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Phylogeography of the freshwater crayfish *Cherax destructor* Clark (Parastacidae) in inland Australia: historical fragmentation and recent range expansion

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The yabby, *Cherax destructor* Clark, is the most widespread species in the most widespread genus of Australian freshwater crayfish. It has a distribution that spans several distinct drainage basins and biogeographical regions within semiarid and arid inland Australia. Here we report a study designed to investigate patterns of genetic variation within the species and hypotheses put forward to account for its extensive distribution using DNA sequences from the mitochondrial 16S rRNA gene region. Results of phylogenetic analyses contradicted previous allozyme data and revealed relatively deep phylogenetic structure in the form of three geographically correlated clades. The degree of genetic divergences between clades (8–15 bp) contrasted with the relatively limited haplotype diversity within clades (1–3 bp). Network-based analyses confirmed these results and revealed genetic structure on both larger and more restricted geographical scales. Nevertheless some haplotypes and 1-step clades had large distributions, some of which crossed boundaries between river basins and aquatic biogeographical regions. Thus both older and more recent historical processes, including fragmentation on a larger geographical scale and more recent range expansion on a local scale, appear to be responsible for the observed pattern of genetic variation within *C. destructor*. These results support elements of alternative hypotheses previously put forward to account for the evolutionary history of *C. destructor* and the origin of its large distribution.

ADDITIONAL KEYWORDS: 16S rRNA – biogeography – mtDNA – network analysis – phylogeny.

INTRODUCTION

The freshwater biogeography of inland Australia is of inherent interest given the age, stability, aridity and low relief of this part of the continent (Unmack, 2001). Considering freshwater-dependent species without drought resistant stages, the region has both narrowly distributed and wide ranging species (Walker, 1981; Ponder, 1986; Unmack, 2001). This suggests that some taxa have been able to persist over reasonable lengths of time and speciate, most probably assisted by the occurrence of aquatic refugia within this otherwise arid landscape (Ponder, 1986), while others have apparently been able to disperse effectively in the relatively recent past. Surprisingly, biogeographical analyses of the aquatic fauna of inland Australia have seen little application of molecular genetic approaches especially those adopting phylogenetic or phylogeographical approaches.

Cherax destructor (the yabby) is the most widespread species of freshwater crayfish in Australia and has a natural range of approximately two million square kilometres, including a significant portion of arid and semiarid inland Australia (Horwitz & Knott, 1995). In addition, its distribution encompasses a number of major drainage basins and biogeographical regions identified for fish (Unmack, 2001). *Cherax destructor* is also an ecologically versatile species, occurring in habitats as diverse as the temporary rivers and lakes in central Australia and the permanent lakes and rivers of the much cooler south-east (Sokol, 1988a, 1988b; Austin, 1996; Lawrence & Jones, 2002).

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The species also adapts readily to artificial environments such as farm ponds, irrigation channels and reservoirs. Thus, the genetics and phylogeography of this freshwater crayfish species are of intrinsic interest both in their own right and in relation to the understanding of the aquatic biogeography of inland Australia.

Effective biogeographical studies depend upon reliable taxonomies. However, inland aquatic species that have been subject to some form of genetic analysis (usually using allozymes) have generally revealed taxonomic complexities including the presence of cryptic species (Musyl, 1990; Musyl & Keenan, 1994; Jerry & Woodland, 1997) thereby complicating biogeographical analyses (Unmack, 2001). In the case of *C. destructor*, its taxonomic classification has been contentious as it is phenotypically variable (Riek, 1969; Sokol, 1988a; Campbell, Geddes & Adams, 1994; Austin, 1996; Austin & Knott, 1996; Austin *et al*., 2003) with as many as four species recognized in what is often referred to as the '*Cherax destructor*' complex. The general consensus is that the yabby is composed of a single species that can be divided into two subspecies (Campbell *et al*., 1994; Austin, 1996; Austin *et al*., 2003) although some authors continue to recognize *Cherax albidus* as a distinct species (Sokol, 1988a; Lawrence & Jones, 2002). *Cherax d. destructor* Clark is widespread throughout central and eastern Australia occurring naturally within the two largest river basins of Australia, the Lake Eyre drainage basin, which is endoreic, and the drainages associated with the Murray–Darling river system (Fig. 1). In the western district of Victoria and the extreme south-east of South Australia, *C. destructor* is represented by *C. d. albidus* Clark, which has a much more limited distribution that is largely restricted to the Glenelg and Wimmera River systems (Fig. 1).

Various hypotheses have been put forward in an attempt to explain the large distribution of *C. destructor* (Sokol, 1988a; Horwitz & Knott, 1995) and these hypotheses can be reduced to two alternatives that can be referred to as the 'relictual' and the 'expanding' distribution models. The former model assumes that *C. destructor* is a relatively 'old' species so that its large distribution could have been achieved over a significant period of time, and that

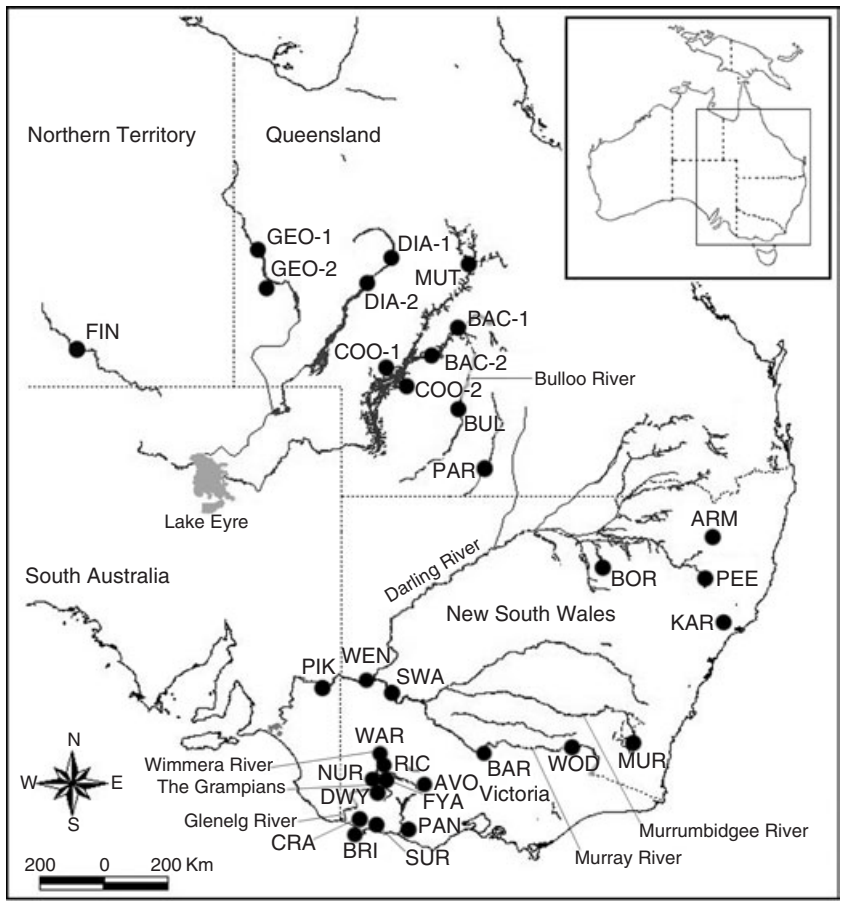

Figure 1. Collection localities for *Cherax destructor destructor* and *C. d. albidus* samples and *C. setosus* in this study (see Table 1 for sample codes).

extensive dispersal would have occurred before the onset of arid conditions in the Pliocene. In contrast, the 'expanding' distribution model postulates that the current distribution of *C. destructor* is at or close to its maximum and that this has been achieved from relatively recent dispersal from the south to the north, west and east during wet periods that connected wetlands and watercourses in the interior of Australia. In addition to natural mechanisms of dispersal, Horwitz & Knott (1995) speculated that significant translocation of *C. destructor* by humans, both before and after European settlement, may not only have contributed to an expanded distribution but also to the genetic homogenization of the species measured in terms of allozyme variation. Campbell *et al*. (1994) also considered that translocations may have had a significant effect on the distribution and population structure of *C. destructor*.

The 'relictual' and 'expanding' distribution hypotheses give different predictions with respect to the extent of genetic divergence and the nature of phylogeographical patterns within *C. destructor*. The 'relictual' hypothesis would be supported by finding deep phylogenetic structure and the 'expanding' distribution model would be favoured either by the absence of variation or the absence of geographical patterns to haplotype diversity. This study therefore aimed to examine genetic diversity in *C. destructor* and to test the above competing hypotheses through partial sequences of the mitochondrial 16S rRNA gene. These data are analysed using both standard phylogeny estimation methods and network analysis (Templeton, 1998).

MATERIAL AND METHODS

Collection of samples

To minimize the potential for misleading genetic and phylogeographical signals caused by sampling populations influenced by translocation, the collection of crayfish from artificial water bodies was avoided. In total, 12 specimens of *C. d. albidus* and 44 of *C. d. destructor* were collected from rivers, creeks, lakes and waterholes in Queensland, New South Wales, South Australia, the Northern Territory and Victoria (Table 1 and Fig. 1). Individuals of *Cherax setosus* (KAR) were obtained from near Karuah, on the central coast of New South Wales, for use as an outgroup (Austin *et al*., 2003). Muscle tissue was dissected from the abdomen or propodus and either preserved in 70–80% ethanol or frozen in liquid nitrogen in the field in 1.5 mL screw top cryogenic vials and subsequently stored at –80 °C. Alternatively, individuals were transported live back to the laboratory then frozen whole at –20 °C until required.

Choice of gene region

A number of recent studies have demonstrated the utility of DNA-based techniques for population genetic and systematic studies of freshwater crayfish (Grandjean, Souty-Grosset & Holdich, 1997; Fetzner & Crandall, 1999; Fetzner & Crandall, 2001; Grandjean & Souty-Grosset, 2001). In particular, direct sequencing of the 16S rRNA region of mitochondrial DNA has proved useful for resolving relationships amongst northern hemisphere freshwater crayfish that show limited allozyme divergence, and shows sufficient variability for population genetic level applications (Crandall, 1997; Fetzner & Crandall, 2001; Grandjean, Bouchon & Souty-Grosset, 2002; Grandjean, Frelon-Raimond & Souty-Grosset 2002). Therefore the 16S rRNA gene is likely to be suitable for investigating both deeper and shallower patterns of phylogeographical relationships and genetic diversity within *C. destructor*. Procedures for DNA extraction, amplification and sequencing were as described by Austin *et al*. (2003).

Statistical analyses

The sequential approach we used was a two-step progression, starting with the examination of phylogenetic structure in the more distant past and working forward in evolutionary time. The first step was to use phylogenetic methods to estimate patterns of relatedness among haplotypes. Although this methodology may sometimes be inappropriate for intraspecific studies (Smouse, 1998; Posada & Crandall, 2001), phylogenetic methods have worked well for many phylogeographical studies and can be viewed as an initial approach that allows comparisons with previous studies (Althoff & Pellmyr, 2002).

Sequences were initially aligned using Clustal X (Thompson *et al*., 1997) and subsequently modified by eye, and then imported into PAUP* (Swofford, 2001) for phylogenetic analyses. Phylogenetic signal was tested using the $g1$ statistic (Hillis & Huelsenbeck, 1992). The null hypothesis that all mutations in the data set are selectively neutral was tested using Tajima's D-test statistic as implemented in DNASP (Rozas & Rozas, 1999).

Three different optimality criteria were used for phylogenetic reconstruction, comprising minimum evolution (ME), maximum likelihood (ML), and maximum parsimony considering all characters unordered and equally weighted (MP) (each as implemented in PAUP*). Under MP and ML optimality criteria, an heuristic search option employing ten replicates of random stepwise addition of taxa and tree bisection-reconnection (TBR) branch swapping was performed. Nodal support was measured utilizing the non-parametric bootstrap method (Felsenstein, 1995)

Table 1. Sample codes, sample size and locality details for each population of *Cherax* sampled in the Northern Territory, Queensland, New South Wales, Victoria and South Australia

Sample code	Locality	*N*	Coordinates
C. d. destructor			
MUT	Muttaburra, Thompson Rive, Queensland	4	144°34′E 22°35′S
GEO-1	Georgina River, Queensland	2	138°30′E 22°00′S
GEO-2	Georgina River, Queensland	2	138°48′E 22°55′S
DIA-1	Diamantina River, Queensland	2	143°02′E 22°24′S
DIA-2	Diamantina River, Queensland	1	141°53′E 22°57′S
BAC-1	Barcoo River, Queensland	1	145°28′E 24°25′S
BAC-2	Barcoo River, Queensland	2	143°20′E 25°15′S
FIN	Finke River, Northern Territory	2	134°12′E 24°00′S
COO-1	Coopers Creek, Queensland	2	142°43′E 25°26′S
COO-2	Coopers Creek, Queensland	1	143°03′E 25°49′S
BUL	Bulloo River, Queensland	2	144°21′E 26°35′S
PAR	Paroo River, Queensland	1	145°03′E 28°10′S
ARM	Armidale, New South Wales	3	151°39′E 30°31′S
PEE	Peel River, New South Wales	1	151°08′E 31°28′S
BOR	Borah Ck, New South Wales	1	149°33′E 31°05′S
MUR	Murrumbidgee River, New South Wales	1	148°50′E 36°00′S
WEN	Wentworth, Darling River, New South Wales	2	141°54′E 34°07′S
PIK	Pike Creek, South Australia	2	140°48′E 34°13′S
SWA	Swan Hill, Victoria	2	143°34′E 35°21′S
AVO	Avoca, Victoria	2	143°28′E 37°06′S
BAR	Barmah State Forest, Victoria	1	144°58′E 36°01′S
WOD	Wodonga, Victoria	2	146°53′E 36°07′S
PAN	Mt Emu Creek, Victoria	4	142°42′E 38°20′S
C. d. albidus			
WAR	Warracknabeal, Victoria	2	142°24′E 36°15′S
RIC	Richardson River, Victoria	2	142°48′E 36°39′S
NUR	Nurrabiel, Victoria	1	141°59′E 36°56′S
FYA	Lake Fyans, Victoria	1	142°37′E 37°09′S
DWY	Dwyers Creek, Victoria	2	142°21′E 37°28′S
CRA	Crawford River, Victoria	2	141°34′E 37°56′S
SUR	Lake Surprise, Victoria	1	141°55′E 38°02′S
BRI	Bridgewater Lakes, Victoria	1	141°25′E 38°19′S
C. setosus			
KAR	Karuah, New South Wales	2	151°43′E 32°36′S

(1000 replicates for ME and MP, 100 replicates for ML). We employed ModelTest (Posada & Crandall, 1998) to select the most likely model of character evolution for ME and ML analysis.

The second step in the sequential approach uses a network-based analysis employing the algorithm of Templeton, Crandall & Sing (1992). This procedure estimated an unrooted haplotype network based upon a 95% plausible set of haplotype linkages. Each 95% plausible set of haplotypes was then converted into a nested design in which haplotypes separated by a single mutation were grouped together in one-step clades (Templeton, 1998). The TCS 1.13 software developed by Clement, Posada & Crandall (2000) was used to construct the haplotype networks. The null hypothesis of no geography–haplotype association for each clade was tested by using the procedures described by Templeton (1998). This involved permutation tests that were conducted separately for each level of the nested cladogram using GEODIS 2.0 (Posada, Crandall & Templeton, 2000).

RESULTS

Phylogenetic analyses

An approximately 440 bp fragment of the 16S rRNA gene region was sequenced from 56 *C. destructor* individuals sampled from 31 locations. All sequences obtained in this study have been submitted to GenBank and accession numbers are provided in Table 2. Forty-nine variable sites were identified, of which 17

Table 2. Distribution of haplotypes among samples and GenBank accession numbers

Haplotypes	Samples	GenBank accession number
1	MUT, ARM, PEE, BOR, WEN	AF500591
2	PIK	AY153855
3	BUL, PAR	AF500595
4	GEO-1, GEO-2, DIA-1, DIA-2, COO-1, COO-2, BAC-1, BAC-2	AF500600
5	FIN	AF500601
6	BAR, SWA, MUR, WOD, AVO	AF500602
7	PAN	AF500605
8	WAR, RIC, NUR, FYA	AF500612
9	DWY	AF500610
10	CRA, SUR, BRI	AY153859
11	KAR	AF500618

were parsimony informative. Aligned sequences of variable sites are presented in Table 3. A total of ten unique haplotypes were identified (excluding the outgroup taxon; see Table 3 and Fig. 1), three from *C. d. albidus* and seven from *C. d. destructor*, with the most divergent differing by 15 base pairs. The average number of nucleotide differences among *C. destructor* haplotypes is 8.3 (1.90%) compared with 35.6 (8.13%) between this species and the outgroup *C. setosus*. A test of neutrality (Tajima, 1989) did not reject the hypothesis that mutations in this DNA fragment are selectively neutral ($D = -1.01$; $P > 0.05$). Results of the skewness test of tree length frequency distribution ($g1$ statistic) indicate that there is significant phylogenetic structure in the data set ($g1 = -1.300$, $P < 0.01$).

The model selected for the ME and ML analyses was HKY, which accommodates differing transition–transversion substitution rates, uneven base frequencies, and among-site substitution rate homogeneity (Hasegawa, Kishino & Yano, 1985). Molecular evolution parameter values estimated by ModelTest are as follows: ti/tv = 2.6441; A = 0.3090; C = 0.1166; T = 0.3455; G = 0.2289.

All three phylogenetic optimality criteria recovered identical tree topologies with only minor differences in bootstrap support, indicating that these methods are relatively insensitive to different assumptions of character evolution for this data set (Fig. 2). All trees recovered three major clades containing samples with non-overlapping geographical distributions. All *C. d. albidus* haplotypes were recovered as a strongly supported clade (100% bootstrap) and have a restricted geographical distribution in western Victoria, largely limited to the Glenelg and Wimmera rivers, which have their headwaters in the Grampians mountain range. All *C. d. destructor* haplotypes were also united into a single clade, although only with moderate bootstrap support (46–64%). The haplotypes from the two subspecies differed by an average of 12.4

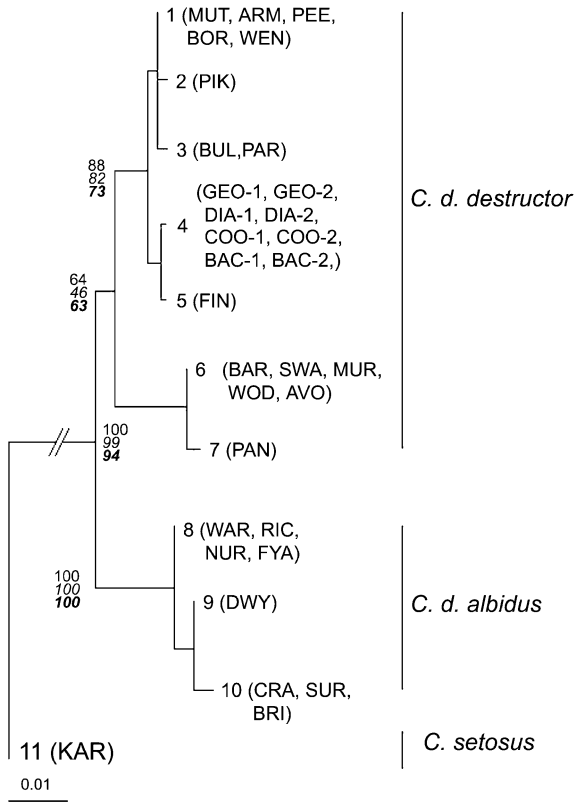

Figure 2. ME estimate of phylogenetic relationships between samples of *Cherax destructor destructor* and *C. d. albidus,* with *C. setosus* used as the outgroup. The numbers at each node represent bootstrap estimates on branches for the minimum evolution (normal font), maximum-parsimony (italic), and maximum-likelihood (bold italic) optimality criteria.

base pairs (2.8%). The samples in the *C. d. destructor* clade, with one exception (PAN), were found throughout the Murray–Darling and Lake Eyre drainage basins and span an enormous distance, with the two

Table 3. Aligned sequences from the mt 16S rRNA gene region from samples of *Cherax destructor* with invariant sites excluded

Haplotype	6	51	65	93	97	108	115	124	136	139	143	151	173	185	190	197	198	199	200	202	227	245	253	270	271	273	276	281	285	291	293	298	307	311	313	314	344	371	373	378	379	380	381	388	389	391	414	415	422	429	432
1	T	A	G	A	T	T	C	G	A	C	G	T	C	A	G	G	T	A	A	–	T	A	T	A	C	C	A	A	T	T	A	A	T	A	A	T	T	A	A	T	T	T	G	C	G	A	T	G	G	A
2	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	–	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	–	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	–	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	.	G	.	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	–	A	.	.	.	.	.	.	.	.	.	.	.	G	.	A	C	C	A	A	T	T	A	A	T	T	T	G	C	G	A	T
6	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	A	C	.	G	–	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	.	.	.	.	.	.	T	.	.	.	.	.	.	G	.	A	C	.	G	–	.	.	.	.	T	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	C	G	.	.	.	.	T	.	.	.	.	.	T	.	.	A	.	T	.	–	.	.	.	.	T	.	.	.	.	.	.	.	.	A	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
9	C	G	.	.	.	.	T	.	.	.	.	.	T	.	A	A	.	T	.	–	.	.	.	.	T	.	.	.	.	.	.	.	.	A	G	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
10	C	G	.	.	.	.	T	.	.	.	.	.	T	.	A	A	.	T	.	–	.	G	.	.	T	.	.	.	.	.	.	.	.	A	G	.	C	.	.	T	.	.	.	.	.	.	.	.	.	.	.
11	.	G	T	G	.	C	T	A	G	.	A	.	T	.	.	.	.	C	.	T	.	.	G	G	T	.	G	G	A	G	G	.	.	.	.	C	.	A	T	G	C	C	C	C	A	A	A	–	C	A	C	G

most wide spaced samples, PAN and MUT being over 1700 km apart.

The samples within the *C. d. destructor* clade are in turn divided between two reasonably well-supported clades, one consisting of a more geographically restricted 'southern' clade (94–100% bootstrap support) and a 'northern' clade (73–88% bootstrap support) with an extremely large distribution through central Australia. The haplotypes from these two clades differed by an average of 9.8 base pairs (2.2%). Representatives of each of these two clades are found within a single river system, the Murray River. The haplotypes from Pike Creek (PIK) and Wentworth (WEN) are from the lower Murray River at or below its confluence with the Darling River and belong to the 'northern' clade, whereas the haplotype from further 'upstream' sampled from the Murray River or its tributaries above the Darling River confluence (SWA, BAR, MUR, WOD and AVO) belong to the 'southern' clade. The Swan Hill (SWA) and Wentworth (WEN) samples were taken from locations that are approximately 200 km apart and the crayfish haplotypes from these locations differ by nine base pairs. In contrast, representatives of the 'northern' clade are found at the extreme west (FIN) and east (ARM, PEE) of the species distribution differ only by two base pairs despite being 1800 km apart and located in two independent drainage basins.

Network-based analyses

Two distinct networks were resolved from the 16S rRNA sequence data (Fig. 3) comprising the *C. d. albidus* samples (Clade 1-4) and the *C. d. destructor* samples (Clade 3-1, Fig. 3), which correspond to the primary bifurcation identified in the preceding phylogenetic analyses. These two individual networks are not joined because divergence between the networks exceeded the 95% confidence limits of parsimonious connection derived from the estimation procedure. Two additional well-differentiated and geographically non-overlapping networks were apparent within the *C. d. destructor* clade, connected by seven mutation steps and corresponding to 'northern' (Clade 2-1, Fig. 3A) and 'southern' (Clade 2-2, Fig. 3B) *C. d. destructor* lineages resolved by the preceding phylogenetic analyses. GEODIS analysis indicated that genetic structuring between these clades is significant ($\chi^2 = 43$, $P < 0.001$). Table 4 presents the results of the nested contingency analysis of haplotype–geographical associations for the nested clades of the haplotype networks shown in Fig. 3. Significant genetic structure was apparent at various geographical scales ($P < 0.05$) and was detected within each of the three main clades. Only in one clade within the *C. d. destructor* network (Clade 1-2) was the null

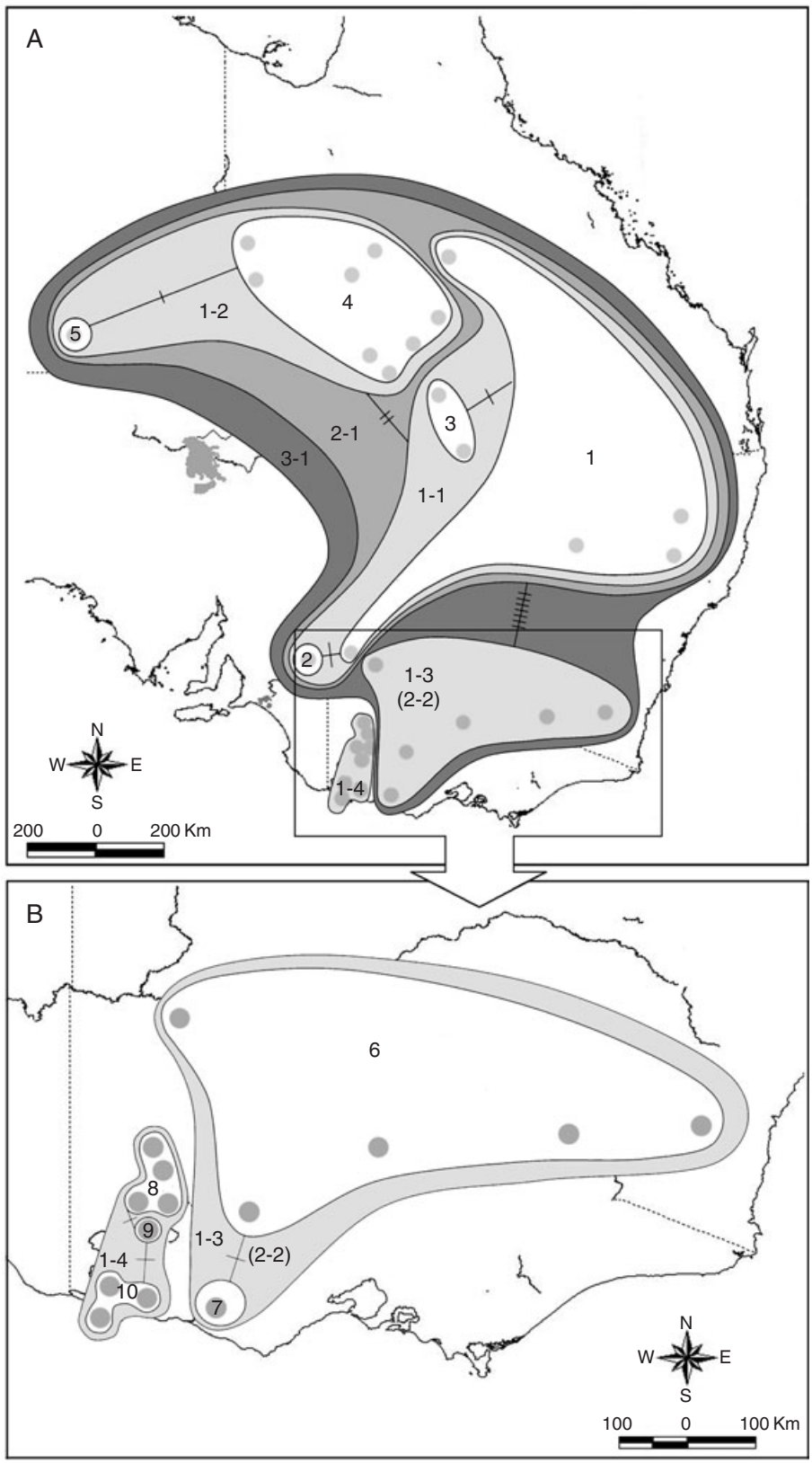

Figure 3. Geographic distribution of 16S haplotypes and haplotype networks for *Cherax destructor destructor* and *C. d. albidus* sampled from 31 locations in (A) eastern Australia and (B) south-eastern Australia.

Table 4. Results obtained from GEODIS for testing geography–haplotype associations

Clade (see Fig. 3)	χ^2	*P*-value
1-1	32	0.003
1-2	15	0.085
1-3	12	0.015
1-4 (*C. d. albidus* network)	24	0.014
2-1	31	0.000
3-1 (*C. d. destructor* network)	43	0.000

hypothesis of no association between clade and geography not rejected (Table 4).

DISCUSSION

Phylogeographical patterns

Cherax destructor shows contrasting deep and shallow phylogenetic patterns based on variation in 16S sequences. The deep phylogeography of *C. destructor* is dominated by the presence of three distinct and geographically isolated clades, although more comprehensive sampling may detect zones of overlap between two of these clades within the Murray River and lower Darling River. These three clades are supported by both the phylogenetic and network analyses. The degree of divergence among these clades (1.8–3.4%) is of note as the 16S rRNA gene is one of the slower evolving mitochondrial genes commonly utilized for phylogeographical analysis. If it is assumed that the 16S rRNA gene in *Cherax* evolves at a similar rate to other decapod crustaceans (i.e. 0.6–0.9% nucleotide substitutions per million years; Schubart, Diesel & Hedges, 1998) the major divisions within *C. destructor* probably occurred well into the Pliocene, as opposed to the recent or Pleistocene epochs. Thus it appears that this species has been a resident of the inland waterways of central and south-eastern Australia for a significant period of time.

While there is clear evidence for a relatively old geographically based pattern of divergence in *C. destructor*, resulting in three distinct and geographically non-overlapping lineages, there is also evidence of more recent dispersal within each of these three lineages. This is evidenced by the large distributions of certain haplotypes and one-step clades within each of the three lineages. In several cases these haplotypes and one-step clades cross catchment basins and trangress boundaries of aquatic bioregions (Unmack, 2001). The extent of this more recent dispersal within these three lineages is variable and in the case of the northern lineage, which covers approximately 75% of the geographical distribution of the species, it is has been extensive. Despite this evidence for relatively recent dispersal, present-day aridity would restrict current gene flow both within and between drainage basins as supported by allozyme frequency difference among populations (Campbell *et al*., 1994; T. T. T. Nguyen, unpubl. data) and the rejection of the null hypothesis of no association between haplotypes and geography for a majority of the clades identified in this study.

The findings of this study are consistent with Austin *et al*. (2003) in their molecular taxonomic re-assessment of the '*C. destructor*' complex of species using 16S sequences based on only limited population sampling within *C. destructor*. More generally, the results of this study are similar to other studies reporting intraspecific variation in freshwater crayfish (Grandjean *et al*., 2000) including *Cherax* (Munasinghe, Murphy & Austin, 2003), which showed a high degree of phylogeographical structure from 16S sequences. Thus the finding of deep phylogeographical patterns to genetic variation in *C. destructor* is very similar to what has been found in *Cherax quiquecarinatus* in Western Australia and freshwater crayfish species such as *Cherax cainii,* which show no detectable interpopulation variation in 16S sequences, are highly unusual (Nguyen *et al*., 2002; Munasinghe *et al*., 2003). The strong phylogeographical patterns to variation of 16S sequences in *C. destructor* contrasts with allozyme studies, which do not support the presence of three clades in *C. destructor* and in fact provides very little evidence of geographical pattern in the distribution of allelic frequencies (Campbell *et al*., 1994; Austin, 1996; Nguyen, 2003).

Relictual and expanding distribution models

From the above discussion the better explanation for the large distribution of *C. destructor* is the 'relictual' model in preference to the 'expanding' distribution model (Horwitz & Knott, 1995). *C. destructor* has clearly been an inhabitant of the inland waterways of Australia for a significant period of time and over this period has acquired an extensive distribution and significant phylogeographical structure. The 'relictual' model which presupposes that *C. destructor* invaded the interior of Australia in the late Tertiary, subsequently survived drought periods in aquatic refugia, and then expanded its distribution during interglacial pluvial periods, is consistent with the level of divergence in 16S sequences among the three clades observed in this study. However there is also evidence to support the 'expanding' distribution model of Horwitz & Knott (1995), involving significant dispersal of *C. destructor* possibly during Pleistocene pluvial periods, but on a more regional scale within each major clade. Thus, a significant portion of the current distribution of *C. destructor* to the north and the west may

have been attained relatively recently as a result of the expansion of the 'northern' clade.

Thus a new and more refined model for the evolution of *C. destructor* in inland Australia is one in which it is assumed that the three main clades reflect the former existence of relict populations that survived in isolated aquatic refugia during arid periods, dating back to the Pliocene. The most likely locations of these refugia, based on the distribution of the older haplotypes within each clade, are the upper reaches of the Darling River for the northern *C. d. destructor* clade, the upper Murray and Murrumbidgee Rivers for the southern *C. d. destructor* clade, and the Wimmera River for the *C. d. albidus* clade. A subsequent pluvial period or periods (probably during the Pleistocene) would have provided opportunities for significant dispersal of *C. destructor* from these refugia, both within and between drainage systems. In the northern lineage this involved dispersal on a much larger scale compared to the other lineages, most probably facilitated by the size and extent of the upper Darling and Lake Eyre drainage basins and major flooding events induced by monsoon weather patterns or the La Niña phase of the El Niño Southern Oscillation in northern and central Australia (Cook, Bunn & Hughes, 2002).

Implications for inland biogeography

The phylogeographical patterns determined in this study are generally at odds with biogeographical or molecular systematic studies of other Australian inland aquatic organisms. In a recent comprehensive biogeographical study of Australia's inland freshwater fish fauna, Unmack (2001) identified regions of endemism and relationships among these regions based on numerical analyses of faunal similarities. *Cherax destructor* occurs in four of the regions of endemism identified by Unmack (2001); south-west Victoria, the Murray–Darling Basin, Bulloo and the Lake Eyre Basin. Other than finding a very close relationship between the Bulloo and Lake Eyre regions, there is very little similarity between the relationships identified among these regions by Unmack (2001) and the phylogeographical patterns apparent within *C. destructor*; the species has transgressed the boundaries between these regions in the relatively recent past, boundaries that apparently have represented significant obstacles to dispersal for a number of inland fishes. Conversely, significant divergence has occurred within one of the zones considered homogenous by Unmack (2001) (i.e. the Murray–Darling basin). Lastly, the phylogenetic analysis of *C. destructor* indicates the south-west region of Victoria has had relatively recent linkages to the Murray River within the last 2–4 million years.

The phylogeographical structure of *C. destructor* contrasts with relationships determined among golden perch (*Macquaria ambigua*) populations sampled from a number of the same drainage basins and river systems by Musyl & Keenan (1994). Their study, based on an allozyme analysis, appears to be the only other that has examined genetic variation in an aquatic species in inland Australia on a similar geographical scale to this study. These authors found that *M. ambigua* populations showed minimal differences within the Murray–Darling system, but detected a cryptic species, closely related to *M. ambigua,* restricted to the Lake Eyre Basin, and identified a population of possibly hybrid origin in the Bullo River. Thus, *M. ambigua* shows patterns that are largely congruent with the analysis of Unmack (2001) and therefore also at odds with our study other than providing support for recent connection between the Lake Eyre Basin or Murray–Darling basin with the Bulloo system.

The lack of correspondence between the above studies may reflect differences in the dispersal capacity and ecology of species and responses to geographical and climatic processes occurring over different time scales. Major patterns of endemism in inland fish probably reflect older biogeographical processes than those invoked for *Cherax*. Unmack (2001) considers that most of the significant patterns detected in his study were established as early as the Miocene, and that Plio–Pleistocene effects on endemism patterns have been minimal. Similarly, the ancestor of the two golden perch taxa is considered to have occupied the inland waterways of central Australia for a significant period of time extending well into the Tertiary (Musyl & Keenan, 1994).

The occurrence of members of the two distinct *C. destructor* lineages in the Murray River based on the present day configuration of these drainage systems and the Unmack (2001) analysis is an anomaly. However, the 'relictual' model described above could account for this situation. Thus if the ancestral form of *C. destructor* was widespread in the late Miocene or early Pliocene, then populations could have been isolated in the head waters of the Darling and Murray Rivers as a result of increasing aridity at the end of the Tertiary period (Bowler, 1982; Frakes, McGowran & Bowler, 1987).

While there is little communality between the studies of Unmack (2001) and Musyl & Keenan (1994) and this study, the close affinities between *C. d. albidus* and *C. d. destructor* and the presence of a significant connection between the Murray and the Wimmera Rivers during the Pliocene is supported by recent nucleotide data obtained from black fish (*Gadopsis* spp.) and spiny crayfish (*Euastacus* spp.). A recent study of sequence variation in the 12S rRNA gene region in the river black fish (Miller *et al*., 2004) revealed a distinct genetic variant, with an almost

identical distribution in south-western Victoria to *C. d. albidus*. This genetic variant formed a sister clade relationship with samples from the Murray–Darling drainage and exhibited a very similar level of divergence from its sister clade (2.8% divergence) to that between the two freshwater crayfish subspecies examined herein. Another species restricted largely to the Grampians region is the spiny freshwater crayfish *Euastacus bispinosus.* This species shows a very close relationship to the Murray River spiny crayfish (*Euastacus armatus*) (Avery & Austin, 1997; Crandall *et al*., 1999). These two nominal species differ by 1.1% divergence in 16S rRNA sequences (Crandall *et al*., 1999) and showed no detectable allozyme differences (Avery & Austin, 1997). Thus given that the 12S rRNA gene tends to evolve at a similar rate to 16S rRNA and that the latter gene may evolve at a slower rate in *Euastacus* compared to other freshwater crayfish (see Crandall *et al*., 1999), the similarities are striking and point to the existence of a significant connection between the Murray and Wimmera rivers, most probably during the Pliocene. It will thus be of interest to examine phylogeographical relationships in other species or pairs of species that are common to both regions.

Translocations and Conservation

Intraspecific or intrageneric translocation issues in relation to the maintenance of genetic diversity in natural populations are becoming an increasingly important issue in relation to freshwater crayfish conservation (Lodge *et al*., 2000; Austin & Ryan, 2002; Nguyen *et al*., 2002). Both Lodge *et al*. (2000) and Austin & Ryan (2002) have reported significant negative impacts arising from translocation of non-indigenous freshwater crayfish populations over relatively small geographical scales. With respect to *C. destructor* the degree to which translocations have influenced the natural range of the species and affected the population structure of natural stocks is thought to be significant. Horwitz & Knott (1995) speculated that significant translocation of *C. destructor* by humans, both before and after European settlement, may not only have contributed to an expanded distribution but also the genetic homogenization of populations. Campbell *et al*. (1994) also considered that translocations may have had a significant effect on the distribution and population structure of *C. destructor* and could have been responsible, in part, for what they described as a chaotic pattern of relationships among the samples of *C. destructor* analysed in their study of allozyme variation. In contrast, the finding of significant population structure and the generally restricted geographical distribution of haplotypes in this study suggests that translocations have probably had little if any influence on the population structure and distribution of natural populations of *C. destructor*, although more intensive sampling is required to verify this. The results of this study, similarly to the allozyme study of Campbell *et al*. (1994), also indicate significant fragmentation of populations and restrictions to gene flow, circumstances conducive to the development of population genetic structure by genetic drift as has been suggested for other crayfish species (Gouin *et al*., 2001). However, unlike the study by Campbell *et al*. (1994) there are clear geographical patterns evident in the distribution of 16S haplotypes.

The failure to identify obvious translocation events in this study, however, does not reduce the need for care in the management of the major components of genetic diversity within *C. destructor*. In fact the results of this study have significant implications for the conservation of wild populations of *C. destructor*. The three major lineages identified in this study represent 'evolutionary significant units' (Moritz, 1994) and therefore warrant appropriate management if genetic diversity is to be preserved. This will be especially important in the extreme south where all three lineages occur in close geographical proximity and where there is a high level of rural development, interest in freshwater crayfish aquaculture and significant recreational fishing activity.

CONCLUSIONS

An analysis of the distribution of 16S rRNA haplotypes within *C. destructor* reveals significant diversity and some intriguing phylogeographical patterns. The data provide support for both older fragmentation and more recent dispersal and is consistent with elements of two competing views; the 'relictual' and 'expanding range' models, put forward to account for the large distribution of this freshwater crayfish species. A new modified model for the evolution of *C. destructor* is proposed on this basis.

The phylogeographical patterns within *C. destructor* show both similarities and differences with biogeographical or genetic studies of other groups of Australian aquatic inland organisms. This study suggests that phylogeographical analysis of mitochondrial genes of widespread species of inland fish and crustaceans have much to offer for understanding processes that have shaped the geographical diversification of the aquatic inland fauna of Australia.

Given the commercial and recreational importance of *C. destructor* and presence of three geographically distinct lineages, a final recommendation for future research is an investigation of the potential for human-mediated translocations for disrupting the genetic integrity of this species. This will be especially critical in the southern part of the distribution of the

species where all three clades occur in close proximity and where significant recreational fishing and aquaculture activities occur.

The natural extension of this study is to examine the finer scale phylogeography and population genetics of *C. destructor* using more intensive sampling. Any such studies would benefit from the use of microsatellite loci, which are being developed for an increasing number of freshwater crayfish species including *Cherax* spp. (e.g. Baker *et al*., 2000).

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