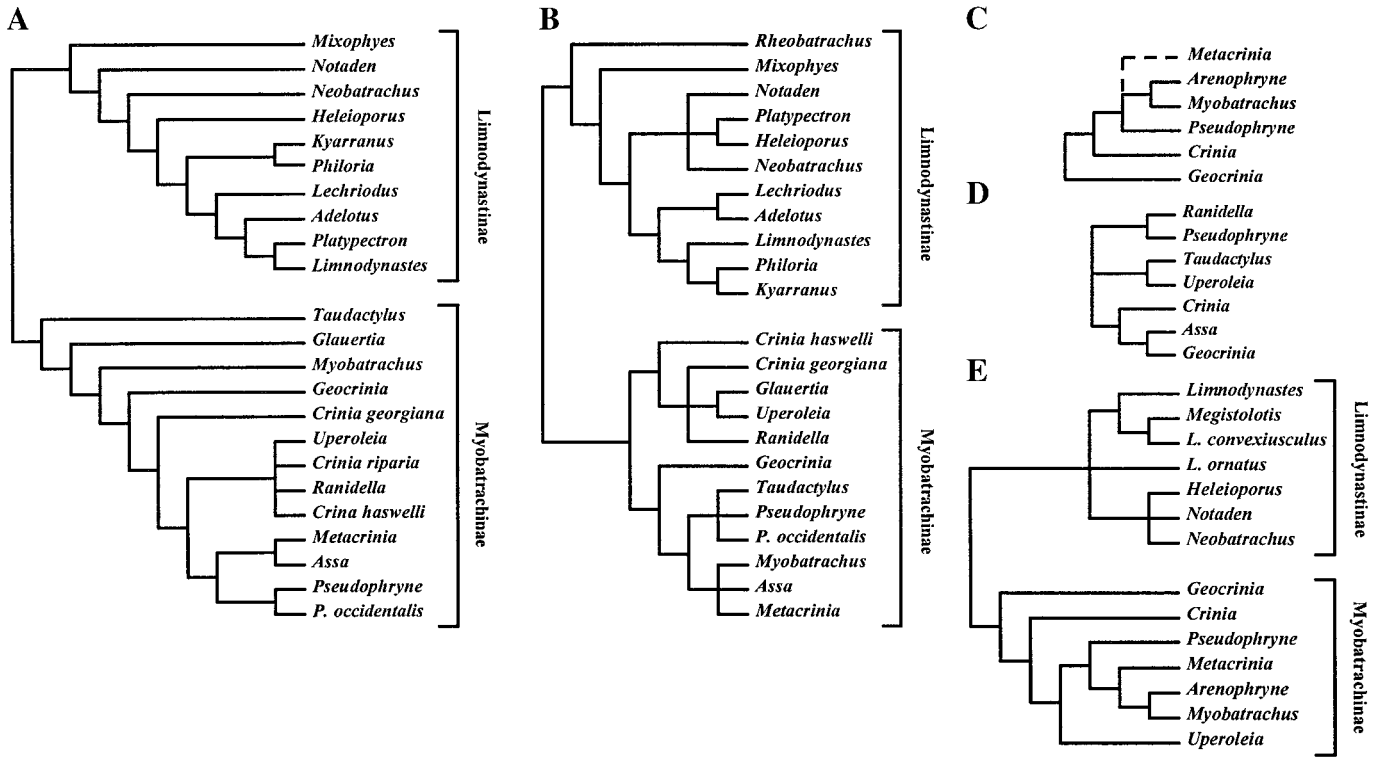

FIG. 1. Previous phylogenetic hypotheses for myobatrachid genera. (A) Phylogeny based on morphological, ecological, and behavioral data (Heyer and Liem, 1976); (B) phylogeny based on the same data as Heyer and Liem (1976), but alternative methods of analysis (Farris *et al.*, 1982); (C) phylogeny based on albumin immunological distance data (Maxson and Roberts, 1985); (D) phylogeny based on morphological, ecological, and behavioral data (Blake, 1973); (E) phylogeny based on albumin immunological distance data (Maxson, 1992).

trachus have not experienced some sort of rearrangement. The monotypic genera *Bryobatrachus* and *Spicospina*, described more recently, have not been part of any phylogenetic work (Rounsevell *et al.*, 1994; Roberts *et al.*, 1997). The taxonomic instability evident in the Myobatrachinae is particularly acute in the genus *Crinia* that has been the subject of considerable taxonomic (Heyer and Liem, 1976; Thompson, 1981; Heyer *et al.*, 1982) and biogeographic (Main *et al.*, 1958; Littlejohn, 1967, 1981; Main, 1968; Barendse, 1984; Roberts and Maxson, 1985a,b, 1988; Watson and Littlejohn, 1985; Roberts and Watson 1993; Littlejohn and Wright, 1997) debate for more than 40 years. For example, *Crinia* was the subject of five major revisions or taxonomic rearrangements between 1972 and 1982 (Tyler, 1972; Blake, 1973; Heyer and Liem, 1976; Thompson, 1981; Heyer *et al.*, 1982). There were 19 species of *Crinia* at the beginning of 1966 and one in 1976, and now there are 14 (Tyler, 1972; Heyer and Liem, 1976; Cogger, 2000). During this time, the genera *Assa*, *Geocrinia*, and *Paracrinia* were described to accommodate species once allocated to *Crinia*, and *Crinia acutirostris* was moved to the genus *Taudactylus* (Tyler, 1972; Blake, 1973; Heyer and Liem, 1976).

Early hypotheses of relationships among *Crinia* species were based on results of hybridization experiments and analyses of male mating call structure: compatible hybrids and higher similarity in male call were presumed to reflect closer relationship (e.g., Main *et al.*, 1958; Littlejohn, 1967; Main, 1968). These models were used to justify biogeographic models of speciation that have been challenged by more recent data (e.g., Daugherty and Maxson, 1982; Barendse, 1984; Roberts and Maxson, 1985a,b, 1988; Roberts and Watson, 1993). Based on morphological and call differences, Main (1957) recognized the distinctness of *C. georgiana* and split the remaining *Crinia* species (excluding those that are now species of *Geocrinia*, *Assa*, or *Taudactylus*) into two species groups, the "*C. signifera* species group" (*C. signifera* and *C. glauerti*) and the "*C. insignifera* species group" (*C. insignifera*, *C. parinsignifera*, *C. pseudinsignifera*, *C. sloanei*, and *C. subinsignifera*). Species described subsequently often did not fit easily into either of these species groups (e.g., *C. riparia*, Littlejohn and Martin, 1964; *C. tinnula*, Straughan and Main, 1966).

The biogeographic and taxonomic debates have been so protracted largely due to the lack of a robust phylogeny covering all species able to test alternative taxonomic scenarios. While there have been a number of attempts to derive phylogenetic hypotheses for *Crinia* using morphology (Blake, 1973; Heyer and Liem, 1976;

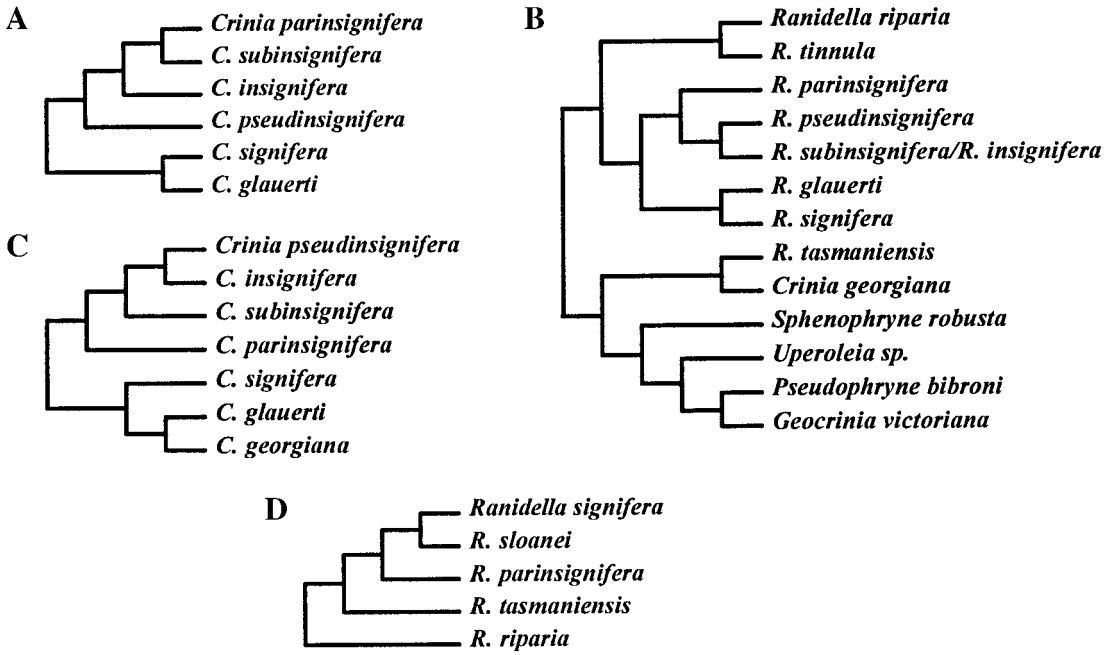

FIG. 2. Previous phylogenetic hypotheses for *Crinia* species—redrawn with the current taxonomic names used. (A) Phylogeny based on a reconstruction of the relationships proposed by Main *et al.* (1958) (as redrawn by Roberts and Maxson, 1985a); (B) phylogeny based on morphological, ecological, and behavioral data (Thompson, 1981); (C) phylogeny based on allozyme data (Barendse, 1984); (D) phylogeny based on *in vitro* and natural hybridization data (Watson and Littlejohn, 1985).

Thompson, 1981; Watson and Littlejohn, 1985), immunological distance (Daugherty and Maxson, 1982; Heyer *et al.*, 1982), and allozyme electrophoresis (Barendse, 1984), little consensus has been reached (Fig. 2). Compounding the problem, no previous studies have included all species of *Crinia*. However, the traditional species groups have remained essentially unchallenged except that the molecular data supported the placement of *Crinia georgiana* in the "*signifera* complex" (Daugherty and Maxson, 1982; Barendse, 1984), and *Ranidella* was synonymized with *Crinia* based on morphological data and the relative degrees of serum albumin similarity with *Ranidella* [*Crinia*] *signifera* (Daugherty and Maxson, 1982; Heyer *et al.*, 1982; Barendse, 1984). The taxonomic status of *Crinia* and its previous members established by Heyer *et al.* (1982), are those widely accepted today (Cogger, 2000).

There has never been an attempt to develop a phylogeny for *Geocrinia*, whose species were also formerly included in the genus *Crinia* (Main, 1957; Blake, 1973). However, there is a clear division with species in the *G. rosea* group (*rosea, lutea, alba,* and *vitellina*), which all have direct development and simple call structures (Roberts and Wardell-Johnson, 1995), and the *G. victoriana* group (*victoriana, laevis,* and *leai*), where all species have terrestrial egg deposition and diphasic calls (Main, 1965; Littlejohn and Harrison, 1985; Harrison and Littlejohn, 1985). Diphasic calls also occur in some *Crinia* species (e.g., Littlejohn and Wright, 1997), suggesting the possibility of convergence or that *Geocrinia* is polyphyletic.

In this study, we utilize sequence data from two regions of the mitochondrial genome (12S rRNA and ND2 genes) to generate a phylogeny for *Crinia* and *Geocrinia* and representatives of all genera in the subfamily Myobatrachinae to supply a unique and independent data set to test previous hypotheses. We sought to address four main questions: (1) Are *Crinia* and *Geocrinia* monophyletic? (2) What are the affinities of species once allocated to *Crinia* including *Geocrinia, Paracrinia, Assa,* and *Taudactylus?* (3) If *Crinia* is not monophyletic, what are the affinities of their members? Finally, (4) What are the relationships among the myobatrachine genera?

MATERIALS AND METHODS

Taxonomic Sampling

Tissue samples from representatives of all genera in subfamily Myobatrachinae were included, but special emphasis was placed on sampling of *Crinia* and *Geocrinia.* For these genera, we sampled all recognized taxa (*sensu* Cogger, 2000), except for *Geocrinia lutea*, for which no samples were available (Table 1). Because we were interested in testing the validity of previous taxonomic hypotheses concerning *Crinia* and *Geocrinia,* we included in our sampling regime *Paracrinia haswelli, Taudactylus acutirostris,* and *Assa darling-*

TABLE 1

Museum Registration Numbers and Locality Data for Taxa Used in This Study

Genus	Species	Museum tissue no.	Voucher no.	Locality
Arenophryne	*rotunda**			
Assa	*darlingtoni**	ABTC 24859		Mt. Warning, NSW
Bryobatrachus	*nimbus**	ABTC 25297		Harzt Mts, TAS
Crinia	*bilingua**	ABTC 13403		Pentecost R., El Questo Stn, WA
Crinia	*deserticola**	ABTC 17752	SAM 45118	Birdsville, QLD
Crinia	*georgiana**	ABTC 62663	WAM R114806	Gungin Gully, 10 km E of Kalamunda, WA
Crinia	*georgiana*			5.4 km E of Cussons Rd on SW Highway, WA
Crinia	*glauerti**	ABTC 62634	WAM R114658	5 km SE Margaret River, WA
Crinia	*glauerti*			Cnr Trent and Middle Rds, ENE of Walpole, WA
Crinia	*insignifera**	ABTC 62741	WAM R115784	Cardup, WA
Crinia	*insignifera*	ABTC 62678	WAM R114932	Yalgorup, WA
Crinia	*parinsignifera**	ABTC 17569	SAMA 42202	22 km E of Wagga Wagga, NSW
Crinia	*pseudinsignifera**			Cnr Railway Pde and SW highway, near Walpole, WA
Crinia	*remota**	ABTC 17181	SAMA 42242	Darwin, NT
Crinia	*riparia**	ABTC 14948		Yudnamatana, SA
Crinia	*signifera* (10)*	ABTC 14924	SAMA 39209	16 km W of Penola, SA
Crinia	*signifera* (11)	ABTC 17180	SAMA 42241	1 km S of Nugent, TAS
Crinia	*signifera* (93)*	ANWC 1706		Kangaroo Island, SA
Crinia	*signifera* (95)	ANWC 1708		Kangaroo Island, SA
Crinia	*signifera* (96)*	ANWC 1709		Kangaroo Island, SA
Crinia	*signifera* (97)	ANWC 1710		Kangaroo Island, SA
Crinia	*signifera* (98)	ANWC 2048		Jerrabombera, ACT
Crinia	*signifera* (99)*			Cann R. valley, between Cann R. and Noorinbee, VIC
Crinia	*signifera* (86)*			Braidwood, NSW
Crinia	*signifera* (87)			Dam pond, Mulligans flat, ACT
Crinia	*signifera* (88)			Dam pond, Mulligans flat, ACT
Crinia	*sloanei**	ABTC 17555	SAMA 42150	E of Albury, NSW
Crinia	*subinsignifera**	ABTC 62565	WAMR114143	14 km E of Mt. Hanett, WA
Crinia	*subinsignifera*			100 m up Pratts Rd, off Sth Coast Highway, WA
Crinia	*tasmaniensis**	ABTC 23114	TMHC 870	Pigsty Ponds, TAS
Crinia	*tinnula**	ABTC 26483		Mungo Brush Myall Lakes NP, NSW
Crinia	sp.*	ABTC 26421		Coffs Harbour area, NSW
Geocrinia	*alba**			"Junction," near Witchcliffe, WA
Geocrinia	*leai**	WAM 115947		Kangaroo Gully, WA
Geocrinia	*leai*	WAM 116137		8 km W of Albany, WA
Geocrinia	*leai*			Cnr Railway Pde and SW Highway, near Walpole, WA
Geocrinia	*laevis**			Mt. Burr, SA
Geocrinia	*rosea**	WAM 114841		Pemberton, WA
Geocrinia	*victoriana**	ABTC 7145		Tanjil Bren, VIC
Geocrinia	*vitellina**			Geo Creek, NW tributary of Spearwood Creek, WA
Metacrinia	*nichollsi**	ABTC 17124	WAMR 106065	9.5 km ENE of Mt. Frankland, WA
Myobatrachus	*gouldi**	ABTC 63391	WAMR115075	Bold Park, Perth, WA
Paracrinia	*haswelli*	ABTC 26440		Lighthouse Beach Port Macquarie, NSW
Paracrinia	*haswelli*	ABTC 26441		Lighthouse Beach Port Macquarie, NSW
Paracrinia	*haswelli**			Cann R. valley, between Cann R. and Noorinbee, VIC
Pseudophryne	*bibroni**			Cann R. valley, between Cann R. and Noorinbee, VIC
Pseudophryne	*bibroni*			Cann R. valley, between Cann R. and Noorinbee, VIC
Pseudophryne	*corroboree**	ANWC 1870		Coree Flat, 2 Stick Rd, Brindabella, ACT
Pseudophryne	*corroboree*	ANWC 1854		Toolong Plain, Snowy Mts, NSW
Taudactylus	*acutirostris**	ABTC 16088	SAMA 41094	Mt. Lewis, QLD
Spicospina	*flammocaerulea**	ABTC 69165		24–30 km NE of Walpole, WA
Uperoleia	*fusca**	ANWC 1994		Tweed River, NSW
Uperoleia	*fusca*	ANWC 1995		Tweed River, NSW
Uperoleia	*rugosa*	ANWC 1843		Shoalwater Bay, QLD
Uperoleia	*rugosa*	ANWC 1844		Shoalwater Bay, QLD
Uperoleia	*rugosa**	ANWC 1845		Shoalwater Bay, QLD
Limnodynastes	*dumerili**			Cann R. valley, between Cann R. and Noorinbee, VIC

Note. Specimens used as representatives in phylogenetic analyses are noted with an asterisk. Numbers in parentheses are reference numbers for the *Crinia signifera* individuals. Museum acronyms as follows: ABTC, Australian Biological Tissue Collection; SAM, South Australian Museum, Adelaide; WAM, Western Australian Museum; ANWC, Australian National Wildlife Collection.

TABLE 2

Details of Primers Used in This Study

Region	Name	Sequence: 5′ > 3′	3′ position	Source
12S	L2519	AAACTGGGATTAGATACCCCACTAT	2519	Richards and Moore, 1996
	H3296	GCTAGACCATKATGCAAAAGGTA	3296	Richards and Moore, 1996
	H3628	GCTGTCTTTACAGGTGGCTGCTTTTAGG	3628	This study
ND2	L4221	AAGGACCTCCTTGATAGGGA	~5780	Macey *et al.*, 1998
	L4437	AAGCTTTCGGGGCCCATACC	5945	Macey *et al.*, 1998
	H4980	ATTTTTCGTAGTTGGGTTTGRTT	6489	Macey *et al.*, 1998
	tRNA-Trp	CTCCTGCTTAGGGCTTTGAAGGC	7041	This study
	tRNA-Asn	CTAAAATRTTRCGGGATCGAGGCC	7167	This study

Note. The letters L and H refer to the light and heavy strands. tRNA-Trp and tRNA-Asn are both heavy strand primers. Values in "3′ position" refer to the position of the 3′ base of the primer in the complete *Xenopus* mtDNA sequence (Roe *et al.*, 1985).

toni because they have been split off from *Crinia* by other authors (Blake, 1973; Heyer and Liem, 1976; Straughan and Main, 1966; Tyler, 1972). When possible, two representatives were sequenced for each species to help identify possible contamination and misidentified specimens, and to provide data on intraspecific variation (Goebel *et al.*, 1999). Given the extreme polymorphism in dorsal coloration and wide distribution of *Crinia signifera* (Parker, 1940; Blake, 1973), we included eleven *Crinia signifera* specimens from six geographic regions to provide a first look at phylogeographic variation in this taxon. *Limnodynastes dumeruli*, a representative of the subfamily Limnodynastinae, was used as an outgroup.

Molecular Data

DNA was extracted from liver or toe samples using a modified CTAB protocol, suspended in TE buffer, and stored at 4°C. We targeted the mitochondrial genes ND2 and 12S rRNA as they have provided good resolution in similar studies of other anurans (Hay *et al.*, 1995; Richards and Moore, 1996; Ruvinsky and Maxson, 1996; Graybeal, 1997; Macey *et al.*, 1998).

Target DNA was amplified using a modified version of the stepdown PCR profile employed by Keogh *et al.* (2000). Primers used to amplify and sequence both 12S rRNA and ND2 are shown in Table 2. Target fragments were amplified in 40 μL reactions, which comprised the following: ~100 ng template DNA, 4 μL 10× reaction buffer, 3 mM $MgCl_2$, 0.5 mM dNTPs, 10 pmol each primer, and 2 units *Taq* DNA polymerase (Life Technologies, Gaithersburg, MD). This reaction was overlaid with 15 μL of mineral oil. Amplification products were visualized by ethidium bromide staining of 1.5% agarose gels.

Templates for sequencing were purified using the BRESAclean DNA purification kit (GeneWorks). Sequencing reactions were done using BigDye Terminator chemistry (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Sequencing reactions were visualized using an ABI 377 Automated Sequencer. DNA sequence data were edited using Sequencher 3.0 (Gene Codes Corporation).

Sequence data for 12S rRNA and ND2 were aligned separately using ClustalX (Thompson *et al.*, 1997). Pairwise and multiple sequence gap opening and extension penalties were set at 50. The multiple alignments were checked by eye, and all ambiguities compared with the original sequences to reduce the possibility of computer or human editing error.

The 12S rRNA secondary structure (Richards and Moore, 1996) was used as a map to designate stem and loop positions. Due to the uncertainty of maintaining alignment in variable length loops, 115 sites were excluded from subsequent analyses, and this represents the same region excluded by Richards and Moore (1996). Approximately 780 bp of sequence data comprising ~505 bp of 12S rRNA, $tRNA^{VAL}$ and ~200 bp of 16S rRNA was obtained for each individual. The ND2 data comprise 676 bp, the first 154 bp of which includes partial sequence of $tRNA^{ILE}$, $tRNA^{GLN}$, and $tRNA^{MET}$. We were unable to obtain the 5′ 123 bp of this fragment for *Geocrinia rosea*, *G. vitellina*, and *Myobatrachus gouldi* and so this small section is missing from our data set for these taxa. All sequences will be deposited on GENBANK upon publication.

Phylogenetic Analyses

All phylogenetic analyses were performed in PAUP* version 4.0b4 (Swofford, 2000). We first performed a partition homogeneity test to assess the congruence of the 12S and ND2 data sets. The amount of phylogenetic information in the individual and combined data sets were estimated with the $g1$ statistic (Hillis, 1991; Hillis and Huelsenbeck, 1992), calculated by examining the tree length distribution of 10,000 randomly generated parsimony trees (excluding the outgroup *Limnodynastes dumerili*).

Once multiple samples of each taxon were confirmed as true representatives of the same species, a single individual was used for all subsequent analyses (noted in Table 1 with an asterisk). To further reduce the

TABLE 3

Jukes–Cantor Interspecific Genetic Distance Matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 *L. dumerili.* 35	—	.16266	.15866	.13494	.13693	.14848	.17673	.15263	.14842	.15045	.15522	.15504	.15246	.17121	.14289	.14487
2 *M. gouldi.* 21	.31357	—	.13101	.13644	.15221	.11545	.09287	.10629	.11144	.12470	.15292	.13871	.14630	.16482	.13494	.12915
3 *A. darlingtoni.* 17	.36389	.38674	—	.13509	.14511	.11755	.14252	.13500	.12913	.12913	.15712	.12196	.09880	.13330	.09707	.09154
4 *U. fusca.* 41	.25563	.21806	.33556	—	.07136	.10629	.15797	.13702	.11535	.10597	.13737	.10641	.11199	.13151	.10664	.10476
5 *U. rugosa.* 42	.28316	.24389	.36160	.16899	—	.11757	.18444	.15878	.13451	.13645	.16522	.12920	.12338	.14123	.11794	.11603
6 *S. flammocaerulea.* 25	.25771	.20072	.34492	.18215	.21310	—	.14626	.13473	.12501	.12887	.14486	.10650	.12301	.14673	.11951	.11761
7 *A. rotunda.* 43	.30316	.13237	.37383	.22712	.26212	.18978	—	.11169	.12459	.12457	.17915	.15634	.16417	.18324	.16450	.15848
8 *M. nichollsi.* 20	.27507	.14771	.36889	.23546	.24166	.20552	.13650	—	.10436	.10810	.17952	.14913	.15242	.16908	.13307	.13114
9 *P. corroboree.* 29	.24527	.17618	.30371	.20326	.22298	.18026	.17651	.15820	—	.03988	.15682	.11943	.12889	.15088	.11576	.11387
10 *P. bibroni.* 27	.29859	.20219	.35671	.19363	.26145	.21319	.23128	.21158	.12235	—	.15889	.11753	.13277	.14694	.12728	.12152
11 *T. acutirostris.* 24	.28810	.33758	.37925	.32611	.33776	.27300	.29264	.27996	.29474	.33315	—	.11774	.15470	.17524	.15070	.14476
12 *P. haswelli.* 26	.25563	.24348	.35680	.21502	.24733	.21502	.21903	.22938	.21700	.24335	.27727	—	.11024	.14331	.11587	.11396
13 *G. leai.* 34	.28977	.24086	.31259	.21700	.23503	.20326	.23103	.22732	.19551	.23720	.29916	.22097	—	.09148	.05732	.05036
14 *G. alba.* 44	.30494	.28856	.36145	.26025	.26911	.24542	.26713	.26320	.24157	.25627	.28950	.25185	.22852	—	.09348	.09162
15 *G. victoriana.* 19	.28375	.27185	.33608	.24160	.29680	.23345	.25406	.24612	.24161	.25821	.28425	.23953	.16741	.25599	—	.00816
16 *G. laevis.* 48	.27464	.27650	.32851	.23100	.27666	.22697	.25360	.24361	.23912	.26619	.29469	.23100	.17463	.25373	.03965	—
17 *G. rosea.* 37	.33820	.28205	.41195	.27573	.29238	.24859	.26057	.25743	.26060	.27682	.37223	.27400	.25598	.19318	.26109	.24559
18 *G. vitellina.* 46	.33555	.26383	.40610	.27073	.29746	.24576	.27360	.27538	.24523	.26128	.32402	.28974	.24587	.06602	.28216	.27123
19 *B. nimbus.* 18	.27269	.25912	.36173	.26425	.30543	.23317	.24963	.24373	.22711	.25375	.31490	.24133	.21512	.24271	.21946	.23519
20 *C. subinsignifera.* 14	.29905	.25162	.36209	.25609	.27301	.23340	.26243	.22974	.23950	.26032	.29076	.27724	.26232	.25902	.27728	.26870
21 *C. glauerti.* 4	.28386	.28196	.34317	.27521	.27310	.24170	.26472	.26083	.26672	.27521	.29469	.29038	.26458	.25858	.27139	.26454
22 *C. parinsignifera.* 6	.27678	.27406	.36400	.27037	.25986	.21898	.26197	.24777	.22898	.25576	.29910	.27250	.24733	.26714	.26667	.26191
23 *C. tinnula.* 16	.25609	.26423	.34554	.23949	.25827	.20557	.24578	.24204	.21543	.24374	.28376	.25189	.25823	.23787	.26494	.25606
24 *C. insignifera.* 5	.29856	.24366	.34727	.25146	.25566	.22697	.25990	.22537	.24116	.25362	.28591	.26613	.25771	.23961	.26454	.25771
25 *C. remota.* 8	.27893	.26899	.36641	.24116	.24531	.21305	.24534	.24158	.22297	.24538	.30577	.23302	.22697	.24368	.25404	.24116
26 *C. georgiana.* 3	.28325	.26404	.36154	.23505	.25562	.23100	.25157	.23343	.24527	.25568	.29025	.27464	.26191	.24791	.27089	.27037
27 *C. deserticola.* 2	.27250	.28655	.34958	.25771	.29639	.23100	.25157	.25191	.24321	.26408	.28153	.27037	.26825	.26248	.27087	.27037
28 *C. riparia.* 9	.28810	.26169	.38172	.26657	.27305	.21933	.24988	.25026	.24571	.26029	.29469	.25398	.24980	.27162	.25227	.24768
29 *C. tasmaniensis.* 15	.26402	.27198	.36873	.25354	.28752	.24321	.26846	.26656	.23912	.24731	.31241	.25771	.24116	.26456	.26031	.25563
30 *C. bilingua.* 1	.28542	.26368	.38112	.24321	.25984	.23100	.26202	.23751	.24733	.23309	.31928	.27250	.24733	.25434	.26665	.26825
31 *C. sloanei.* 13	.30811	.27154	.37447	.26876	.26033	.24786	.27113	.24420	.25830	.27101	.30132	.25410	.26672	.25676	.27144	.25826
32 *C. pseudinsignifera.* 91	.29415	.24846	.34489	.25563	.27041	.23100	.26413	.23140	.23912	.25988	.29244	.27464	.25354	.25012	.26880	.26402
33 *Crinia sp.* 12	.28371	.29023	.36949	.25600	.27953	.22335	.24784	.23589	.22335	.26242	.30801	.25603	.25188	.25675	.27556	.27080
34 *C. signifera.* 10	.28325	.26569	.35668	.24733	.26616	.23302	.23110	.21143	.23100	.25572	.30126	.26613	.25146	.23085	.26455	.26191

Note. ND2 above the diagonal and 12S rRNA below. Numbers after each species name correspond to sample numbers in Table 1.

number of taxa included in the analyses, we broke our analyses into two parts due to the large number of individuals included in our sampling regime. The first of these included single representatives of each species, except for *C. signifera* (representatives are marked with asterisks in Table 1). In these analyses, *C. signifera* was represented by five of the 11 individuals, from four different geographic localities. *Limnodynastes dumerili* was used to root the tree. The second set of analyses was limited to *C. signifera,* but included all individuals, and *Bryobatrachus nimbus* was used to root the tree.

Phylogenies for each set of analyses were constructed using maximum-parsimony and maximum-likelihood methods. The large number of taxa and consequent large number of possible trees required heuristic searches be used for all the parsimony analyses. To reduce the probability of finding suboptimal trees, each search was replicated 30 times under the random-stepwise and tree-bisection-reconnection branch swapping options of PAUP* 4.0. The actual transition/transversion ratios (Ti/Tv) were estimated for each data set and the combined data set via maximum-likelihood. Ti/Tv ratios of 2 and 5 were used in the parsimony analyses to approximate and overestimate the actual Ti/Tv ratio to examine the effect on tree topology. The parsimony trees were bootstrapped with 1000 pseudoreplicates, and bootstraps above 70% were judged as strong support (Hillis and Bull, 1993). We also used successive approximations based on the rescaled consistency index to assess the effect of reweighting on tree topology. Maximum-likelihood analyses were conducted using the actual Ti/Tv under the conservative HKY85 model (Hasegawa *et al.*, 1985).

RESULTS

With all taxa and individuals included, the ND2 data set comprised 677 bp of which 383 were variable and of these 322 informative under parsimony. After the exclusion of unalignable regions, the 12S data set comprised 621 bp of which 266 were variable and of these 195 informative under parsimony. Thus, the combined data set comprised 1298 included base pairs of which 649 were variable and 517 informative under parsimony. A partition-homogeneity test did not reject the null hypothesis that the data were homogeneous ($P >$

TABLE 3—*Continued*

17	18	19	20	21	22	23	23	25	26	27	28	29	20	31	32	33	34
.17966	.17122	.16869	.14682	.15275	.15687	.14507	.15275	.14518	.15276	.17724	.14683	.15654	.15504	.14677	.15504	.16280	.14715
.14656	.16689	.15850	.15454	.15457	.15501	.14880	.15853	.14088	.15457	.18930	.15055	.16864	.14513	.14861	.15876	.15855	.13506
.12176	.13142	.13492	.13306	.13890	.14130	.13523	.13305	.11588	.13892	.16700	.12342	.14273	.12353	.12338	.13714	.14287	.11406
.14485	.13345	.14086	.12156	.12153	.12565	.13141	.12925	.11610	.12346	.14283	.13315	.12919	.12560	.11579	.12559	.12736	.12189
.15878	.14915	.15666	.13505	.13308	.14913	.13729	.14091	.14122	.13698	.15667	.14488	.15464	.15102	.14282	.14313	.14293	.13540
.14289	.14283	.13859	.12506	.12699	.13110	.12908	.13084	.12536	.12316	.16444	.12318	.14446	.13692	.11743	.13292	.13280	.11961
.16463	.18750	.16433	.15850	.17064	.17323	.17091	.15847	.15666	.16055	.19764	.15850	.17660	.16281	.15254	.16683	.17886	.15677
.14117	.17320	.14271	.14872	.16071	.16515	.15493	.15071	.14698	.15269	.18318	.14675	.15457	.15896	.14478	.15692	.16474	.14301
.14661	.15492	.13672	.12518	.13286	.13316	.12541	.13095	.12535	.12324	.15047	.12711	.14847	.13306	.12320	.13313	.12904	.11392
.14858	.14699	.14263	.13099	.13289	.13709	.12543	.13680	.12153	.12904	.15446	.12714	.15246	.13308	.12707	.13898	.13101	.11774
.16722	.16705	.16299	.15681	.15082	.15113	.16515	.16284	.15506	.15481	.18129	.15479	.17116	.16528	.15086	.16310	.15680	.15310
.14095	.13351	.14122	.13148	.13929	.13960	.13559	.13928	.12021	.12954	.15715	.12761	.14123	.12792	.11991	.13555	.14520	.12020
.09523	.08967	.11556	.11933	.12315	.11213	.11191	.12314	.11767	.11555	.14647	.10801	.12506	.12720	.11933	.12712	.12703	.10261
.06805	.03325	.14503	.14483	.14483	.13912	.14306	.15078	.14908	.13503	.16684	.14286	.14896	.16509	.15076	.15298	.14677	.14311
.09171	.09350	.11387	.11963	.11965	.12001	.10844	.12345	.11797	.11774	.15087	.11205	.12158	.12960	.12158	.12745	.13119	.10477
.08620	.09164	.11579	.11773	.11394	.11429	.10655	.12153	.11228	.11584	.14493	.11017	.11967	.12380	.11586	.12551	.12925	.09916
—	.07159	.13534	.12741	.12937	.12564	.13142	.13126	.13740	.11593	.15099	.11212	.14913	.15925	.13518	.13532	.13316	.12378
.16973	—	.15097	.14682	.14485	.13915	.14111	.15279	.15510	.13504	.17501	.13895	.15096	.16716	.14880	.15097	.14481	.14116
.30351	.28500	—	.07870	.08594	.10470	.08610	.07869	.09898	.07692	.14269	.08961	.08046	.10832	.08230	.08610	.10073	.07710
.32545	.28723	.22545	—	.02308	.05568	.03324	.00816	.07168	.01475	.07686	.03150	.08049	.06626	.02141	.00653	.04516	.03326
.36279	.29550	.23157	.11734	—	.06271	.03664	.02812	.07708	.02475	.08591	.03830	.08231	.06804	.03150	.02984	.05383	.03670
.33370	.29257	.23729	.15633	.17687	—	.04877	.06096	.09181	.04697	.08422	.05748	.10658	.09174	.05743	.05919	.04875	.04537
.29288	.27687	.22547	.12778	.15639	.13119	—	.03836	.08094	.03322	.09338	.03833	.09339	.07892	.04521	.04008	.03662	.03330
.31411	.27647	.21915	.02262	.11203	.14345	.11541	—	.07706	.01974	.08227	.03489	.08229	.07160	.02643	.01479	.05035	.03666
.29773	.28179	.20931	.16188	.19785	.16717	.16375	.15613	—	.06989	.09905	.08249	.09718	.04874	.06104	.07896	.08793	.07340
.33391	.27938	.22116	.10507	.13656	.15613	.14005	.09308	.16532	—	.07148	.03150	.08412	.06804	.02308	.02142	.04001	.02988
.31897	.29176	.26213	.16373	.17497	.19359	.16001	.15430	.18976	.16532	—	.08409	.13485	.09529	.07866	.08422	.09506	.08252
.30931	.30348	.23149	.14196	.16006	.15451	.13474	.13293	.16742	.15270	.17863	—	.08960	.07700	.03830	.03833	.04689	.02650
.33131	.30153	.20730	.22930	.24987	.22297	.23746	.22497	.23912	.24733	.27250	.20938	—	.10272	.08591	.08789	.11762	.09539
.30588	.27653	.24134	.17864	.19593	.17838	.17679	.16532	.13986	.17650	.19167	.19003	.21502	—	.04871	.07348	.09340	.06104
.32543	.29267	.21558	.08500	.14189	.16015	.14368	.07334	.15828	.12418	.19012	.14189	.22941	.17309	—	.02814	.05383	.02821
.32496	.29488	.21518	.01653	.11201	.15430	.12940	.02412	.15613	.10323	.15796	.13115	.22097	.17089	.08655	—	.05213	.04010
.29858	.29549	.25407	.15288	.17680	.16371	.11543	.14007	.17300	.15085	.17669	.15822	.26019	.18432	.18246	.14365	—	.04009
.30557	.25575	.23319	.13111	.16193	.14705	.14363	.11524	.17463	.12921	.16163	.14178	.22297	.16347	.11718	.13275	.16737	—

.05), thus all the analyses we present are based on the combined data set.

The distributions of the 10,000 randomly generated trees from each of the 12S, ND2, and combined data sets were left skewed, indicating sufficient hierarchical phylogenetic signal in the data (Hillis, 1991; Hillis and Huelsenbeck, 1992): ND2 $g1 = -0.443$, $P < 0.01$; 12S rRNA $g1 = -0.375$, $P < 0.01$; combined g1 = −0.362, $P < 0.01$. Our ND2 data translated into amino acids without any stop codons and our 12S sequence data are congruent with the 12S sequence published by Richards and Moore (1996), so we assume that the target genes were amplified rather than paralogues. We present Jukes-Cantor (1969) genetic distances among taxa in Tables 3 and 4 for comparison with other studies.

TABLE 4

Jukes–Cantor Intraspecific Genetic Distance Matrix for *Crinia signifera*

	1	2	3	4	5	6	7	8	9	10	11
1 *C. signifera*.VIC.99	—	.00816	.01145	.00816	.00816	.01145	.00817	.01641	.01145	.00980	.00980
2 *C. signifera*.NSW.86	.07976	—	.00325	.00000	.00000	.00816	.00983	.01475	.00980	.00816	.00816
3 *C. signifera*.ACT.87	.07319	.01196	—	.00325	.00325	.01145	.01312	.01807	.01310	.01145	.01145
4 *C. signifera*.ACT.88	.07319	.01046	.00446	—	.00000	.00816	.00983	.01475	.00980	.00816	.00816
5 *C. signifera*.ACT.98	.08307	.00896	.01196	.01046	—	.00816	.00983	.01475	.00980	.00816	.00816
6 *C. signifera*.TAS.11	.08973	.08141	.07811	.07811	.08141	—	.01479	.02308	.01807	.01641	.01641
7 *C. signifera*.SA.10	.09983	.07647	.07319	.07319	.07319	.06344	—	.01314	.00819	.00655	.00655
8 *C. signifera*.KI.93	.08307	.06506	.06183	.06183	.06830	.06344	.04905	—	.00816	.00652	.00652
9 *C. signifera*.KI.95	.09140	.07319	.06993	.06993	.07319	.06344	.04905	.01196	—	.00163	.00163
10 *C. signifera*.KI.96	.09308	.07483	.07156	.07156	.07483	.06506	.05063	.01347	.00446	—	.00000
11 *C. signifera*.KI.97	.08806	.06993	.06668	.06668	.06993	.06022	.04589	.01196	.00297	.00446	—

Note. ND2 above the diagonal and 12S rRNA below. Numbers after species names correspond to sample numbers in Table 1.

A Ti/Tv ratio of 2.10 was estimated via maximum-likelihood for the combined data set (2.20 for ND2 only and 1.94 for 12S only). Parsimony analysis with a Ti/Tv ratio of 2 resulted in a single most parsimonious tree (Fig. 3: length = 4111, CI = 0.31, RI = 0.56, RC = 0.20, HI = 0.67), that is identical to the tree produced in the maximum-likelihood analysis. Parsimony analysis with a Ti/Tv ratio of 5 also resulted in a single most parsimonious tree (figure not shown: length = 7182, CI = 0.32, RI = 0.61, RC = 0.22, HI = 0.68), but this tree differed slightly from the 2 Ti/Tv tree in the arrangement of a single branch within the genus *Crinia*. In the 5 Ti/Tv analyses, the *Crinia* clade comprising *C. parinsignifera* and allies forms a sister group to the *Crinia* clade comprising *C. georgiana* and allies, rather than to the *Crinia* clade comprising *C. riparia* and *C. signifera*. Thus, the topology of our myobatrachine tree is highly consistent between different phylogenetic procedures and the only difference occurs at a single branch with no bootstrap support. To test for saturation at third codon positions, we did additional analyses with ND2 third codons removed, but this did not change the topology of trees generated in any of the various analyses (Fig. 3).

Taudactylus acutirostris forms a well-supported sister group to the rest of the myobatrachine genera. *Paracrinia haswelli* forms a second sister group to the other genera, but this branch is not supported by bootstrap values. The recently described genus *Spicospina* forms a well-supported clade with *Uperoleia* and together they form a (somewhat weakly supported) sister group to the very strongly supported clade comprising *Pseudophryne, Metacrinia, Myobatrachus,* and *Arenophryne. Pseudophryne* forms the well-supported sister clade to the other genera in the group. *Metacrinia* forms the well-supported sister group to a clade comprising *Myobatrachus* and *Arenophryne.*

The genus *Assa* forms the sister group to *Geocrinia* with a bootstrap value of 94%. The monophyly of *Geocrinia* is supported by a high bootstrap value, and the genus comprises two well-supported lineages with *G. leai, G. victoriana,* and *G. laevis* on the one hand and *G. alba, G. rosea,* and *G. vitellina* on the other. All nodes are supported by exceptionally high bootstrap values.

Our analyses demonstrate that the genus *Crinia* is not monophyletic if the recently described genus *Bryobatrachus* is excluded. *Bryobatrachus nimbus* and *Crinia tasmaniensis* form a very well-supported clade and together they are the sister clade to the rest of *Crinia*. The rest of *Crinia* comprises a series of well-supported clades: *C. remota* and *C. bilingua; C. deserticola; C. georgiana, C. glauerti, C. sloanei, C. insignifera, C. subinsignifer*a, and *C. pseudinsignifera;* and *C. parinsignifer*a, *C. tinnula, C.* sp.; *C. riparia,* and *C. signifera*. The relationship of *C. parinsignifera* to the *C. tinnula* and *C.* sp. is less strongly supported, but nonetheless this relationship consistently appears in all the phylogenetic analyses we performed. As mentioned above, analyses with a Ti/Tv ratio of 5 placed the *Crinia georgiana* clade and the *C. parinsignifera* clade as sister groups. To examine these relationships further, we also performed analyses that included only *Crinia* and *Bryobatrachus* (with *Limnodynastes* as the outgroup), but included all individuals we sequenced (Table 1). These analyses did not produce different topologies from those already shown in Fig. 3. However, given that the actual Ti/Tv ratio of our data set was 2.1, we prefer the topology shown in Fig. 3, but acknowledge that this branch is weakly supported.

Analyses of just the *C. signifera* samples resulted in a single consistent topology. The 2 Ti/Tv ratio parsimony analysis of the *C. signifera* resulted in two most parsimonious trees (strict consensus tree shown in Fig. 4: length = 583, CI = 0.65, RI = 0.69, RC = 0.54, HI = 0.35) and all alternative analyses resulted in the same tree. The tree shows fully resolved relationships among all included taxa. However, strong bootstrap support is entirely restricted to the branches relating individuals from the same geographic region. Despite the lack of strong bootstrap support, there is a clear phylogeographic pattern evident among the samples with individuals from southeastern Australia forming a group (Tasmania, Victoria, NSW, Australian Capital Territory), those from Kangaroo Island in South Australia forming a group (with some substructure evident on the island), and an individual from mainland South Australia forming the sister group to the rest of the samples. Regional divergences range from 4.6 to 10% for ND2 and 0.83 to 1.8% for 12S, with the highest variation between Victoria and SA (ND2), and Kangaroo Island and Tasmania (12S) (Table 4).

DISCUSSION

Based on the combined 12S and ND2 data set and a summary of results from our phylogenetic analyses, we show in Fig. 5 a mitochondrial gene tree that represents a conservative summary of phylogenetic relationships among the Myobatrachinae. Only branches corroborated by all analytical methods and/or with bootstrap support of 70% or more are shown. We base our discussion on this tree. The topology of our strongly supported summary tree does not fully corroborate any previous phylogeny based on other types of data. This is partly because no previous phylogeny has been constructed that included representatives from every currently recognized myobatrachine genus (redrawn in Fig. 2 in the Introduction). Excluding *Taudactylus* and *Paracrinia,* the other ten myobatrachine genera comprise three major clades based on our data: *Assa* and *Geocrinia; Spicospina, Uperoleia, Pseudophryne, Metacrinia, Myobatrachus,* and *Arenophryne;* and *Bryobatrachus* and *Crinia*. We consider each major group in turn.

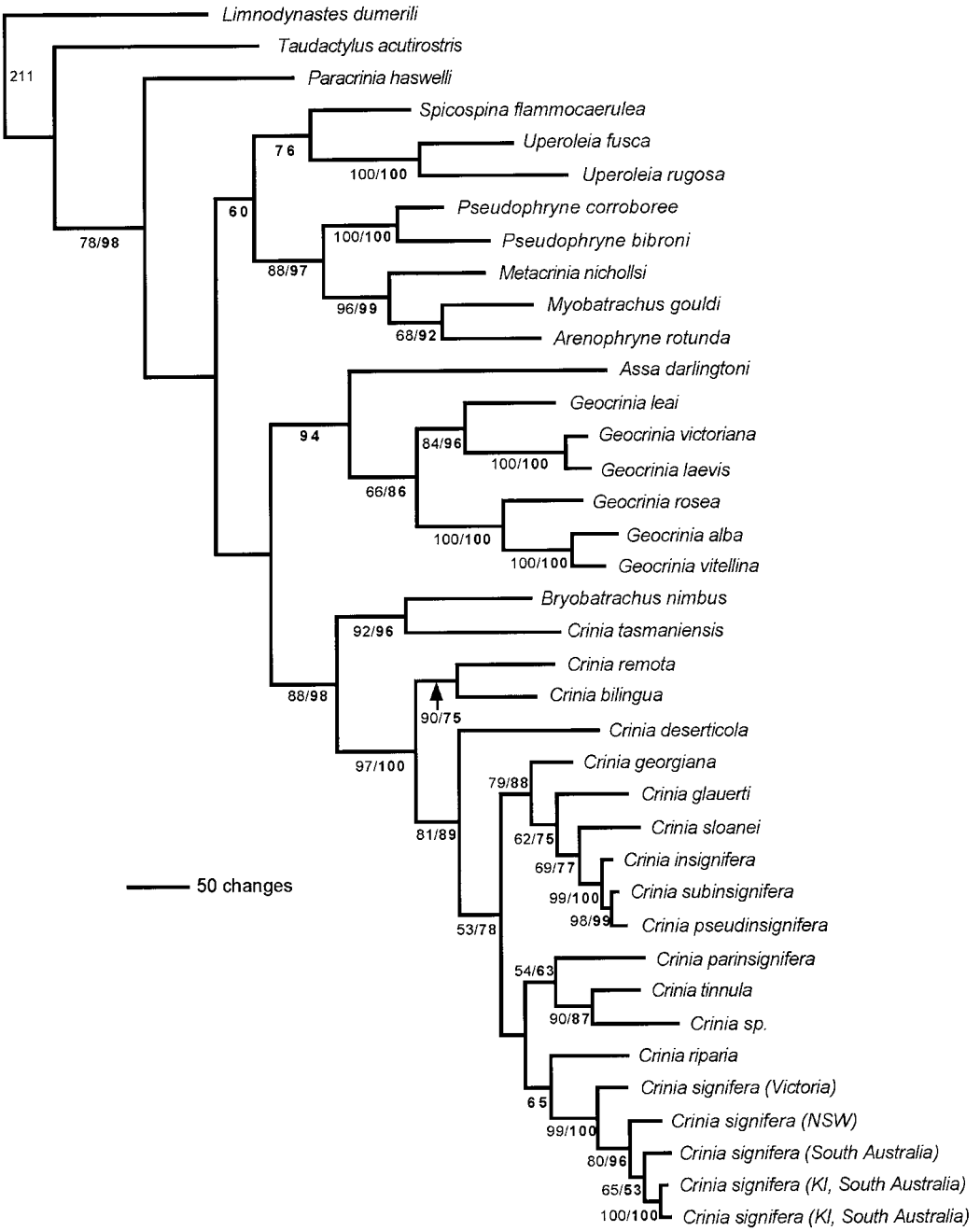

FIG. 3. Single most parsimonious tree resulting from analysis with of the combined ND2 and 12S data sets, with a transition/transversion ratio of 2. Maximum-likelihood analysis with the transition/transversion ratio estimated from the data produced the identical tree. Numbers on nodes represent bootstrap values for 1000 pseudoreplicates before (plain next) and after one round of successive approximations (bold text) based on the rescaled consistency index.

Taudactylus and Paracrinia

In our analyses, *Taudactylus*, as represented by *T. acutirostris*, forms the strongly supported sister group of the other myobatrachine genera. This corroborates the phylogenetic position of *Taudactylus*, first identified by Heyer and Liem (1976), but contradicts Blake (1973) and Farris *et al.* (1982). Our data did not clearly resolve the relationship of the monotypic *Paracrinia haswelli* to the rest of the myobatrachine genera. While Fig. 3 shows *P. haswelli* as an additional sister group to the rest of the myobatrachine genera, this branch has no bootstrap support. However, it is clear from our data that *Paracrinia* is not closely allied to the morphologically similar *Crinia* and *Geocrinia*, as previous studies

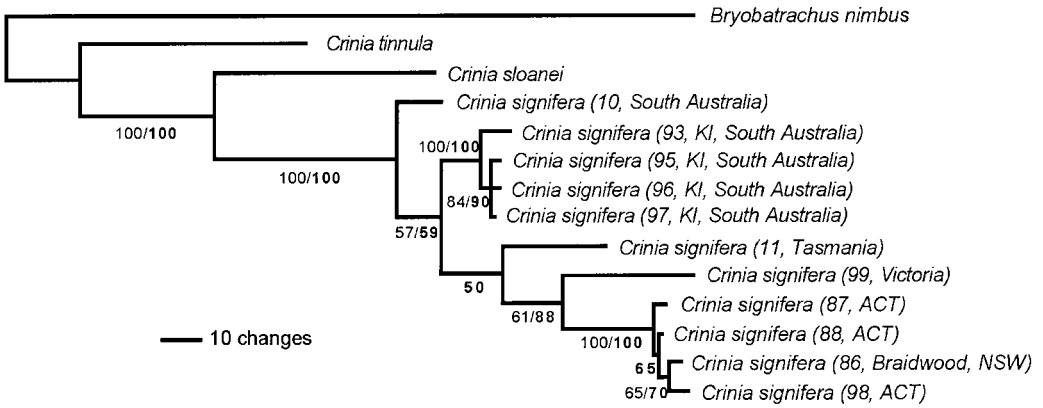

FIG. 4. This phylogeny for *Crinia signifera* populations is a strict consensus of two single most parsimonious trees resulting from an analysis with a transition/transversion ratio of 2. A parsimony analysis with ti/tv ratio of 5, maximum-likelihood with ti/tv estimated from the data, and a distance analysis with the Kimura 3-parameter substitution model all resulted in the same tree.

suggest (Blake, 1973; Heyer and Liem, 1976; Farris *et al.*, 1982). Previous authors have allied *Paracrinia* with a variety of other taxa, particularly *Crinia georgiana* (e.g., Blake, 1973), but this is also clearly incorrect (Fig. 5).

Spicospina, Pseudophryne, and Allies

Our data support a monophyletic clade comprising the speciose genera *Uperoleia* and *Pseudophryne,* and the four monotypic genera *Metacrinia, Myobatrachus, Arenophryne,* and *Spicospina.* While the branch supporting this clade is relatively weakly supported (60% bootstrap support), there is strong additional evidence to suggest that this topology is correct. With the exception of *Spicospina,* which was then not described, our phylogeny fully corroborates the phylogeny presented by Maxson (1992), based on immunological distance data.

In their description of the monotypic *Spicospina flammocaerulea,* Roberts *et al.* (1997) used karyotype and one-way immunological distance data to add the genus to the phylogeny developed by Maxson (1992). They suggested that *Spicospina* lay between *Uperoleia* and the other genera in this clade, about equidistant from *Uperoleia* and *Pseudophryne,* as we have found here. *Pseudophryne* was tentatively identified as the sister taxon to *Uperoleia* in an osteological study (Davies, 1989), but other studies based on morphological data did not recognize this relationship (Fig. 1; Blake, 1973; Heyer and Liem, 1976; Farris *et al.*, 1982).

Myobatrachus and *Arenophryne* are the only Australian frogs that burrow forward, and they both show extreme morphological adaptations for this behavior (Maxson and Roberts, 1985). Our phylogeny shows that they are closely related. These genera also share with each other, and *Metacrinia,* a highly derived breeding biology and they are all confined to southwest Australia (Maxson and Roberts, 1985; Cogger, 2000). Previous authors have been divided between over whether the monotypic *Metacrinia nichollsi* should be recognized as a distinct genus (Heyer and Liem, 1976; Tyler *et al.*, 1981; Farris *et al.*, 1982; Maxson and Roberts, 1985; Barker *et al.*, 1995) or placed in synonymy with *Pseudophryne* (Blake, 1973). Our data strongly support a monotypic *Metacrinia* distinct from *Pseudophryne.* This view is also supported by immunological comparisons of serum albumin, breeding biology, and morphological data (Roberts and Maxson, 1989).

Assa and Geocrinia

Our data strongly support the sister group relationship of the monotypic *Assa* and *Geocrinia.* Only one of three previous hypotheses of relationship (that included *Assa*) suggested the same affinities (Blake, 1973). The other two studies both showed a close relationship between *Assa* and *Metacrinia* (Heyer and Liem, 1976) or to both *Metacrinia* and *Myobatrachus* (Farris *et al.*, 1982).

Based on overall morphological similarity, *Crinia* and *Geocrinia* are nearly identical (Blake, 1973). At the generic level, all previous phylogenetic studies that have included *Geocrinia* have also included *Crinia* due to their perceived close relationship (Blake, 1973; Heyer and Liem, 1976; Thompson, 1981; Daugherty and Maxson, 1982; Farris *et al.*, 1982; Maxson, 1992). Our data very strongly support the monophyly of *Geocrinia,* but our data also clearly demonstrate the phylogenetic distinctiveness of *Geocrinia* and *Crinia.*

The phylogenetic relationships shown in Fig. 5 generally support the three recognized *Geocrinia* species groups: *G. victoriana* and *G. laevis, G. rosea* (and *G. lutea*), and *G. alba* and *G. vitellina,* with *G. leai* possibly in a group by itself (Blake, 1973). An immunological distance study was equivocal on the affinities of *G. leai,* showing that the species is highly distinct from both the *G. rosea* group and the *G. laevis*/*G. victoriana* pair (Roberts and Maxson, 1985b). *Geocrinia alba* and *G.*

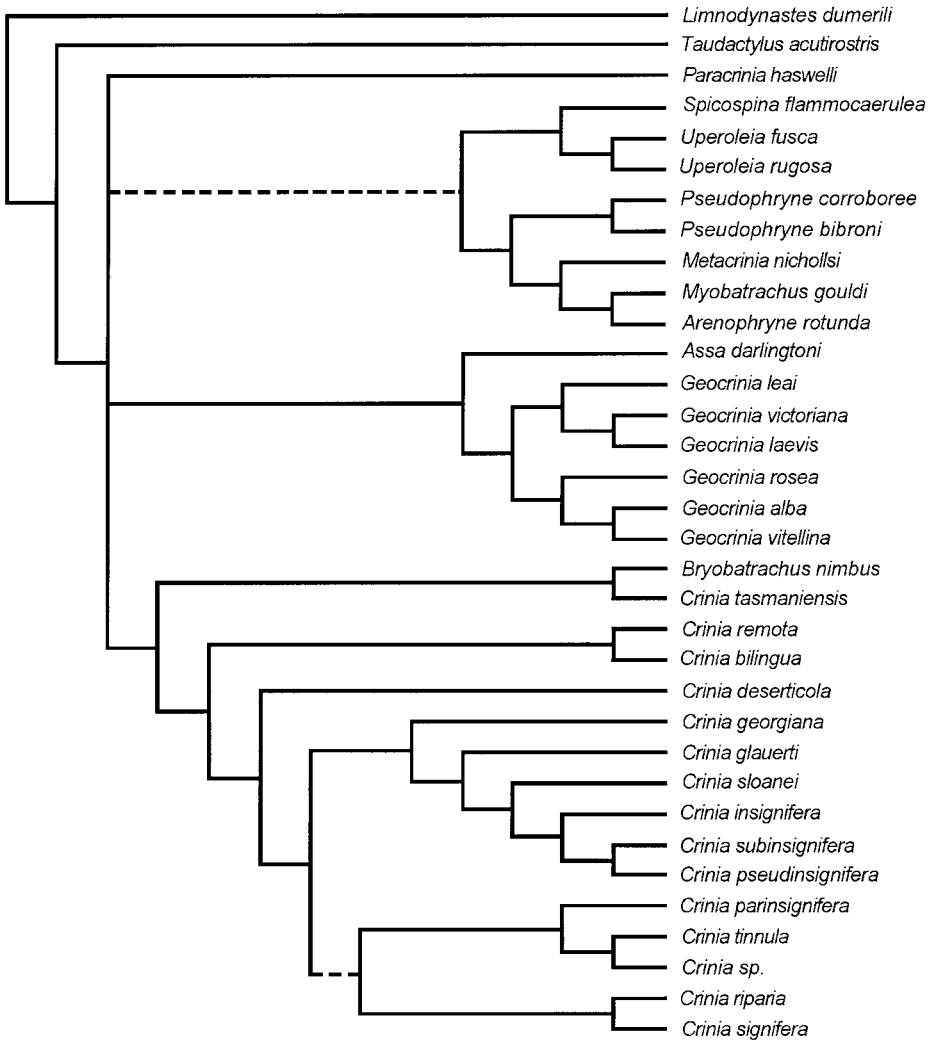

FIG. 5. Conservative summary of the phylogenetic relationships among the myobatrachine frog species included in this study based on the combined ND2 and 12S data set. Only nodes with strong bootstrap support and/or corroboration between analytical methods are illustrated. Branches with dotted lines are less well supported but nonetheless consistent based on alternative phylogenetic methods.

vitellina were described by Wardell-Johnson and Roberts (1989; see also Roberts *et al.*, 1990; Wardell-Johnson and Roberts, 1993), and can be distinguished from *G. rosea* and *G. lutea* by the absence of a black chin in males and by advertisement call structure (Roberts *et al.*, 1990; Roberts and Wardell-Johnson, 1985). Our data divide *Geocrinia* into two strongly supported lineages: (a) *G. leai, G. victoriana,* and *G. laevis,* and (b) *G. rosea, G. alba,* and *G. vitellina.* These lineages can also be recognized by similarities in call structure and the level of direct development exhibited by their members. *Geocrinia leai, G. victoriana,* and *G. laevis* share diphasic calls and terrestrial egg deposition with aquatic tadpoles, while *G. rosea, G. lutea, G. alba,* and *G. vitellina* share simpler pulsed calls and terrestrial egg deposition with nonfeeding tadpoles confined to a terrestrial nest (Roberts *et al.*, 1990; Roberts, 1993).

Bryobatrachus nimbus and Crinia tasmaniensis

In this study, *Bryobatrachus nimbus* and *Crinia tasmaniensis* form a distinct clade, and together they form the sister group to the rest of *Crinia.* Both of these relationships are supported by bootstrap values nearing 100%. *Crinia tasmaniensis* has been consistently recognized as the most distinctive member of *Crinia* based on both morphological (Littlejohn, 1970; Blake, 1973; Heyer and Liem, 1976; Thompson, 1981) and molecular (Daugherty and Maxson, 1982) data. Heyer and Liem (1976) described the genus *Australocrinia* to accommodate *C. tasmaniensis* and *C. riparia.* A later phenetic analysis of morphological data led to the sinking of *Australocrinia* and the return of both species to *Ranidella* (now *Crinia*), but continued recognition of the derived morphology of *C. tasmaniensis* (Thompson, 1981).

In their description of the monotypic genus *Bryobatrachus* for the new species *B. nimbus*, Rounsevell *et al.* (1994) pointed out the morphological similarity between it and *C. tasmaniensis*, however, they noted that the call of *B. nimbus* is similar to that of *Crinia signifera*, its reproductive mode similar to some *Geocrinia*, and the structure of the hyoid most closely resembles that of *Rheobatrachus*. Rounsevell *et al.* (1994) concluded they were unable to identify the sister taxon to *Bryobatrachus* based on the phenetic comparison they presented. Our data very strongly support the monophyly of a clade comprising *B. nimbus* and *C. tasmaniensis*, thus making *Crinia* paraphyletic. *Bryobatrachus* is characterized by two autapomorphies: fusion of vertebrae VII and VIII and direct development of the eggs and larvae (Rounsevell *et al.*, 1994). *Bryobatrachus* was distinguished from *Crinia* (*sensu* Blake, 1973) and *Ranidella* by Rounsevell *et al.* (1994). However, if *Crinia* and *Ranidella* are synonymized, then none of the characters listed by Rounsevell *et al.* (1994) unequivocally excludes *Bryobatrachus* from *Crinia* (*sensu lato*). Given this, we here synonymize *Bryobatrachus* with *Crinia* pending further investigation of the morphological distinctiveness of this clade.

Crinia

Crinia (including *B. nimbus*) form a monophyletic clade with nearly 100% bootstrap support. Our phylogeny clearly shows that the affinities of species previously allocated to *Crinia* and later placed in other genera (*Assa darlingtoni*, *Geocrinia* species, *Paracrinia haswelli*, and *Taudactylus acutirostris*) do not lie closely with *Crinia*.

The inclusion of all known *Crinia* species in this phylogenetic analysis radically changes our view of relationships in this genus. With the placement of *C. parinsignifera* in a species group with species sharing calls with high pulse repetition rates, Main's (1957) "*insignifera*" group, is not supported. The sets of relationships suggested by models of speciation in southwestern and southeastern Australia involving major migrations and isolation events across Australia or between the Australian mainland and Tasmania (Main *et al.*, 1958; Littlejohn, 1967; Littlejohn and Watson, 1985; Roberts and Maxson, 1985a,b, 1988; Roberts and Watson, 1993) are also not supported. For example, Main *et al.* (1958) argued that *C. pseudinsignifera* and *C. insignifera* were sister taxa because they hybridized. Their closest relative in eastern Australia was claimed to be *C. parinsignifera*, but in our phylogeny (Fig. 5), it is *C. sloanei*—a species not known in 1957 (Littlejohn, 1958). Similarly, the claim that *C. signifera* and *C. glauerti* are sister taxa (Littlejohn, 1959; Littlejohn and Wright, 1997) is also rejected by our phylogeny—these two species are not even in the same major clades within *Crinia* (Fig. 5).

Crinia remota and *C. bilingua* form a well-supported sister clade to *C. deserticola*, which in turn forms the sister group to the rest of *Crinia*. These three species have not been included in any previous phylogenetic study of *Crinia*. However, it is worth pointing out the distributions of these taxa relative to the rest. *Crinia remota* and *C. bilingua* are the only *Crinia* species found in northern Australia and *C. deserticola* is found in central and northern Australia (*C. remota* also inhabits southern New Guinea); all the other *Crinia* are in southern Australia (Cogger, 2000). The remaining *Crinia* species comprise three major clades: *C. insignifera, C. glauerti, C. georgiana, C. sloanei, C. subinsignifera, C. pseudinsignifera; C. parinsignifera, C. tinnula, C. sp.;* and *C. signifera* and *C. riparia*. The relationship between these three major clades is not well supported by our data. The summary tree we present in Fig. 5 shows what we believe to be the most likely arrangement for these clades, derived from the consistency between three of the four *Crinia*-only analyses.

Despite the assertion that *C. georgiana* is distinct from the "*C. signifera*" complex, our data clearly nest *C. georgiana* within a *Crinia* clade, rather than as a sister species to the rest of *Crinia*. *Crinia georgiana* forms the sister taxon to *C. glauerti* and four other *Crinia* species. A close relationship between *C. georgiana* and *C. glauerti* has been suggested only once before, in a phylogeny constructed using allozyme electrophoresis (Barendse, 1984). The placement of *C. georgiana* well within *Crinia* contradicts all of the traditional views of the divide between *Ranidella* (the "*signifera* species complex") and *Crinia* (*C. georgiana*). Girard (1853) originally separated *Crinia* because the only two *Crinia* at the time, *C. georgiana* and *C. signifera*, exhibited the presence and absence of vomerine teeth, respectively. However, Girard (1853) did not formally raise *Ranidella* to full generic status, as he did not have a *C. georgiana* specimen available for comparison with *C. signifera*. Throughout the nine years of the separation of *Ranidella* from *Crinia* from Blake (1973) to Heyer *et al.* (1982), no other nonlabile features were provided to support this relationship. To repeat Daugherty and Maxson (1982), "*C. georgiana* represents a lineage which, like *R. riparia*, has undergone relatively rapid morphological evolution following a divergence from other species of *Ranidella*."

Our data show that *C. insignifera*, *C. subinsignifera*, and *C. pseudinsignifera* are very closely related, and this corroborates previous studies. Both *C. subinsignifera* and *C. pseudinsignifera* were described from call races of *C. insignifera* (Littlejohn, 1957; Main, 1957), and the three species are distinguishable only by male call (Littlejohn, 1957; Cogger, 2000). In the morphological analyses by Thompson (1981), *C. insignifera* and *C. subinsignifera* were inseparable. *Crinia pseudinsignifera* and *C. subinsignifera* are the mostly closely related species in our study, with an average genetic distance

of only 1.2% for both genes. *Crinia pseudinsignifera,* and *C. subinsignifera* are sympatric in the southwest of Australia (Littlejohn, 1959; Tyler *et al.*, 1994) with rare F1 hybrids (Roberts, unpub. data), but *C. pseudinsignifera* and *C. insignifera* have parapatric distributions separated by a narrow hybrid zone with a variety of hybrid call phenotypes consistent with back and/or intercrossing (Bull, 1978; Backwell and Bull, 1978). Laboratory hybridizations (including F1 hybrids and backcross products) of *C. pseudinsignifera* and *C. insignifera* found no evidence of hybrid inviability (Bull, 1979).

Crinia parinsignifera and Allies

All previous studies that examined the relationships of *C. insignifera, C. pseudinsignifera,* and *C. subinsignifera* also included *C. parinsignifera,* and together these four species formed a monophyletic group in these studies (e.g., Main *et al.*, 1958; Thompson, 1981; Barendse, 1984). However, our data clearly show that *C. parinsignifera* is instead closely allied to *C. tinnula* and a possible undescribed species. This result was consistent, regardless of how the data were analyzed. Biogeographically, our hypothesis of relationships for *C. parinsignifera* is more parsimonious as *C. parinsignifera* and *C. tinnula* both occur in eastern Australia, while *C. insignifera, C. pseudinsignifera,* and *C. subinsignifera* are all western Australian species (Cogger, 2000).

Our sampling of *Crinia signifera* from throughout its range revealed what may be a new taxon in the Coffs Harbour region of New South Wales (*Crinia sp.* in Figs. 3 and 5). A specimen initially identified as one of five *C. signifera* collected on the same day from the same area forms a clade with *C. tinnula,* also collected from the same area, and *C. parinsignifera.* Further morphological and molecular analyses will be reported elsewhere.

Crinia riparia and C. signifera

Our data suggest that *C. riparia* is the sister species to *C. signifera,* corroborating the hypothesis first put forward by Littlejohn and Martin (1964). While Blake's (1973) morphological data suggest a closer relationships between *C. riparia* and *C. tasmaniensis,* and Heyer and Liem (1976) described the genus *Australocrinia* to accommodate these two species (later sunk by Thompson, 1981), the immunological distance data of Daugherty and Maxson (1982), like ours, support the close relationship of *C. riparia* to *C. signifera.* Odendaal and Bull (1980) suggested that *C. riparia* arose from *C. signifera* through adaptation to life in the fast flowing creeks in the Flinders ranges to which *C. riparia* is restricted.

Our preliminary data on the phylogeography and intraspecific genetic differences within *Crinia signifera* demonstrate that there is strong phylogeographic structure between and within geographic regions. Specimens from Tasmania and southeastern Australia (Victoria, NSW, ACT) form a clade distinct from specimens from mainland South Australia and Kangaroo Island off South Australia. The average genetic distance, based on the combined data set between the major biogeographic regions we sampled, ranged from 2.7 to 5.9%. This level of genetic difference is larger than between any combination of *C. insignifera, C. pseudinsignifera,* and *C. subinsignifera* and is also only 1% lower than the divergence between *C. insignifera* and *C. sloanei,* which are distinct species restricted to western and eastern Australia, respectively, with a likely several million years separation (Roberts and Maxson, 1985b). Littlejohn (1964) reported geographic variation in male advertisement over a similar range, suggesting the possibility of looking at correlated patterns in genetic and behavioral evolution. Clearly, thorough sampling of *C. signifera* from throughout its range could be a fruitful area for future research.

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