CSIRO PUBLISHING

www.publish.csiro.au/journals/mfr *Marine and Freshwater Research*, 2004, **55**, 165–172

Deep phylogenetic structure has conservation implications for ornate rainbowfish (Melanotaeniidae : *Rhadinocentrus ornatus*) in Queensland, eastern Australia

Timothy J. Page[A,B], *Suman Sharma*[A] *and Jane M. Hughes*[A]

[A]Cooperative Research Centre for Freshwater Ecology, Centre for Riverine Landscapes, Faculty of Environmental Sciences, Griffith University, Nathan Campus, Qld. 4111, Australia.
[B]Corresponding author. Email: t.page@griffith.edu.au

Abstract. The freshwater fish, *Rhadinocentrus ornatus* Regan, 1914, has a patchy distribution through coastal drainages of Queensland and New South Wales, eastern Australia. Isolated populations of *R. ornatus* are found on several islands, as well as in a disjunct northern population 350 km from its nearest conspecific population. Deoxyribonucleic acid was extracted and sequenced for the mitochondrial ATPase gene to describe the geographic and genetic subdivision within the species. Four major clades were identified. These clades diverged between two and seven million years ago and so represent long-term divisions and possible units of conservation. There are conservation implications in that the narrow and localised distribution of *R. ornatus* overlaps with an area of large-scale land clearing, high human population and threats from introduced exotic fish. A particularly high centre of *Rhadinocentrus* diversity in the Tin Can Bay area of Queensland presents some interesting questions about the evolution of the genus *Rhadinocentrus.*

Extra keywords: ATPase, mitochondrial DNA, phylogeography, wallum.

Introduction

Allopatric populations of obligate freshwater fish of the same species are prone to localised differentiation and adaptation, as well as extinction, by the very nature of their specific habitat requirements and presumed limited dispersal ability. Species spread across a fragmented landscape thus present a conservation management challenge because of the putative high diversity, both intra- and interspecific, that is potentially at risk. Conservation genetic studies can provide a useful, cheap and quick way of elucidating magnitudes of gene flow between populations over evolutionary and recent time (Moran 2002). This can complement other sources of information (ecology, geography, morphology, behaviour) in the assignment of possible broad intraspecific units of conservation (i.e. evolutionarily significant unit, ESU) as a delineation of biodiversity (Moritz 1994; Vrijenhoek 1998; Fraser and Bernatchez 2001; Moran 2002).

Many studies have used maternally inherited mitochondrial DNA (mtDNA) to describe genetic and geographic divisions in freshwater fish species, owing to its relatively rapid divergence and lack of recombination. These have focused on biogeographic explanations of present diversity, both recognised and cryptic (McGlashan and Hughes 2000; McGuigan *et al*. 2000; Hurwood and Hughes 2001) and delineation of management units (Zhu *et al*. 1998; Alves *et al*. 2001; Grunwald *et al*. 2002).

Rhadinocentrus ornatus Regan, 1914 (Melanotaeniidae) is a small, iridescent obligate freshwater fish with recognised colour variation in its second dorsal and anal fins (Hansen 1992). It is the only species currently recognised within its genus. Although also found in rainforest streams, it is mostly a denizen of 'wallum', which is low, sandy, coastal heathland of south-east Queensland (QLD) and northern New South Wales (NSW) (Arthington *et al*. 1994). It is often locally abundant in slow and unpolluted, tannin-stained, acidic (pH 5.0–6.8) creeks and lakes (Hansen 1992; Allen *et al*. 2002). *Rhadinocentrus ornatus* has a narrow, patchy distribution that encompasses barrier sand islands, coastal creeks and lakes of eastern Australia from 30°S to 22°S (Australia New Guinea Fishes Association – South-east Queensland Regional Group (ANGFA-SEQRG) 1992; Arthington *et al*. 1994; Morris *et al*. 2001). Its range is nearly continuous amongst the unconnected coastal river basins from the Coffs Harbour area of NSW (30°S, Clarence River basin) in the south to Fraser Island (25°S) in the north (Fig. 1). At the Fraser Island/Tin Can Bay area, there is a break in the distribution of *R. ornatus* of over 350 km (encompassing eight river basins) to a disjunct northern population in the Byfield area (Water Park Creek basin; Marshall 1988).

The patchy and restricted coastal range of *R. ornatus* overlaps with a region undergoing a very high level of development, including large-scale housing projects and land

 10.1071/MF03139 1323-1650/04/020165

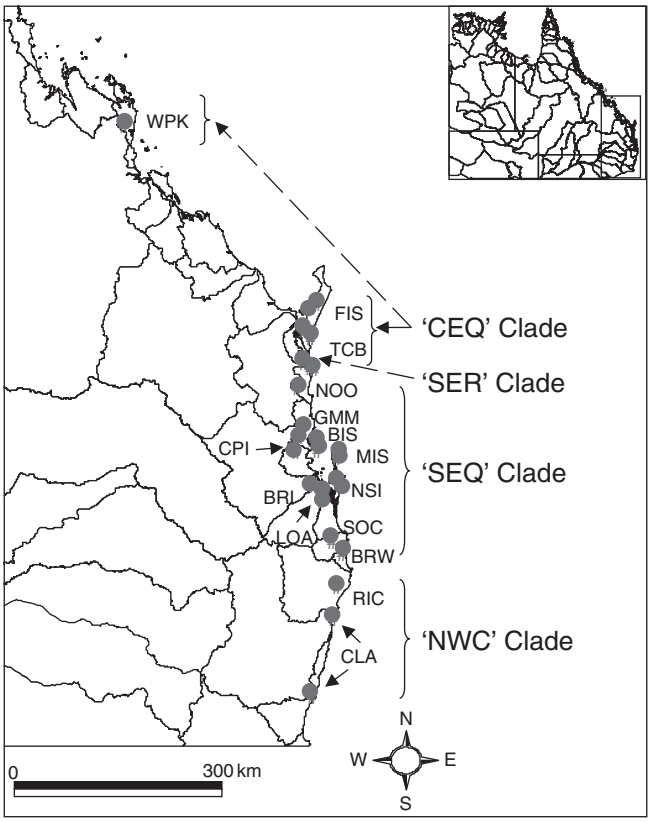

Fig. 1. *Rhadinocentrus ornatus* sampling sites (with river basin borders and basin codes from Table 1; Geoscience Australia 1997) and geographic ranges of major clades. CEQ, Central-east Queensland; SER, Searys Creek; SEQ, south-east Queensland; NWC, New South Wales.

clearing for forestry and agriculture (Arthington *et al*. 1994; Young and Dillewaard 1999). In concert with development comes increased water pollution and habitat disturbance, which can further restrict and fragment populations of environmentally-sensitive freshwater fish (Arthington and Milton 1983; Arthington and Hughes 1996). A third and linked threat to *R. ornatus* is direct competition from invasive exotic species, in particular the mosquitofish, *Gambusia holbrooki* (Girard, 1859), which competes with *R. ornatus* for resources (Arthington and Marshall 1999), adversely affects native fish reproduction (Howe *et al*. 1997) and eats both fish eggs (Aarn *et al*. 1997) and larvae (Ivantsoff and Aarn 1999).

As a consequence of such threats, the distribution of *R. ornatus* within some basins has contracted recently, with its present range in the Brisbane River system limited to one small creek, where it was once found widely (McKay and Johnson 1990). *Rhadinocentrus ornatus* was previously listed as 'restricted' in 1987 by the Australian Society of Fish Biology, but was delisted in 1992 (Arthington *et al*. 1994) following the discovery of the northern Byfield population, which greatly extended its known range.

The aim of this study is to describe the level and geographic partitioning of genetic variation of *R. ornatus* within Queensland, with the objective of defining possible conservation units. The null hypothesis is that *R. ornatus* represents a single conservation unit, as this is how conservation legislation would treat it. The downgrading of its conservation status after the large extension of its range to Byfield can only be justified if this population is closely allied genetically to southern ones and if there is little genetic structure within the southern QLD or northern NSW populations. Even in the absence of genetic data this would seem unlikely, given the degree of geographic isolation of the northern Byfield and various island populations, and the marine barrier to present day dispersal between adjacent coastal drainages (Unmack 2001). If deep (i.e. ancient) phylogenetic and geographic structure becomes evident through molecular systematics, then the conservation status of *R. ornatus* must be reconsidered in the light of a more detailed description of its genetic diversity.

Materials and methods

Sample collection

Specimens were collected from 27 sites in Queensland, eastern Australia, encompassing 12 of the 13 Queensland river basins (as defined in Geoscience Australia (1997); after separating 'Tin Can Bay' from the 'Noosa' Basin) that have been reported to host *R. ornatus*, including four offshore islands (Fig. 1, Table 1). This covers the entire latitudinal range of *R. ornatus* in Queensland. Fish were captured with a small seine net, dip-net or baited box trap. Either fin clips or whole fish were taken and frozen in liquid nitrogen or preserved in 95% ethanol. A further four ethanol-preserved specimens from four sites in three NSW basins, representing the northern and southern range of *R. ornatus* in NSW, were provided by the Australian Museum (Sydney, Australia) for comparison.

Deoxyribonucleic acid extraction and PCR amplification

Genomic DNA was extracted using a modified version of a CTAB-phenol/chloroform extraction (Doyle and Doyle 1987). A fragment of the mtDNA ATP synthase subunits 8 and 6 genes was PCR amplified using primers ATP8.2L8331 and COIII.2H9236 (ATP8.2L8331: 5′-AAA GCR TYR GCC TTT TAA GC-3′; COIII.2H9236: 5′-GTT AGT GGT CAK GGG CTT GGR TC-3′) (S. McCafferty, unpublished data) with the following cycling conditions: 3 min at 95°C; 40 cycles of 30 s at 94°C, 30 s at 47°C, 45 s at 72°C, then 7 min at 72°C. Most amplifications were 50 μL reactions on a Geneamp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) of 1 μL template DNA, 2 μL of primers (10 pmol of each), 25 μL Taq PCR Master Mix (Qiagen, Melbourne, Australia) and 20 μL dd$H_2$0. All individuals were sequenced with primer ATP8.2 with a BigDye version 1.1 Terminator (Applied Biosystems) sequencing reaction and the sequences were produced on an ABI Prism 377 Sequencer (Amersham Biosciences, Little Chalfont, UK) at Griffith University. Selected individuals from the major areas were also sequenced in the other direction with the reverse primer COIII.2 to check sequence accuracy.

Sequence analysis

A total of 115 ATPase sequences of *R. ornatus* was added to an outgroup sequence of the sympatric rainbowfish, *Melanotaenia duboulayi* (Castelnau, 1878), from Spring Creek, Brisbane (lodged under GenBank Accession AY452258). An aligned dataset of 40 unique

Table 1. *Rhadinocentrus ornatus* sample sites, Basin Codes, sample size and major clades

Sample site	Site code	Basin	Basin code	Latitude (S)	Longitude (E)	*n*	Clades
Sandy Creek, Byfield	by	Waterpark	WPK	22°49′	150°39′	9	CEQ
Bowarrady Creek	bw	Fraser Island	FIS	25°07′	153°09′	3	CEQ
Coongul Creek	cg	Fraser Island	FIS	25°11′	153°06′	3	CEQ
Rocky Creek	rc	Fraser Island	FIS	25°28′	153°00′	3	CEQ
Alligator Creek	ag	Fraser Island	FIS	25°29′	152°59′	3	CEQ
Gerowweea Creek	ge	Fraser Island	FIS	25°35′	153°05′	3	CEQ
Govi Creek	go	Fraser Island	FIS	25°35′	153°05′	3	CEQ
Snapper Creek	sn	Tin Can Bay	TCB	25°54′	153°01′	6	CEQ
Searys Creek	se	Tin Can Bay	TCB	25°58′	153°04′	6	SER, CEQ
Freshwater Lake	fw	Tin Can Bay	TCB	25°59′	153°08′	12	CEQ
Kin Kin Creek	kk	Noosa	NOO	26°14′	152°54′	5	SEQ
Mellum Creek	me	Glasshouse Mtns/Maroochy	GMM	26°48′	152°59′	8	SEQ
Coochin Creek	co	Glasshouse Mtns/Maroochy	GMM	26°51′	152°57′	10	SEQ
Coonowrin Creek	cw	Glasshouse Mtns/Maroochy	GMM	26°53′	152°57′	8	SEQ
Middle Swamp	ms	Bribie Island	BIS	26°57′	153°07′	1	SEQ
Site B	sb	Bribie Island	BIS	27°02′	153°10′	1	SEQ
Waraba Creek	wa	Caboolture/Pine	CPI	27°04′	152°54′	2	SEQ
Honeyeater Lake	hl	Moreton Island	MIS	27°05′	153°26′	1	SEQ
Eager's Creek	ec	Moreton Island	MIS	27°09′	153°25′	4	SEQ
Aranarawai Creek	ar	Stradbroke Island	NSI	27°27′	153°27′	3	SEQ
Blue Lake Creek 1	bl	Stradbroke Island	NSI	27°32′	153°29′	3	SEQ
Blue Lake Creek 2	bo	Stradbroke Island	NSI	27°32′	153°28′	2	SEQ
Spring Creek	sc	Brisbane	BRI	27°31′	153°06′	1	SEQ
Eprapah Creek	ep	Logan–Albert	LOA	27°35′	153°14′	2	SEQ
Tingalpa Creek	tc	Logan–Albert	LOA	27°36′	153°12′	5	SEQ
California Creek	cc	Logan–Albert	LOA	27°40′	153°15′	1	SEQ
Upper Currumbin Creek	uc	South Coast	SOC	28°14′	153°21′	3	SEQ
Cudgera Creek*	cu	Brunswick	BRW	28°24′	153°29′	1	SEQ
Wollongbar*	wo	Richmond	RIC	28°49′	153°25′	1	NWC
Bundjalung NP*	bu	Clarence	CLA	29°14′	153°22′	1	NWC
Ulidarra National Park*	ul	Clarence	CLA	30°16′	153°07′	1	NWC
Total						115	

* NSW specimen from Australian Museum.

R. ornatus haplotypes (lodged under GenBank Accession numbers AY452203–AY452242) of 495 bp was produced with Sequencher 4.1.2 (Gene Codes 2000), corresponding to positions 7943–8437 of the *M. lacustris* Munro, 1964 mtDNA genome (accession number AP004419; Miya *et al*. 2003). ModelTest version 3.06 (Posada and Crandall 1998) was used to select the most appropriate nucleotide substitution model. Maximum-likelihood (ML) and maximum-parsimony (MP) analyses were performed in PAUP* version 4.0b10 (Swofford 2002) using the suggested ModelTest parameters (for ML) and bootstrapped 1000 times for MP and 500 for ML. Bremer Decay indices were calculated for the MP analyses in TreeRot version 2 (Sorenson 1999). Clock-like molecular evolution was tested using a Likelihood Ratio Test in PAUP*. A distance matrix was calculated in PAUP* using the suggested model of molecular evolution. Net divergence times between clades were calculated using a correction for within-clade polymorphism (Avise 1994) and rate of sequence divergence for fish ATPase genes of 1.3% per million years (Bermingham *et al*. 1997).

Results

Within the *R. ornatus* ATP sequences, 91 bases were variable, with 67 parsimony informative. ModelTest selected Hasegawa, Kishino, Yano and gamma (HKY + G) (Hasegawa *et al*. 1985) as the most appropriate model for ML analysis (gamma: 0.360, Ti/Tv ratio: 6.266, invariable sites: 0). The likelihood ratio test could not reject clock-like evolution in the *R. ornatus* ATP sequences ($P = 0.554$).

Tree topologies

Maximum likelihood and MP both suggest four major clades (Fig. 2), with three clades strongly supported (Fig. 2, 71–99% bootstrap) and one slightly less so (NWC, 65%). These clades correspond to: (*i*) 'CEQ' (Central-east Queensland): a widely dispersed clade that incorporates the highly isolated northernmost population in Byfield (Waterpark basin) south to Tin Can Bay and Fraser Island basins; (*ii*) 'SER' (Searys Creek): a divergent clade identified at only one site, sympatric with CEQ in Searys Creek (Tin Can Bay); (*iii*) 'SEQ' (south-east Queensland): a large clade concentrated in south-eastern QLD from the Noosa River south to Cudgera Creek in NSW (nine QLD basins: Noosa, Glasshouse Mountains/Maroochy, Bribie Island, Caboolture/Pine, Moreton Island, Stradbroke

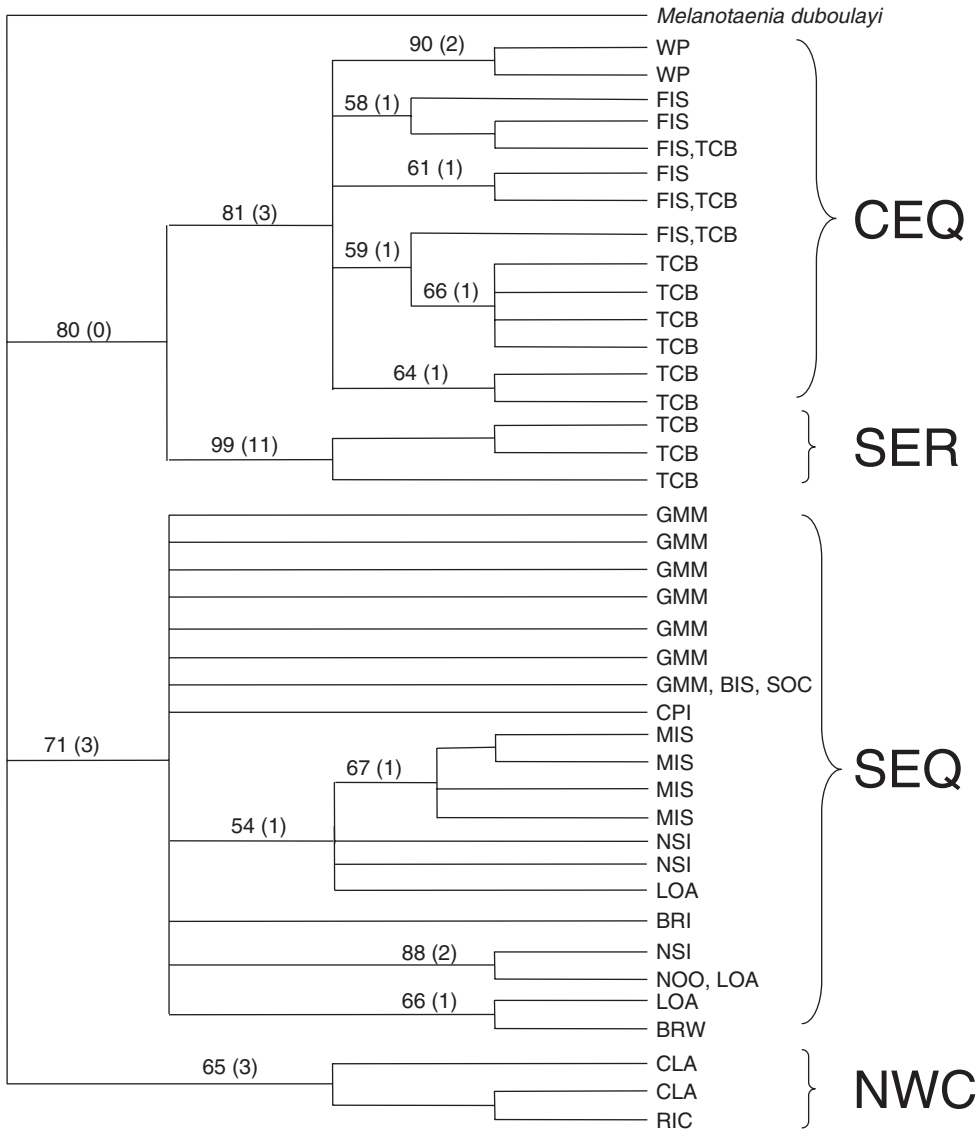

Fig. 2. Maximum-likelihood bootstrap cladogram (majority rule) of ATPase dataset showing major *R. ornatus* clades and basin codes (Table 1). Bootstrap values from maximum-likelihood analysis and Bremer decay indices from maximum-parsimony analysis in parenthesis.

Island, Brisbane, Logan–Albert, South Coast, and one NSW basin: Brunswick); (*iv*) 'NWC' (New South Wales Clade): a clade from two NSW basins (Richmond, Clarence).

Clade sequence variation

Mean ATP sequence variation within each major clade ranged from 0.54 to 0.98% and corrected net mean divergence rates between clades ranged from 6.57 to 8.99%, with the exception of SEQ *v.* NWC, which only diverged by 3.15% (Table 2). This equates to a divergence time of 2.42 million years (MY) between the more closely related SEQ and NWC, and between 5.06 and 6.92 MY (Table 2) for every other pairwise

Table 2. ATPase sequence divergence and calculated divergence times between major clades

Corrected net mean divergence rates below diagonal (standard error) and calculated mean divergence times in million years above diagonal (standard error)

	CEQ	SER	SEQ	NWC
CEQ		5.89 (0.05)	5.06 (0.02)	6.10 (0.05)
SER	7.65% (0.07)		6.89 (0.04)	6.92 (0.13)
SEQ	6.57% (0.03)	8.96% (0.05)		2.42 (0.03)
NWC	7.93% (0.07)	8.99% (0.17)	3.15% (0.04)	

CEQ = central-east Queensland; SER = Searys Creek; SEQ = south-east Queensland; NWC = New South Wales.

analysis between clades (assuming 1.3% divergence per MY, Bermingham *et al*. 1997). A separate analysis (not displayed) of another mtDNA gene, *16S* rDNA (accession numbers AY452243-AY452257) from exemplars of each ATPase clade (*Melanotaenia duboulayi* as an outgroup, accession number AY461521) recovered three clades: CEQ, SER, SEQ/NWC, which are congruent with the above analysis and highlight the close relationship between SEQ and NWC.

Discussion

Phylogenetic groupings and centers of diversity

Analyses reveal four divergent clades within *R. ornatus*. The times of ATPase divergence between the CEQ, SER and SEQ/NWC clades are all in the order of 5–7 MY (Table 2), placing their divergence to some time in the Miocene. This therefore represents long-term isolation of these mitochondrial lineages. The dates of divergence between CEQ, SER and SEQ/NWC are all in the same range and so it is not currently possible to state which one is basal. A 'star phylogeny' relationship (i.e. all diverged roughly simultaneously) seems most likely.

A sympatric species, the freshwater shrimp *Caridina indistincta* Calman, 1926, has also displayed a similar pattern of high mitochondrial lineage diversity (Chenoweth and Hughes 2003). It displays coalescence dates in a similar range (6–11 MY) in the Miocene, which was a period of progressive aridity in Australia (Frakes *et al*. 1987). Significant genetic differences within both *R. ornatus* and *C. indistincta* may reflect enforced allopatry between populations and their subsequent genetic divergence due to the increasing large barriers of dry land or salt water between isolated freshwater habitats.

The distinct, non-overlapping delineation of geographic distributions of three of the four major clades suggests that the differences between them are not merely the retention of ancient haplotypes, or one would not expect geographically structured monophyly. This is not the case for the SER clade, which is sympatric with CEQ at one site (Searys Creek). It is not currently clear whether: (*i*) SER represents an ancient lineage from Searys Creek, which has recently been joined by the CEQ lineage; (*ii*) SER has colonised Searys Creek from elsewhere and its relatives have subsequently gone extinct; (*iii*) other SER populations remain unsampled; (*iv*) the pattern is due to stochastic incomplete lineage sorting within a stable Searys Creek population (very unlikely over this length of time); and (*v*) cryptic sympatric species within *R. ornatus*. Another possibility is that the divergent SER sequences are not mtDNA ATP gene sequences but instead a nuclear gene copy. This explanation is unlikely given that another mt gene (*16S* rDNA) for SER individuals displays exactly the same pattern and relative level of divergence as ATP.

A simple isolation by distance model is not a sufficient explanation for the differences between clades since the genetic divergence between Byfield and Tin Can Bay (~400 km) is only ~1%, whereas the difference between Tin Can Bay and Noosa (~30 km) is more than 6%. There appears to have been a complex history of *R. ornatus* colonisation and recolonisation between regions and river basins by different lineages at different times. McGlashan and Hughes (2002) studied another atheriniform, *Pseudomugil signifer*, and revealed large genetic differences among populations in eastern Queensland and northern NSW; Wong *et al*. (2004) refined the data and identified a phylogeographic break between the Pine and Mary basins, which may be congruent with the break identified here at Tin Can Bay. In contrast, an earlier study on the sympatric freshwater fish, *Nannoperca oxleyana* Whitley, 1940, found no deep break between Tin Can Bay, Noosa and Glasshouse Mountain basins, and in fact Searys Creek (Tin Can Bay) shared haplotypes with both the Noosa River and Mellum Creek (Glasshouse Mountains) (Hughes *et al*. 1999).

There is also potentially significant geographic structuring of genetic divergence at smaller scales within the northernmost clade, CEQ. This disjunct clade is spread over ~475 km of coastal habitat from Byfield (Waterpark Basin) in the north to Tin Can Bay in the south, with *R. ornatus* apparently absent in the eight river basins between the two. This may be due to localised extinctions, unsuitable habitat, or merely as yet undiscovered populations. It may also be the product of a particular large-scale biogeographic process, given that other freshwater fish (*Pseudomugil mellis*, *Gobiomorphus australis*) also have disjunct northern populations in the Fitzroy freshwater fish biogeographic region (Unmack 2001), which incorporates the Waterpark Basin. The northern Byfield population is monophyletic with respect to its southern CEQ conspecifics (Fig. 2). This, and an ATP divergence estimate of 720 000 years (standard error 22 450 years) between Byfield and southern CEQ, indicates the colonisation of this range may have been relatively recent.

Conservation implications

The centre of diversity in the Melanotaeniidae is east of Wallace's Line in New Guinea and northern Australia. Of the four melanotaeniid genera found in Australia, *Iriatherina* and the speciose *Melanotaenia* also occur in New Guinea and share species across the Torres Strait (McGuigan *et al*. 2000, Allen *et al*. 2002). The two melanotaeniid genera endemic to Australia, *Cairnsichthys* and *Rhadinocentrus*, are both monotypic and have restricted ranges (Allen *et al*. 2002). Zhu (1995) recovered *R. ornatus* as basal to all the other genera of Australasian rainbowfish in a *12S* rDNA analysis. As *R. ornatus* is the sole representative of a basal genus, it has a unique and ancient phylogenetic history, and thus warrants particular attention.

The clades identified here (CEQ, SER, SEQ, NWC) represent putative evolutionarily significant units due to their reciprocal monophyly of mtDNA and the significant

length of evolutionary time each lineage represents. In fact each *R. ornatus* lineage appears to be older than entire suites of species within the genus *Melanotaenia* (Zhu 1995; McGuigan *et al*. 2000). The within-CEQ clade localised at Byfield may also warrant some management consideration. *R. ornatus* is not currently listed as a threatened species in Queensland or New South Wales state conservation legislation, although it is recognised locally in south-east Queensland as a 'significant' species in Brisbane and Logan–Albert (Queensland Government 1996; Brisbane City Council 2000). Morris *et al*. (2001) recommended an increase in the conservation status of *R. ornatus* to 'Rare' in Queensland (Queensland Government 2001) and for an increase in protection under the Federal *Environment Protection and Biodiversity Conservation Act 1999* (Commonwealth of Australia 2003). The effect of this change in Queensland would be the enforced consideration of environmental impacts for development applications in areas that contain *R. ornatus*. The change in *EPBCA* status would afford some level of protection on Federal government land and in the international wildlife trade. Both *EPBCA* and Queensland Government status changes seem reasonable given the data presented here, but some form of protection in New South Wales may also be warranted after more extensive sampling there. The intraspecific ESUs delineated here should be considered if any translocations are planned, and may eventually require separate listings if further work shows reproductive isolation.

Moving individuals from one population to another is a common method to counteract local extinctions (Salgueiro *et al*. 2003), but should only be done with a sound knowledge of genetic and geographic structure within a species (Allendorf *et al*. 2001). One reason for this is because inappropriately translocated lineages can result in outbreeding depression and even extinction of resident lineages, thus reducing rather than increasing variation (Hughes *et al*. 2003). It has also been demonstrated that the highly localised adaptations in some freshwater fish mean they do not thrive when moved or interbred with other populations (Moran 2002). In practical terms, the implications are that translocations should not be attempted between areas that host different *R. ornatus* lineages.

If the aim is to preserve biodiversity, then potential ESUs highlighted above will need to be considered in any future natural resource management plans. Fortunately many areas of high diversity in *R. ornatus* fall under the protection of various National Parks. The CEQ clade can be found in National Parks at Byfield, Tin Can Bay and Fraser Island (both Great Sandy National Park); SEQ in many parks, including the Great Sandy National Park (Cooloola Section), Bribie and Moreton Island National Parks, and Blue Lake National Park on Stradbroke Island; NWC in Broadwater and Yuragyir National Parks in NSW (Morris *et al*. 2001). The SER clade has currently only been identified in Searys Creek, which although it falls within a National Park, is a very popular recreation site and so could be at risk. Although some sites may fall under protection via land tenure, this does not guarantee safety for the species, because native freshwater fish have gone locally extinct in protected areas in the past (Arthington and Hughes 1996). In addition, increased tourism presents a potential threat (Hadwen *et al*. 2003) and many of the populations are not located in protected areas.

Rhadinocentrus ornatus shares much of its range with the IUCN red-listed Oxleyan pygmy perch, *Nannoperca oxleyana*, and the vulnerable honey blue-eye, *Pseudomugil mellis* Allen and Ivantsoff, 1982 (Morris *et al*. 2001), and so any measure implemented to protect one species would likely serve as an umbrella for the protection of others. These 'indicator' species can serve as proxies for the many unsampled, sympatric species whose intraspecific genetic structure is currently unknown (Simberloff 1998). A more general protection of wallum as a threatened habitat would have a similar effect.

Future considerations

Mitochondrial gene trees will not always accord with species and population trees (Avise 1994), although congruence is more likely over the relatively large time scales presented here for *R. ornatus*. A strict ESU definition (*sensu* Moritz 1994) requires not only reciprocal monophyly for mtDNA, but also 'significant divergence in nuclear allele frequencies'. Further studies on nuclear DNA (allozymes, microsatellites, nuclear sequences) will inform at both higher and lower phylogenetic levels, revealing smaller scale population differences and thus can confirm and refine the conservation status of *R. ornatus* and the different lineages (Salgueiro *et al*. 2003). Such studies could also reveal whether these ESUs may represent cryptic species within *R. ornatus*, each of which would require separate conservation listings, thus affording greater levels of protection, than are currently suggested by mtDNA alone.

Previous morphological studies (Hansen 1992; Aarn and Ivantsoff 1996) have identified a north/south differentiation of body pigmentation, which correspond with this study's genetic differentiation between CEQ/SER and SEQ/NWC clades. Future morphological and ecological studies could use this new genetic information as a framework to separate the influences of local environment, ecology and genetic lineage (Leiper 1985). Such a wide dataset would contribute much to taxonomic and conservation designations of *R. ornatus*.

More intensive field surveys may locate new populations and clades of *R. ornatus*. The many 'empty' basins between Tin Can Bay and Byfield may well harbour undiscovered populations, although no predicted high or medium quality habitat for *R. ornatus* has been identified between Fraser Island/Tin Can Bay and Byfield (Queensland CRA/RFA Steering Committee 1997).

Assessment of the genetic structure and distribution of *R. ornatus* and other freshwater species in eastern Australia will help to explain the complex biogeographic processes

which likely underlie the observed diversity in other species (e.g. Oxleyan pygmy perch, Hughes *et al*. 1999; *Caridina indistincta*, Chenoweth and Hughes 2003). Phylogeographic congruence between a suite of species will likely reflect large-scale processes, with differences due to species-specific factors, such as dispersal ability (Avise 1994). One intriguing question worth further consideration is whether the triangle of high *Rhadinocentrus* diversity focused on Tin Can Bay reflects this taxon's geographic centre of evolution and speciation, rather than the centre being further north as is the case for most of the Melanotaeniidae (Allen *et al*. 2002).

Acknowledgments

We would like to thank the following people for help in the field: Kaye Stuart; Gio Carini, Ben Cook, James Fawcett, Mia Hillyer, Joel Huey, Carl Murray (Griffith University); Jon Marshall, Alisha Steward (Qld. Department of Natural Resources and Mines); James Diana (University of Michigan); Peter Fugelli (University of Qld.); Dave Hurwood (Qld. University of Technology); John Evans (SCRUB) and Allan Tonks. Specimens were provided by: Alicia and Brian Toon, Tim Marsden (Qld. Department of Primary Industries), Mick Smith (Ecosystem Health Monitoring Project), Mick McGrouther (Australian Museum). Jing Ma and Rodney Eastwood helped with laboratory and analysis work. Chris Marshall and Mark Kennard helped with fish questions. We also thank two anonymous referees for their helpful comments. Funding was provided by the CRC for Freshwater Ecology, Australian Postgraduate Award (TJP) and Tangalooma Marine and Research Foundation (TJP). Fish were sampled under Queensland National Parks and Wildlife Service and Department of Primary Industries permits.

References

Aarn, and Ivantsoff, W. (1996). Descriptive anatomy of *Rhadinocentrus ornatus* (Osteichthyes: Melanotaeniidae). *Icthyological Exploration of Freshwaters* **7**, 41.

Aarn, Ivantsoff, W., and Hansen, B. (1997). East coast sympatry: differential developmental characteristics of two rainbowfish and a blue-eye (Teleostei: Atheriniformes: Melanotaeniidae and Pseudomugilidae) of eastern Australia, with a discussion of adult habitat preferences. *Fishes of Sahul* **11**, 507–519.

Allen, G. R., Midgley, S. H., and Allen, M. (2002). 'Field Guide to the Freshwater Fishes of Australia.' (Western Australian Museum: Perth, Australia.)

Allendorf, F. W., Leary, R. F., Spruell, P., and Wenburg, J. K. (2001). The problems with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution* **16**, 613–622. doi:10.1016/S0169-5347(01)02290-X

Alves, M. J., Coelho, H., Collares-Pereira, M. J., and Coelho, M. M. (2001). Mitochondrial DNA variation in the highly endangered cyprinid fish *Anaecypris hispanica*: importance for conservation. *Heredity* **87**, 463–473. doi:10.1046/J.1365-2540.2001.00929.X

ANGFA-SEQRG (1992). *Rhadinocentrus ornatus* survey records. *Fishes of Sahul* **7**, 317–318.

Arthington, A. H., and Hughes, J. M. (1996). Conservation status of *Nannoperca oxleyana* and recovery plan. In 'Recovery plan for the Oxleyan Pygmy Perch *Nannoperca oxleyana*'. (Ed. A. H. Arthington.) pp. 97–117. (Centre for Catchment and In-stream Research, Griffith University: Nathan, Australia.)

Arthington, A. H., and Marshall, C. J. (1999). Diet of the exotic mosquitofish, *Gambusia holbrooki*, in an Australian lake and potential for competition with indigenous fish species. *Asian Fisheries Science* **12**, 1–16.

Arthington, A. H., and Milton, D. A. (1983). Effects of urban development and habitat alterations on the distribution and abundance of native and exotic freshwater fish in the Brisbane region, Queensland. *Australian Journal of Ecology* **8**, 87–101.

Arthington, A. H., Chandica, A. L., and Marshall, C. J. (1994). 'Geographic Information System and Distribution Maps for the Honey Blue-eye, *Pseudomugil mellis*, and Other Freshwater Fishes in South-eastern Queensland.' Final Report to the Australian Nature Conservation Agency Endangered Species Program. Vol. 2. (Centre for Catchment and In-stream Research, Griffith University: Nathan, Australia.)

Avise, J. C. (1994). 'Molecular Markers, Natural History and Evolution.' (Chapman & Hall: New York, USA.)

Bermingham, E., McCafferty, S., and Martin, A. P. (1997). Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. In 'Molecular Systematics of Fishes'. (Eds T. Kocher and C. Stepien.) pp. 113–126. (Academic Press: New York, USA.)

Brisbane City Council (2000). 'Brisbane City Plan 2000, Vols 1 and 2.' (Brisbane City Council: Brisbane, Australia.)

Chenoweth, S. F., and Hughes, J. M. (2003). Speciation and phylogeography in *Caridina indistincta*, a complex of freshwater shrimps from Australian heathland streams. *Marine and Freshwater Research* **54**, 807–812. doi:10.1071/MF02168

Commonwealth of Australia (2003). 'Environment Protection and Biodiversity Conservation Act 1999 – Incorporating Amendments up to Act No, 88 of 2003.' (Commonwealth of Australia: Canberra, Australia.)

Doyle, J. J., and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of leaf tissue. *Phytochemistry Bulletin* **19**, 11–15.

Frakes, L. A., McGowran, B., and Bowler, J. M. (1987). Evolution of Australian environments. In 'Fauna in Australia'. (Eds G. R. Dyne and D. W. Walton.) pp. 1–16. (CSIRO: Canberra, Australia.)

Fraser, D. J., and Bernatchez, L. (2001). Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology* **10**, 2741–2752. doi:10.1046/J.1365-294X.2001.T01-1-01411.X

Gene Codes (2000). 'Sequencher version 4.1.1 b1.' (Genecodes Corporation: Ann Arbor, MI, USA.)

Geoscience Australia (1997). 'Australia's River Basins 1997.' Available at: http://www.ga.gov.au/. (Department of Industry, Tourism and Resources: Canberra, Australia.)

Grunwald, C., Stabile, J., Waldman, J. R., Gross, R., and Wirgin, I. (2002). Population genetics of shortnose sturgeon *Acipenser brevirostrum* based on mitochondrial DNA control region sequences. *Molecular Ecology* **11**, 1885–1898. doi:10.1046/J.1365-294X.2002.01575.X

Hadwen, W. L., Arthington, A. H., and Milton, D. A. (2003). The impact of tourism on dune lakes on Fraser Island, Australia. *Lakes & Reservoirs: Research and Management* **8**, 15–26.

Hansen, B. (1992). A forgotten jewel – *Rhadinocentrus ornatus. Fishes of Sahul* **7**, 313–318.

Hasegawa, M., Kishino, H., and Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **21**, 160–174.

Howe, E., Howe, C., Lim, R., and Burchett, M. (1997). Impact of the introduced poeciliid *Gambusia holbrooki* (Girard, 1859) on the growth and reproduction of *Pseudomugil signifer* (Kner, 1865) in Australia. *Marine and Freshwater Research* **48**, 425–434.

Hughes, J. M., Goudkamp, K., Hurwood, D., Hancock, M., and Bunn, S. E. (2003). Translocation causes extinction of a local population of the freshwater shrimp *Paratya australiensis* (Atyidae). *Conservation Biology* **17**, 1007–1012. doi:10.1046/J.1523-1739.2003.01636.X

Hughes, J. M., Ponniah, M., Hurwood, D., Chenoweth, S., and Arthington, A. (1999). Strong genetic structuring in a habitat specialist, the Oxleyan pygmy perch *Nannoperca oxleyana. Heredity* **83**, 5–14. doi:10.1038/SJ.HDY.6885390

Hurwood, D. A., and Hughes, J. M. (2001). Historical interdrainage dispersal of eastern rainbowfish from the Atherton Tableland, north-eastern Australia. *Journal of Fish Biology* **58**, 1125–1136. doi:10.1006/JFBI.2000.1523

Ivantsoff, W., and Aarn (1999). Detection of predation on Australian native fishes by *Gambusia holbrooki*. *Marine and Freshwater Research* **50**, 467–468.

Leiper, G. (1985). *Rhadinocentrus ornatus* – rare colour forms. *Fishes of Sahul* **2**, 82–83.

Marshall, J. C. (1988). An extension of the known range of *Rhadinocentrus ornatus. Fishes of Sahul* **5**, 196–197.

McGlashan, D. J., and Hughes, J. M. (2000). Reconciling patterns of genetic variation with stream structure, earth history and biology in the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae). *Molecular Ecology* **9**, 1737–1751. doi:10.1046/J.1365-294X.2000.01054.X

McGlashan, D. J., and Hughes, J. M. (2002). Extensive genetic divergence among populations of the Australian freshwater fish, *Pseudomugil signifer* (Pseudomugilidae), at different hierarchical scales. *Marine and Freshwater Research* **53**, 897–907.

McGuigan, K., Zhu, D., Allen, G. R., and Moritz, C. (2000). Phylogenetic relationships and historical biogeography of melanotaeniid fishes in Australia and New Guinea. *Marine and Freshwater Research* **51**, 713–723. doi:10.1071/MF99159

McKay, R., and Johnson, J. (1990). The freshwater and estuarine fishes. In 'The Brisbane River: A source book for the future'. (Eds P. Davie, E. C. Stock and D. L. Choy.) pp. 153–168. (Australian Littoral Society: Brisbane, Australia.)

Miya, M., Takeshima, H., Endo, H., Ishiguro, N. B., Inoue, J. G., *et al.* (2003). Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **26**, 121–138. doi:10.1016/S1055-7903(02)00332-9

Moran, P. (2002). Current conservation genetics: building an ecological approach to the synthesis of molecular and quantitative genetic methods. *Ecology of Freshwater Fish* **11**, 30–55. doi:10.1034/J.1600-0633.2002.110105.X

Moritz, C. (1994). Defining 'Evolutionary Significant Units' for conservation. *Trends in Ecology & Evolution* **9**, 373–375. doi:10.1016/0169-5347(94)90057-4

Morris, S. A., Pollard, D. A., Gehrke, P. C., and Pogonoski, J. J. (2001). 'Threatened and potentially threatened freshwater fishes of coastal New South Wales and the Murray–Darling Basin.' (NSW Fisheries: Sydney, Australia.)

Posada, D., and Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics (Oxford, England)* **14**, 817–818. doi:10.1093/BIOINFORMATICS/14.9.817

Queensland CRA/RFA Steering Committee (1997). 'Stage II—Assessment of Habitat Quality for Priority Species in South-east Queensland Bioregion.' (Department of Natural Resources: Indooroopilly, Australia.)

Queensland Government (1996). 'Nature Conservation (Protected Areas) Regulation 1994—Reprint No. 3.' (Queensland Government: Brisbane, Australia.)

Queensland Government (2001). 'Nature Conservation (Wildlife) Regulation 1994—Reprint No. 2C.' (Queensland Government: Brisbane, Australia.)

Salgueiro, P., Carvalho, G., Collares-Pereira, M. J., and Coelho, M. M. (2003). Microsatellite analysis of genetic population structure of the endangered cyprinid *Anaecypris hispanica* in Portugal: implications for conservation. *Biological Conservation* **109**, 47–56. doi:10.1016/S0006-3207(02)00132-5

Simberloff, D. (1998). Flagships, umbrellas, and keystones: is single-species management passé in the landscape era? *Biological Conservation* **83**, 247–257. doi:10.1016/S0006-3207(97)00081-5

Sorenson, M. D. (1999). 'TreeRot version 2.' (Boston University: Boston, MA, USA.)

Swofford, D. L. (2002). 'PAUP* Version 4.0b5—Phylogenetic Analysis Using Parsimony (*and Other Methods).' (Sinauer Associates: Sunderland, MA, USA.)

Unmack, P. J. (2001). Biogeography of Australian freshwater fishes. *Journal of Biogeography* **28**, 1053–1089. doi:10.1046/J.1365-2699.2001.00615.X

Vrijenhoek, R. C. (1998). Conservation genetics of freshwater fish. *Journal of Fish Biology* **53**, 394–412. doi:10.1006/JFBI.1998.0830

Wong, B. B. M., Keogh, J. S., and McGlashan, D. J. (2004). Current and historical patterns of drainage connectivity in eastern Australia inferred from population genetic structuring in a widespread freshwater fish *Pseudomugil signifer* (Pseudomugilidae). *Molecular Ecology* **13**, 391–401. doi:10.1046/j.1365-294X.2003.02085.x

Young, P. A. R., and Dillewaard, H. A. (1999). Southeast Queensland. In 'The Conservation Status of Queensland's Bioregional Ecosystems'. (Eds P. Sattler and R. Williams.) (Environmental Protection Agency: Brisbane, Australia.)

Zhu, D. (1995). 'Patterns of Mitochondrial DNA Evolution and Phylogenetic Relationships in Australian Rainbowfishes (Pisces: Melanotaeniidae) and Inferred Phylogeny in Atheriniformes.' PhD thesis. (University of Queensland: Brisbane, Australia.)

Zhu, D., Degnan, S., and Moritz, C. (1998). Evolutionary distinctiveness and status of the endangered Lake Eacham rainbowfish (*Melanotaenia eachamensis*). *Conservation Biology* **12**, 80–93. doi:10.1046/J.1523-1739.1998.96330.X

Manuscript received 10 September 2003; revised 24 November 2003; accepted 22 December 2003.