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Molecular phylogeny and zoogeography of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in Australia

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The evolutionary history and biogeography of freshwater-dependent taxa in Australia is of intrinsic interest given the present-day aridity of this continent. *Cherax* is the most widespread and one of the most species-rich of Australia's nine freshwater crayfish genera. The phylogenetic relationships amongst 19 of the 23 Australian *Cherax* were established from mitochondrial DNA sequences representing the 12S rRNA and 16S rRNA gene regions. The relationships among species support an initial east–west separation, followed by a north–south divergence in eastern Australia. Molecular clock estimations suggest that these divergences date back to the Miocene. The phylogenetic relationships support endemic speciation within geographical regions and indicate that long-distance dispersal has not led to recent speciation as previously hypothesized. This new evolutionary scenario is consistent with the climatic history of Australia and the evolutionary history of other similarly distributed freshwater-dependent organisms in Australia. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **81**, 553–563.

ADDITIONAL KEYWORDS: 12S rRNA – 16S rRNA – biogeography – endemism – Miocene – molecular clock – mtDNA.

INTRODUCTION

The historical biogeography of widespread Australian freshwater-dependent species is of intrinsic interest, given the present-day inland aridity, the long isolation of the continent and its relatively recent collision with Asia (Unmack, 2001). However, historical biogeographical studies of Australian freshwater-dependent taxa with widespread distributions are relatively rare. Most of those that have been undertaken have been descriptive, including the assembling of species lists and the analysis of levels of endemism in order to define biogeographical provinces (McDowall, 1981; Walker, 1981; Williams, 1981; Williams & Allen, 1987). By far the most comprehensive of these studies is that of Unmack (2001) involving patterns of endemism in Australian freshwater fish species. Unmack (2001) identified regions of endemism and then estimated relationships among these regions using numerical methods, thus providing a framework for testing biogeographical hypotheses in other widespread taxa of inland aquatic environments.

Cherax is the most widespread and species-rich of Australia's nine freshwater crayfish genera. The genus has three distinct centres of diversity in Australia: the south-west of Western Australia, the south-east of Queensland and the Cape York region in the north (Austin & Knott, 1996; Austin, 1996; Munasinghe, Murphy & Austin, 2003; Munasinghe, Burridge & Austin, 2004). The genus also occurs widely across southern New Guinea (Fig. 1).

Robust phylogenetic information is considered highly desirable if not essential for historical biogeographical studies (Cracraft, 1994; Schuh, 2000). The taxonomy of *Cherax* (Riek, 1967, 1969) has proven to be complicated by a high degree of morphological vari-

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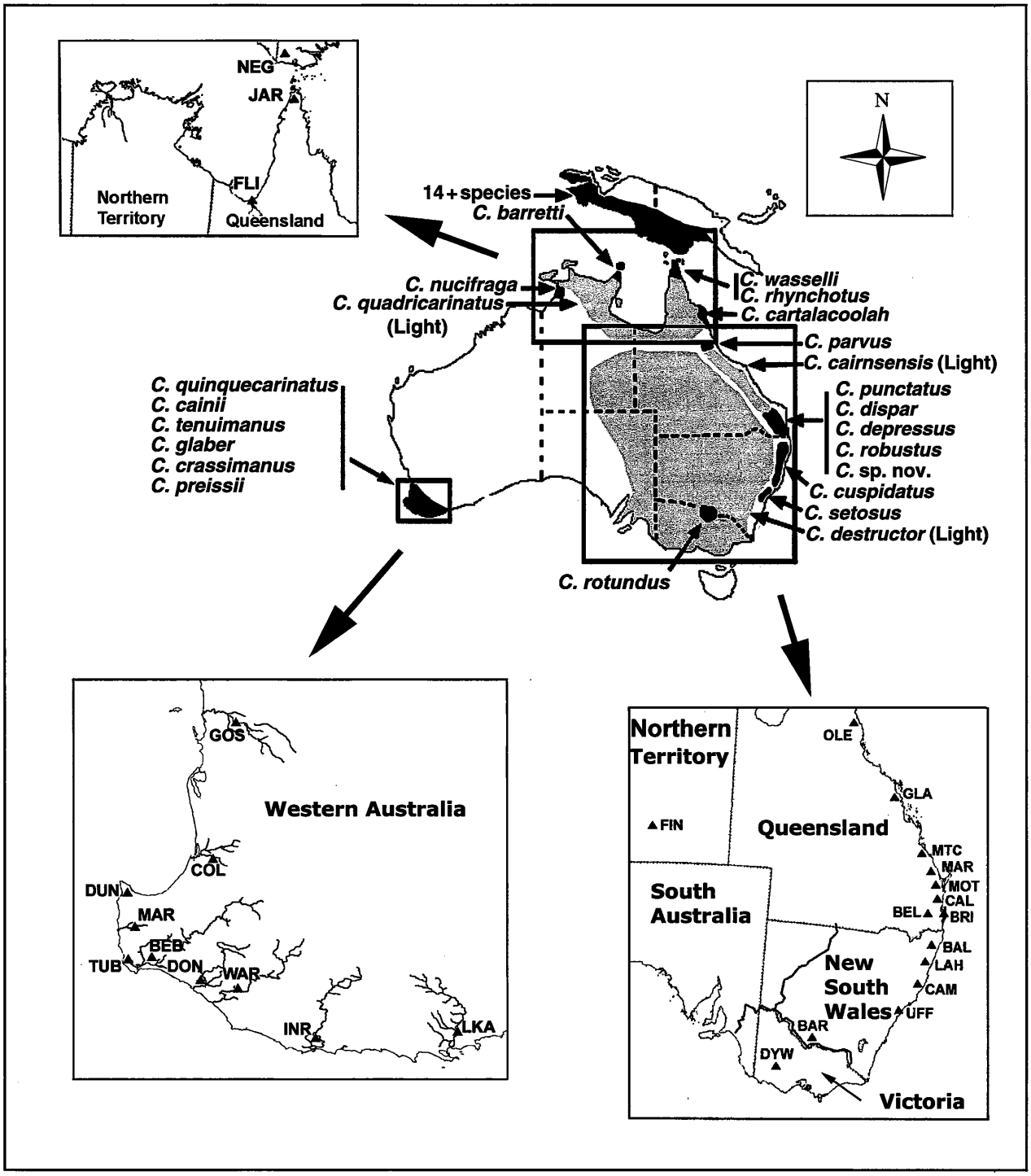

Figure 1. Geographic distribution of *Cherax* species and sample collection sites for this study.

ability, especially within the geographically widespread species, and as such cannot be used as a proxy for phylogeny. A series of taxonomic and molecular studies published over the last two decades (Patak & Baldwin, 1984; Patak, Baldwin & Lake, 1989; Austin, 1995b; Crandall, Lawler & Austin, 1995; Austin & Knott, 1996; Austin, 1996; Crandall *et al.*, 1999) indicate that significant revisions to Riek's (1967, 1969) taxonomies are required. Riek (1969) was the first to suggest relationships among *Cherax*, by establishing

species groups and in some cases suggesting relationships among these groups. While Riek (1969) did not consider explicitly the biogeographical implications of these relationships, his placement of taxa from different geographical regions together within particular species groups suggests that long-distance dispersal has occurred in the evolution of the genus. In contrast, Austin (1995a) undertook a preliminary study of the phylogenetic relationships within the genus *Cherax* using cladistic analysis of morphological and allozyme data. While both data sets contained relatively high levels of homoplasy, the resulting phylogenetic trees suggested that endemic speciation within each of the major geographical regions may be a better model for explaining the evolutionary history of *Cherax* in Australia.

The objective of this study was to utilize DNA sequence data to investigate the phylogeographical relationships of *Cherax* throughout Australia and to test the phylogenetic hypotheses of Riek (1969) and Austin (1995a). These results are then evaluated within the context of the zoogeography of other freshwater-dependent organisms and the known climatological and geological history of the Australian continent. While Crandall *et al.* (1999; 2000) analysed a number of *Cherax* species during broader studies of parastacid crayfish systematic relationships using mtDNA 16S rRNA (16S) sequences, the inferred relationships differed among studies and the type of analysis, and also showed conflicts with both Riek's (1969) and Austin's (1995a) studies. More recently, 16S and 12S rRNA (12S) sequence data have been collected and used to investigate systematic relationships within *Cherax* from the south-west of Western Australia (Munasinghe *et al.*, 2003) and northern and eastern Australia (Munasinghe *et al.*, 2004) and these are analysed jointly herein.

MATERIAL AND METHODS

Individuals were analysed from 29 locations representing 20 *Cherax* species as delineated by Riek (1967, 1969) and subsequently modified by the findings of Austin (1996), Austin & Knott (1996), Austin & Ryan (2002), Munasinghe *et al.* (2003, 2004) from south-western, eastern and northern Australia, and a sample of a species of uncertain taxonomic identity from New Guinea. *Geocharax falcata* (Colac, Victoria) was used as the outgroup (Austin, 1995a; Crandall *et al.*, 1999). Wherever possible, each species was represented by individuals from at least two geographical locations, especially those with wider geographical distributions. However, not all of the individuals analysed by Munasinghe *et al.* (2003, 2004) were included in the analysis owing to computational constraints. Total DNA was extracted from muscle tissue using the protocol described by Crandall *et al.* (1999). Voucher specimens were preserved in 95% EtOH and are held within the Deakin University crustacean collection. Sample localities are illustrated in Figure 1 and the details of the sampling localities are given in Table 1.

The data set comprised approximately 365-bp and 545-bp fragments of the 12S rRNA and 16S rRNA genes respectively (Munasinghe *et al.*, 2003, 2004). PCR was carried out in 50 μL reactions consisting of, 0.8 μM of each primer, 1× reaction buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP, 1 unit of *Taq* polymerase and 4 μL of DNA extract. A fragment of the mitochondrial 12S rRNA gene was amplified with the oligonucleotide primers L1085 5′CAAACTAGGATTAGATACCC3′ and H1478C 5′GAGAGGCGACGGGCGTATGTGT3′ (Kitaura, Wada & Nishida, 1998) and a fragment of the mitochondrial 16S rRNA gene was amplified with the oligonucleotide primers 1471 5′CCT GTTTANCAAAAACAT3′ and 1472 5′AGA TAGAAACCAACCTGG3′ (Crandall *et al.*, 1995). All PCR reactions were carried out using a Corbett PC-960 Thermal Cycler. They were started at 95°C for 5 min and terminated with 72°C for 5 min and 25°C for 1 min. PCR conditions for 12S rRNA gene consisted of 32 cycles of 30 s at 94°C, 1 min at 50°C and 1 min at 72°C. Thermocycling conditions for the 16S rRNA gene comprised 30 cycles of 30 s at 95°C, 30 s at 50°C and 30 s at 72°C. Two microlitres of the resulting PCR product was electrophoresed on an agarose gel and visualized with ethidium bromide to verify product size and estimate concentration against the Promega DNA/*Hae*III marker. The remaining product was purified using a QIAGEN QIAquick PCR purification kit. The samples were sent to the Australian Genome Research Facility, University of Queensland, for sequencing. Sequencing reactions followed the standard protocols for ABI 377 sequencers using ABI big dye terminator kit (Perkin-Elmer). For each sample, sequence reactions were performed using both primers. Chromatograms were viewed using EDITVIEW and edited using SEQPUP (Gilbert, 1997). GenBank accession numbers for sequences are given in Table 1.

Nucleotide sequences were aligned using CLUSTAL X (Thompson *et al.*, 1997) and then adjusted by eye. Phylogenetic analyses were carried out using PAUP* v.4.0b10 (Swofford, 2000) unless otherwise stated. The congruence between the two data sets was assessed under different weighting schemes using the partition homogeneity test (Farris *et al.*, 1994). Phylogenetic signal within the data set was assessed using $g1$ statistics (Hillis & Huelsenbeck, 1992).

Phylogenetic relationships were estimated using three different optimality criteria, Minimum Evolution (ME), Maximum Likelihood (ML) and Maximum

Table 1. Sample codes, collecting localities and GenBank accession numbers for sequences

Species	Code	Location	12S rRNA	16S rRNA
C. cainii Austin & Ryan (2002)	WAR	Warren River, Western Australia	AY492782	AY395852
C. cairnsensis Riek (1969)	GLA	4 km South-west of Gladstone, Queensland	AY191732	AY191761
	MTC	7 km north-east of Mt. Charlton, Queensland	AY191733	AY191762
C. crassimanus Riek (1967)	DON	Donnelly River, Western Australia	AF492780	AF492806
	TUB	Turner Brook, Western Australia	AF492779	AF492805
C. cuspidatus Riek (1969)	BAL	49 km south-west of Ballina, New South Wales	AY191724	AY191753
	CAM	1 km north of Camden Haven River, New South Wales	AY191723	AY191750
Cherax sp. nov.	LAH	2 km west of Lake Haiwatha, New South Wales	AY191720	AY191748
	BEL	Bell Bird Forest park, 15 km north-west of Brisbane	AY191727	AY191756
C. d. destructor Clark (1936)	FIN	Finke River, Northern Territory	AY191738	AY191765
C. d. albidus Clark (1936)	DYW	Dywers Creek, Grampians, Victoria	AY191740	AY191767
C. dispar Riek (1951)	CAL	5 km south of Caloundra turn off, New South Wales	AY191734	AY191771
	MAR	11 km north-west of Maryborough, Queensland	AY191735	AY191772
C. depressus Riek (1951)	BEL	Bell Bird Forest park, 15 km north-west of Brisbane	AY191731	AY191760
C. glaber Riek (1967)	DUN	Blackwood River, Western Australia	AY211981	AY211980
C. parvus Short & Davie (1993)	OLE	O'leary Creek, north-east Queensland	AY191729	AY191757
C. preissii (Erichson, 1846)	COL	Collie River, Western Australia	AF492776	AF492807
	LKA	Kalgan River, Western Australia	AF492778	AF492808
C. punctatus Clark (1936)	MOT	Mount Mothar, Queensland	AY191728	AY191758
C. quadricarinatus (von Martens, 1868)	FLI	Flinders River, Queensland	AY191744	AY191773
C. quinquecarinatus (Gray, 1845)	BEB	Brennan Bridge, Scott River, Western Australia	AF492773	AF492804
	GOS	Canning River, Western Australia	AF492772	AF492802
	INR	Inlet River, Western Australia	AF492774	AF492803
C. robustus Clark (1941)	BRI	Bribie Island	AY191730	AY191759
C. rhynchotus Riek (1951)	JAR	Jardine River, Queensland	AY191746	AY191774
C. rotundus Clark (1941)	BAR	Barmah Forest, Victoria	AY191743	AY191768
C. setosus Riek (1951)	UFF	Uffington State Forest, New South Wales	AY191741	AY191770
C. tenuimanus (Smith, 1912)	MAR	Margaret River, Western Australia	AF492784	AF492810
Cherax sp. (New Guinea)	NEG	Southern New Guinea	AY191747	AY191775
Geocharax falcata Clark (1936)	–	Colac Lake, Victoria	AF492785	AF492811

parsimony (MP). The model of sequence evolution employed during ME and ML analyses was determined using MODELTEST v.3.04 (Posada & Crandall, 1998). For MP and ML analyses, a heuristic search option employing ten random stepwise additions and Tree Bisection and Reconnection (TBR) branch swapping was performed. Confidence in the resulting relationships was assessed using non-parametric bootstrapping (Felsenstein, 1985) with 100 replicates for ML and 1000 for ME and MP analyses.

In order to test previously established phylogenetic hypotheses for the genus *Cherax* (Riek, 1969; Austin, 1995a) (Fig. 2A and 2B, respectively), the optimal constrained and unconstrained ML trees were tested for a significant difference in likelihood scores using the Shimodaira & Hasegawa (1999) method. This allows for comparison against a topology specified a posteriori. We removed several species from our dataset to maintain consistency with the taxonomic sampling of Riek (1969) and Austin (1995a) and in each instance re-estimated the optimum model of sequence evolution using MODELTEST.

RESULTS

Both the 12S and 16S gene regions contained significant phylogenetic signal with $g1 = -0.66$ (12S) and $g1 = -0.51$ (16S) ($P < 0.01$). The pairwise sequence divergence levels among samples ranged from 2.6 to 23.2% for 12S and 4.4 to 17.3% for 16S (a table of pairwise distances will be supplied upon request). The partition homogeneity test failed to reject phyloge-

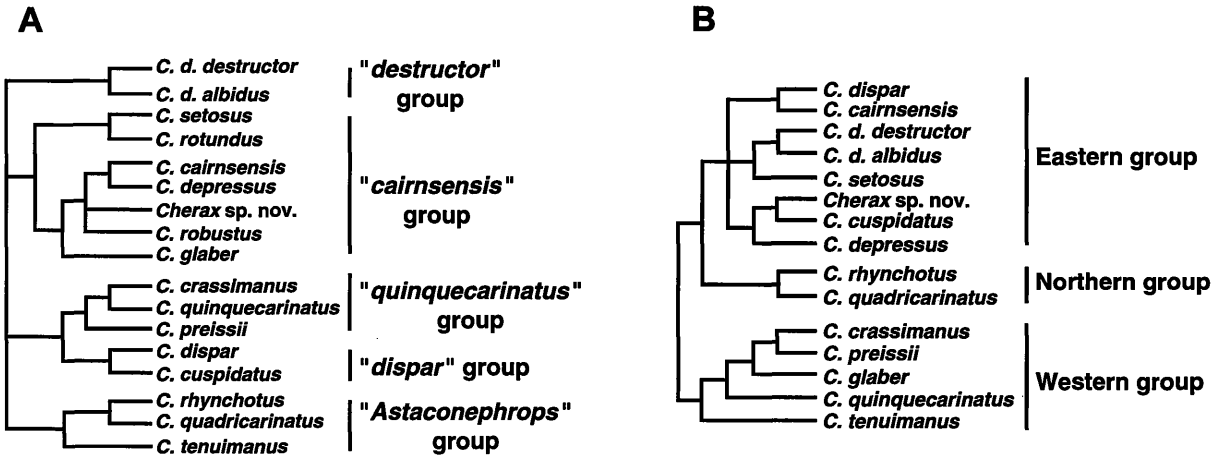

Figure 2. Hypotheses proposed by (A) Riek (1969) and (B) Austin (1995a) for the phylogenetic relationships within *Cherax*. The taxon names and groups are modified according to the current study and other recent studies (Austin & Knott, 1996; Austin, 1996; Austin & Ryan, 2002; Austin *et al.*, 2003; Munasinghe *et al.*, 2003, 2004).

netic congruence of the 12S and 16S data sets ($P = 0.15$) when employing a Tr1:Tv2 step matrix, but rejected congruence when employing equal weights ($P = 0.00$). Thus, the two data sets were combined for subsequent analyses under the former condition. The combined data set consists of 910 bp, containing 385 variable sites of which 277 are parsimony informative. K81uf + I + G was identified as the optimum model of sequence evolution for the combined data set using the MODELTEST program (base frequencies: A = 0.3338, C = 0.0911, G = 0.1957, T = 0.3794, gamma distribution parameter = 0.7585, substitution rate matrix: A-C = 1.00, A-G = 9.7773, A-T = 1.4522, C-G = 1.4522, C-T = 9.7773, G–T = 1.00).

The phylogenetic relationships estimated under the three different optimality criteria are consistent with each other with only a few minor exceptions. The shortest tree recovered by the MP analysis was 1055 steps. The consistency and retention indices of this tree were 0.522 and 0.696, respectively. The log likelihood score for the resultant ML tree was –lnL = 6801.956. The ME tree derived from the combined data set is given in Figure 3. The species of *Cherax* cluster into three distinct clades according to geographical region. The primary dichotomy reflects the major geographical disjunction within the genus, between the species restricted to the extreme south-west of Western Australia and all other species from eastern and northern Australia. Monophyly of the south-western species received 81–99% bootstrap support and monophyly of northern and eastern species received 85–93% support (Fig. 3). The levels of genetic divergence between the major clades are substantial, averaging 15% (uncorrected divergence).

The south-western species were divided into two relatively well-supported clades, one with *C. tenuimanus*, *C. cainii* and *C. quinquecarinatus*, and the other with *C. preissii*, *C. glaber* and *C. crassimanus*. The 15 eastern and northern species were subdivided into two clades, with monophyly of the eastern clade receiving 74–84% bootstrap support, and monophyly of the northern clade receiving 99–100% bootstrap support, with an average divergence of 14% (uncorrected) between species of the two clades. The 'eastern group' was consistently divided into three well-supported clades (Fig. 3): (1) *C. cuspidatus* and *C.* sp. nov., (2) *C. destructor*, *C. rotundus* and *C. setosus* and (3) *C. depressus*, *C. cairnsensis* and *C. punctatus*. *Cherax dispar* is quite divergent and forms a basal relationship with groups (2) and (3). The placement of *C. robustus* (BRI) and *C. parvus* (OLE) are inconsistent. Both MP and ME analyses place *C. robustus* in a basal position to other eastern species, while ML groups it with *C. cuspidatus* and *Cherax* sp. nov. MP and ML analyses place *C. parvus* within the *C. cairnsensis* lineage while ME placed it with the *C. destructor* samples. In addition, while most of the lineages (described above) within the 'eastern group' receive relatively high bootstrap support for their inclusion (Fig. 3), the relationships amongst these lineages are poorly resolved with most receiving less than 50% bootstrap support.

The specific phylogenetic hypotheses of both Riek (1969) and Austin (1995a) are inconsistent with the nucleotide data obtained in this study. The Shimodaira & Hasegawa (1999) test indicated that the two previously established phylogenetic hypotheses were significantly inferior to our unconstrained topology ($P < 0.05$, Table 2). However, it is noteworthy that while there are a number of differences in shallower

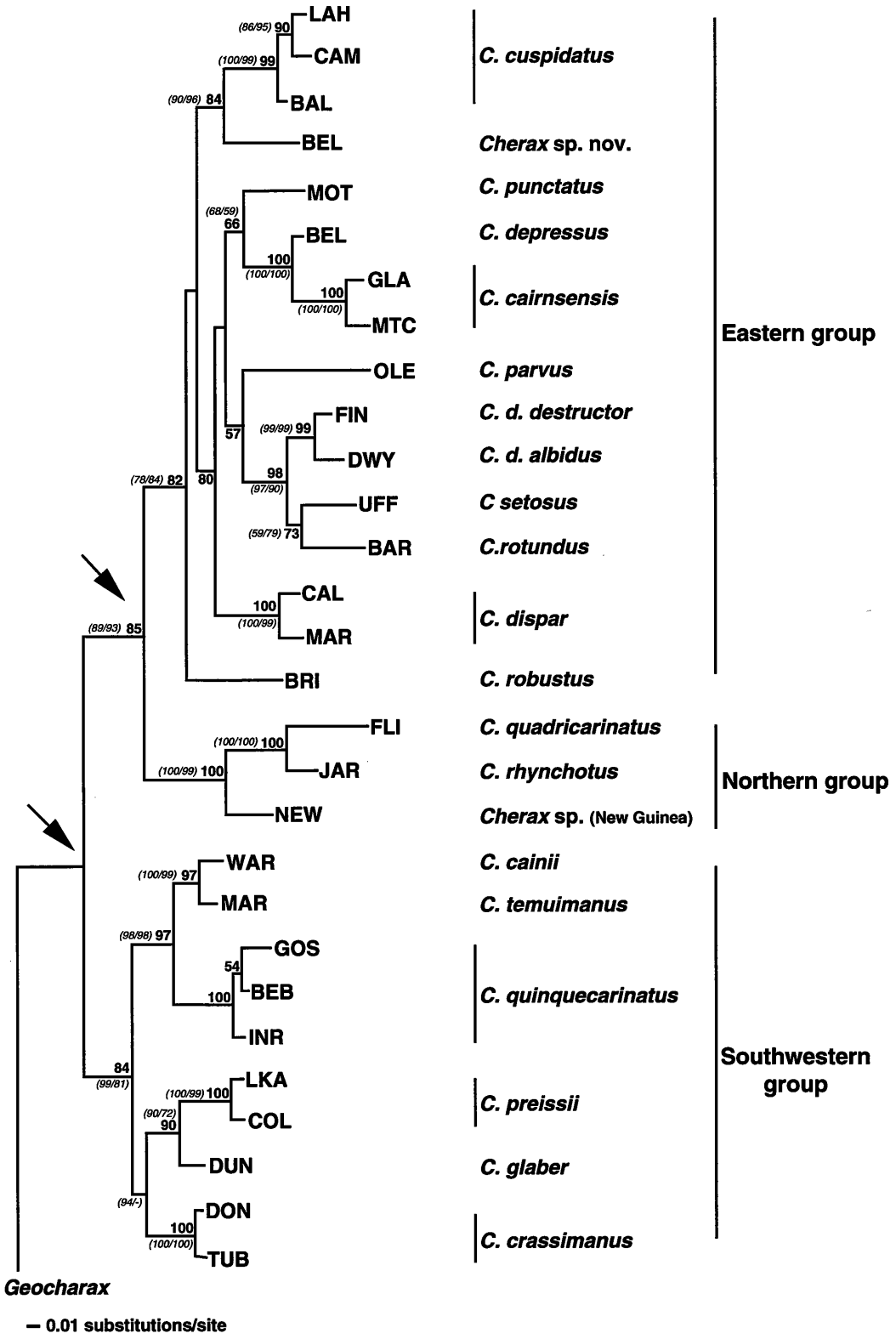

Figure 3. The Minimum Evolution tree derived from combined data set (12S + 16S rRNA), estimated using K81UF + I + G model of evolution. Bootstrap values for the ME, MP searches are based on 1000 replicates and for the ML search is based on 100 replicates. The bootstrap values (>50%) in bold are for ME analyses and for MP and ML analyses are given in parentheses (MP/ML). Arrows indicate nodes for which divergence times were estimated.

Table 2. Tests of alternative topologies following the method of Shimodaira & Hasegawa (1999). Taxa not represented in the topologies of Riek (1969) and Austin (1995a) were removed to facilitate comparisons with our data set. The abbreviations of taxa are given in Table 1

Test	Taxa removed	Model used	Tree	Length (–ln L)	*P*
Riek (1969) vs. Unconstrained	OLE, MOT, UFF, NEW	HKY + I + G	Riek (1969) Unconstrained	6462.61888 5963.11024	0.000
Austin (1995a) vs. Unconstrained	OLE, MOT BRI, NEW	HKY + G	Austin (1995a) Unconstrained	5289.41097 5206.79329	0.000

relationships amongst species in the analysis by Austin (1995a), the deeper relationships, with respect to the recovery of three geographically correlated clades, are entirely consistent with the tree presented in Figure 3.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

The mitochondrial DNA sequence data presented here strongly support the existence of three geographically correlated clades within *Cherax,* consisting of western, eastern and northern species groups (Figs 1, 3). This outcome and the relationships between species within these major groups is in conflict with the phylogenetic relationships proposed by Riek (1969) and also to some extent with those of Austin (1995a). The finding that the primary phylogenetic division within the 20 *Cherax* species is between those from the south-west of Western Australia and those from elsewhere, is contrary to Riek's (1969) views, but not those of Austin (1995a). Riek's (1969) phylogenetic arrangements were based upon external morphological characteristics, which divided the eastern species into three groups that also contained taxa from the south-west (Fig. 2A). Other studies, however, have proposed major differences between western and eastern *Cherax* groups based upon immunological data (Patak *et al.*, 1989) and allozyme data (Austin, 1995a, 1996; Austin & Knott, 1996). Furthermore, numerical cladistic analyses of a morphological data set assembled by Austin (1995a), which included attributes emphasized by Riek (1969), and traits associated with mouth parts, sternal keel, gill and gastric mill, also split *Cherax* into western and eastern/northern groups. Thus, it would appear that the traits emphasized by Riek (1969), which include features of the head and body and claw shape are susceptible to convergence or parallel evolution and therefore represent poor characters for phylogenetic analyses. This is consistent with the observations of Austin & Knott (1996), who found a high degree of variation in these traits within and between species of Western Australian *Cherax,* and strong correlation between such traits and habitat (e.g. swamps vs. rivers). Indeed, within *Cherax* there is considerable scope for parallel or convergent evolution in morphology with respect to habitat variation. Within each of the three major geographical regions where *Cherax* occur, species exploit a range of environments ranging from semipermanent swamps to permanent rivers, streams and creeks.

The grouping of all currently defined *Cherax* species from eastern Australia into a well-supported monophyletic lineage (to the exclusion of *Cherax* from northern Australia) is also contrary to Riek (1969), who depicted close relationships between some of the western and eastern species (*C. glaber* placed in the *'punctatus'* group and affinities between *'quinquecarinatus'* and *'dispar'* groups) (Fig. 2A). The 16S and 12S-based groupings are consistent with aspects of analyses of allozyme data presented by Austin (1995a). That study presented two trees derived from a numerical cladistic analysis, one with a monophyletic lineage consisting of the three northern species (*C. quadricarinatus*, *C. rhynchotus* and *C. wasselli*) embedded within a lineage containing the eastern species. The other tree suggested a sister group relationship between the northern and eastern species. Additional analyses using a phenetic approach and greater population sampling also supported the latter arrangement (Austin, 1996).

Unlike the western and eastern groups, for which we analysed individuals of all currently recognized species, our northern group only contained representatives from two of the six currently recognized species from northern Australia. However, by pooling the results of this study with those of Austin (1996), which also included samples of *C. wasselli* from Cape York, it appears likely that the northern species of *Cherax* are a monophyletic group which forms a sister group relative to the eastern Australian species. This hypothesis needs to be tested by greater taxon sampling from across northern Australia and also from New Guinea. The New Guinean freshwater crayfish fauna appears to show a close relationship with *Cherax* from northern Australia on the basis of this study and the findings by Austin (1986, 1996) that *C. quadricarinatus*

and *C. rhynchotus* occur in both localities. The well-supported phylogenetic relationships determined for *Cherax* species provide an appropriate framework for a zoogeographical analysis, which is considered below for *Cherax* in Australia.

ZOOGEOGRAPHY AND EVOLUTIONARY HISTORY

The phylogeny presented in this study, although not fully resolved, provides new insights into patterns of speciation and the role of dispersal in determining the evolution of *Cherax*. Specifically, we found that the western species *C. glaber* and *C. tenuimanus* are more closely related to each other than to morphologically similar species in northern and eastern Australia, and that the eastern *C. dispar* is more closely related to other eastern species than to western species. This indicates that long-distance dispersal has played a more minor role in the recent diversification of this genus than suggested by Riek (1969). Furthermore, the phylogenetic division of *Cherax* into three geographically correlated clades favours a localized endemic model of speciation. This is a more robust finding for the south-western and eastern regions, as the phylogenetic analyses included all known species from these areas. As there is incomplete taxon sampling for the northern region, endemic speciation is more cautiously inferred.

The monophyly of *Cherax* species from the south-west of Western Australia and the extent of the divergence of this lineage from the eastern *Cherax* species is consistent with zoogeographical relationships determined for other organisms from this region. The south-west of Australia is noted for the endemism of its flora and fauna, especially within its freshwater-dependent species (Moore, 1961; Carlquist, 1974; Walker, 1978; Hopper, 1979; Unmack, 2001). In a comprehensive analysis of levels of endemism of inland fish species, Unmack (2001) found this region to have a very high level of endemism and to be highly distinctive, but with closest affinities to south-eastern Australia. He considered that increasing aridity and the formation of the Nullarbor Plain during the Miocene (14–16 Mya) would have made subsequent east–west migration of freshwater-dependent fish unlikely. Similar conclusions have been reached from phylogenetic and biogeographical studies of anurans by Barendse (1984) and Roberts & Maxon (1985). These authors reject hypotheses mostly associated with multiple east to west invasions during the Pleistocene in favour of endemic speciation. Barendse (1984) and Roberts & Maxon (1985) applied a molecular clock to allozyme and immunological data and suggested the major east–west divergence events occurred in the Miocene or Pliocene.

There is considerable debate concerning the reliability and accuracy of molecular clocks (Swofford *et al.*, 1996). Nevertheless, they are frequently applied to estimate divergence times in studies of evolutionary history in a diversity of organisms (Cunningham, Blackstone & Buss, 1992; Caccone *et al.*, 1994; Ponniah & Hughes, 1998; Jarman & Elliott, 2000; Hedin, 2001). While recognizing the validity of criticisms directed towards molecular clocks (Hillis, Moritz & Mabel, 1996), clock calibrations determined for other decapod groups of 0.9% divergence per million years for 16S rRNA sequences (Schubart, Diesel & Hedges, 1998) places the primary divergence between eastern and western *Cherax* species (9.7–17.3%) at approximately 10–19 Mya, well into the Miocene epoch. This result is very similar to the timing of major east–west divergence events for anurans estimated by Barendse (1984) and Roberts & Maxon (1985). It also supports the view of Unmack (2001), who suggests that there is little evidence for the Pleistocene divergence events that have been hypothesized by other workers (Savage, 1973; Watson & Littlejohn, 1985; Roberts & Watson, 1993). Although less well supported as a consequence of incomplete taxonomic sampling, a second significant phylogenetic split has occurred between eastern and northern *Cherax* species. This divergence is dated at almost the same time as the east–west disjunction (Fig. 3) with genetic distances of 11.3–16.2% corresponding to 12–18 Mya.

These results require that *Cherax* had a greater dispersal capacity and a widespread distribution during the Miocene followed by range reductions, fragmentations and speciation via vicariance, which is in accord with palaeoclimatological and geological evidence. Climates were considerably wetter across southern and inland Australia during the Miocene than they are today (Williams & Allen, 1987). Australian landscapes in the early Tertiary were characterized by temperate and tropical rain forests (Kemp, 1981). However, mid-Miocene (15 Mya) vertebrate fossils from inland Australia are dominated by aboreal marsupials and aquatic animals (e.g. crocodiles) (Hope, 1982). Since this time the botanical and vertebrate fossil record documents gradual change from floras and faunas of wetland humid environments to those adapted to arid conditions by the end of the Pliocene (Hope, 1982; Kershaw *et al.*, 1994). Also, based upon the modelling of plant growth in relation to environmental factors (Nix, 1982) and on geological evidence (Bowler, 1982), aridity has been increasing since the mid-Miocene.

During the Pleistocene the Australian environment was dominated by glacial cycles which led to significantly wetter climates during interglacial periods and more arid environments during glacial maxima (Bowler, 1982). While these cycles would have undoubtedly had a significant effect upon the distribution of aquatic species, they are unlikely to have

been responsible for the majority of speciation within *Cherax*. Similar conclusions have been reached for other freshwater-dependent groups in which speciation is considered to have been mostly pre-Pliocene (Barendse, 1984; Unmack, 2001).

In considering the biogeography of eastern and northern *Cherax*, the Great Dividing Range (GDR) and the divide between northern and southern catchment basins represent significant barriers to *Cherax* dispersal. This is a similar observation to Unmack (2001), who established a line of 'endemicity' for freshwater fish that runs from north-western Australia, across northern Australia, and southward down the eastern coast along the GDR. The highest diversity within *Cherax* falls within south-eastern Queensland. This is an area of high richness but low endemism for freshwater fish (Unmack, 2001). A number of freshwater fish species found in south-east Queensland are also found in catchments to the west of the GDR. In contrast, there are five species of *Cherax* endemic to south-eastern Queensland (*C.* sp. nov., *C. depressus*, *C. punctatus*, *C. robustus* and *C. dispar*) but only *C. cairnsensis*, which occurs to the north, extends beyond this subprovince. To the south, the subprovince of north-east New South Wales identified by Unmack (2001) contains two species of *Cherax*, *C. cuspidatus* and *C. setosus*. While *C. cuspidatus* is closely related to *C.* sp. nov. from south-east Queensland, *C. setosus* is the only member of the *'destructor'* species group to occur east of the GDR. Thus, the GDR appears to have been a much more significant barrier to the movement and dispersal of *Cherax* than to freshwater fish. Nevertheless, the phylogenetic relationships determined for eastern *Cherax* indicated that there have been at least two dispersal events across the GDR that have led to speciation. The first, from east to west, led to the establishment of the *'destructor'* lineage and most likely occurred towards the end of the Miocene. The second, more recent, dispersal event occurred from west to east, most likely during the Pliocene. This took place in the vicinity of the Hunter River in the Newcastle region and led to the speciation of *C. setosus* and *C. rotundus*. The relatively recent transfer of other aquatic species across the divide in this region has also been documented. For example Jerry & Woodland (1997) considered that the catfish *Tandanus tandanus* (Mitchell, 1838) had dispersed across the divide in this region in the recent past.

Although the discussion outlined above suggests that freshwater crayfish have relatively limited powers of migration, there are clear circumstances under which they are able to disperse over significant distances both within and between catchments. For example *C. quadricarinatus* occurs throughout the northern province defined for fish by Unmack (2001), and *C. destructor*, even more impressively, is found in three separate provinces in inland and southern Australia (central Australia, Murray Darling and Bass provinces).

CONCLUSION

This study has contributed significant new knowledge about the phylogenetic relationships amongst the majority of Australian *Cherax* species using 16S and 12S rRNA sequences. Phylogeny estimation supports three geographically correlated clades consisting of a south-western group, which is the sister to northern and eastern clades of *Cherax*. This phylogeny supports a model of endemic speciation within geographical regions. Thus, significant morphological and ecological divergence has occurred in parallel within the major lineages of *Cherax*. It is also suggested that the wide distribution of *Cherax* was achieved during the Miocene, with fragmentation and vicariance associated with increasing aridity, a major cause of speciation within the genus.

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