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Phylogeography of a threatened freshwater fish (*Mogurnda adspersa*) in eastern Australia: conservation implications

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Abstract. Phylogeography is a field that has the potential to provide an integrative approach to the conservation of threatened species. The southern purple spotted gudgeon, *Mogurnda adspersa*, is a small freshwater fish that was once common and widely distributed throughout south-eastern Australia. However, habitat alteration has dramatically reduced the size and the range of Murray–Darling Basin populations, which are now classified as endangered. Here patterns of genetic structure and evolutionary history of *M. adspersa* in southern Queensland and the Murray–Darling Basin are elucidated using three regions of the mitochondrial DNA, the ATPase 6 and 8 and the control region. Murray–Darling Basin populations are characterised by lineages with highly localised endemism, very low genetic diversity and restricted gene flow. Phylogenetic reconstructions show that Murray–Darling Basin populations comprise a monophyletic clade that possibly originated by range expansion from the coast around 1.6 million years ago. It is proposed that the divergent Murray–Darling Basin clade is of high conservation priority and requires separate management. The present study further exemplifies the role of drainage rearrangement in driving evolutionary diversification in Australian freshwater fishes, an historical process with profound implications for conservation management.

Additional keywords: biogeography, conservation genetics, gudgeon, mitochondrial DNA.

Introduction

Conservation strategies now acknowledge not only the importance of conserving biodiversity at the species level but also of maintaining the genetic diversity within these species. Genetic diversity gives species the ability to cope with environmental change and promotes long-term evolutionary potential. A critical factor in maintaining genetic diversity is the effective population size (N_e), which is influenced by both historical and contemporary processes, particularly gene flow (Lande and Barrowclough 1987). Cessation of gene flow is common in small and geographically isolated populations and tends to reduce N_e (Hedrick 1992). This in turn can result in inbreeding depression (Hedrick and Kalinowski 2000; Keller and Waller 2002; Woodworth *et al*. 2002). Inbreeding depression reduces aspects of the fitness of an individual, including birthweight and survival, reproductive ability and resistance to disease (Hedrick 1992; Hedrick and Kalinowski 2000; Keller and Waller 2002). Inbreeding depression has been identified as a threatening process in many populations, including freshwater fish (Frankham *et al*. 2002; Keller and Waller 2002). Conservation of these threatened populations can be informed by phylogeographic studies, the integration of genealogy and geography.

Australia is a relatively ancient and geologically stable continent. Approximately 90 million years ago, one of the most prominent geological features of the Australian landscape was formed: the Great Dividing Range (Wellman 1979; Ollier 1982; White 1994). The Great Dividing Range extends along the east coast of Australia, separating coastal drainages from those inland. Many aquatic species that would have previously been continuously distributed were isolated in catchments east and west of the Great Dividing Range. The influence of this vicariant factor in evolutionary diversification is supported by findings of marked genetic and phylogeographic structure between coastal and inland populations of several eastern Australian freshwater fish. In some cases, fish populations have been designated as separate species, such as the Murray cod (*Maccullochella peeli*), Eastern freshwater cod (*Maccullochella ikei*) and Mary River cod (*M. p. mariensis*) (Rowland 1993). In others, populations were recognised as genetically distinct units (Moritz 1994*b*), such as Golden perch (*Macquaria ambigua*) (Musyl and Keenan 1992), the flyspecked hardyhead (*Craterocephalus stercusmuscarum*) (McGlashan and Hughes 2001), rainbowfishes (*Melanotaenia duboulayi* and *Melanotaenia fluviatilis*) (Crowley *et al*. 1986), eel-tailed catfish (*Tandanus tandanus*) (Musyl and Keenan 1996) and bony herring (*Nematalosa eribi*) (Allen *et al*. 2002). The recognition of these historically isolated lineages aids management considerations aimed at maintaining genetic diversity.

The southern purple spotted gudgeon (*Mogurnda adspersa*, Castelnau 1878) is a small freshwater Eleotrid fish and is one of six species of *Mogurnda* found in Australia (Allen *et al*. 2002). *Mogurnda adspersa* was once common and widely

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distributed throughout south-eastern Australia, including the Murray–Darling Basin (MDB) (Queensland (Qld), New South Wales (NSW), Victoria (Vic.) and South Australia (SA)) and coastal catchments in northern NSW and Qld (Llewellyn 1983; Wager and Jackson 1993). During the early 1980s, populations within the MDB experienced rapid and dramatic reductions in both range and abundance (Harris and Gehrke 1997). The impacts of habitat loss (owing to urban and agricultural development), introduced species (carp, redfin and *Gambusia*) and river regulation (dam construction and altered flow regimes) have all contributed to the species decline in the MDB (Harris and Gehrke 1997). Presently the species remains common in coastal Qld (Boxall *et al*. 2002) but its distribution within the MDB is restricted to a few rivers on the NSW/Qld border, including the Condamine, Dumaresq, MacIntyre, Severn (Wager and Jackson 1993) and Gwydir, and a recently discovered population in the Macquarie catchment (NSW DPI Fisheries, unpubl. data). As a result of the secure status of populations in coastal Qld, both the International Union for Conservation of Nature and Natural Resources (IUCN) 2000 red list and the Australian Society for Fish Biology (ASFB) consider the species as a whole as being of 'Lower risk' (least concern). However, the MDB populations are classified as endangered under the *NSW Fisheries Management Act 1994*.

The New South Wales Department of Primary Industries is undertaking recovery planning for the MDB populations of *M. adspersa*. This includes the development of a captive breeding program to enable translocations and reintroductions. In this strategy, individuals from a larger (or captive) population with greater genetic variability are introduced to smaller populations to attempt to recover genetic variation and reduce inbreeding (Storfer 1999). Translocation is considered in many conservation recovery programs, but without adequate knowledge about the ecology, demography and current and historical population genetic structure (phylogeographic structure) of threatened populations these programs have limited success (Philippart 1995; Storfer 1999).

To date, the only phylogeographic study of *M. adspersa* has been on the secure populations from the north-east coast of Qld (Hurwood and Hughes 1998). In that study, genetic variation was determined using mtDNA (ATPase 6 and 8) and 19 haplotypes were identified, most of which were site-specific. Most of the genetic variation was distributed within drainages rather than among drainages, and overall, the species showed low gene flow in its northernmost distribution (Hurwood and Hughes 1998). Nonetheless, no information is available about the genetic structure of the endangered MDB populations.

In the present study, we elucidate patterns of population genetic structure in *M. adspersa* along the entire distribution of the species using three regions of the mitochondrial genome: ATPase 6 and 8 and the control region. We also investigate the evolutionary history of *M. adspersa* based on a molecular phylogenetic reconstruction. Our main objective is to understand phylogeographic patterns within the MDB. This information is not only useful to assess the role of historical geomorphologic processes in the evolution of small freshwater fish, but is also essential towards the development and implementation of appropriate strategies for the conservation management of the endangered MDB populations.

Methods

Sampling

We obtained samples of *Mogurnda adspersa* from the endangered MDB (Tenterfield, Bingara and Wuluuman Creek) and 'non-endangered' coastal Qld (Kilcoy) populations (Fig. 1 and Table 1). Non-destructive sampling methods were used (fyke nets, bait traps, spotlighting and backpack electrofishing), and small tissue samples were taken from the caudal fin, placed in 70% ethanol and stored at 4°C until use. *Mogurnda adspersa* samples were also obtained from Deepwater River, Farm Creek, Inverell, Severn River, the Condamine catchment near Toowoomba, and Mary River. Other species of *Mogurnda* were also obtained from Glen Helen Gorge, Finke River, Northern Territory (*Mogurnda larapintae*); Dalhousie Springs, South Australia (*Mogurnda thermophila*); and the Barcoo River, Queensland (*Mogurnda clivicola*).

Laboratory procedures

DNA was extracted from the fin tissue using a modified salting out method (Sunnucks and Hales 1996). Three gene regions of the mitochondrial genome were amplified: ATPase 6 and 8 and the control region. The primers ATP8.2 (5′ AAAGCRTYRGCCTTTTAAGC) and CO3.2 (5′ GTTAGTGGTCAKGGGCTTGGRTC) (Sivasundar *et al*. 2001) were used to amplify ~950 base pairs (bp) of the ATPase 6 and 8 genes. The reaction contained: 1 μL template DNA, 2 μL of primers (4.8 pmol each), 0.4 μL Taq DNA polymerase (Applied Biosystems, Madison, WI, USA), 4 μL dNTPs (125 μM each), 3 μL MgCl, 4 μL buffer and ddH_2O to a final volume of 40 μL. Amplification was carried out using a touch down polymerase chain reaction (PCR) program: 94°C for 3 min, 94°C for 30 s, 61°C for 45 s, 72°C for 1 min, 94°C for 30 s, 59°C for 45 s, 72°C for 1 min, 94°C for 30 s, 57°C for 45 s, 72°C for 1 min, 94°C for 30 s, 55°C for 45 s, 72°C for 1 min followed by 26 cycles at 94°C for 30 s, 53°C for 45 s, 72°C for 1 min, and a final extension of 72°C for 5 min.

Approximately 400 bp of the control region was amplified using primers A (5′ TTCCACCTCTAACTCCCAAAGCTAG) and E (5′ CCTGAAGTAGGAACCAGATG) (Lee *et al*. 1995) in the following reaction: 1 μL template DNA, 2 μL of primers (4.8 pmol each), 0.4 μL Taq DNA polymerase (Applied Biosystems), 6 μL dNTPs (187.5 μM each), 3.2 μL MgCl, 5 μL buffer and ddH_2O to a final volume of 40 μL. This region was amplified using a step up PCR program: 94°C for 2 min, then six cycles at 94°C for 30 s, 45°C for 1 min, 72°C for 1 min, followed by 32 cycles at 94°C for 30 s, 58°C for 1 min 72°C for 1 min and a final extension of 72°C for 3 min.

Polymerase chain reaction products were purified using a GENECLEAN III Kit (Qbiogene, Inc., Carlsbad, CA, USA) and DNA sequencing was carried out by an automated sequencing system (ABI 377) according to manufacturer's directions.

Data analysis

All control region and ATPase 6 and 8 sequences were cleaned and aligned using SEQUENCHER 4.1 (Gene Codes Corporation, MI 2000, Ann Arbor, MI, USA) and submitted to GenBank (Accession numbers DQ219317–39, EF548159–63).

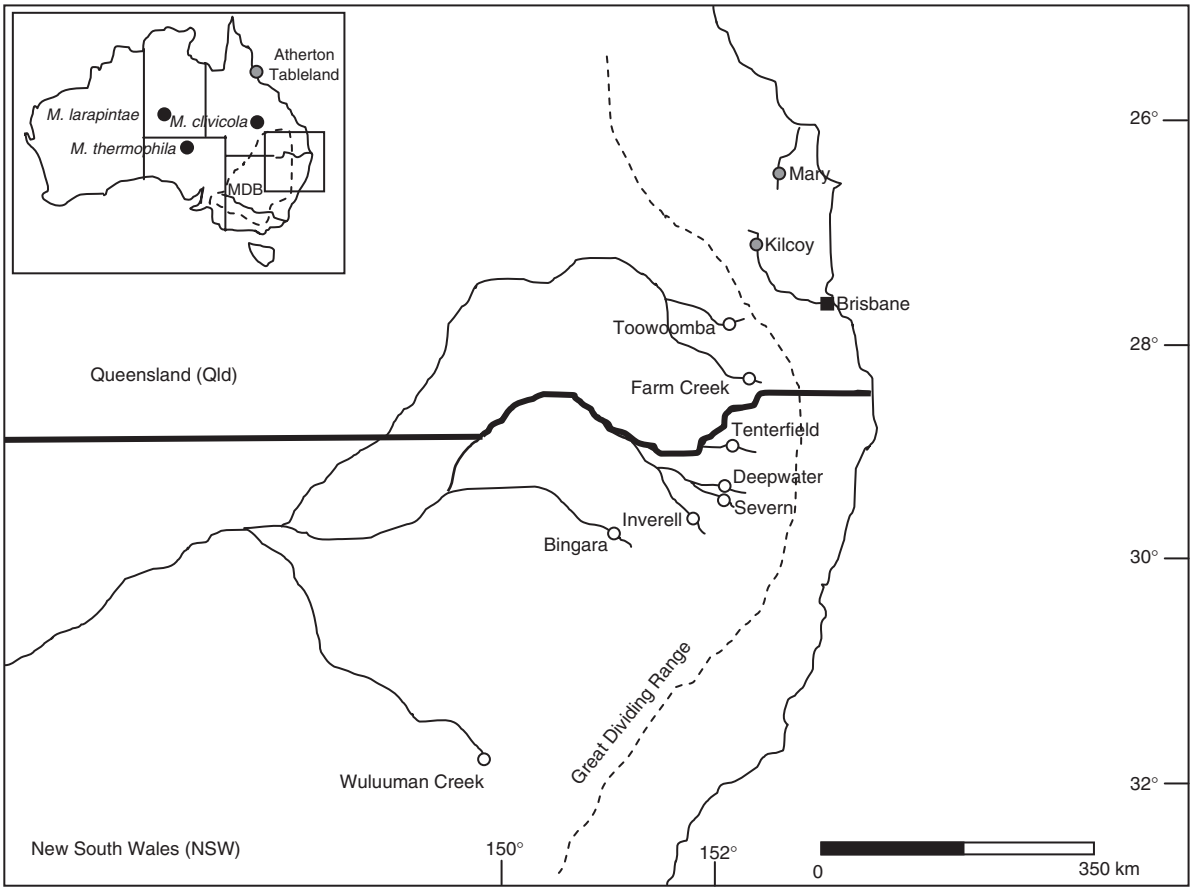

Fig. 1. Map of Australia showing the sampling locations of *Mogurnda* species. Inset map shows the collection sites for *Mogurnda adspersa* in eastern Australia.

Table 1. Frequencies of composite mtDNA haplotypes of *Mogurnda adspersa* in each sampling location

Haplotypes represent 1141 bp of mtDNA sequence that includes 339 bp of the control region and 802 bp of ATPase 6 and 8 (GenBank Accession numbers DQ219317–39, EF548159–63)

Haplotype	Location and sample size									
	Mary River (9)	Kilcoy (10)	Toowoomba (7)	Farm Creek (2)	Tenterfield (17)	Deepwater River (3)	Severn River (2)	Bingara (14)	Inverell (4)	Wuluuman Creek (17)
A	–	–	–	0.5	1.0	–	–	0.6	–	–
B	–	–	–	–	–	–	–	–	–	1.0
C	–	–	–	–	–	–	1.0	–	–	–
D	–	–	–	–	–	1.0	–	–	–	–
E	–	–	–	–	–	–	–	0.4	1.0	–
F	–	–	–	0.5	–	–	–	–	–	–
G	–	–	1.0	–	–	–	–	–	–	–
H	–	0.3	–	–	–	–	–	–	–	–
I	–	0.4	–	–	–	–	–	–	–	–
J	–	0.1	–	–	–	–	–	–	–	–
K	–	0.1	–	–	–	–	–	–	–	–
L	–	0.1	–	–	–	–	–	–	–	–
M	0.8	–	–	–	–	–	–	–	–	–
N	0.2	–	–	–	–	–	–	–	–	–

Nineteen haplotypes of *M. adspersa* from Atherton Tableland (northern Qld) containing 700 bp of the ATPase 6 and 8 regions (GenBank Accession numbers AF046101–AF046119) (Hurwood and Hughes 1998) were also included to enable a phylogenetic analysis across the species range. Analysis of the control region and ATPase 6 and 8 separately yielded similar results to the combined dataset (the results of which are shown here). The most likely model of DNA substitution for the combined control region and ATPase 6 and 8 dataset was assessed using MODELTEST 3 (Posada and Crandall 1998). The selection was based on a series of hierarchical likelihood ratio tests conducted over 56 models of nucleotide substitution. The selected model was the Tamura-Nei distance with a gamma distribution of shape parameter = 0.4237 (TrN+G). This model corrects for multiple hits, taking into account the differences in substitution rate between nucleotides and the inequality of nucleotide frequencies (Tamura and Nei 1993). The TrN+G model was used for subsequent analyses of genetic diversity using ARLEQUIN and phylogenetic analyses using PAUP* 4.0b10 (Swofford 2003).

Levels of population subdivision were quantified using the hierarchical partitioning of molecular variance (AMOVA). The following levels were investigated: (i) within and among populations in the MDB; (ii) within and among populations in coastal Qld; and (iii) within and between the two groups, MDB and coastal Qld.

Phylogenetic reconstructions were carried out using maximum likelihood and neighbour joining methods starting from consensus sequences for each sample. *Mogurnda clivicola*, *Mogurnda larapintae* and *Mogurnda thermophila* were used as outgroup taxa. The strength of tree nodes was determined by bootstrap analysis. This section of the analysis was conducted with ATPase 6, 8 sequences only to make possible the inclusion of Hurwood and Hughes (1998) dataset.

Genealogical relationships within *M. adspersa* were investigated by constructing a haplotype network with the statistical parsimony method implemented in TCS (Clement *et al*. 2000) using data from the three mtDNA genes. The statistical parsimony method links first haplotypes with the smaller number of sequence differences, providing high resolution for inferring relationships among recently diverged populations. It also estimates haplotype outgroup probabilities, allowing inference of the most ancient haplotype in the sample.

Results

Genetic variation

We obtained and confidently aligned 339 bp of the control region and 802 bp of ATPase 6 and 8 for 85 *Mogurnda adspersa* samples (Table 1). There were four control region and 13 ATPase 6, 8 haplotypes. Of the 339 bp of the control region, six sites were variable with a Ts:Tv of 2.0. Of the 802 bp of ATPase 6 and 8, 28 sites were variable with a Ts:Tv of 3.7. The resulting 1141 bp of mtDNA had 34 variable positions and a Ts:Tv of 3.3. Sequence comparison of the three combined mtDNA regions revealed very low levels of genetic variation within populations and a total of 14 haplotypes. Most haplotypes were population specific (12 out of 14 haplotypes). The coastal Qld population of Kilcoy showed the highest genetic variability with five haplotypes. Six of the eight MDB populations were fixed for a single haplotype, the remaining two showed two haplotypes.

Table 2. Results of AMOVA for populations of *Mogurnda adspersa*
MDB: Murray–Darling Basin; Qld: Queensland

Level of partitioning	F-statistics
Among MDB populations	$\theta_{ST} = 0.86$, $P < 0.001$
Among coastal Qld populations	$\theta_{ST} = 0.40$, $P < 0.001$
Between MDB and coastal Qld populations	$\theta_{CT} = 0.79$, $P = 0.02$
Among populations within the MDB and coastal Qld	$\theta_{SC} = 0.97$, $P < 0.001$
Among all populations	$\theta_{ST} = 0.88$, $P < 0.001$

There was marked genetic structure across the study area (Table 2). There was a significantly high level of differentiation between the MDB and coastal Qld ($\theta_{CT} = 0.79$, $P = 0.02$), among populations within the MDB and coastal Qld ($\theta_{SC} = 0.88$, $P < 0.001$), and among all populations ($\theta_{ST} = 0.97$, $P < 0.001$). When tested separately, there was a higher level of differentiation among the MDB populations ($\theta_{ST} = 0.86$, $P < 0.001$) than the coastal Qld populations ($\theta_{ST} = 0.40$, $P < 0.001$).

Phylogenetic relationships, phylogeographic structure and demographic history

Neighbour-joining and maximum-likelihood methods produced trees with very similar topologies. Our molecular phylogenetic analysis revealed four well-supported major clades in *M. adspersa*: two represented by the northern Qld populations, a third by southern Qld and a fourth by MDB populations (Fig. 2). The northern Qld lineages appear to be older, showing much higher sequence divergences (up to 4.14%) than those found in southern Qld (0.85%) or in the MDB (0.71%) populations. Divergence values between the northern Qld and MDB populations are high, ranging from 3.71 to 5.43%. Of note is the grouping of *Mogurnda clivicola* with the northern Qld haplotypes of *M. adspersa*, a result that suggests taxonomic uncertainty in the genus.

Marked phylogeographic structure was found in *M. adspersa* (Fig. 3), with the distinctive separation of coastal and MDB populations in divergent phylogroups. Southern Qld and MDB phylogroups are at least 19 mutational steps apart. Within the Murray–Darling, haplotype A appears ancestral, being the most common and widespread (Farm Creek (Qld), Tenterfield and Bingara (NSW)). Haplotype A probably acted as the source lineage from which other MDB haplotypes have recently originated. Despite reduced levels of divergence in the MDB, moderate phylogeographic structure was detected. For instance, the closely related haplotypes C, D and E are found in northern NSW (Severn River, Deepwater River, Bingara, and Inverell), and the remaining haplotypes B, F and G in isolated populations in central NSW (Wuluuman Creek) and southern Qld (Condamine and Farm Creek). In southern coastal Qld, haplotypes H to L are found in Kilcoy and are separated by seven mutational steps to haplotypes M and N from the Mary River.

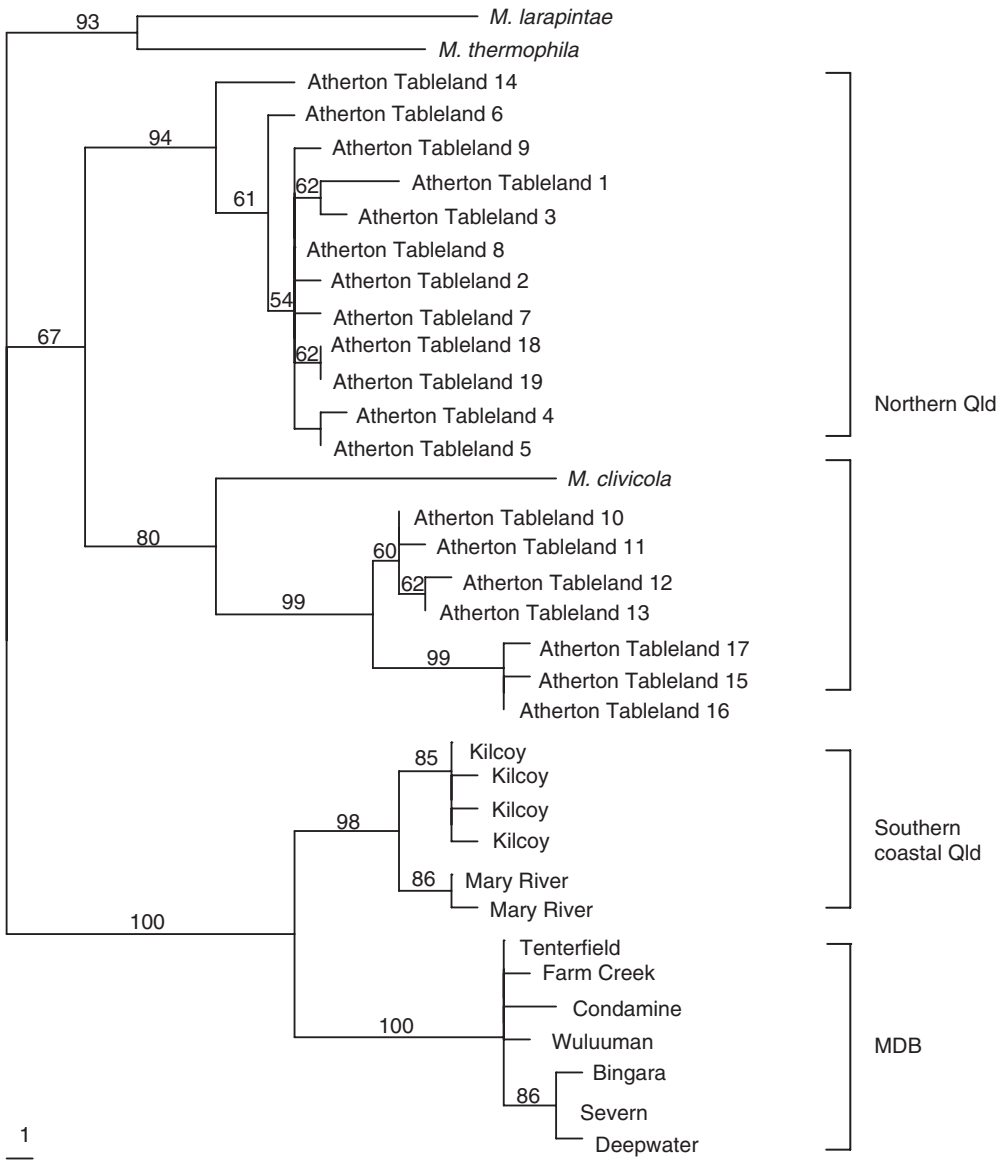

Fig. 2. Maximum likelihood tree showing relationships among *Mogurnda adspersa* populations based on 700 bp of the mtDNA ATPase 6 and 8. The tree was constructed using a Tamura-Nei genetic distance with a gamma distribution (details in the text). The outgroups are *Mogurnda* species from central Australia. Bootstrap values greater than 50% are presented above the branches and the scale bar represents one nucleotide change. Three clades are indicated; northern Queensland (Qld), southern coastal Qld, and Murray–Darling Basin (MDB) populations.

Discussion

Evolutionary history and phylogeography of Mogurnda adspersa

Here we present evidence for marked levels of phylogeographic structure in *Mogurnda adspersa*, a small freshwater fish of conservation concern, from south-eastern Australia. We identify four phylogroups: two from northern coastal Qld (Atherton Tableland), one from southern coastal Qld (Kilcoy and Mary River) and the fourth composed of small and isolated populations from the MDB. Two phylogroups, southern coastal Qld and the MDB, form a distinct and highly divergent clade compared with that from northern Qld (sequence divergences of up to 5.4%). Assuming a tentative molecular clock for the mtDNA ATPase 6 and 8 dataset of 1.4% per million years (Bermingham *et al*. 1997), the separation between these clades took place ~2.6–3.9 million years ago. The most likely geographic process that may have facilitated expansion and subsequent divergence of coastal populations during that period is drainage rearrangement at headwaters caused by tectonic activity or erosion (Unmack 2001).

Although the southern coastal Qld and the MDB phylogroups are more related to each other (sequence divergences of 2.00–2.57%), it appears that the MDB populations are evolutionarily younger and have colonised the Murray–Darling river system in

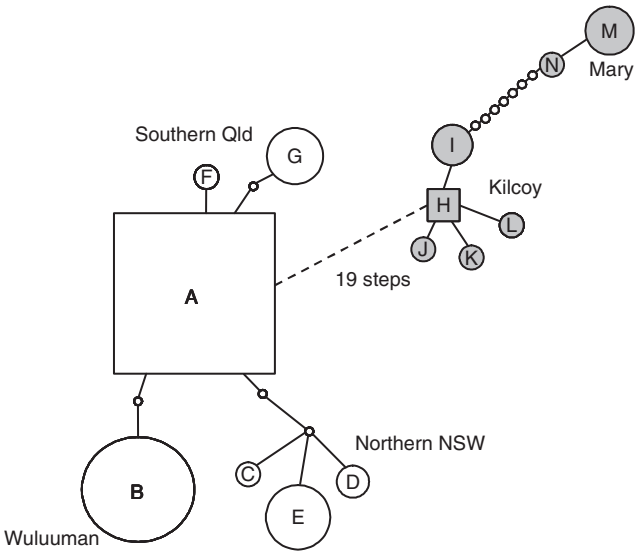

Fig. 3. Network showing the relationships among *Mogurnda adspersa* populations based on mtDNA control region and ATPase 6, 8 haplotypes. Letters correspond to haplotypes as in Table 1 and the size of ovals and squares to the frequency of a particular haplotype in the sample. Haplotypes separated by single lines are one mutation apart, and small circles along lines represent missing haplotypes (not sampled or extinct). Squares are haplotypes considered as ancestral based on coalescent criteria. Shaded ovals are haplotypes found on the coast and clear ones from the Murray–Darling Basin. The minimum number of steps between lineage A (MDB) and H (southern coastal Qld) is 19 (not supported by statistical parsimony).

the relatively recent past (Fig. 2). Using the ATPase molecular clock, we obtain a time since separation of the southern coastal Qld and the MDB phylogroups of around 1.4–1.8 million years. The Great Dividing Range was formed ~90 million years ago (Veevers 1984), and is the major biogeographical barrier between coastal and inland populations. However, given that this event greatly pre-dates the separation of MDB and coastal populations of *M. adspersa*, a more recent biogeographical scenario must be proposed. There are several sites along the Great Dividing Range that are of lower elevation and could represent possible pathways between inland and coastal drainages (Unmack 2001). For *M. adspersa*, the headwaters of the Fitzroy and Burnett Rivers in Qld and the Clarence River in NSW are potentially important for understanding phylogeographic patterns. Some of the processes that could have accounted for the phylogeographic structure observed in *M. adspersa* are drainage rearrangement via river capture, beheading or diversion (Bishop 1995; McGlashan and Hughes 2000, 2001; Waters and Wallis 2000). In a geologically complex drainage system like the Clarence, a combination of these and other historical events may have provided the chance for *M. adspersa* to cross the Great Dividing Range (Haworth and Ollier 1992). Recent drainage rearrangement in the Clarence River area has been suggested as being responsible for the divergence of *Craterocephalus stercusmuscarum* populations (McGlashan and Hughes 2001) at a similar period (around 1 million years) to that for *M. adspersa*. Although the coastal–inland phylogeographic break occurs in several Australian freshwater fish, the mechanisms by which species have crossed the divide are still largely speculative. Comparative phylogeographic studies of codistributed freshwater taxa using a combination of nuclear, mtDNA and geomorphological datasets could provide a powerful framework for investigating the biogeographic history of the eastern Australian aquatic fauna (e.g. Carini *et al*. 2006; Huey *et al*. 2006; Hughes and Hillyer 2006). This is a promising area of interdisciplinary research that could also become an integral component of the management of freshwater fish in eastern Australia.

Conservation implications

The MDB populations of *M. adspersa* are genetically and evolutionarily distinct from those found elsewhere in Australia, including those from coastal Qld. All maternal lineages found in the MDB show reciprocal monophyly and appear to have evolved independently from other *Mogurnda* lineages during a period of not less than 1 million years. The evolutionary distinctiveness and geographic segregation of MDB populations warrant their classification as an Evolutionary Significant Unit (ESU) (Ryder 1986; Dizon *et al*. 1992; Moritz 1994*a*, 1994*b*, 1995; Vogler and Desalle 1994; Bernatchez 1995; Legge *et al*. 1996; Crandall *et al*. 2000; Beheregaray *et al*. 2003). An ESU is a biological unit with a distinct, long-term evolutionary history that should be managed separately (Ryder 1986). Future studies assessing morphological and functional diversity in *M. adspersa* from the MDB should be conducted to verify if the phylogeographic differentiation reported here is consistent with ecological divergence (*sensu* Crandall *et al*. 2000).

The pattern of genetic structure and variability detected here for the MDB populations also indicates that they are of high conservation priority and that their current listing as an 'endangered population' (NSW Fisheries Scientific Committee) is warranted. Within the MDB, most populations were fixed for a single maternal lineage; this contrasts markedly with the higher diversity observed in the more abundant 'non-endangered' southern coastal Qld population (seven haplotypes identified from only nineteen individuals). AMOVA also indicates that there is a higher level of genetic variability within populations in coastal Qld compared with the MDB ($\theta_{ST} = 0.40$, $P < 0.001$ and $\theta_{ST} = 0.86$, $P < 0.001$ respectively). The finding of reduced genetic diversity in endangered species or populations compared with their non-endangered counterparts is common (Frankham 1995; Barrowclough *et al*. 1999; Parsons *et al*. 2002).

The decline in range and abundance of MDB populations in the 1980s would have reduced their population size and genetic variability, and restricted opportunities for gene flow. Limited gene flow among MDB populations is indicated by the specificity of haplotypes to single populations and geographic locations, and the particularly high population differentiation ($\theta_{ST} = 0.86$, $P < 0.001$) compared with other levels of the AMOVA. This combination of factors is potentially a better explanation for the high sorting and low variability in the mtDNA dataset than assuming strong male-biased dispersal. The latter is unlikely to be the case for this species as Hurwood and Hughes (1998) found restricted gene flow in northern Qld populations using both mitochondrial and biparentally inherited allozyme data.

Lack of gene flow among populations is a driving force in the reduction of genetic diversity, which in turn renders species

more susceptible to extinction (Frankham *et al*. 2002). Management actions for such species include: increasing population size through translocation, rehabilitation and protection of habitats and captive breeding programs. These options have been considered by NSW Department of Primary Industries. Although the remnant populations of *M. adspersa* continue to be monitored and protected under the *Fisheries Management Act 1994*, further management is required. Management actions for such species include: rehabilitation and protection of habitats; increasing population size and genetic rescue through translocation; or captive breeding and reintroductions into areas where populations have become extinct.

The present study has determined that the MDB populations of *M. adspersa* represent an ESU and require separate management from the coastal populations. As a consequence, inter-basin genetic rescue is not recommended. The limited genetic divergence among populations within the MDB suggests that intra-basin genetic rescue could be an option (Tallmon *et al*. 2004). Current recovery efforts are focussed on a captive breeding and reintroduction program into catchments where *M. adspersa* is known to be locally extinct. This program has been designed to ensure that genetic diversity is augmented by using broodstock from all available remnant MDB populations. This serves to 'artificially' restore gene flow among populations. Hence, the reintroduced fish will show greater potential to establish new populations and adapt. However, habitat restoration and protection of remnant populations to encourage natural recruitment and recovery are also equally important management considerations. The results of the present study demonstrate the importance of phylogeographic studies to the development of integrated conservation strategies fundamental to threatened species management.

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