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NOTE

A genetic investigation on translocation of Australian commercial freshwater crayfish, *Cherax destructor*

T.T.T. Nguyen[a]

School of Ecology and Environment, Deakin University, Warrnambool, VIC 3280, Australia

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Abstract – The Australian freshwater crayfish, *Cherax destructor* is cultured commercially and has been translocated throughout much of Australia. Previous investigation on *C. destructor* using 16S rRNA sequences of samples collected from natural environments has revealed a significant phylogeographic structure in this species with three well supported geographically non-overlapping clades, namely 'northern' *C. d. destructor*, 'southern' *C. d. destructor* and *C. d. albidus*. Movement of individuals beyond their natural range of distribution may have adverse effects on genetic integrity of the species. In the present study, aspects of translocations of the species were genetically investigated. Sequences of the 16S rRNA gene region of the mitochondrial DNA (mtDNA) were obtained from samples collected in nine quasi-natural waterbodies, supplemented with sequences of samples obtained from 31 natural waterbodies examined in a previous study. Results of phylogeographic analysis provide evidence that certain haplotypes from major clades of *C. destructor* have been translocated. The findings of this study have important implications for the conservation and management of genetic diversity within *C. destructor*.

Key words: *Cherax destructor* / Freshwater crayfish / Translocation / Conservation / 16S rRNA

1 Introduction

The translocation of aquatic organisms is an issue of increasing concern to many conservationists (Claudi and Leach 2000). The substantial negative effects of conspicuous intercontinental translocations of exotic aquatic species up on local biodiversity and ecosystem function have been well documented (Claudi and Leach 2000; Sala et al. 2000). Less well appreciated, but more subtle and potentially insidious, are small scale local translocations involving genetically distinct conspecific individuals or those from closely-related species.

Detecting intra-specific translocations is a difficult task because of the frequent a lack of morphological differentiation among populations, and/or genetic data regarding population structure prior to translocation. Nevertheless, studies of several freshwater species used for stocking have revealed a number of instances where genetically distinct forms have become mixed and led to large-scale introgression and consequent loss of intra-specific genetic diversity (Hindar et al. 1991; Rhymer and Simberloff 1996; Hughes et al. 2003).

In recent years, freshwater crayfish have increasingly attracted the attention of conservationists (Horwitz 1995; Crandall 1998; Fetzner and Crandall 2002). Recent estimates are that as much as 70% of the approximately 600 known crayfish species worldwide are under threat (Taylor 2002). Crayfish are especially vulnerable to the detrimental effects of both long distance and localised translocations as they are easy to transport, survive for long periods out of water and readily adapt to new environments. Many species are popular targets of recreational fishers, bait collectors, the ornamental fish trade and aquaculturists (Huner 1994, 1997), and are widely translocated (Morrissy and Cassells 1992; Lörtscher et al. 1997; Gouin et al. 2001; Grandjean et al. 2001; Perry et al. 2001a,b; Nguyen et al. 2002). Impacts of interspecific hybridisation between indigenous and non-indigenous crayfish leading to the loss of genetic diversity has also been highlighted in North America (Perry et al. 2001a, 2002), and Australia (Austin and Ryan 2002; Nguyen et al. 2002).

Cherax destructor, commonly referred to as the "yabby", is the most widespread and best-known of Australia's freshwater crayfish. The species is ecologically versatile, occurring in habitats ranging from the ephemeral rivers and lakes in central Australia to the permanent lakes and rivers of the much cooler southeast (Sokol 1988a,b). It is also one of the principal cultured freshwater crayfish in Australia, contributing 73% of the total freshwater crayfish production between 1996 and 2000 (Piper 2000). It is widely stocked into artificial waterbodies by farmers and commonly used as bait by recreational fishers.

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[a] Corresponding author: `thuy.nguyen@enaca.org`

Thus, *C. destructor* is highly vulnerable to translocation, and consequently feral or cultured stocks of this species have become established outside its natural range in Tasmania (Elvey et al. 1996), and Western Australia.

Hybridisation and introgression are of particular concern given the effects on crayfish biodiversity resulting from hybridisation between resident and introduced freshwater crayfish in North America (Perry et al. 2001a, 2002), and in Australia (Austin and Ryan 2002). To date, literature concerning the loss of genetic diversity due to translocation is only interspecific, such as in the case of the progressive extirpation of an indigenous form of *Orconectes propinquus* by the introduction of *O. rusticus* in northern Wisconsin (Perry et al. 2001a,b). Similarly, Nguyen et al. (2002) documented the translocation of *C. cainii* into the Margaret River in southwestern Western Australia and Austin and Ryan (2002) and Bunn (personal communication) also reported evidence for almost complete displacement of the indigenous *Cherax tenuimanus* by *C. cainii* in the lower Margaret River after only 14 years since its introduction into this river system.

A molecular investigation of *C. destructor* using 16S rRNA sequences of samples collected from natural environments revealed significant phylogeographic structure in this species with three well supported geographically non-overlapping clades (Nguyen et al. 2004; Nguyen et al. 2005), these being 'northern' *C. d. destructor*, 'southern' *C. d. destructor* and *C. d. albidus*. In the present study, sequences from the 16S rRNA gene region of *C. destructor* individuals sampled from nine quasi-natural waterbodies (e.g. aquaculture and farm ponds, irrigation channels or artificially constructed waterbodies within townships) were obtained to evaluate the extent to which the genetic components within the species have been established in relation to the translocations, leading to a comparison of the distribution of haplotypes sampled from both natural and quasi-natural waterbodies.

2 Materials and methods

A total of 22 crayfish were collected from ten quasi-natural waterbodies, between March 2000 to February 2002 using drop nets, fish traps or dip nets. Details of localities are provided (Table 1, Fig. 1). Tissue samples were generally acquired from crayfish that had been transported live to the laboratory and frozen or preserved in 80% ethanol. Procedures for DNA extraction, amplification and sequencing were as described by Nguyen et al. (2002).

The data set used in the present study includes 22 sequences, together with 50 additional sequences of *C. destructor* collected from natural locations in a previous study (Nguyen et al. 2004). Methods of sequence alignment and tree reconstruction also followed Nguyen et al. (2004), i.e. three inference methods were employed, including minimum evolution (ME), maximum parsimony (MP) and maximum likelihood (ML). The sequences were analysed using heuristic search algorithm, employing tree bisection-reconnection (TBR) branch swapping and 100 relicates of random sequence addition. Nodal supports were estimated using the non-parametric bootstrap method (Felsenstein 1985) with 1000 replicates for ME and MP, and 500 replicates for ML. The most likely model of character evolution for ME and ML analysis was selected using program ModelTest (Posada and Crandall 1998). The sequence of *C. setosus* (SET) was used as an outgroup.

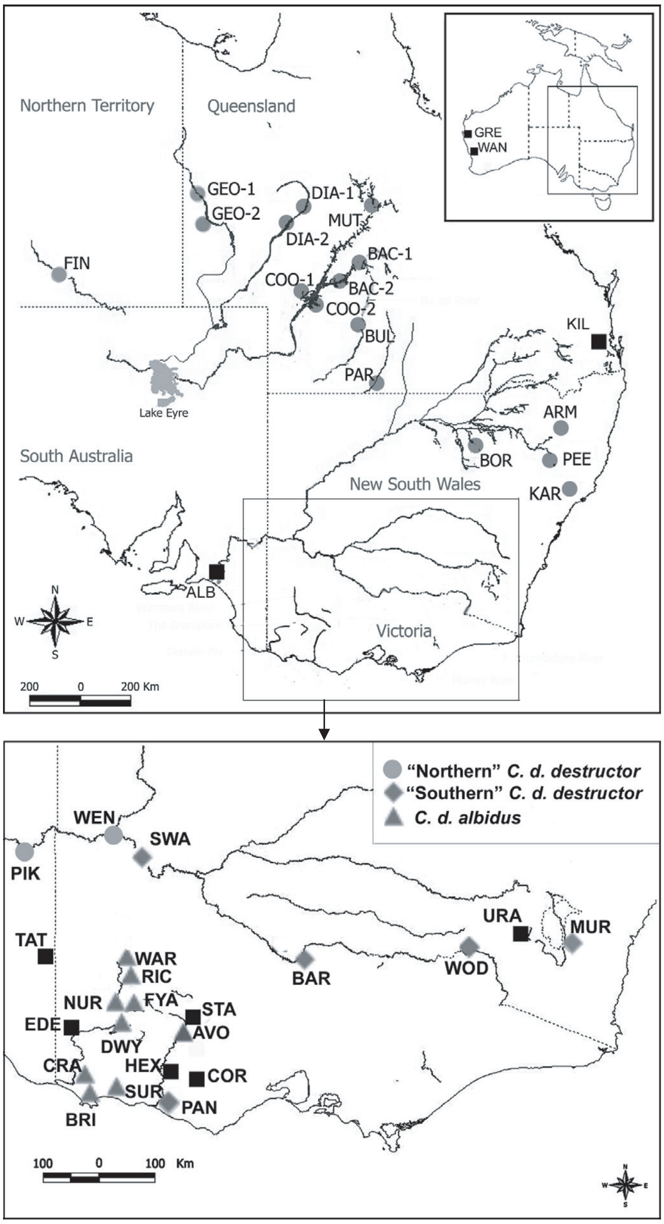

Fig. 1. Sampling localities of *Cherax destructor*. Grey dots are localities of samples from natural waterbodies analysed by Nguyen et al. (2004), those in black are samples obtained in this study.

3 Results and discussion

A total of 11 unique haplotypes were identified within *C. destructor*, containing 22 variable sites. Ten of these haplotypes were recorded from natural populations (Nguyen et al. 2004). Only four haplotypes were identified from quasi-natural waterbodies or translocated samples, of which only one

Table 1. Codes, sample sizes (*n*), and localities for *Cherax destructor* obtained from quasi-natural waterbodies in the present study, and from natural waterbodies of Nguyen et al. (2004).

Sample code	Locality	*n*	Coordinates
Samples obtained in the present study			
ALB	Lake Albert, South Australia	2	139°22’E; 35°42’S
COR	Corrangamite, Victoria	3	143°30’E; 38°22’S
EDE	Edenhope, Victoria	2	141°18’E; 57°03’S
GRE	Greenough, Western Australia	2	115°48’E; 31°45’S
HEX	Hexham, Victoria	2	142°51’E; 38°00’S
KIL	Kilcoy, Queensland	2	152°33’E; 26°56’S
STA	Stawell, Victoria	2	142°58’E; 37°05’S
TAT	Tatiara Creek, South Australia	3	140°46’E; 36°18’S
URA	Urana, New South Wales	2	146°15’E; 35°20’S
WAN	Wanneroo, Western Australia	2	114°44’E; 28°56’S
Samples obtained by Nguyen et al. (2004)			
“Northern” *C. d. destructor*			
ARM	Armidale, New South Wales	3	151°39’E; 30°31’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
BOR	Borah Creek, New South Wales	1	149°33’E; 31°05’S
BUL	Bulloo River, Queensland	2	144°21’E; 26°35’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
GEO-1	Georgina River, Queensland	2	138°30’E; 22°00’S
GEO-2	Georgina River, Queensland	2	138°48’E; 22°55’S
MUT	Muttaburra, Thompson River, Queensland	4	144°34’E; 22°35’S
PAR	Paroo River, Queensland	1	145°03’E; 28°10’S
PEE	Peel River, New South Wales	1	151°08’E; 31°28’S
PIK	Pike Creek, South Australia	2	140°48’E; 34°13’S
WEN	Wentworth, Darling River, New South Wales	2	141°54’E; 34°07’S
“Southern” *C. d. destructor*			
AVO	Avoca, Victoria	2	143°28’E; 37°06’S
BAR	Barmah State Forest, Victoria	1	144°58’E; 36°01’S
MUR	Murrumbidgee River, New South Wales	1	148°50’E; 36°00’S
PAN	Panmure, Mt Emu Creek, Victoria	4	142°42’E; 38°20’S
SWA	Swan Hill, Victoria	2	143°34’E; 35°21’S
WOD	Wodonga, Victoria	2	146°53’E; 36°07’S
C. d. albidus			
BRI	Bridgewater Lakes, Victoria	1	141°25’E; 38°19’S
CRA	Crawford River, Victoria	2	141°34’E; 37°56’S
DWY	Dwyers Creek, Victoria	2	142°21’E; 37°28’S
FYA	Lake Fyans, Victoria	1	142°37’E; 37°09’S
NUR	Nurrabiel, Victoria	1	141°59’E; 36°56’S
RIC	Richardson River, Victoria	2	142°48’E; 36°39’S
SUR	Lake Surprise, Victoria	1	141°55’E; 38°02’S
WAR	Warracknabeal, Victoria	2	142°24’E; 36°15’S

(ALB) was not recorded from natural populations (GenBank Accession Number: AY153856).

All three reference methods (ME, MP and ML) recovered identical tree topologies with only minor differences in bootstrap support (Fig. 2), which showed similar topology to the tree obtained by Nguyen et al. (2004) for samples from natural locations only. None of the populations were polymorphic, except for the samples TAT and EDE.

Within *C. d. albidus*, of the three haplotypes identified, only one (haplotype 8, Fig. 1) has been translocated from the northern part of its distribution. The translocations have extended the distribution on a massive continental scale as this haplotype also characterises *C. d. albidus* from the southwest of Western Australia (GRE and WAN). Morrissy and Cassells (1992) claimed, based on anecdotal evidence, that the initial *C. d. albidus* introduction originated from the northern range of this subspecies distribution. The findings of the present study are consistent with this claim.

With respect to *C. d. destructor*, there is evidence for the translocation of two haplotypes from the ‘northern’ lineage. One of these (haplotype 1), the most common haplotype within the ‘northern’ lineage, has been translocated to the eastern side of the Great Dividing Range in southeast Queensland (KIL) and to a significant distance in the south, to the extent it is now

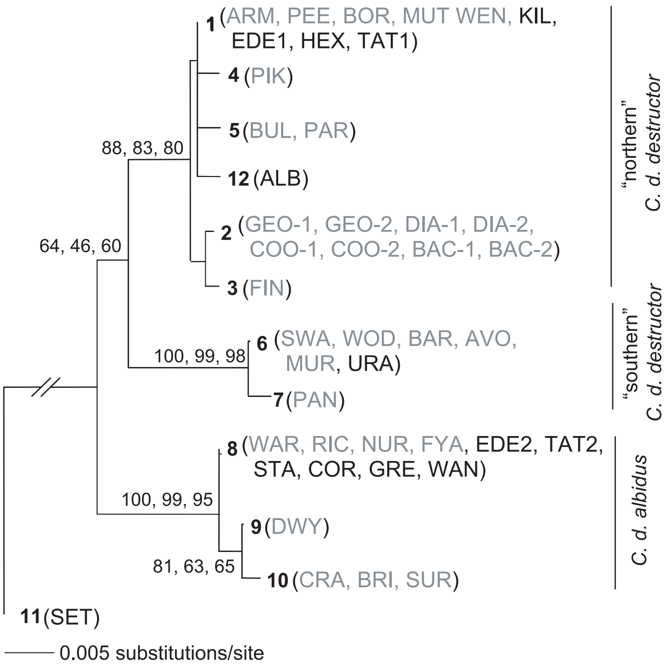

Fig. 2. The minimum evolution (ME) tree showing relationships between *Cherax destructor* samples. The number at each node represents bootstrap values for ME and maximum parsimony (MP) (based on 1000 replicates), and maximum likehood ML (500 replicates). Samples in grey are from natural waterbodies obtained by Nguyen et al. (2004).

found within (EDE) and on the margin (TAT and HEX) of the distribution of *C. d. albidus*.

Evidence was found for the mixing of crayfish at two sites; at Tatiara Creek (TAT), near and possibly outside the distributional limits of *C. d. albidus*, and from an aquaculture farm well within the distribution of *C. d. albidus* (EDE). Two haplotypes were found at each of these sites, representing haplotypes 1 and 9, which differ from each other by 11 bp (2.52%). This finding is consistent with Campbell et al. (1994) and Nguyen et al. (2005) who studied the population at Tatiara Creek (TAT) using morphology, allozyme and RAPD analysis, which showed characteristics of both subspecies, consistent with their mixing at this location. Further, allozyme data are consistent with random mating over a 10-year period, indicating these two subspecies can freely interbreed in the wild. These results and an investigation of inheritance of molecular markers in *C. destructor* (Nguyen and Austin 2004) also support the reproductive compatibility of the two subspecies, as they can interbreed and produce fertile F_1 hybrids. Consequently, if the two subspecies are mixed as a result of translocation, the most likely outcome will be introgression.

The present findings of three genetically distinct forms of *C. destructor* occurring in close proximity, and of two mixed populations of *C. d. albidus* and 'northern' *C. d. destructor* highlights the fact that region-specific genetic diversity of these subspecies may potentially be lost as a consequence of competitive displacement and/or introgression. For *C. destructor*, in contrast to translocations of other freshwater crayfish (Perry et al. 2001a; Austin and Ryan 2002), introgression may be more likely than displacement because *C. d. destructor* and *C. d. albidus* are conspecific.

The negative impacts of translocations at intra-specific level in Australian aquatic fauna were recently highlighted by Hughes et al. (2003), based on work on the freshwater shrimp, *Paratya australiensis.* In this species, one indigenous population became extinct only seven years after a translocation event, indicating a need for more caution in the context of *C. destructor* in the current study.

The translocations documented in the present study represent a potential threat to the genetic diversity of *C. destructor*. Data from the present study provide strong evidence that several haplotypes representing both *C. d. destructor* and *C. d. albidus* have being translocated and as a result, the distribution of the two subspecies now overlaps. This increases the probability of mixing of non-indigenous forms with indigenous local forms. If the genetic integrity of this species is to be conserved, the three primary clades will need to be managed separately and 'inter-clade' translocation minimised (Nguyen et al. 2005).

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